The Analyst

The Analytical Journal
of The Chemical Society

A monthly international publication dealing with all branches of analytical chemistry

Volume 105 No 1248 Pages 193–304 March 1980

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

Published by The Chemical Society

Editorial: The Director of Publications, The Chemical Society, Burlington House, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001

Advertisements: Advertisement Department, The Chemical Society, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-734 9864

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

Volume 105 No 1248 March 1980

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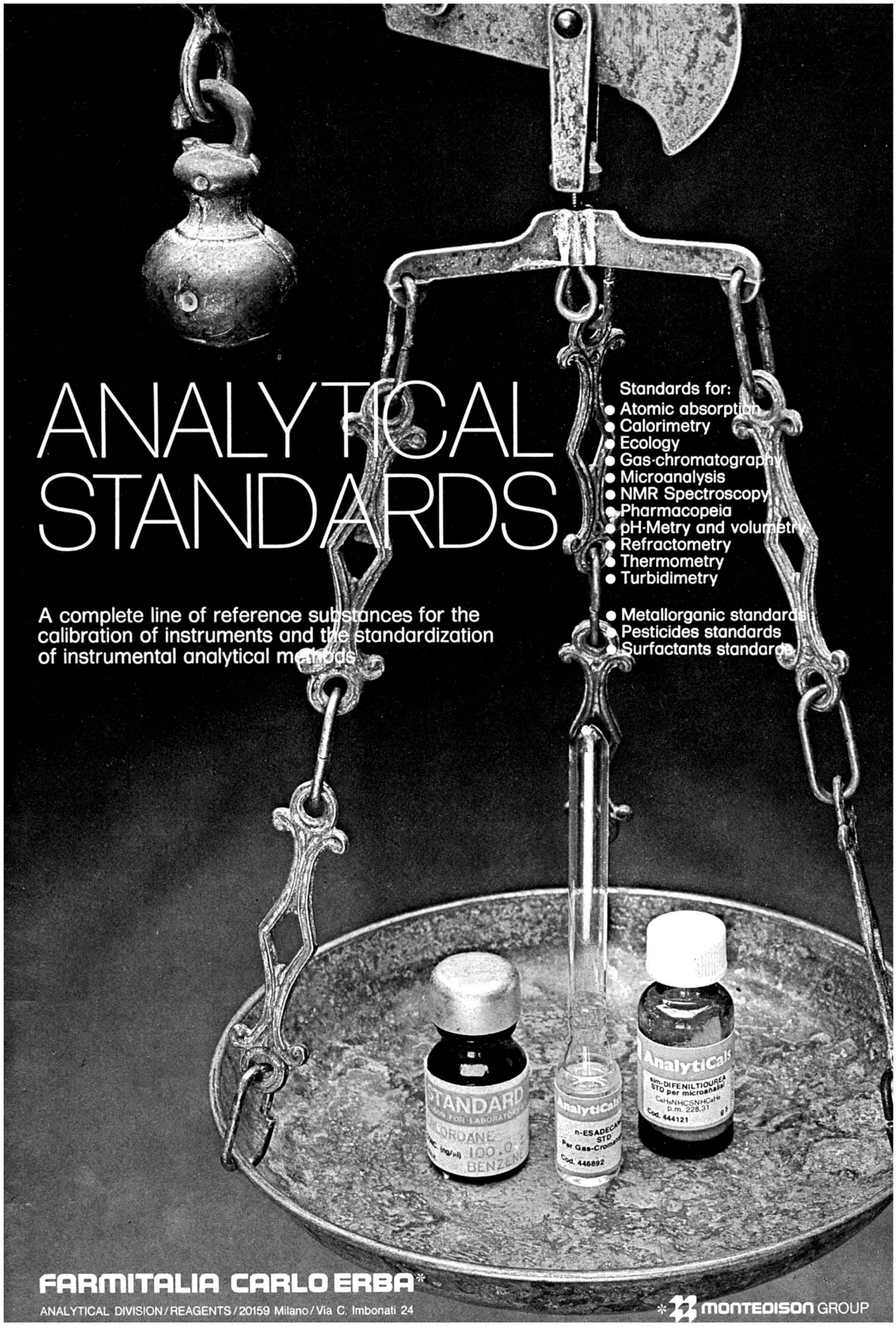
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Summaries of Papers in this Issue

Spectrophotometric Determination of Microgram Amounts of Calcium in Waters and Foods Using Diphenylglyoxal Bis(2-Hydroxybenzoyl Hydrazone)

The synthesis, characteristics and analytical applications of diphenylglyoxal bis(2-hydroxybenzoyl hydrazone) are described. This compound reacts with calcium(II) at pH 12 to produce a yellow complex ($\lambda_{max.}$ = 432 nm, ϵ = 1.76 × 10^4 l mol^{-1} cm^{-1}); another complex (1 : 1) can be detected at pH 7.80. Dipyridylglyoxal bis(2-hydroxybenzoyl hydrazone) also reacts with calcium and both reagents are compared. A sensitive and selective spectrophotometric method is proposed for the determination of calcium using diphenylglyoxal bis(2-hydroxybenzoyl hydrazone). Interferences have been investigated and when masking agents are added common cations do not interfere. The yellow calcium(II) complex has been used for the determination of calcium in waters and foods. The results are compared with those obtained using glyoxal bis(2-hydroxyanil).

Keywords: *Calcium determination*; *spectrophotometry*; *diphenylglyoxal bis-(2-hydroxybenzoyl hydrazone)*; *waters*; *foods*

M. SILVA and M. VALCÁRCEL

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

Analyst, 1980, **105**, 193–202.

Spectrophotometric Determination of Cobalt with Biacetyl Mono(2-pyridyl)hydrazone

The absorbance *versus* pH graphs and stoicheiometry of the complexes formed by biacetyl mono(2-pyridyl)hydrazone (BPH) with cobalt(II), nickel(II), copper(II) and iron(II) have been studied. A method for the spectrophotometric determination of cobalt has been devised. The orange - red chelate is formed at acidities between 2 and 10^{-6} M (pH 6), in aqueous ethanolic solution, and has $\lambda_{max.}$ at 505 nm with a molar absorptivity of 2.3 × 10^4 l mol^{-1} cm^{-1}. The method has been applied to the determination of cobalt in nitrates and has been compared with other pyridylhydrazone procedures for the spectrophotometric determination of cobalt.

Keywords: *Biacetyl mono(2-pyridyl)hydrazone reagent*; *cobalt determination*; *spectrophotometry*

A. G. ASUERO and M. M. RODRIGUEZ

Department of Applied Chemical Analysis, Faculty of Pharmacy, University of Seville, Seville-4, Spain.

Analyst, 1980, **105**, 203–208.

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Precision Spectrophotometric Determination of Chromium in Chromite Ores and Ferro-chrome

The chemical and manipulative variables have been examined in detail in order to develop a precise and direct spectrophotometric determination of chromium in chromite ores and in ferro-chrome. Samples are sintered with sodium peroxide in zirconium crucibles for 3 h at 510 ± 10 °C, then leached, filtered, acidified with perchloric acid and reduced with hydrazine hydrate. Chromium is determined from the absorbance of the chromium(III) aquo ion at 410 and 578 nm. The procedure has been evaluated using standard chrome ores, ferro-chrome alloys and a series of Sudanese and industrial ferro-chrome samples. The results are in good agreement with, and of comparable precision to, published data and to those obtained by titrimetric assay.

Keywords: *Precision spectrophotometry*; *chromium determination*; *chromite ores*; *ferro-chrome alloys*

D. THORBURN BURNS and M. E. M. ABDEL AZIZ

Department of Analytical Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland.

Analyst, 1980, **105**, 209–216.

Selective Colorimetric Detection of Carboxylic Acids

A selective colour reaction for the detection of carboxylic acids has been developed. Acetic anhydride in the presence of ammonium carbonate, sodium carbonate and the sodium salts of acetic, formic, oxalic and pyruvic acids is used as the reagent. Amongst 39 acids, citric, isocitric, α-keto-glutaric and oxaloacetic acids can be detected selectively.

Keywords: *Carboxylic acid detection*; *colorimetry*; *acetic anhydride reagent*

H. S. RATHORE, M. N. AKHTAR and S. K. SHARMA

Chemistry Section, Zakir Husain College of Engineering and Technology, Aligarh Muslim University, Aligarh-202001, India.

Analyst, 1980, **105**, 217–221.

Use of Orthogonal Function in Differential Spectrophotometry

An orthogonal function method has been applied to the differential spectrophotometric determination of acetazolamide and hydrochlorothiazide in tablets. The error resulting from setting the wavelength scale during the application of the orthogonal function to conventional spectrophotometry is thereby minimised. The results of assay using the proposed method [$p_{2(r)}$ method] are more precise than those obtained with the p_2 method.

Keywords: *Pharmaceutical analysis*; *differential ultraviolet spectrophotometry*; *orthogonal function*

M. ABDEL-HADY ELSAYED, YOUSRY M. ELSAYED and HASSAN ABDINE

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

Analyst, 1980, **105**, 222–226.

Tungsten Filament Vaporiser and Oxyhydrogen Flame for Optical-emission Spectrometry

The vaporisation of a sample from a tungsten filament into a hydrogen atmosphere with subsequent combustion in oxygen provides a novel source for optical-emission spectrometry. The burner and ancillary equipment are described. Some of the factors affecting sensitivity, particularly in relation to flame background, are discussed. Results are presented for calcium, chromium, copper, iron, lead, lithium, magnesium, manganese, nickel, silver, strontium and thallium. The precision is about 5% for a 3-μl sample of total element content between 10 pg (for lithium) and about 7 ng (for lead).

Keywords: *Flame-emission spectrometry*; *tungsten filament vaporiser*; *micro-samples*; *oxyhydrogen flame*

R. L. WARREN

Institute of Nuclear Medicine, Middlesex Hospital Medical School, Mortimer Street, London, W1N 8AA.

Analyst, 1980, **105**, 227–233.

Effect of Tetraphenylphosphonium Chloride on D.C. and Differential-pulse Polarograms of Synthetic Food Colouring Matters

Tetraphenylphosphonium chloride (TPPC) has a pronounced effect on the d.c. and differential-pulse (d.p.) polarograms of some synthetic food colouring matters. The d.c. half-wave potentials and d.p. peaks of two pyrazole azo colouring matters, tartrazine and Yellow 2G, and of the triphenylmethane colouring matter Brilliant Blue FCF, are shifted to more negative potentials on the addition of TPPC. The mean limiting diffusion currents and peak currents of these and certain other synthetic colouring matters are altered significantly on its addition, the changes being greater in d.p. polarography. For both d.c. and d.p. polarograms the changes occur at TPPC concentrations up to 100 μg ml^{-1}; above this concentration half-wave and peak potentials and limiting diffusion and peak currents change very little in most instances. The implication of these effects on the use of polarography for the determination of synthetic food colouring matters is discussed.

Keywords: *Tetraphenylphosphonium chloride*; *food colouring matters*; *differential-pulse polarography*

A. G. FOGG and D. BHANOT

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU.

Analyst, 1980, **105**, 234–240.

Determination of Nitrate and Nitrite in Meat Products by Using a Nitrate Ion-selective Electrode

A procedure for the determination of nitrate and nitrite in meat products by a direct potentiometric method with a nitrate ion-selective electrode is presented. The soluble nitrate and nitrite were extracted from the meat products with a Soxhlet extractor using borax buffer solution (pH 9). The effect of pH on the recovery of nitrite and nitrate was studied. Results obtained by the proposed method were compared with those obtained by recommended spectrophotometric methods. Storage of samples before analysis was also studied.

Keywords: *Nitrate determination*; *nitrite determination*; *meat analysis*; *potentiometry*; *nitrate ion-selective electrode*

K. K. CHOI and K. W. FUNG

Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong.

Analyst, 1980, **105**, 241–245.

Simple and Rapid Determination of Iodine in Milk by Radioactivation Analysis

Iodine has been determined in milk samples by radioactivation analysis by using a Van de Graaff accelerator. The iodine is separated from the irradiated sample with an iodine-loaded resin. The limits of detection of iodine by gamma-ray spectrometry and beta-counting are 10 and 5 μg, respectively. The precision of the proposed method is $\pm 5\%$ when the iodine content exceeds 0.01 p.p.m.

Keywords: *Iodine determination*; *milk*; *radioactivation analysis*

S. OHNO

National Institute of Radiological Sciences, 9–1, 4-chome, Anagawa, Chiba-shi, Japan.

Analyst, 1980, **105**, 246–250.

Determination of Residual Methyl Bromide in Fumigated Commodities Using Derivative Gas - Liquid Chromatography

A sensitive method for the determination of residual methyl bromide in fumigated foodstuffs is described. Methyl bromide in extracts was converted stoicheiometrically into methyl iodide by reaction with sodium iodide and analysed using gas - liquid chromatography with electron-capture detection. Residual methyl bromide was readily detected at the 10 μg kg^{-1} level in a variety of foodstuffs.

Keywords: *Methyl bromide determination*; *fumigation*; *residues analysis*; *derivative gas - liquid chromatography*

R. J. FAIRALL and **K. A. SCUDAMORE**

Ministry of Agriculture, Fisheries and Food, Slough Laboratory, London Road, Slough, Berkshire, SL3 7HJ.

Analyst, 1980, **105**, 251–256.

Assay of Carbaryl in Honey Bees (*Apis mellifera*) by High-performance Liquid Chromatography

A method for detecting and measuring carbaryl in poisoned honey bees (100 ng per bee) using high-performance liquid chromatography is described.

Clean-up of extracts on a Florisil column removed all substances that interfered with fluorescence detection and most that affected ultraviolet detection at 215 nm.

Gas chromatography of carbaryl derivatives was not consistently useful because bee constituents interfered either with the formation of derivatives or with detection. Only the *N*-acetyl derivative was formed quantitatively in the presence of cleaned-up bee extracts, but the nitrogen-specific detector was sometimes, and the electron-capture detector always, subject to interference from bee constituents remaining after clean-up.

Keywords: *Carbaryl residues*; *high-performance liquid chromatography*; *gas chromatography*; *honey bees*

K. A. LORD, G. R. CAYLEY, L. E. SMART and R. MANLOVE

Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ.

Analyst, 1980, **105**, 257–261.

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Spectrophotometric Determination of Microgram Amounts of Calcium in Waters and Foods Using Diphenylglyoxal Bis(2-hydroxybenzoyl Hydrazone)

M. Silva and M. Valcárcel

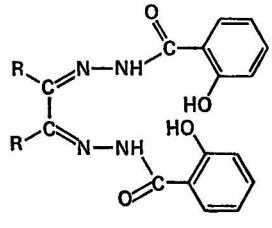
Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Córdoba, Spain

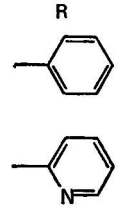
The synthesis, characteristics and analytical applications of diphenylglyoxal bis(2-hydroxybenzoyl hydrazone) are described. This compound reacts with calcium(II) at pH 12 to produce a yellow complex ($\lambda_{max.}$ = 432 nm, ϵ = 1.76×10^4 l mol^{-1} cm^{-1}); another complex (1 : 1) can be detected at pH 7.80. Dipyridylglyoxal bis(2-hydroxybenzoyl hydrazone) also reacts with calcium and both reagents are compared. A sensitive and selective spectrophotometric method is proposed for the determination of calcium using diphenylglyoxal bis(2-hydroxybenzoyl hydrazone). Interferences have been investigated and when masking agents are added common cations do not interfere. The yellow calcium(II) complex has been used for the determination of calcium in waters and foods. The results are compared with those obtained using glyoxal bis(2-hydroxyanil).

Keywords: *Calcium determination*; *spectrophotometry*; *diphenylglyoxal bis-(2-hydroxybenzoyl hydrazone)*; *waters*; *foods*

This work forms part of a systematic investigation of the use of α-bisaroyl hydrazones as analytical reagents. Only the bis(4-hydroxybenzoyl hydrazones) of glyoxal, methylglyoxal and dimethylglyoxal have been described as colorimetric and fluorimetric reagents for the determination of calcium(II), cadmium(II), lanthanum(III) and bismuth(III),[1] but the monoaroyl hydrazones have been more widely used in chemical analyses.[2] Pyridine-2-carbaldehyde 2-hydroxybenzoyl hydrazone has been investigated as a spectrophotometric reagent for the determination of nickel and zinc,[3] vanadium(V)[4] and iron(II).[5]

The preparation and properties of the bis(2-hydroxybenzoyl hydrazones) of diphenylglyoxal (BSHB) and dipyridylglyoxal (BSHP) are described in this paper.





R	Compound
	BSHB
	BSHP

The calcium(II) - BSHB complex is of great interest and several spectrophotometric methods for the determination of trace amounts of calcium in waters and foods have been reported. The over-all results are discussed and compared with those obtained using the classical spectrophotometric reagent for calcium, glyoxal bis(2-hydroxyanil) (GBHA) [di(2-hydroxyphenylimino)ethane].[6–10]

A search of the literature revealed few colorimetric methods for the determination of calcium in comparison with other metal ions. Sandell quoted five methods in detail.[11] Marczenko[12] reported only two main methods, using GBHA and murexide.[13–15] Other methods for the spectrophotometric determination of calcium include the use of azo reagents: Chloro-

phosphonazo III[16,17]; Calcichrome III[18–20]; Arsenazo I[21,22]; Arsenazo III[23,24]; Eriochrome Black T[25–27]; calmagite[28]; Acid Chrome Blue K[29]; calcon[30]; and Azo-azoxy BN.[31] Some complexone derivatives have also been employed: calcein[32]; metalphthalein[33]; thymolphthalexone[34]; bromo-oxine[35,36]; and TTA [4,4,4-trifluoro-1-(2-thienyl)butane-1,3-dione].[37]

Experimental

Apparatus

The pH measurements were carried out with a Philips PW 9408 pH meter, equipped with a combined glass - calomel electrode. The absorption spectra were recorded on a Unicam SP 8000 spectrophotometer and measurements at fixed wavelengths were recorded on a Beckman DU spectrophotometer in the ultraviolet region and on a Coleman 55 (digital) spectrophotometer in the visible region. Silica or glass (according to the wavelength required) 1-cm path length cells were used. The infrared spectra were recorded on a Perkin-Elmer, Model 577, spectrophotometer.

Reagents

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

Diphenyl- and dipyridylglyoxal bis(2-hydroxybenzoyl hydrazone) reagent solutions. Prepare 0.1% *m/V* solutions in dimethylformamide - ethanol (1 + 1). These solutions are stable for at least 1 week.

Glyoxal bis(2-hydroxyanil) (Merck) stock solution. Prepare a 0.05% *m/V* solution in methanol.

Standard calcium solution, 1 mg ml^{-1}. Dissolve 2.498 g of calcium carbonate (Carlo Erba), which has been dried at 110 °C, in 40 ml of 2 M hydrochloric acid. Remove any carbon dioxide by boiling, and dilute the solution to 1 l with distilled water in a calibrated flask.

Procedure

Synthesis of bisaroyl hydrazones

Equimolecular amounts of 2-hydroxybenzoylhydrazide (Ega Chemie), diphenylglyoxal (Merck) or dipyridylglyoxal (Ega Chemie) were mixed in ethanol - water (1 + 1); several drops of concentrated hydrochloric acid were added and the mixture was refluxed for 30 min. The white BSHB and yellow BSHP compounds were separated by filtration. The products obtained were washed with hot ethanol - water (1 + 1).

The elemental analysis results were as follows. Calculated for BSHB ($C_{28}H_{22}N_4O_4$), C 70.29%, H 4.60% and N 11.71%; found, C 70.0%, H 4.6% and N 11.5% (yield 53% and melting-point above 300 °C). Calculated for BSHP ($C_{26}H_{20}N_6O_4$), C 65.00%, H 4.16% and N 17.50%; found, C 64.9%, H 4.2% and N 17.3% (yield 47% and melting-point 281–282 °C).

Determination of calcium with BSHB

The sample solution must be neutral (pH 6–8) and contain 12.5–50 μg of calcium. Add 10 ml of the BSHB solution to the sample solution in a 25-ml calibrated flask and then 3 ml of 0.1 M sodium hydroxide solution and dilute with distilled water to the mark. Measure the absorbance of the solution at 432 nm against a reagent blank.

If the analytical group I–III metals (hydrogen sulphide scheme) are present, add approximately 10 mg of potassium cyanide or 0.2 ml of mercaptoacetic acid, in order to mask small amounts of these metals.

Other experimental procedures

Calcium has been determined by the BSHB method in waters and foods. The pre-treatments required by the various samples are described below.

Waters. Filter sample waters before the determination in order to remove any suspended matter.

Egg. Add a sample of the egg yolk or white and 20 ml of 10% *m/V* sodium carbonate solution to a porcelain capsule and evaporate to dryness at 100–105 °C. Transfer the capsule, while hot, into a furnace at 500 °C for 1 h. Cool, add a few drops of distilled water, break

up the residue with the flat end of a glass rod, and cover the capsule with a watch-glass; slowly add 10 ml of nitric acid (1 + 3), while stirring, filter, wash the charred material thoroughly with distilled water and collect the filtrate in a 100-ml calibrated flask.

Milk. To 10 ml of milk, in a 100-ml Erlenmeyer flask, add slowly and with constant stirring 10 ml of 20% *m/V* trichloroacetic acid solution. Place the flask in a boiling water-bath for 30 min. Cool, filter, wash with distilled water and collect the filtrate in a 100-ml calibrated flask.

Cheese. To a 1.5–3.5-g sample of grated cheese in a 100-ml Erlenmeyer flask, add 10 ml of 40% *m/V* urea solution and 5 ml of 4 N hydrochloric acid and warm gently. Transfer the mixture into a porcelain capsule and evaporate to dryness on a sand-bath at 100–105 °C and proceed as for egg.

Orange juice. Evaporate 50 ml of juice, in a porcelain capsule, to dryness on a sand-bath at 100–105 °C. Place the capsule in a furnace at 500 °C for 1 h. Cool, add 10 ml of distilled water and 5 ml of concentrated hydrochloric acid and evaporate to dryness. Extract the residue with 25 ml of hydrochloric acid (1 + 9), heat for 15 min and filter.

Beer. Add 50 ml of beer and 4 g of sodium carbonate to a porcelain capsule and evaporate to dryness, then proceed as for orange juice.

Results and Discussion

Analytical Properties of the Reagents

The solubilities of the BSHB and BSHP reagents were measured in various solvents, and were found to be very low in organic solvents, except for dimethylformamide. In dimethylformamide the solubilities of BSHB and BSHP are 32.65 and 14.95 g l^{-1}, respectively. In, for example, chloroform, ethanol, benzene and nitrobenzene the solubilities are less than 1.0 g l^{-1}.

The infrared spectra of the BSHB and BSHP were obtained using potassium bromide discs. Both spectra are analogous and selected infrared absorption bands were assigned to stretching vibrations of the NH bond (3200 cm^{-1}), the C=N bond (1660 cm^{-1}), the OH bond (1160 cm^{-1}) and the C=O bond (1680 cm^{-1}) for both bis(2-hydroxybenzoylhydrazones).

When measuring the ultraviolet spectra a water - dimethylformamide (3 + 2) medium was used in order to prevent the precipitation of the reagents and their complexes. In this medium a BSHB solution of 1.4×10^{-4} M shows absorption maxima at 305 and 325 nm. The ultraviolet spectrum of the BSHP for a 2×10^{-5} M solution shows two analogous absorption maxima at 305 and 320 nm. In both instances the ultraviolet spectra of these reagents show bathochromic shifts in an alkaline medium ($\lambda_{max.} = 370$ nm).

The Phillips and Merritt method[38] was used for the determination of the ionisation constants; the average p*K* value found for the diphenylglyoxal bis(2-hydroxybenzoylhydrazone) was 8.20 ± 0.05. This behaviour may be caused by de-protonation of the hydroxyl groups.

TABLE I

CHARACTERISTICS OF BSHB COMPLEXES IN SOLUTION

	Buffer solution, pH 4.7			Buffer solution, pH 9.8		
Metal ion	$\lambda_{max.}$/ nm	Molar absorptivity/ 10^3 l mol^{-1} cm^{-1}	Colour of complex	$\lambda_{max.}$/ nm	Molar absorptivity/ 10^3 l mol^{-1} cm^{-1}	Colour of complex
Pb(II) ..	435	13.4	Yellow	450	29.0	Yellow
Cu(II) ..	455–480	4.8	Brown	430	4.2	Yellow
Ni(II) ..	425	3.1	Yellow	445	5.7	Yellow
Co(II) ..	410	5.1	Orange	465	1.6	Orange
Bi(III) ..	470	17.7	Orange	460	20.5	Orange
Sn(II) ..	450	22.5	Orange - yellow	450	10.5	Orange - yellow
Fe(II) ..	425	3.5	Brown - red	450	2.3	Brown - red
UO_2(II) ..	440	19.6	Yellow	445	19.5	Yellow
Ti(IV) ..	460	15.3	Orange	460	5.0	Orange
In(III) ..	440	46.0	Yellow	440	45.8	Yellow
Ca(II) ..				432	17.5	Yellow
Mg(II) ..				430	1.0	Yellow
Sr(II) ..				430	5.5	Yellow
Ba(II) ..				430	1.7	Yellow

BSHP gave two dissociation constants in the medium used, with average values of 3.27 ± 0.05 and 7.85 ± 0.05. The first pK value may be that of the protonated pyridine nitrogen atom and the second that of the hydroxyl groups.

Solutions (0.1% m/V) of the reagents were stable for at least 1 week. The C=N groups of both reagents are resistant to hydrolysis at any pH.

The reactions of the reagents with 30 cations at various pH values were investigated in a medium containing 60% V/V of dimethylformamide and 40% V/V of water. The characteristics of the most important BSHB and BSHP complexes in solution are shown in Tables I and II.

TABLE II

CHARACTERISTICS OF BSHP COMPLEXES IN SOLUTION

	Buffer solution, pH 4.7			Buffer solution, pH 9.8		
Metal ion	$\lambda_{max.}$/nm	Molar absorptivity/10^3 l mol^{-1} cm^{-1}	Colour of complex	$\lambda_{max.}$/nm	Molar absorptivity/10^3 l mol^{-1} cm^{-1}	Colour of complex
Pb(II) ..	385	14.9	Yellow	450	20.5	Yellow
Cu(II) ..	380	47.0	Yellow	445	51.5	Yellow
Ni(II) ..	380	46.4	Yellow	425	10.0	Yellow
Co(II) ..	375	96.6	Yellow	460	15.3	Orange
Bi(III) ..	390–470	12.2	Orange	465	20.6	Orange
Sn(II) ..	450	2.3	Orange - yellow	450	1.1	Yellow
Fe(II) ..	630	5.7	Green	600	1.7	Brown
UO_2(II) ..	450	14.3	Yellow	450	15.2	Yellow
Ti(IV) ..	460	17.5	Orange	460	15.3	Orange - yellow
In(III) ..	440	45.5	Yellow	440	33.0	Yellow
Ca(II) ..				435	10.0	Yellow
Mg(II) ..				435	0.6	Yellow
Sr(II) ..				435	3.0	Yellow
Ba(II) ..				435	1.0	Yellow

Study of the Calcium(II) - BSHB Complex in Solution

When dilute solutions of calcium(II) and BSHB were mixed, a soluble yellow complex was obtained. The absorption spectrum of the calcium(II) - BSHB system (Fig. 1) shows that the wavelength of maximum absorbance is 432 nm.

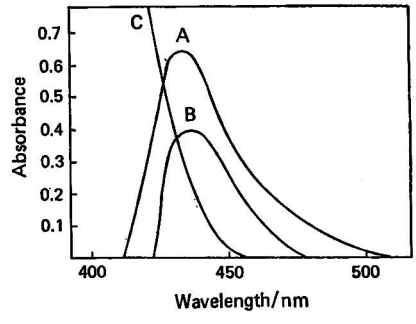


Fig. 1. Absorption spectra of the calcium complexes. Concentration of calcium(II), 1.5 p.p.m.; and concentration of reagents, 6.25 × 10^{-4} M. A, Calcium(II) - BSHB complex in dimethylformamide - ethanol (1 + 1) aqueous medium at pH 12; B, calcium(II) - BSHP complex in dimethylformamide - ethanol (1 + 1) aqueous medium at pH 9.8; and C, reagent blank at the same concentration.

Stability of the complex

At alkaline pH in aqueous dimethylformamide - ethanol solution the complex forms immediately and the absorbance remains stable for at least 4 h.

Glyoxal bis(2-hydroxyanil), one of the best spectrophotometric reagents for determining calcium, has been employed in this work as a standard reagent for the measurement of calcium in several samples; this compound forms a soluble red complex with calcium in alkaline media.[6] In order to compare both complexes, the stability of the calcium(II) - GBHA system was studied. The absorbance at 455 nm of a solution of the red complex decreases continually with time. The absorption spectrum of GBHA shows a bathochromic shift after 1–3 h. Fig. 2 shows this behaviour for both reagents and their complexes with calcium.

Using X-ray diffraction and infrared spectroscopy, Lindstrom and Milligan[39] have shown that instability of the calcium(II) - GBHA complex is due to the following: the hydrolysis of GBHA, which is present in sufficient excess, the alkaline medium catalysing the reaction to yield glyoxal and *o*-aminophenol; the low stability constant of the calcium(II) - GBHA complex; the formation of the anion of glycollic acid, $OH–CH_2–COO^-$, from glyoxal, which is catalysed by the OH^- anion; and the formation of the calcium(II) - glycolate complex.

The greater stability of the calcium(II) - BSHB system is due to the stability of the chelate, and to the resistance of the C=N bonds to hydrolysis.

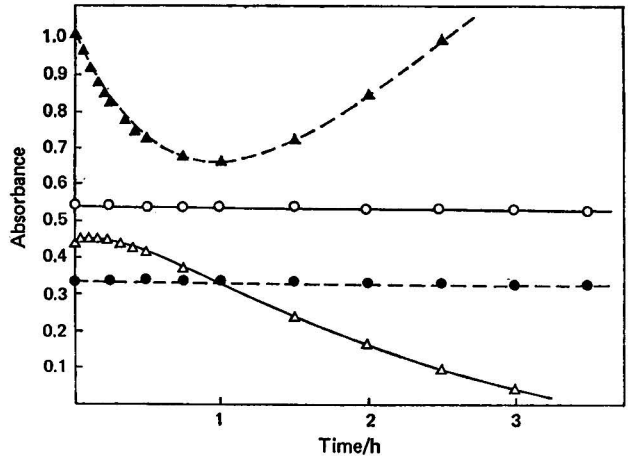


Fig. 2. Stability of diphenylglyoxal bis(2-hydroxybenzoylhydrazone), glyoxal bis(2-hydroxyanil) and their calcium complexes: ●, BSHB solution, concentration 1.4×10^{-5} M (λ = 370 nm); ○, calcium(II) - BSHB complex, calcium concentration 3.12×10^{-5} M (λ = 432 nm); ▲, GBHA solution, concentration 10^{-3} M (λ = 455 nm); and △, calcium(II) - GBHA complex, calcium concentration 2.5×10^{-5} M (λ = 516 nm).

Influence of pH

The influence of pH was studied using a series of solutions in the pH range 7–13. The absorbance was maximum and constant over the alkaline region and showed two possible optimum pH ranges of 7.4–8.2 and 9.0–12.5 (Fig. 3). The latter range was chosen because of the greater sensitivity it offered.

Effect of reagent concentration, ionic strength and proportions of the dimethylformamide - ethanol mixture

The absorbance of the complex was studied as a function of the molar ratio of BSHB to calcium(II). A 15-fold molar excess of the reagent over calcium was necessary in order to obtain the maximum absorbance value.

The ionic strength of the solution does not affect the absorbance of the calcium(II) - BSHB system. The same constant absorbance measurements were obtained when 5 ml of 0.5 M potassium nitrate solution or 5 ml of 0.5 M potassium chloride solution were added.

At alkaline pH the solubility of BSHB is greater, and a medium containing 40% *V/V* of dimethylformamide - ethanol (1 + 1) and 60% *V/V* of water was chosen for further experimental work as the yellow complex does not precipitate in this medium.

The results obtained are in total agreement if dimethylformamide is used alone in place of the dimethyformamide - ethanol (1 + 1) mixture. Ethanol cannot be used alone because the complex and the excess of reagent precipitates. Hence, it is proposed to use the dimethylformamide - ethanol (1 + 1) solvent mixture, which will also reduce the cost owing to the use of the cheaper ethanol as part of the mixture in place of the more expensive dimethylformamide alone.

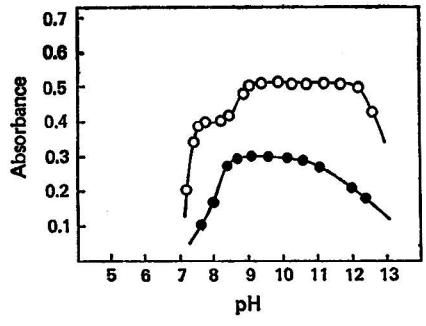


Fig. 3. Effect of pH on the formation of calcium complexes. Concentration of calcium(II), 3.12×10^{-5} M: ○, with BSHB at 432 nm; and ●, with BSHP at 435 nm.

Nature of the complex

The stoicheiometry of the chelates in solution has been determined by Job's (Fig. 4), Yoe Jones's and slope-ratio methods at both pH ranges in which the absorbance remains constant (see Fig. 3). At pH 7.80 (triethanolamine - hydrochloric acid buffer) the formation of two complexes with stoicheiometric ratios of metal to ligand of 2:3 and 1:1 has been observed. However, at pH 12 only one complex species, with a metal to ligand ratio of 2:3, has been found, which is probably favoured by an excess of reagent and by the high pH. A study of the retention of these chelates on an anion-exchange resin showed that, under these experimental conditions, the complexes were anionic.

In both stoicheiometric complexes the reagent acts as a quadrivalent ligand. The presence of the double aroylhydrazone contiguous chain was essential for the formation of the calcium(II) chelates with BSHB, as the 2-hydroxybenzoyl hydrazone of benzaldehyde does not give rise a complex with calcium(II).

Study of the Calcium(II) - BSHP Complex in Solution

The absorption spectrum of the calcium(II) - BSHP complex is illustrated in Fig. 1. The absorption maximum of the complex occurs at 435 nm. The yellow complex is formed immediately at alkaline pH and the absorbance at 435 nm is stable for at least 4 h.

Fig. 3 shows the influence of pH on the calcium(II) - BSHP system. The optimum pH range is 8.5–10.5. A volume of not less than 5 ml of 0.1% *m/V* reagent solution is required in order to obtain the maximum development of the yellow complex.

The ionic strength does not affect the absorbance and the same proportions of dimethylformamide and ethanol were used as for the calcium(II) - BSHB complex.

In order to determine the composition of the yellow complex, continuous-variation and molar-ratio methods were carried out. The results indicated a calcium to BSHP ratio of 2:3 (Fig. 4). The complex was retained by anion-exchange resin, and therefore this system is anionic in alkaline solution.

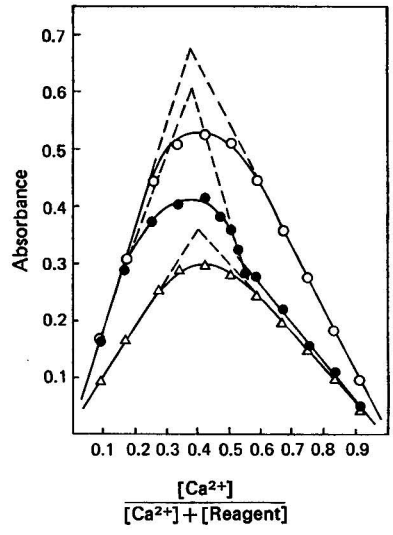


Fig. 4. Stoicheiometry of the complexes. Calcium(II) - BSHB complex (λ = 432 nm): concentration of calcium(II), 6×10^{-4} M; ○, pH 12; ●, pH 7.80. △, Calcium(II) - BSHP complex (λ = 435 nm) at pH 9.8: concentration of calcium(II), 5×10^{-4} M.

Spectrophotometric Determination of Calcium with BSHP

In an alkaline medium, the calcium(II) - BSHP system obeys Beer's law from 0.75 to 3.00 p.p.m. of calcium. The molar absorptivity is $1.03 \pm 0.05 \times 10^4$ l mol^{-1} cm^{-1}. A Ringbom plot shows that the range for minimum error in the determination is 1.25–2.50 p.p.m. of calcium. The mean values of results from 11 samples each containing 2.0 p.p.m. of calcium gave the relative error (P = 0.05) of the method as $\pm$0.32%.

The effect of diverse ions on the determination of 2.0 p.p.m. of calcium using BSHP was studied under the experimental conditions used. Strontium, barium, magnesium and most of the analytical group I–III metals (hydrogen sulphide scheme) interfere down to 2 p.p.m. The anions EDTA and oxalate also give rise to serious interferences.

The use of masking agents in the BSHP method to eliminate interferences has not been introduced because the interferences of other species were much more significant when masking agents were used than when they were not and because the BSHB method has a greater sensitivity and is more selective.

Spectrophotometric Determination of Calcium with BSHB

Characteristics of the method

Under the optimum conditions used for the formation of the calcium complex, Beer's law is obeyed between 0.5 and 2.0 μg ml^{-1} of calcium and the molar absorptivity at 432 nm is $1.76 \pm 0.14 \times 10^4$ l mol^{-1} cm^{-1}. The sensitivity of the method, according to Sandell, is 0.0023 μg cm^{-2}. The optimum concentration range, evaluated by Ringbom's method, is 0.5–1.5 p.p.m. of calcium. The mean values of results from 11 samples each containing 0.5 p.p.m. of calcium gave the relative error (P = 0.05) of the method as $\pm$ 0.34%.

Table III shows a comparative study of the calcium(II) - BSHB and calcium(II) - BSHP chelates in solution. The properties are analogous, although the sensitivity of the reaction with BSHB is better. The interferences are not shown in this table, but the calcium(II) - BSHB system is subject to less interferences than the calcium - BSHP system.

TABLE III

COMPARISON OF CALCIUM COMPLEXES WITH BOTH BISHYDROXYBENZOYL HYDRAZONES

Compound	$\lambda_{max.}$/nm	Optimum pH	Stoicheiometry, metal to ligand ratio	Molar absorptivity/ 10^3 l mol^{-1} cm^{-1}
BSHB	432	9.0–12.5	1:1, 2:3	17.6
BSHP	435	8.5–10.5	2:3	10.4

Effect of diverse ions

The effect of various amounts of 50 diverse ions on 12.5 μg of calcium per 25 ml of solution was investigated. The tolerance limits showed that calcium can be determined in the presence of a large number of ions (Table IV).

TABLE IV

TOLERANCE LIMITS IN THE DETERMINATION OF 12.5 μg PER 25 ml OF CALCIUM(II) WITH BSHB

Ions tolerated	Tolerance limit/ μg per 25 ml
K(I), Na(I), NH_4(I), Mg(II), Sr(II),* Sb(III),† Al(III),† W(VI),† Ni(II), I^-, SCN^-, $B_4O_7^{2-}$, CN^-, SO_4^{2-}, NO_3^-, Br^-, tartrate, ClO_4^-, BrO_3^-, S^{2-}, AsO_4^{3-}, CO_3^{2-}	2500
UO_2(II)	1250
Ba(II), Mn(II),* V(V)	625
PO_4^{3-}, citrate, F^-, SO_3^{2-}, Mo(VI)	250
Li(I), Rb(I), $S_2O_3^{2-}$	125
Hg(II), Sn(II), Bi(III), Be(II), Ag(I), Tl(I), $C_2O_4^{2-}$	50
Pb(II), Cd(II), La(III)	25

* Centrifuge the sample prior to measuring the absorbance.
† 0.25% *m*/*V* BSHB solution.

Ascorbic acid, mercaptoacetic acid (MAA) and triethanolamine (TEA) could be used as masking agents, so their tolerance limits were studied. Ascorbic acid (100 g l^{-1}), mercaptoacetic acid (10 g l^{-1}) and triethanolamine (20 g l^{-1}) did not interfere.

TABLE V

ELIMINATION OF INTERFERENCES OF FOREIGN IONS IN THE DETERMINATION OF CALCIUM WITH BSHB BY ADDITION OF MASKING AGENTS

Foreign ion	Tolerance limit/μg per 25 ml: Without masking agent	Tolerance limit/μg per 25 ml: With masking agent	Masking agent
Ag(I)	50	2500	CN^-, 100 μg ml^{-1}
Pb(II)	25	2500	TEA, 20 g l^{-1}
Tl(I)	50	1250	CN^-, 100 μg ml^{-1}
Hg(II)	50	2500	CN^-, 100 μg ml^{-1}
Cu(II)		2500	MAA, 10 g l^{-1}
Bi(III)	50	2500	MAA, 10 g l^{-1}
Pd(II)		625	CN^-, 100 μg ml^{-1}
Cd(II)	25	2500	MAA, 10 g l^{-1}
Sn(II)	50	2500	MAA, 10 g l^{-1}
Pt(IV)		625	CN^-, 100 μg ml^{-1}
Mo(VI)	250	1250	Ascorbic acid, 100 g l^{-1}
V(V)	625	2500	CN^-, 100 μg ml^{-1}
Zr(IV)		2500	MAA, 10 g l^{-1}
La(III)	25	1250	MAA, 10 g l^{-1}
Fe(II)		625	CN^-, 100 μg ml^{-1}
Be(II)	50	1250	F^-, 10 μg ml^{-1}
In(III)		2500	MAA, 10 g l^{-1}
Zn(II)		2500	MAA, 10 g l^{-1}
Co(II)		250	TEA, 20 g l^{-1}
Cr(III)		2500	MAA, 10 g l^{-1}
Li(I)	125	2500	MAA, 10 g l^{-1}
Rb(I)	125	2500	MAA, 10 g l^{-1}

Good results were obtained by masking the interfering ions with potassium cyanide and mercaptoacetic acid (Table V). The most serious interference was from EDTA, which interfered at the 1.0 p.p.m. level.

Determination of calcium in waters and foods

The proposed method was satisfactorily applied to the determination of calcium in waters and foods. Aliquots of the prepared solutions, different in each sample according to the concentration of calcium, were analysed as described. The results were compared (Table VI) with those obtained by the glyoxal bis(2-hydroxyanil) spectrophotometric method.

TABLE VI

RESULTS OF THE DETERMINATION OF CALCIUM IN WATERS AND FOODS BY THE USE OF BSHB AND GLYOXAL BIS(2-HYDROXYANIL) (GBHA) METHODS

Sample	Calcium found*	
	BSHB method	GBHA method
Swamp water	20.8 ± 0.1 p.p.m.	20.2 ± 0.1 p.p.m.
Mineral water	19.9 ± 0.1 p.p.m.	19.6 ± 0.1 p.p.m.
Orange juice	59.0 ± 0.1 p.p.m.	58.0 ± 0.1 p.p.m.
Beer	33.0 ± 0.1 p.p.m.	32.0 ± 0.1 p.p.m.
Egg white	115.0 ± 0.2 p.p.m.	118.0 ± 0.2 p.p.m.
Egg yolk	0.152 ± 0.002%	0.150 ± 0.001%
Milk	0.109 ± 0.001%	0.108 ± 0.001%
Cheese	0.701 ± 0.002%	0.698 ± 0.002%

* Average of 5 separate determinations.

Conclusions

It has been shown that the sensitivity of both spectrophotometric techniques is similar ($\epsilon \approx 1.8 \times 10^4$ l mol^{-1} cm^{-1}). The interferences of the BSHB method are similar and in many instances much more advantageous than those from the GBHA method, which requires extraction with Azo-azoxy BN. Therefore, the BSHB procedure does not require a preliminary extraction step.

The calcium(II) - BSHB system offers a greater stability than that of the calcium(II) - GBHA complex. The analytical data provided by our method show this to be the case. The stability of the BSHB solutions permits the same reagent blank solution to be used for at least 1 d. With the GBHA method a blank solution has to be prepared at the same time each sample is made.

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Received *July* **20***th*, **1979**
Accepted *October* **10***th*, **1979**

Analyst, March, 1980, Vol. 105, pp. 203–208

Spectrophotometric Determination of Cobalt with Biacetyl Mono(2-pyridyl)hydrazone

A. G. Asuero and M. M. Rodriguez

Department of Applied Chemical Analysis, Faculty of Pharmacy, University of Seville, Seville-4, Spain

The absorbance *versus* pH graphs and stoicheiometry of the complexes formed by biacetyl mono(2-pyridyl)hydrazone (BPH) with cobalt(II), nickel(II), copper(II) and iron(II) have been studied. A method for the spectrophotometric determination of cobalt has been devised. The orange - red chelate is formed at acidities between 2 and 10^{-6} M (pH 6), in aqueous ethanolic solution, and has $\lambda_{max.}$ at 505 nm with a molar absorptivity of 2.3×10^4 l mol^{-1} cm^{-1}. The method has been applied to the determination of cobalt in nitrates and has been compared with other pyridylhydrazone procedures for the spectrophotometric determination of cobalt.

Keywords: *Biacetyl mono(2-pyridyl)hydrazone reagent*; *cobalt determination*; *spectrophotometry*

The analytical use of pyridylhydrazone compounds in colour-forming reactions with metal ions is well known,[1–3] and these compounds are very useful as spectrophotometric and spectrofluorimetric reagents and as acid - base indicators. After a preliminary study of pyridylhydrazones derived from α-diketones as analytical reagents,[2–5] we selected biacetyl mono(2-pyridyl)hydrazone (BPH) for the spectrophotometric determination of cobalt for several reasons: (*i*) the high solubility of BPH and its complexes in aqueous media; (*ii*) the fact that the absorption of BPH at the $\lambda_{max.}$ of the cobalt complex is negligible even for a great excess of reagent; (*iii*) the reactions of BPH with cobalt ion are superior in sensitivity to those of biacetyl bis(2-pyridyl)hydrazone (BBPH) and biacetyl monoxime (2-pyridyl)-hydrazone (BMPH); (*iv*) BPH is the most selective of the reagents tested in colour reactions; and (*v*) the BPH method is the most economical. Therefore, in this paper, BPH is introduced as a valuable reagent for the spectrophotometric determination of cobalt.

Experimental

Solutions

Biacetyl mono(2-pyridyl)hydrazone was used as a 0.25% *m/V* solution in ethanol.

A standard solution of cobalt(II) (4.9283 mg ml^{-1}) was prepared from cobalt nitrate hexahydrate and was standardised by EDTA titration.[6] Working solutions were prepared by appropriate dilution. The other standard metal-ion solutions were available from previous investigations in this laboratory.

The solutions of metals for interference tests were prepared by weighing the required amounts of the nitrates and adding water and the appropriate amount of nitric acid until complete dissolution was obtained.

Apparatus

The apparatus used was identical with that described previously.[3]

Procedures

Absorbance versus *pH graphs*

Absorbance *versus* pH graphs for the complexes were obtained with the reagent in excess. The solutions were always prepared with the same order of operations: the metal, addition of reagent, adjustment of ionic strength (2.5 ml of 1 M potassium chloride solution per 25 ml) and adjustment of acidity. No buffer was used.

Stoicheiometry of the complexes

The molar-ratio[7] and slope-ratio[8] methods and the method of continuous variation[9,10] were used to try to ascertain the nature of the complexes formed at pH 7 and 10. The

medium was 20% *V/V* ethanol; the concentration of the stock solutions of metal ions and BPH was 1–2 × 10^{-4} M, various volumes being taken, and the pH was controlled by the addition of 5 ml of 1 M ammonium acetate solution or 5 ml of pH 10 borate buffer solution. The order of addition of reagents was metal ion, reagent, buffer and diluent. As a buffer was used, the ionic strength was not adjusted.

Photometric determination of trace amounts of cobalt with BPH

Transfer up to 20 ml of the sample solution, previously adjusted to pH 4.5 (not more than 65 μg of cobalt), by pipette into a 25-ml calibrated flask. Add 1–5 ml of the 0.25% *m/V* solution of BPH in ethanol. Mix the solutions thoroughly and allow the mixture to stand for 10 min (unless otherwise stated). Dilute the mixture to the mark with appropriate amounts of ethanol, perchloric acid and water to give final concentrations within the ranges 0.1–1 M in acid and 4–50% *V/V* in ethanol. Measure the absorbance at 505 nm in a glass cell of 10-mm path length against distilled water.

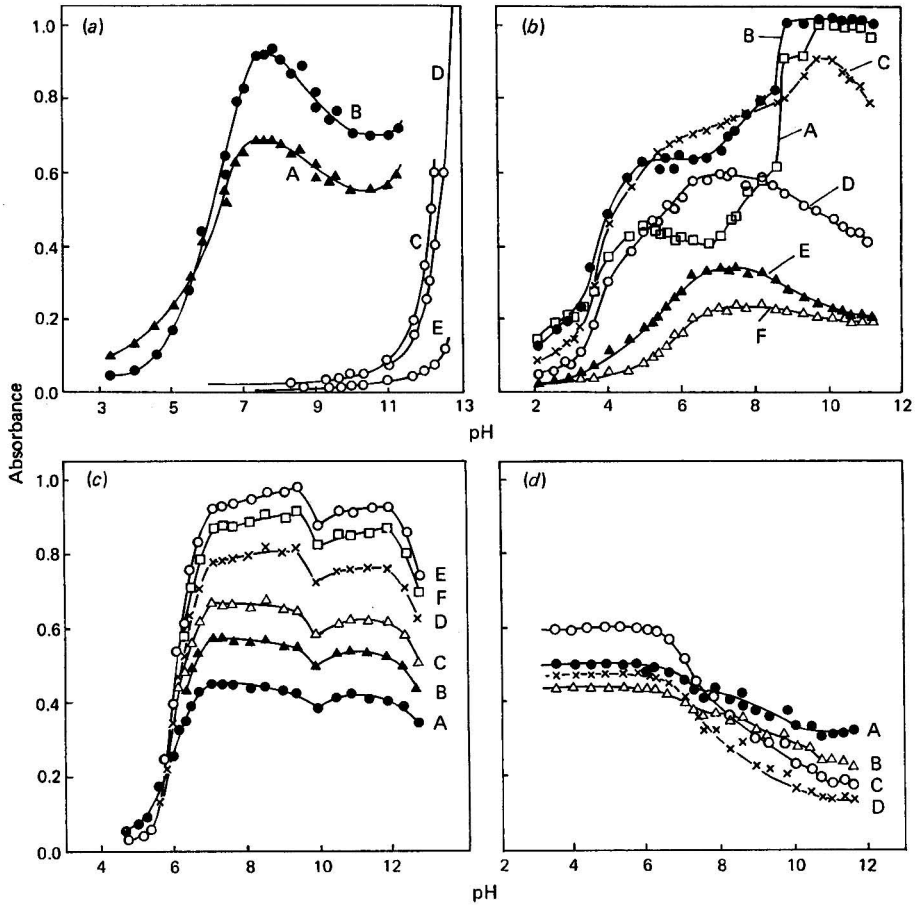

Fig. 1. Absorbance *versus* pH graphs for the metal ion - BPH complexes. (*a*) Ni(II), 2 p.p.m.: absorbance at (A) 425 nm and (B) 445 nm. C, D and E: reagent at 430, 440 and 470 nm, respectively. C_R = 5.65 × 10^{-4} M in 4% aqueous ethanol. (*b*) Cu(II), 3 p.p.m.: absorbance at (A) 430 nm, (B) 450 nm, (C) 470 nm, (D) 490 nm, (E) 510 nm and (F) 530 nm. C_R = 5.65 × 10^{-4} M in 4% aqueous ethanol. (*c*) Fe(II), 10 p.p.m., plus 0.1 g of ascorbic acid: absorbance at (A) 600 nm, (B) 615 nm, (C) 625 nm, (D) 650 nm, (E) 675 nm and (F) 685 nm. C_R = 8.47 × 10^{-4} M in 6% aqueous ethanol. (*d*) Co(II), 2 p.p.m.: absorbance at (A) 440 nm, (B) 465 nm, (C) 505 nm and (D) 535 nm. C_R = 5.65 × 10^{-4} M in 4% aqueous ethanol.

A calibration graph prepared using the same procedure (1 ml of the 0.25% *m/V* BPH solution, 20% *V/V* ethanol, 1 M perchloric acid) was rectilinear, passing through the origin, in the range 5–65 μg of cobalt, with a slope of 0.008 absorbance unit per microgram of cobalt.

The effect of various ions was studied. The general procedure was followed except that the solutions of foreign ions were added before the reagent solution. When a large amount of interfering cation was used, the samples were first prepared as nitrates in a 50-ml beaker because of precipitation. After adding acid the precipitate dissolved and the samples were then transferred into a 25-ml calibrated flask and diluted to the mark with water, followed by spectrophotometric measurement.

Results and Discussion

Absorbance *versus* pH Graphs

From the shapes of absorbance *versus* pH graphs (Fig. 1) for the metal ion - BPH complexes, it seems that two different complexes are formed with each of nickel, copper and cobalt. The apparent pH values for the development of maximum absorbance, determined by spectrophotometric titrations,[3] are approximately 4.8, 5.6, 7.1 and 7.6 for the cobalt(II), copper(II), nickel(II) and iron(II) complexes, respectively. The apparent pH for the formation of the cobalt(II) benzil mono(2-pyridyl)hydrazone complex[11] is approximately 7.5. However, on adding acid only the cobalt complex remained unchanged. Exploratory experiments indicated that the reagent forms precipitates with copper(I) and palladium(II).

Solvent-extraction Tests

Solvent-extraction tests indicated that the complexes formed in basic medium are neutral.

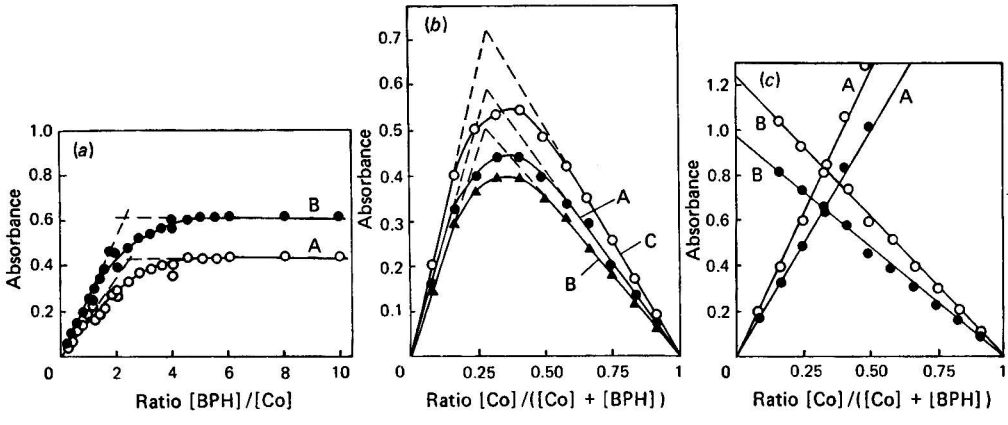

Fig. 2. Composition of cobalt - BPH complex at pH 7 (ammonium acetate). (*a*) Composition determined by the molar-ratio method; cobalt(II) concentration 3.4×10^{-5} M. Absorbance at (A) 465 nm and (B) 505 nm. (*b*) Composition determined by Job's method; concentration of cobalt(II) plus ligand 7.63×10^{-5} M. Absorbance at (A) 440 nm, (B) 465 nm and (C) 505 nm. (*c*) Composition determined by the slope-ratio method: A, concentration of cobalt(II) variable and ligand 1.27×10^{-3} M and B, concentration of ligand variable and cobalt 1.27×10^{-3} M. Absorbance at (○) 505 nm and (●) 535 nm.

Stoicheiometry of the Complexes

The method of continuous variation failed with the complexes of nickel(II), copper(II) and iron(II) at pH 7 and also failed for iron and zinc at pH 10. The slope-ratio method also failed with iron(II) and zinc(II) because the complexes were not formed when the metal ion was in excess. The data obtained for cobalt(II) (Fig. 2) indicated a ratio of metal to ligand of 1:2, the same as that found by Pflaum and Stucker[11] for the cobalt(II) benzil mono(2-pyridyl)hydrazone complex. The formation constant of the cobalt complex was not calculated by the Harvey and Manning method[8] because the complex is very strong. If EDTA was added after cobalt complexation no interference was observed. At higher pH,

a new cobalt complex appeared in the solution with maximum absorption at 450 nm and a very broad shoulder between 460 and 530 nm. The molar-ratio method indicated a 1:4 Ni - BPH complex at pH 7, whereas the slope-ratio method was not conclusive. Both the molar-ratio and continuous-variation methods indicated a 1:2 Ni - BPH complex at pH 10. The molar-ratio method for iron(II) was not conclusive at pH 10.

Spectrophotometric Determination of Cobalt with BPH

Spectra measured at acidities between 2 and 10^{-6} M (pH 6) (in 20% *V*/*V* ethanol) indicated the formation of a single complex having maxima at 440 and 505 nm. The complex was stable for at least 24 h in 1 M perchloric acid (in 20% ethanol), for a ratio of cobalt to BPH of 1:35. The spectrum of the cobalt - BPH complex was recorded in various solvents miscible with water (Fig. 3). In a basic solvent such as dimethylformamide, the complex formed at high pH in aqueous medium was present.

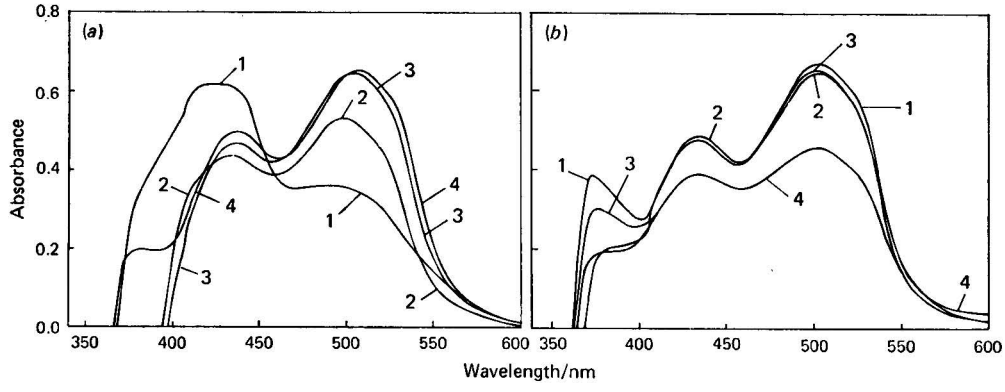

Fig. 3. Spectrum of the cobalt - BPH complex in various solvents miscible with water. (*a*) Solvent: 1, dimethylformamide; 2, formic acid; 3, acetic acid; 4, tetrahydrofuran. (*b*) Solvent: 1, acetone; 2, ethanol; 3, methanol; 4, dioxan. Cobalt, 2 p.p.m.; $C_R = 5.65 \times 10^{-4}$ M.

The optimum range for accurate determination, as evaluated from a Ringbom plot, is 0.3–1.2 p.p.m. of cobalt. The sensitivity of the colour reaction is 0.0025 μg cm^{-2} (at 505 nm) and the molar absorptivity is 2.35×10^4 l mol^{-1} cm^{-1}. The precision of the method was checked by measuring the absorbance of ten samples, each series containing a final concentration of 0.3, 0.8 and 1.2 p.p.m. of cobalt. The method gave relative errors of 0.54, 0.48 and 0.39%, respectively.

TABLE I

EFFECT OF VARIOUS IONS ON DETERMINATION OF COBALT BY THE RECOMMENDED PROCEDURE

Concentration of cobalt: 0.3 p.p.m.

No interference up to 1.2×10^4 p.p.m.	Interfering ions and approximate tolerance levels, p.p.m.		
Tartrate, SO_4^{2-}, PO_4^{3-}, AsO_4^{3-}, Br^-, I^-, Cl^-, alkali metals, alkaline earth metals	$C_2O_4^{2-}$ (10)	Sb^{3+} (6)	Ag^+ (60)
	SCN^- (10)	Al^{3+} (12)	Zr^{4+} (60)
	$S_2O_3^{2-}$ (5)	Ga^{3+} (30)	Cu^{2+} (100)*
	NO_2^- (5)	Cr^{3+} (240)	Ni^{2+} (100)*
	Cr(VI) (60)	V(V) (100)	Fe^{2+} (100)*
	$P_2O_7^{4-}$ (60)	Mn^{2+} (1200)	
	F^- (5000)		

* 5 ml 0.25% BPH.

Results of the interference studies are given in Table I. The tolerance towards a foreign ion was taken as the largest amount that caused an error of not more than 5% in the absorbance of cobalt alone (0.3 p.p.m.). However, greater amounts of these metal ions can be tolerated if a larger amount of reagent is employed and/or the solutions are allowed to stand at room temperature long enough for the displacement reaction to occur. As expected, negative errors in the determination of cobalt occurred in the presence of larger amounts of nickel, copper and iron than indicated in Table I. As no standard samples were available, a series of recovery experiments were carried out by adding standard cobalt solution to samples of various nitrates and carrying out the analysis as described under Procedures, with the exception that the samples were allowed to stand for 2.5 h before perchloric acid was added. Small amounts of cobalt (7.5–60 μg) were correctly determined when added to nitrates (Table II).

TABLE II

ANALYSIS OF COBALT IN NITRATES

Cation*	Amount added, p.p.m.	Co added, p.p.m.	Approximate metal to Co ratio	Absorbance values (samples)	Co found, p.p.m.	Comments
Zinc ..	6000	0.32	1:20000	0.124, 0.127, 0.117	0.31	2 ml of 0.25% BPH
	6000	1.19	1:5000	0.461, 0.476, 0.478	1.17	
Cadmium ..	12000	0.32	1:40000	0.145, 0.127, 0.127	0.33	
	12000	1.19	1:10000	0.489, 0.490, 0.488	1.22	
Mercury ..	12000	0.32	1:40000	0.125, 0.123, 0.127	0.31	
	12000	1.19	1:10000	0.484, 0.456, 0.478	1.17	
Lead ..	6000	0.32	1:20000	0.127, 0.125, 0.125	0.35	
	12000	1.19	1:10000	0.470, 0.471, 0.477	1.17	
	12000	0.32	1:40000	0.124	0.34	
Uranyl ..	1200	0.27	1:4000	0.107	0.26	5 ml of 0.25% BPH
	6000	0.27	1:20000	0.103, 0.100, 0.097	0.25	
	6000	1.08	1:6000	0.383, 0.396, 0.402	0.97	
Iron(III) ..	900	0.27	1:3000	0.099, 0.108, 0.112	0.26	
	900	1.08	1:800	0.338, 0.340, 0.352	0.85	

* With the exception of mercury all of the precipitates initially formed were dissolved on adding perchloric acid.

Conclusion

Many organic reagents have been proposed for the absorptiometric determination of cobalt. Recent papers have recommended the use of several pyridylhydrazones (Table III) (which have been claimed to offer advantages over nitroso reagents) and some azo compounds containing halogen-substituted pyridine.[16] The reagents containing sulphur as a ligand atom, most of which are less sensitive than the above, include quinoxaline-2,3-dithiol.[17] With such reagents it could be said that the spectrophotometric determination of cobalt in the presence of large amounts of nickel and other transition metal ions is not an especial

TABLE III

CHARACTERISTICS OF COBALT - PYRIDYLHYDRAZONE COMPLEXES

Reagent	Optimum pH	$\lambda_{max.}$/nm	Molar absorptivity/ l mol^{-1} cm^{-1} × 10^{-4}	Concentration of ethanol in sample, %	Relative reagent cost	Reference
Pyridine-2-aldehyde 2-quinolylhydrazone*	6.5–11.5	510	3.00	20	5	12
2-Benzoylpyridine 2-pyridylhydrazone	3.85–11.6	478	2.93	50	3.5	13
Benzil mono(2-pyridyl)hydrazone ..	2 M HCl–pH 8	535	2.74	60	0.6	11
2,2'-Dipyridyl 2-pyridylhydrazone	3–11	480	3.20	8	19	14
	20% $HClO_4$	500	4.20			
Pyridine-2-aldehyde 2-pyridylhydrazone and eosin	5.4–5.8	547	7.80	7+3†	1.5	15
Biacetyl monono(2-pyridyl)-hydrazone	2 M HCl–pH 6	505	2.35	4–25	1	This work

* Included for the sake of comparison.
† Chloroform - acetone.

problem nowadays, which makes the justification for another method for cobalt difficult. Results of spectrophotometric measurements of the cobalt chelates show that the reactions with higher relative molecular mass hydrazones are more sensitive than those with BPH, as they possess a more extended π-system. The values of $\lambda_{max.}$ for the orange - red chelates, however, do not differ significantly. In spite of its greater molar absorptivity, benzil mono-(2-pyridyl)hydrazone is not superior to BPH as a reagent for cobalt, and there are three good reasons for preferring the latter. Firstly, the cobalt - BPH complex has a low apparent pH, which leads to greater selectivity, secondly, a lower excess of reagent is required for complex formation, and thirdly, BPH and its metal complexes have high solubilities in aqueous ethanolic media. Further advantages of BPH include its solubilities in water (0.01%) and ethanol (greater than 4%, at room temperature), its relatively low cost and its high selectivity and sensitivity.

One of us (M.M.R.) expresses his gratitude to Professor Dr. F. Pino for giving him the opportunity to carry out these investigations.

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Received *July 23rd*, 1979
Accepted *September 5th*, 1979

Analyst, March, 1980, Vol. 105, pp. 209–216

Precision Spectrophotometric Determination of Chromium in Chromite Ores and Ferro-chrome

D. Thorburn Burns and M. E. M. Abdel Aziz

Department of Analytical Chemistry, The Queen's University of Belfast, Belfast, BT9 5AG, Northern Ireland

The chemical and manipulative variables have been examined in detail in order to develop a precise and direct spectrophotometric determination of chromium in chromite ores and in ferro-chrome. Samples are sintered with sodium peroxide in zirconium crucibles for 3 h at 510 ± 10 °C, then leached, filtered, acidified with perchloric acid and reduced with hydrazine hydrate. Chromium is determined from the absorbance of the chromium(III) aquo ion at 410 and 578 nm. The procedure has been evaluated using standard chrome ores, ferro-chrome alloys and a series of Sudanese and industrial ferro-chrome samples. The results are in good agreement with, and of comparable precision to, published data and to those obtained by titrimetric assay.

Keywords: *Precision spectrophotometry; chromium determination; chromite ores; ferro-chrome alloys*

Accurate assays of chromite ores are necessary because discrepancies of the order of 1% on a single 25-tonne truck load have significant financial implications for both the buyer and seller. At present there is pressure to change from the accepted classical gravimetric and titrimetric assay procedures for major constituents of ores owing to skilled manpower shortages and to rising costs, even in developing countries. Earlier work on the precision spectrophotometric determination of major metallic components in oxides and oxide mixtures[1] indicated the potential of the technique, but only a few applications have been reported.[2,3]

Direct spectrophotometric methods of analysis using conventional equipment are rapid and simple but give precisions below those of classical procedures. Differential spectrophotometric methods can, under favourable circumstances, give results similar to those attainable by classical methods but they are susceptible to stray light problems and require considerably more time and greater operator skills.[4] Dinnin[5] failed in an attempt to apply differential spectrometry to chromite analyses and expressed doubts if such a method could be made to work.

This paper describes the direct precision spectrophotometric determination of chromium in chrome ores and ferro-chrome and the precautions necessary at each stage of the analysis in order to achieve the required over-all precision and accuracy. The main problems were encountered in the decomposition and filtration stages and in optimising the solution conditions prior to spectrophotometric measurement.

To allow the accurate and precise assay of ores the component of interest must be dissolved completely and any matrix effects overcome. A survey of procedures for the decomposition of chromite ores showed that many were unnecessarily complicated and time consuming[6–8] and others were inefficient.[9–14] Sodium peroxide is an excellent general-purpose oxidising alkaline flux, its main disadvantage being its corrosive properties. Porcelain,[15,16] iron,[13,15,17–20] silver,[21] nickel,[15,22,23] zirconium[5,24] and vitreous graphite[25] crucibles have been used for the sodium peroxide fusion of chromite ores. Studies of their relative resistance to corrosion has shown that zirconium is the best material for peroxide fusions at temperatures above 550 °C.[26,27]

The decomposition of minerals can also be effected by sintering with a reagent at lower temperatures, which reduces attack on crucibles.[28,29] Differential thermal analytical studies have shown that most minerals decompose with sodium peroxide between 250 and 300 °C. For chromite and other reasonably resistant materials the size of the endothermic peak indicates their resistivity. The efficiency of sintering is controlled by the relative amount of reagent to sample, the temperature and the time of sintering. Rafter[28] suggested that 1.2–3.0 g of sodium peroxide was suitable for the decomposition of 0.2–1.0 g of sample, with heating at 480 ± 10 °C for 7 min in platinum crucibles; these conditions have not been found satisfactory for the decomposition of chromite ores.

Problems were encountered at the filtration stage. Apart from excessive filtration times (under gravity, 10 h), small amounts of solid were present in the filtrate, which caused light scatter at short wavelengths, even when using anti-creep filter-paper.[30]

In common with earlier workers, difficulties were encountered when using chromium(VI) solutions acidified with sulphuric acid[31,32] or perchloric acid[33,34] owing to complex solution equilibria. More recent work,[35] however, has indicated isosbestic points at 320 and 345 nm so that the quantitative spectrophotometric use of acidic solutions of chromium(VI) may still be possible.

The final procedure is based on the absorption of chromium(III) following reduction of chromium(VI) with hydrazine hydrate, which was used to avoid the adventitious addition of complexing anions.

Experimental

Reagents and Apparatus

Except when unavailable, all reagents were of analytical-reagent grade.

Sodium peroxide.

Sulphuric acid, 97.5–100% *m/m*.

Perchloric acid, 72% *m/m*.

Hydrazine hydrate, $NH_2NH_2.H_2O$, 99–100%. Laboratory grade.

Potassium dichromate.

Ammonium dichromate.

Volumetric glassware. This was of grade A specification and calibration.

Absorbance Measurements

Spectra were recorded in the ultraviolet and visible ranges using a Unicam SP 8000 spectrophotometer. The quantitative measurements were made using a Unicam SP 3000 spectrophotometer and 4-cm silica cells. The instrumental absorbance discrimination is 0.001 up to 1.2 absorbance unit and 0.01 absorbance unit thereafter. The linearity of response was checked by using calibrated neutral density filters (C. Davis Keller Ltd.) (in 0.1 absorbance unit steps from 0.20 up to 0.80 absorbance unit).

Solutions were kept at 25 ± 0.1 °C until required for measurement.

TABLE I

SUMMARY OF SAMPLE DECOMPOSITION EXPERIMENTS

Crucible	Amount of Na_2O_2/g	Temperature/°C	Time	Recovery, %	Comments
Zirconium ..	5	600–700	5 min	100	(1) Light scatter problems due to attack of zirconium crucible. (2) Violent reactions with ferro-chrome samples, particularly those with high carbon content.
Platinum or zirconium ..	3	480 ± 10 (sintering)	7 min	75	
Platinum or zirconium ..	3	480 ± 10 (sintering)	3 × 7 min	95	
Platinum or zirconium ..	3	480 ± 10 (sintering)	3 h	98	
Platinum or zirconium ..	5	370 ± 10 (sintering)	6 h	100	(1) Efficient decomposition but very slow. (2) Negligible attack on platinum crucibles even when left overnight.
Zirconium ..	5	510 ± 10 (sintering)	3 h	100	Platinum crucibles severely attacked.

Preliminary Studies

It was initially intended to determine both chromium and iron in a single solution obtained by acid dissolution of the sintered samples. The absorbance due to iron(II) species depended critically on the solution conditions, including the excess of reducing agent, and it was therefore necessary to separate iron and chromium prior to their determination.

Sample Decomposition

Samples were dried and weighed as recommended by Yoshimori.[36] Various decomposition conditions were examined, as summarised in Table I, from fusion at 600–700 °C to sintering at temperatures of 370–510 °C for various periods of time. Efficient decomposition with only slight attack on the zirconium crucible was obtained by sintering at 510 ± 10 °C for 3 h using 5 g of sodium peroxide with 0.6–0.8 g of finely powdered chromite ore or 0.3–0.4 g of ferro-chrome powder or flake.

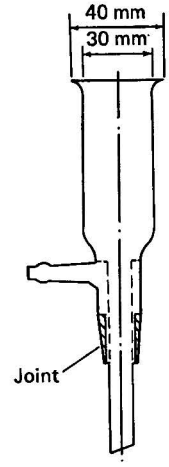

Fig. 1. Filtration adaptor.

Filtration

Following sintering, it is necessary to leach the samples and separate the insoluble hydrous iron oxide from the chromium(VI) solution. This precipitate is difficult and time consuming to filter and wash by gravity (10 h), despite the use of anti-creep (edge-silanised) filter-papers, and light scatter remained a problem. Glass-fibre papers (GF/C) and a special Büchner adapter (Fig. 1) allowed rapid filtration and washing directly into the volumetric flasks used for reduction, etc.

TABLE II

EFFECT OF SCATTERED LIGHT

Decomposition process: fusion at 600–700 °C. Filter-paper: Whatman 542.

Sample	Chromium content, %	Experimental result at 578 nm, %	Calculated result at 410 nm, %	Experimental result at 410 nm, %
BCS 366 ..	74.6*	75.1	75.3	75.2
BCS 204/4 ..	71.9*	72.5	72.7	72.7
Reg 1330 ..	58.2†	58.7	58.9	58.9
Reg 1830 ..	57.7†	58.6	59.0	58.7
Reg 1841 ..	68.8†	70.1	70.7	70.4
Reg 3283 ..	68.8†	69.1	69.2	69.20
Lot 167 ..	59.9†	60.4	60.7	60.6
G 129 ..	67.0†	68.5	69.1	69.0

* Chromium content: certificate value.
† Chromium content: supplied value.

The higher apparent chromium contents as calculated from absorbance measurements at 410 nm compared to those from measurements at 578 nm can be attributed to a wavelength-dependent absorbance from light scattering. The variation of the contribution by scatter to the absorbance of a solution with wavelength is complex.[37] For small particles of diameter (d) less than $\lambda/3$, the scattered intensity is proportional to $1/\lambda^4$ (Rayleigh scattering) and for $d \approx \lambda$ the scattered component is proportional to $1/\lambda^2$ (Clausius scattering). For very large particles the absorbance due to the particles is independent of λ. The present results showed a $1/\lambda$ dependence, indicating $d > \lambda$, which is consistent with the fact that the GF/C glass-fibre filters can pass particles up to 1.2 μm, *i.e.*, 2–3λ.

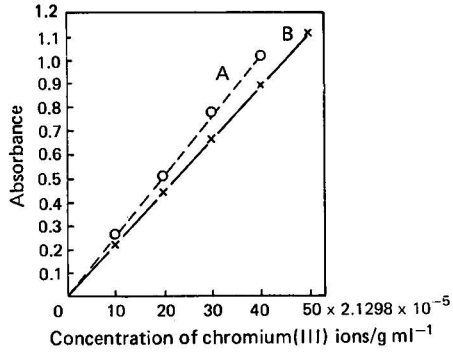

Fig. 2. Beer's law graph for chromium(III): A, 410 nm; and B, 578 nm.

As absorbances are additive the apparent chromium content at 410 nm (R_{410}) can be calculated from the certificate value for a sample (V) and the assay result at 578 nm (R_{578}) as follows:

$$R_{410} = V + 1.41\ (R_{578} - V)$$

The data in Table II, obtained using the finalised solution conditions, indicate that the apparent high results when filtration is not efficient are consistent with large particle scatter. Determination at two wavelengths serves as a check on filtration efficiency; the results should agree within two standard deviations on a single set.

TABLE III

VARIATION OF SPECIFIC ABSORPTIVITY OF CHROMIUM(III) IN $[Cr(H_2O)_6]^{3+}$ WITH DIFFERENT CONCENTRATIONS OF $K_2Cr_2O_7$ (7.2% *m/m* $HClO_4$ AND 0.15% *V/V* $N_2H_4.H_2O$)

	Specific absorptivity/ml g^{-1} cm^{-1}	
$K_2Cr_2O_7$/g ml^{-1}	410 nm	578 nm
1.6040×10^{-3}	301.3	259.0
8.5191×10^{-4}	299.8	256.9
6.3880×10^{-4}	299.9	256.4
4.2502×10^{-4}	298.2	255.7
2.1215×10^{-4}	298.6	257.9

Solution Conditions

During initial experiments it was noted that chloride ion, added with the reducing agent, hydroxylammonium chloride, caused anomalous results that were additional to the effect of scattered light noted when filtration was inefficient. This problem was circumvented by using hydrazine hydrate.

Beer's law was obeyed up to an absorbance value of 1.2 (Fig. 2 and Table III). The specific absorptivity of the chromium(III) aquo ion was found to be independent of perchloric acid concentration above 14.4% *m/m* (Table IV). The amount of hydrazine hydrate is not critical provided that it is greater than 0.075 *V/V* (Table V).

TABLE IV

VARIATION OF SPECIFIC ABSORPTIVITIES OF CHROMIUM(III) IN $[Cr(H_2O)_6]^{3+}$ WITH DIFFERENT CONCENTRATIONS OF $HClO_4$ (0.15% *V/V* $N_2H_4.H_2O$ AND 1.5050×10^{-4} g ml^{-1} OF $K_2Cr_2O_7$)

	Specific absorptivity/ml g^{-1} cm^{-1}	
$HClO_4$, % *m/m*	410 nm	578 nm
1.8	308.1	262.0
3.6	302.6	260.1
7.2	296.9	256.2
14.4	298.9	256.4
18.0	298.9	256.4

Specific Absorptivity of Chromium(III) in 14.4% *m/m* Perchloric Acid at 25 °C

It was observed that the specific absorptivities determined by reduction of potassium dichromate were slightly higher and considerably more precise (six results) than those determined starting with Specpure chromium metal (Table VI). The errors were considered to be due to the loss of chromium as chromyl chloride during the dissolution of the metal in hot concentrated perchloric acid.

TABLE V

VARIATION OF SPECIFIC ABSORPTIVITY OF CHROMIUM(III) IN $[Cr(H_2O)_6]^{3+}$ WITH DIFFERENT CONCENTRATIONS OF $N_2H_4.H_2O$ [1.5058×10^{-3} g ml^{-1} OF $K_2Cr_2O_7$ AND 7.2 *m/m* $HClO_4$ (72%)]

	Specific absorptivity/ml g^{-1} cm^{-1}	
$N_2H_4.H_2O$ (5%), % *V/V*	410 nm	578 nm
0.050	297.1	258.3
0.075	298.9	258.3
0.100	289.0	258.3
0.200	299.0	258.4
0.250	298.9	258.3

As potassium perchlorate is not very soluble, it may precipitate on long standing. This problem can be overcome by the use of ammonium dichromate, which is available in AnalaR grade. The standard solutions and solutions obtained from chromite ores were stable and mean results (six values) identical to within $\pm 0.1\%$ of chromium were attained for solutions stored at ambient temperature for 1 month (Tables VII and VIII).

TABLE VI

SPECIFIC ABSORPTIVITIES OF CHROMIUM(III) AQUO ION IN 14.4% *m/m* $HClO_4$ AT 25 °C

	Specific absorptivity/ml g^{-1} cm^{-1}	
Standard reagent	410 nm	578 nm
Chromium beads	295.2 ± 1.6	253.3 ± 1.2
Potassium dichromate	299.0 ± 0.1	257.0 ± 0.1

TABLE VII

STABILITY OF AMMONIUM DICHROMATE STANDARD SOLUTIONS

Absorbance measurements	Specific absorptivity of chromium(III)/ml g^{-1} cm^{-1} 410 nm	578 nm
After 1 h	298.1 ± 0.4	256.1 ± 0.3
After 1 month	298.2 ± 0.4	256.3 ± 0.3

Recommended Method

A. Chromium ores

1. Finely grind the sample to pass a test sieve of nominal aperture size 100 μm and dry for 1 h at 110 °C.
2. Transfer 0.6–0.8 g of the sample, weighed to the nearest 0.01 mg, into a 25-ml zirconium crucible.
3. Mix thoroughly with 5 g of dry sodium peroxide, cover the crucible and sinter the contents at 510 ± 10 °C for 3 h in an electric muffle furnace.
4. Allow the crucible to cool, place it in a 250-ml tall-form Pyrex beaker, leach the sintered mass with 50 ml of water, cover the beaker immediately with a watch-glass, and leave for about 1 h for the hydrogen peroxide to be decomposed.
5. Filter the solution, under a differential pressure of approximately 6 cmHg, through a 5.5 cm diameter glass-microfibre GF/C filter placed in a Büchner funnel connected with an adaptor to a calibrated 250-ml volumetric flask in which 50 ml of perchloric acid (72% *m/m*) had previously been placed.
6. Rinse the crucible, beaker and watch-glass and scrub them with a rubber policeman, then filter and wash the total residue on the filter thoroughly with hot distilled water.
7. Reduce the filtrate with 4 ml of hydrazine hydrate (5% *V/V*), swirl the volumetric flask until the vigorous evolution of nitrogen bubbles has ceased, thermostat at 25 ± 0.1 °C and dilute with distilled water to the mark. Mix thoroughly.
8. Measure the absorbance immediately after stabilisation at 25 ± 0.1 °C at 410 and 578 nm against distilled water using a 4-cm cell.
9. Carry out fusion and reagent blank measurements.
10. Calculate the chromium(III) oxide content of the ore using a pre-determined specific absorptivity (ml g^{-1} cm^{-1}) of chromium(III) at 410 and 578 nm and express the result as chromium(III) oxide.

TABLE VIII

STABILITY OF CHROMITE SAMPLE SOLUTIONS: DETERMINATION OF CHROMIUM(III) OXIDE CONTENTS

Sample	Chromium(III) oxide content, % 410 nm After 2 h	410 nm After 1 month	578 nm After 2 h	578 nm After 1 month
Ingessana Hills (III)	55.5 ± 0.3	55.7 ± 0.3	55.6 ± 0.2	55.4 ± 0.2
Ingessana Hills (D)	57.9 ± 0.2	58.0 ± 0.3	57.8 ± 0.1	57.8 ± 0.1
Chromite sample (II)	49.1 ± 0.1	49.3 ± 0.1	49.2 ± 0.1	49.2 ± 0.2

B. Ferro-chrome alloys

1. Transfer 0.3–0.4 g of ferro-chrome flakes or, preferably, powder, weighed to the nearest 0.01 mg, into a zirconium crucible.
2. Continue steps 3 to 9 as described in A.
3. Calculate the chromium content of the alloy using a pre-determined specific absorptivity (ml g^{-1} cm^{-1}) of chromium(III) at 410 and 578 nm and express the result as chromium.

Results and Discussion

The assay results are shown in Tables IX and X. In each instance the standard deviation is based on six results.

TABLE IX

RESULTS OF ANALYSIS OF ALLOYS AND ORES

Sample type	Sample	Stated chromium content, %	Experimental result, %	
			410 nm	578 nm
Ferro-chrome alloys ..	BCS 366	74.6 ± 0.1*	74.8 ± 0.3	74.6 ± 0.2
	BCS 204/4	71.9 ± 0.04*	71.4 ± 0.1	71.5 ± 0.1
Chrome ore	BCS 308	41.5 ± 0.2*	41.9 ± 0.2	41.6 ± 0.1
	Student sample 49g G	53.4*	54.8 ± 0.2	54.5 ± 0.2
	Student sample 49f	43.1*	43.5 ± 0.1	43.4 ± 0.1
Ferro-chrome	Reg 1330	58.2†	59.5 ± 0.4	59.0 ± 0.2
	Reg 1830	57.7†	57.8 ± 0.2	57.9 ± 0.3
	Reg 1841	68.8†	69.1 ± 0.4	69.1 ± 0.3
	Reg 3283	68.8†	68.9 ± 0.4	68.8 ± 0.3
	Lot 167	59.9†	60.6 ± 0.2	60.4 ± 0.2
	G129	67.0†	67.4 ± 0.1	67.1 ± 0.1

* Certificate value.
† Supplied value.

The precisions of the results are in all instances comparable to those attained by careful titrimetric procedures for similar analyses, where a coefficient of variation of 0.32% was found for a single analyst and 0.51% for between-laboratory results.[38] The accuracy of the present results is in accord with certificate values except for one "Student sample," which is not a reference material. Results are determined at two wavelengths as a check on filtration efficiency; the results on chrome ores should agree to within ±0.1% of chromium.

TABLE X

RESULTS OF ANALYSIS OF SUDANESE CHROMITE SAMPLES

Sample	Chromium content, %	
	410 nm	578 nm
Ingessana Hills (II)	49.1 ± 0.1	49.2 ± 0.1
Ingessana Hills (D)	57.9 ± 0.2	57.8 ± 0.1
Chromite sample (III)	55.5 ± 0.3	55.6 ± 0.2
Gabaneit Mine NT1	56.7 ± 0.2	56.5 ± 0.1
Gabaneit Centre NT5	56.3 ± 0.2	56.2 ± 0.1
Gabaneit South NT14	57.3 ± 0.3	57.2 ± 0.3
Jebel Jam Mine JC40	52.9 ± 0.2	52.7 ± 0.2
Bayomi Lease BMC2	54.6 ± 0.3	54.5 ± 0.2
Jebel El Tawila T	45.5 ± 0.3	45.6 ± 0.2

The solution conditions adopted avoid the problems of sulphate-bridged polymeric chromium species[39] and spectrochemical effects of anions coordinated to monomeric chromium(III).[40] Hydrazine hydrate has the further advantage that neither it nor its decomposition products absorb in the visible or ultraviolet regions.

It is possible to determine iron after dissolution of the hydrous oxide in concentrated hydrochloric acid.[41]

We thank the Industrial Research and Consultancy Institute (Sudan) for financial support for M. E. M. Abdel Aziz, the British Steel Corporation for the gift of analysed samples of ferro-chrome and Dr. I. M. Babiker (Geological Department, Sudan) for providing samples of Sudanese chromite ores.

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Received *July 27th*, 1979
Accepted *September 25th*, 1979

Analyst, March, 1980, Vol. 105, pp. 217–221

Selective Colorimetric Detection of Carboxylic Acids

H. S. Rathore, M. N. Akhtar and S. K. Sharma

Chemistry Section, Zakir Husain College of Engineering and Technology, Aligarh Muslim University, Aligarh-202001, India

A selective colour reaction for the detection of carboxylic acids has been developed. Acetic anhydride in the presence of ammonium carbonate, sodium carbonate and the sodium salts of acetic, formic, oxalic and pyruvic acids is used as the reagent. Amongst 39 acids, citric, isocitric, α-keto-glutaric and oxaloacetic acids can be detected selectively.

Keywords: *Carboxylic acid detection*; *colorimetry*; *acetic anhydride reagent*

A solution of citric acid in acetic anhydride has been used for the selective detection of tertiary amines.[1] Feigl[2] remarked that the chemistry of the colour test seems to be complicated and has not been elucidated. The solution of citric acid in acetic anhydride also gives colours with salts of tertiary amines, quaternary ammonium salts, salts of potassium, rubidium, caesium, strontium and barium, and salts of alkali and alkaline earth metals with organic acids. However, the colour reaction between citric acid - acetic anhydride and salts of alkali metals has not previously been studied for the detection or determination of carboxylic acids. An attempt has been made to use this reaction for the selective detection of certain carboxylic acids and α-ketoglutaric acid in particular.

Experimental and Results

Materials

All chemicals and reagents were of analytical-reagent grade. An aluminium heating block maintained at 165 ± 2 °C was employed. Aqueous or ethanolic solutions of the test substances (1%) were prepared. When it was not possible to prepare a 1% solution, a saturated solution was used. Dowex 1-X8 anion-exchange resin (20–50 US mesh) was converted into the carbonate, formate, acetate and oxalate forms by treatment with a 1 M aqueous solution of sodium carbonate, formic acid, acetic acid or oxalic acid, respectively. The resins obtained were washed with distilled water and dried at room temperature (25 °C).

General Procedure

Two or three drops of the solution of the test substance (1 mg) were evaporated to dryness in a micro test-tube by cautious direct heating in order to eliminate all moisture, cooled to room temperature and then two or three drops of acetic anhydride were added. The colour that developed was noted, then the contents of the tube were heated at 165 ± 2 °C in the heating block for 5 min and the colour that subsequently developed was also noted. The results obtained are given in Table I.

Two or three drops of the solution of the test substance and an equal volume of a 1% aqueous solution of ammonium carbonate, sodium carbonate, sodium pyruvate, sodium formate, sodium acetate or sodium oxalate were evaporated to dryness in a micro test-tube and the above procedure was followed. The results obtained are given in Table I. Limits of detection are given in Table II.

The above procedure was then carried out but in place of the aqueous solutions of salts, 8–10 anion-exchange resin beads in the carbonate, formate, acetate or oxalate form were used. The limits of detection are given in Table II.

Detection and Semi-quantitative Determination of Carboxylic Acids in the Presence of Foreign Substances

As a result of this study, it was concluded that sodium acetate - acetic anhydride was the best reagent for the detection of carboxylic acids. It was used for the detection of carboxylic acids, using α-ketoglutaric acid as a representative compound in the presence of large amounts of foreign substances.

TABLE I

DETECTION OF ACIDS USING DIFFERENT REAGENTS AT 165 ± 2 °C

Colours: NC = no colour; V = very; L = light; O = orange; R = red; Y = yellow; G = green; B = blue; Br = brown.

Acid	Ac_2O	Ac_2O + ammonium carbonate	Ac_2O + sodium carbonate	Ac_2O + sodium pyruvate	Ac_2O + sodium formate	Ac_2O + sodium acetate	Ac_2O + sodium oxalate
Acetic	NC	NC	NC	LY	NC	NC	NC
Ascorbic	LY	O	R	R	R	R	O
Benzoic	NC	NC	NC	O	NC	NC	NC
Cinnamic	NC	NC	VLY	LY	NC	NC	NC
Formic	NC	NC	NC	Y	NC	NC	NC
Gallic	NC	NC	O	LY	NC	NC	NC
Glyoxalic	NC	LO	VLY	VLY	NC	NC	NC
Lactic	NC	NC	NC	LO	NC	NC	NC
Nicotinic	NC	NC	NC	LY	NC	NC	NC
m-Nitrobenzoic	NC	NC	NC	LY	NC	NC	NC
p-Nitrobenzoic	NC	NC	NC	VLY	NC	NC	NC
Pyruvic	NC	NC	NC	NC	NC	NC	NC
Salicylic	NC	LY	NC	LY	NC	NC	VLY
Adipic	NC	NC	NC	O	NC	NC	NC
Fumaric	NC	NC	LY	R	NC	NC	NC
Glutaric	NC	NC	NC	LY	NC	NC	NC
α-Ketoglutaric	LG	VLY - LY*	RG	GB	RG	GB	GR
Maleic	NC	VLY	NC	R	LO	NC	NC
Malic	NC	NC	VLY	LY	VLY	NC	NC
Malonic	LO	O	VLY	Y	LY	LY	O
Oxalic	NC	NC	NC	VLY	NC	NC	NC
Oxaloacetic	LBr	Br	Br	Br	Br	Br	Br
Phthalic	NC	NC	NC	LY	Y	NC	NC
Succinic	NC	NC	NC	Y	Y	NC	NC
Tartaric	LY	NC	LY	O	O	Y	Y
Citric	LR	R	R	R	R	R	R
Isocitric	LR	R	R	R	R	R	R
Alanine	NC	NC	NC	NC	NC	NC	NC
Argenine.HCl	LY	Y	NC	O	O	NC	NC
Aspartic	NC	NC	NC	LR	LR	NC	NC
l-Cystine	NC	NC	NC	O	O	NC	NC
Cysteine.HCl	LY	NC	NC	LY	LY	NC	NC
Glutamic	NC	NC	NC	NC	NC	NC	NC
Glycine	LY	Y	Y	LY	LY	NC	NC
l-Lysine	NC	NC	NC	LY	LY	NC	NC
Barbituric	VLY	VLY	VLY	R	LY - Y*	NC	NC
Boric	NC	NC	NC	NC	NC	NC	NC
Sulphamic	LR	NC	NC	LY - O*	O	NC	NC
Uric	NC	NC	NC	VLY	VLY	NC	NC

* The former refers to the colour at room temperature (25 °C) and the latter at 165 ± 2 °C.

Detection of α-ketoglutaric acid

A known volume of the aqueous test solution (250 μg), two or three drops of sodium acetate solution (2 mg) and a known volume of a solution of the interfering substances (as listed in Table III) were evaporated to dryness in a micro test-tube in order to eliminate all moisture, then cooled to room temperature. Two or three drops of acetic anhydride were added and the solution was heated at 165 ± 2 °C. The results are given in Table III.

Semi-quantitative determination of α-ketoglutaric acid

Known volumes of the solution of the test substance and 8–10 resin beads in the acetate form were placed in different test-tubes and the above procedure was followed. The results obtained are given in Table IV. The colours of solutions of unknown concentration were developed in the same way and then the nature and intensity of the colours were compared with those obtained from the solutions of known concentration.

TABLE II

LIMITS OF DETECTION OF SOME ACIDS (IN MICROGRAMS) USING DIFFERENT REAGENTS AT 165 ± 2 °C

The colour developed was the same when either the salt solutions or the ion-exchange resin beads were used.

Acid	Ac_2O + sodium carbonate		Ac_2O + sodium formate		Ac_2O + sodium acetate		Ac_2O + sodium oxalate	
	Solution phase	Resin phase	Solution phase	Resin phase	Solution phase	Resin phase	Solution phase	Resin phase
Ascorbic	50	40	50	50	100	100	100	100
Barbituric	250	250	100	100	100	100	100	100
Citric	20	20	10	5	20	50	60	60
Isocitric	100	100	20	20	50	50	50	50
α-Ketoglutaric	50	40	20	20	20	50	50	50
Oxaloacetic	80	100	70	70	100	100	100	100
Tartaric	500	500	100	100	150	150	150	150

Behaviour of Other Compounds

Various organic compounds (1–2 mg) were tested by the recommended procedure and were found not to interfere. The following compounds were tested: *amines*, aniline, diethanolamine, dimethylamine, diphenylamine and trimethylamine; *amides*, acetamide, benzamide, and salicylamide; *alcohols*, butan-1-ol, ethanol, methanol and propan-1-ol; *aldehydes*, benzaldehyde, *o*-nitrobenzaldehyde, salicylaldehyde and vanillin; *carbohydrates*, glucose, lactose and sucrose; *esters*, ethyl formate; *ethers*, diethyl ether; *heterocyclic bases*, indole, nicotine and pyridine; *hydrocarbons and their derivatives*, benzene, carbon tetrachloride, chlorobenzene, nitrobenzene, light oil and *o*-toluidine; *ketones*, acetone and acetophenone; *phenols and their derivatives*, catechol, hydroquinone, quinolin-8-ol, phenol and resorcinol.

TABLE III

DETECTION OF α-KETOGLUTARIC ACID (250 μg) IN THE PRESENCE OF INTERFERING SUBSTANCES

Substance	Amount added/μg	Colour*
Bovine serum albumin (BSA)	50	BrG
	150	BrG
	500	BrG
Alanine	50	YG
	150	YG
	1000	YG
Aspartic acid	50	RG
	150	RG
	1000	RG
Glutamic acid	50	G
	150	G
	1000	BrG
Oxaloacetic acid	50	BrG
	150	BrG
	1000	BrG
Pyruvic acid	50	BrG
	150	BrG
	1000	BrG

* Abbreviations as in Table I.

The following compounds (1–2 mg) were found to interfere; the colours developed are given in parentheses: cinnamaldehyde (light yellow), *p*-nitrophenol (light yellow), pyrrole (red), thiourea (yellow) and urea (yellow).

Discussion

From Table I it can be seen that acetic anhydride alone gives colours with various acids, but the sensitivity of these colour reactions is low and they cannot be used for analytical purposes.

Acetic anhydride in the presence of ammonium carbonate gives colours with 12 of the acids. Acetic anhydride in the presence of sodium carbonate gives colours with 14 of the acids, with higher intensity than those with ammonium carbonate. Acetic anhydride in the presence of sodium pyruvate gives colour with all of the acids except pyruvic, glutamic and boric acids and it therefore cannot be used for the selective detection or determination of the acids. Acetic anhydride in the presence of sodium formate gives colours with 20 of the acids, in the presence of sodium acetate with 7 of the acids and in the presence of sodium oxalate with 8 of the acids.

Hence acetic anhydride in the presence of sodium acetate or sodium oxalate is more selective than in the presence of sodium formate. Table II shows the following order of sensitivity of the colour reactions of acetic anhydride in the presence of the different sodium salts: sodium formate > sodium carbonate > sodium acetate > sodium oxalate. Thus, acetic anhydride in the presence of sodium formate can be used for sensitive detection and in the presence of sodium acetate or oxalate it can be used for selective detection. For example, ketodicarboxylic acids (α-ketoglutaric and oxaloacetic) can be distinguished from ketomonocarboxylic acids (glyoxalic and pyruvic). Perhaps the ketomonocarboxylic acids do not react under the conditions used. Similarly, hydroxytricarboxylic acids (citric and isocitric) acids can be distinguished from hydroxydicarboxylic acids (malic and tartaric).

Acetic anhydride in the presence of sodium acetate gives a greenish blue colour with α-ketoglutaric acid, which is different to those of the remaining 38 compounds (Table I). The limit of detection of α-ketoglutaric acid is 20 μg (Table II). At low concentrations (less than 100 μg) it gives a brownish blue colour instead of greenish blue, and then cannot be distinguished from oxaloacetic acid by this procedure. However, aspartic, glutamic, glutaric and pyruvic acids, alanine and BSA give no colour and do not interfere in the procedure (Table III).

Table IV shows that anion-exchange resin beads in the acetate form in the presence of acetic anhydride can be used for the semi-quantitative determination of α-ketoglutaric acid. It is of interest that pyruvic acid does not interfere with this determination of α-ketoglutarate, nor does oxaloacetate when the keto acid concentration is not extremely low. These observations, together with the finding that substantial amounts of BSA also do not interfere, suggests the possible use of this reaction in the assay of transaminases such as glutarate oxaloacetate transaminase and glutarate pyruvate transaminase.

TABLE IV

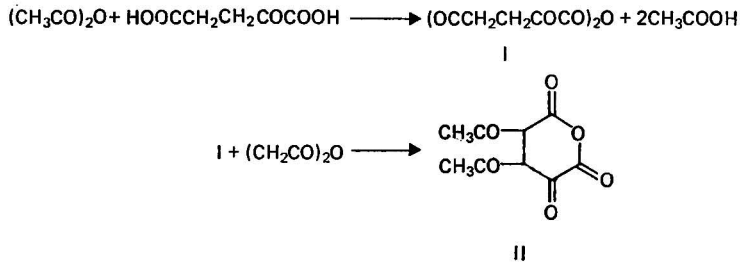
SEMI-QUANTITATIVE DETERMINATION OF α-KETOGLUTARIC ACID BY THE USE OF ANION-EXCHANGE RESIN BEADS IN THE ACETATE FORM AND ACETIC ANHYDRIDE AT 165 ± 2 °C

Amount of α-ketoglutaric acid added/μg	Colour of resin beads*
50	LBr
150	Br
250	BrG
350	BrG
500	G

* Abbreviations as in Table I.

The results show that acetic anhydride alone reacts with acids very slowly, even at high temperature (165 °C), whereas in the presence of salts it reacts very rapidly at 165 °C. Higher acids, such as ketodicarboxylic and hydroxytricarboxylic acids, give dark colours. It is known[3] that the higher acid anhydrides can be prepared by heating a mixture of the acid and acetic anhydride. These observations suggest that in the colour reaction higher acids react with acetic anhydride to give a higher acid anhydride. The anhydride so formed

reacts with acetic anhydride to give a coloured product and salts act only as catalysts. The following reaction scheme may be proposed for the acetic anhydride - α-ketoglutaric acid reaction in the presence of a salt:



The large conjugated system of structure **II** seems to be responsible for the colour.

We thank Professor M. Qureshi, Head, Chemistry Section, for providing research facilities and Dr. Saleem Uddin, Reader, Biochemistry Department, for helpful suggestions. M. N. A. is grateful to U.G.C. (India) for providing financial assistance.

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Received, *August 29th*, 1979
Accepted *October 1st*, 1979

Analyst, March, 1980, Vol. 105, pp. 222–226

Use of an Orthogonal Function in Differential Spectrophotometry

M. Abdel-Hady Elsayed,* Yousry M. Elsayed and Hassan Abdine

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt

An orthogonal function method has been applied to the differential spectrophotometric determination of acetazolamide and hydrochlorothiazide in tablets. The error resulting from setting the wavelength scale during the application of the orthogonal function to conventional spectrophotometry is thereby minimised. The results of assay using the proposed method [$p_{2(r)}$ method] are more precise than those obtained with the p_2 method.

Keywords: *Pharmaceutical analysis*; *differential ultraviolet spectrophotometry*; *orthogonal function*

Differential spectrophotometric methods[1–3] were developed in order to improve the precision of the results of determinations of the main components of given samples. This improvement can be achieved through the appropriate expansion of the absorbance scale by using a solution of suitable concentration in the reference cell. In this instance the relative absorbance value (A_r), assuming the validity of Beer's law, is directly proportional to the concentration:

$$A_r = \alpha (C_x - C_r) \qquad (1)$$

C_x and C_r are the concentrations of the analysed solution and the reference solution, respectively, and α, a constant, is the specific absorbance of the blank.

The orthogonal function method[4] has been used for the correction of the interfering substances in the determination of the main components of pharmaceutical preparations.[5] In the application of the orthogonal function method to spectrophotometric analysis, the absorbance, A, is replaced by the coefficient of orthogonal function, p_j. Hence, subject only to the usual Beer - Lambert law limitations, a given coefficient is exactly proportional to concentration:

$$p_j = \alpha_j C \qquad (2)$$

In order to extract the coefficient of a given polynomial from an absorption curve, it is necessary to obtain absorbances at a number of equally spaced wavelengths. Therefore, to extract the coefficient of the quadratic polynomial p_2, we need, say, six absorbance measurements at six equally spaced wavelengths. By plotting the quadratic coefficient p_2 at different intervals *versus* λ_m (the mean of the set of wavelengths) a convoluted curve is obtained.[6] The optimum wavelength range is selected in order to maximise the coefficient p_2. Also, λ_m is better sited on a broad peak or minimum than on a narrow peak. The sensitivity of the coefficient to over-all shifts in the spectrophotometer wavelength calibration is thereby minimised.

On application of the orthogonal function to differential spectrophotometry, A_r and α are replaced with $p_{j(r)}$ and α_j, respectively. Thus,

$$p_{j(r)} = \alpha_j (C_x - C_r) \qquad (3)$$

where $p_{j(r)}$ is the coefficient obtained from the convoluted differential curve. This theory constitutes the basis of this paper.

* Present address: Department of Pharmacy, University of Nigeria, Nsukka, Nigeria.

Experimental

Reagents

Acetazolamide standard solution. A 1 mg ml^{-1} solution of acetazolamide (Cid Company, Cairo, Egypt) in ethanol.

Hydrochlorothiazide standard solution. A 1 mg ml^{-1} solution of hydrochlorothiazide (Elnile Company for Pharmaceutical and Chemical Industries) in ethanol.

Tablets. Cidamox (250 mg of acetazolamide per tablet) (Cid Company) and Hydrazide K (50 mg of hydrochlorothiazide and 300 mg of potassium chloride) (Elnile).

Instrument

A Prolabo photoelectric spectrophotometer with 1-cm silica cells was used.

Determination of Optimum Concentration of Reference Solution

Prepare a set of solutions in 0.1 N sulphuric acid containing 0.1, 0.3, 0.5 and 1.9 mg per 100 ml of acetazolamide or hydrochlorothiazide. Carry out five sets of measurements for each compound. Select reference solutions of concentrations 0.7, 0.9, 1.1, 1.3 and 1.5 mg per 100 ml for the differential absorbance measurements.

Preparation of Standard Calibration Graphs

Prepare a set of solutions in 0.1 N sulphuric acid containing 0.3, 0.5, 0.7, . . ., 1.8 mg per 100 ml of acetazolamide or hydrochlorothiazide. Measure the absorbance (A value) of these solutions at equally spaced wavelengths (p_2 method), using 0.1 N sulphuric acid as the blank. Measure the absorbance (A_r value) at $\lambda_{max.}$ (A_r method) or at equally spaced wavelengths [$p_{2(r)}$ method], using a solution containing 1.3 mg per 100 ml (for acetazolamide) or 0.9 mg per 100 ml (for hydrochlorothiazide) as the blank.

Assay for Tablets

Powder and mix 20 tablets. Weigh accurately an amount equivalent to one tablet. Next extract the active ingredient with ethanol (4 × 25 ml). Dilute the ethanolic solution with

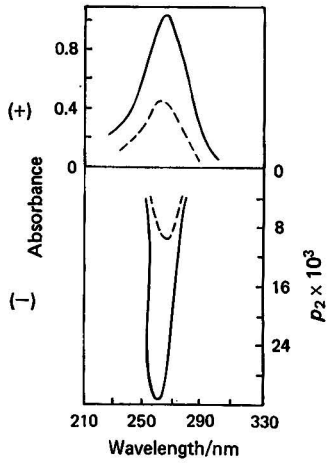

Fig. 1. Positive scale: A curve (solid line); and A_r curve (broken line). Negative scale: p_2 curve (solid line); and $p_{2(r)}$ curve (broken line) derived therefrom. Sample, 2.3 mg-% of acetazolamide in 0.1 N sulphuric acid.

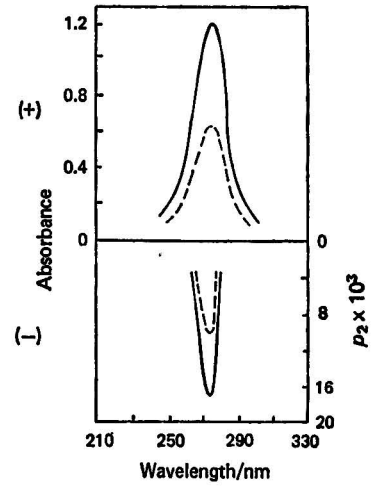

Fig. 2. Positive scale: A curve (solid line); and A_r curve (broken line). Negative scale: p_2 curve (solid line); and $p_{2(r)}$ curve (broken line) derived therefrom. Sample, 2 mg-% of hydrochlorothiazide in 0.1 N sulphuric acid.

0.1 N sulphuric acid as necessary in order to carry out the spectrophotometric measurements. Measure the A value and the A_r value as described above for the different methods.

Results and Discussion

Differential Spectrophotometric Method (A_r Method)

In the differential spectrophotometric determination the A_r values of acetazolamide and hydrochlorothiazide solutions in 0.1 N sulphuric acid were measured at the corresponding $\lambda_{max.}$ values, as shown in Figs. 1 and 2 (acetazolamide at 264 nm, hydrochlorothiazide at 270 nm).

In each of the five sets of experiments the relative absorbance values (A_r), when plotted against concentration, yielded linear graphs. By use of the method of least squares,[7] the equations of the best lines fitting the experimental points were determined, together with the standard deviation of the slopes, S_b.[7] The best results when employing differential spectrophotometry can be obtained by using the reference solution for a set of experiments that gives the lowest S_b value.[2] Therefore, the concentrations of the reference solutions used for the differential measurement of acetazolamide and hydrochlorothiazide were 1.3 and 0.9 mg per 100 ml, respectively (Tables I and II).

To check for the validity of the equations (with lowest S_b value, Tables I and II) the laboratory-made tablets of acetazolamide and commercial tablets for both compounds were analysed using the A_r method. The results are given in Table III.

TABLE I

DETERMINATION OF OPTIMUM CONCENTRATION OF REFERENCE SOLUTION FOR ACETAZOLAMIDE

Experiment No.	Concentration in reference solution/ mg per 100 ml	Linear calibration graph* $\pm A_r = a + bC$	$s_b \times 10^3$
1	0.7	$-0.3215 + 0.4486C$	5.59
2	0.9	$-0.4433 + 0.4750C$	3.31
3	1.1	$-0.4977 + 0.4550C$	3.98
4	1.3	$-0.5824 + 0.4520C$	2.50
5	1.5	$-0.6766 + 0.4516C$	3.24

* In this investigation, the reproducibility of any calibration graph was checked by taking three different amounts, from each of which three different concentrations were prepared.

Orthogonal Function Method (p_2 Method)

For the application of an orthogonal function to the spectrophotometric analysis of acetazolamide and hydrochlorothiazide the quadratic polynomial p_2 was chosen (Figs. 1 and 2). The absorbance values for different concentrations in 0.1 N sulphuric acid were measured over the wavelength range 256–276 nm at 4-nm intervals (λ_m, 266 nm) for acetazolamide, and over the wavelength range 268–278 nm at 2-nm intervals (λ_m, 273 nm) for hydro-

TABLE II

DETERMINATION OF OPTIMUM CONCENTRATION OF REFERENCE SOLUTION FOR HYDROCHLOROTHIAZIDE

Experiment No.	Concentration in reference solution/ mg per 100 ml	Linear calibration graph* $\pm A_r = a + bC$	$s_b \times 10^3$
1	0.7	$-0.4336 + 0.6277C$	3.37
2	0.9	$-0.5454 + 0.6284C$	2.63
3	1.1	$-0.6623 + 0.6180C$	5.28
4	1.3	$-0.7948 + 0.6175C$	2.91
5	1.5	$-0.9357 + 0.6170C$	3.54

* See footnote to Table I.

chlorothiazide. The wavelength range mentioned above was chosen as the analytical set of wavelengths because the corresponding p_2 value is a maximum. The quadratic coefficient, p_2, was calculated from

$$p_2 = [(+5)\,A_1 + (-1)\,A_2 + (-4)\,A_3 + (-4)\,A_4 + (-1)\,A_5 + (+5)A_6]/84$$

in which A_1, A_2, A_3, . . . are the absorbance values for the compound at the corresponding wavelengths mentioned above, the numbers in parentheses are obtained from the work of Fisher and Yates[8] and the divisor is the normalising factor.

Within the concentration range 0.3–1.8 mg per 100 ml for acetazolamide and hydrochlorothiazide, p_2 *versus* C shows a linear relationship. The corresponding calibration graphs can be described by the following equations:

$$p_2 \times 10^3 = +0.08357 - 9.1587C \text{ for acetazolamide}$$

$$p_2 \times 10^3 = -0.0479 - 9.4800C \text{ for hydrochlorothiazide}$$

The validity of the above equations was tested by analysing laboratory-made tablets of acetazolamide and commercial tablets of both compounds (Table III).

TABLE III

ASSAY RESULTS FOR ACETAZOLAMIDE AND HYDROCHLOROTHIAZIDE TABLETS

Tablet	Mean* recovery† ± standard deviation, %				F-value‡
	A_r method	p_2 method	$p_{2(r)}$ method	A method	
Acetazolamide (laboratory prepared) . .	101.57 ± 1.39 (4.23)§	100.78 ± 2.34 (0.94)	99.92 ± 0.81 (0.28)	101.68 ± 0.90 (5.28)	8.35
Cidamox	100.74 ± 1.21 (2.29)	99.69 ± 2.78 (0.32)	100.67 ± 1.03 (1.84)	102.10 ± 1.27 (4.68)	7.28
Hydrazide K	96.38 ± 1.80 (7.52)	96.99 ± 3.21 (2.65)	95.79 ± 0.95 (12.53)	98.68 ± 0.90 (3.03)	11.41

* Mean of 14 determinations (A_r method) and 8 determinations (other methods)

† Recovery for commercial tablets is expressed as a percentage of the label claim.

‡ The calculated F-value for which the theoretical value at the 5% level equals 3.79; the F-value is determined to compare the p_2 and $p_{2(r)}$ methods.

§ The figures in parentheses are the calculated t values, for which the theoretical value ($\alpha = 0.05$) for seven degrees of freedom = 2.365 and for thirteen degrees of freedom = 2.160.

Application of Orthogonal Functions to Differential Spectrophotometry: the Proposed Method [$p_{2(r)}$ Method]

The error involved in the p_2 method, as indicated by the relatively high standard deviation, can be attributed to the wavelength-setting errors as absorbance measurements are usually made on the slopes of the absorption curves.[9] Moreover, for a given error in setting the wavelength scale, the resultant absorbance error increases with the slope of the absorption curve at the wavelength of measurement. Therefore, any procedure that diminishes the slope will also reduce such error.

The coefficient $p_{2(r)}$ was calculated by measuring the absorbance values of a sample solution at a set of wavelengths equally spaced against a reference solution of optimum concentration. When the concentration of reference solution becomes equal to that of sample solution negligible slope is encountered on the spectrum and this is termed the balance point.[10] In fact, the slope of the spectrum decreases with increasing concentration of reference solution until the balance point is reached. In the course of determining the orthogonal function coefficient (p_2 method) most of the absorbance measurements are made on the slope of the absorption curves, so that a major proportion of the variance of a coefficient must arise from wavelength-setting error. An optimum set of experimental conditions must be worked out to find the optimum concentration of the reference solutions. With the optimum concentration of the reference solutions known (Tables I and II), the orthogonal function can be applied to differential spectrophotometry. The optimum experimental conditions for the application of the orthogonal function method to the differential spectrophotometric determination of acetazolamide and hydrochlorothiazide are presented in Table IV.

In order to measure the accuracy of each method [A_r, p_2 and $p_{2(r)}$ methods] the true amount of the drug should be known. This amount can be found by the analysis of tablets prepared

TABLE IV

OPTIMUM EXPERIMENTAL CONDITIONS FOR THE APPLICATION OF AN ORTHOGONAL FUNCTION TO THE DIFFERENTIAL SPECTROPHOTOMETRY [$p_{j(r)}$] METHOD

Experimental parameter	Acetazolamide	Hydrochlorothiazide
Reference solution concentration/mg per 100 ml	1.3	0.9
Wavelength range/nm	256–276	268–278
Wavelength interval/nm ..	4	2
Median wavelength/nm	266	273
Concentration range/mg per 100 ml	1.5–2.7	1.1–2.3
Regression equation	$p_{2(r)} \times 10^3 = 11.4898–8.9204C$	$p_{2(r)} \times 10^3 = 7.6634–8.5884C$

in the laboratory by adding a specified amount of tablet base (composed of commercial lactose 60 g, calcium carbonate 30 g, starch 4 g, gelatin 3 g and talc 3 g) to a known amount of the drug. The laboratory-prepared tablets were also assayed by the A method (traditional spectrophotometric method). Commercial tablets (Cidamox and Hydrazide K) were analysed after powdering and mixing 20 tablets of only one batch by each of the four methods (Table III). The accuracy of each method is determined by calculation of the t value. For laboratory-prepared tablets and Cidamox, the calculated value of t in the p_2 and $p_{2(r)}$ methods does not exceed the theoretical value at the 95% confidence level, whereas in the A_r and A methods the calculated value is higher than the theoretical value. While the p_2 and $p_{2(r)}$ methods give assay results not significantly different from the true or label value, indicating their high accuracy, the A_r and A methods give assay results significantly different from the true value. Therefore, the irrelevant absorbance is corrected mathematically by the use of either the p_2 or $p_{2(r)}$ method. All of the methods for Hydrazide K give results significantly different from the true value, which suggests that the tablet does not really contain 100% of the label claim.

With the $p_{2(r)}$ method a low standard deviation is obtained. The F-test shows that the precision of the $p_{2(r)}$ method is better than that of the p_2 method.

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Received *May 22nd*, 1979
Accepted *September 26th*, 1979

Analyst, March, 1980, Vol. 105, pp. 227–233

Tungsten Filament Vaporiser and Oxyhydrogen Flame for Optical-emission Spectrometry

R. L. Warren

Institute of Nuclear Medicine, Middlesex Hospital Medical School, Mortimer Street, London, W1N 8AA

The vaporisation of a sample from a tungsten filament into a hydrogen atmosphere with subsequent combustion in oxygen provides a novel source for optical-emission spectrometry. The burner and ancillary equipment are described. Some of the factors affecting sensitivity, particularly in relation to flame background, are discussed. Results are presented for calcium, chromium, copper, iron, lead, lithium, magnesium, manganese, nickel, silver, strontium and thallium. The precision is about 5% for a 3-μl sample of total element content between 10 pg (for lithium) and about 7 ng (for lead).

Keywords: *Flame-emission spectrometry*; *tungsten filament vaporiser*; *micro-samples*; *oxyhydrogen flame*

In the determination of sodium and potassium at the picogram level it was found that the introduction of such a small sample into a physically small flame provided a greater proportion of emission of sample radiation over the emission of the flame itself than was the case when the flame was larger.

The problem of the introduction of the sample into a small flame was solved by volatilising the sample from an electrically heated filament into the air supply of a pre-mixed air - hydrogen flame. The brief emission produced by the sample in the flame was integrated by means of a monochromator - photomultiplier combination monitoring the appropriate spectrum line in the flame, the period of integration being controlled by a timer circuit. This method and its application to the analysis of cochlear endolymph was described by Bosher and Warren.[1] Subsequent similar applications of this technique have been reported by Haljamöe and Wood[2] and Grime and Vickers.[3]

Certain advantages of the technique suggested a possible wider application, even where sample size was not a restriction. Linearity can be assessed using only one concentration of standard solution by the application of multiples of the measured aliquot to the filament. Similarly, sample matrix effects can be investigated by comparison of the level of signal obtained when sample and standard are simultaneously vaporised from the filament, as opposed to the sum of the signals that they produce separately. The introduction of the sample vapour into the flame does not cause any significant alteration in the combustion processes, for example by dilution and consequent cooling, as occurs during the aspiration of aqueous aerosols. Advantage can also be taken of the fact that in some instances the temperature at which different elements are evaporated from the filament is sufficiently marked that, by appropriate adjustment of the filament current, a thermal fractionation can be obtained. Initial drying of the sample, and subsequent ashing if required, can be effected by similar control, and following vaporisation of the element any sample residue can be removed by a final, higher temperature heating of the filament.

The initial limitation on the application of the original method to other elements was imposed by the use of a platinum filament and an air - hydrogen flame; the melting-point of the former would not allow vaporisation of such elements as calcium or magnesium, nor would the temperature of the latter be suitable for their excitation.

Experimental

A new burner - filament combination has been developed, together with a four-channel filament power supply and timer circuit to control the duration of heating in each channel and the period of integration of the sample emission.

The system uses a tungsten filament operating in an atmosphere of pure hydrogen. The stream of hydrogen passing over the filament is ignited by a pilot oxyhydrogen flame as it

emerges from the burner. The pilot hydrogen supply is then cut off and the flame supported by the oxygen supply that had previously supplied the pilot flame.

Access to the filament for application of the sample is provided by a hinged door fitted with a transparent silica window, which permits observation of the behaviour of the sample on the filament. The door is sealed with a rubber O-ring and secured with a screw latch. Before opening the door the hydrogen supply to the filament is cut off, then nitrogen is substituted and allowed to flush out the filament compartment. Fig. 1 shows a photograph of the burner with the door open and a cross-sectional diagram is shown in Fig. 2. The filament, F, is formed from a 65 mm length of 0.04 × 0.7 mm tungsten ribbon, bent in the form shown. A 12-mm length at each end of the ribbon is bent back on itself to ensure that the maximum voltage drop occurs across the centre V portion. These double thickness ends of the filament are clamped between split 8 mm diameter brass terminals (E) set 20 mm apart, which pass through gas-tight PTFE insulated bushes in the back plate of the filament compartment. The terminals are connected to the power supply by cable rated at 20 A a.c. current. In a pure hydrogen atmosphere the filament operates at white heat with a current of about 16 A. Filament life has not been assessed; it is in excess of 1000 heating cycles and its destruction has so far been caused only by inadvertent overheating in an oxidising atmosphere or by mechanical mishandling.

The burner body is screwed into the top of the filament compartment and is constructed so that the main stream of hydrogen emerges at the centre of three pilot flame jets (B) at the top of the burner. These jets are angled towards the centre line and are formed from 10 mm long, 0.6 mm internal diameter stainless-steel tubes pressed into the body of the burner. Oxygen and by-pass hydrogen are supplied through the concentric tube mixer, as is shown in Fig. 2. A water-cooled jacket surrounds the upper 27 mm of the burner body. A 160 × 33 mm diameter transparent silica chimney is placed 25 mm above the top of the burner. This chimney has a steadying influence on the flame as well as providing a safeguard against inadvertently reaching across the flame, which is not readily seen, being small and relatively non-luminous. By lowering the chimney and providing an adequate pure air supply the flame can be enclosed and isolated from the laboratory atmosphere, as in the original design. This has only proved desirable for very low level sodium determinations.

Gas flow-rates are monitored and adjusted by means of flow meters fitted with needle valves. The consumption of gas, depending on the particular element being determined, is oxygen 0.6–0.8, pilot hydrogen 0.15–0.20, main hydrogen 0.35–0.45 and nitrogen 0.8 l min^{-1}.

The burner is positioned about 60 mm before the entrance slit of the monochromator and at the centre of curvature of a 56 mm focal length concave mirror placed on the opposite side of the burner to the entrance slit. The aperture of this mirror and the position of the flame then ensure that the monochromator aperture is adequately filled. The monochromator was adapted from a design by Perry[4] and is of the Littrow type, using a 60° quartz prism with a refracting face 17.8 cm long by 11.4 cm high. The relevance of the use of a monochromator of such aperture is discussed in a later section.

The photomultiplier is an EMI, Type 6256 SA, the output of which is connected to the input of a Model 610C Keithley electrometer, which, in the coulombmeter mode, is used to integrate the flame emission. A Honeywell Electronik 194 pen recorder is driven by the output of the electrometer in order to provide a permanent record of the integrated signals. As a coulombmeter the electrometer functions as a current integrator or charge measuring device capable of integrating current of pulse width down to about 100 ns. The duration of the input to the electrometer during integration (channel 3) is controlled by the timer circuit shown in Fig. 3. It also controls the duration of heating of the filament in the other channels. The required current in each is provided by four separate, variable transformers, T_1, which regulate the input to a single, low voltage, high current transformer T_2, the secondary winding of which is connected to the filament. The duration of each stage of heating is determined by four separate, variable resistance - capacitor combinations (R_1 and C_1) in the timer circuit. For the purpose of determining the required level and duration of passage of filament current in each channel appropriate switching is incorporated to enable the power supplies and timer circuits to be manually adjusted separately, independently of each other. For clarity, this switching is omitted from Fig. 3. Similarly, only one channel is shown in the circuit diagram.

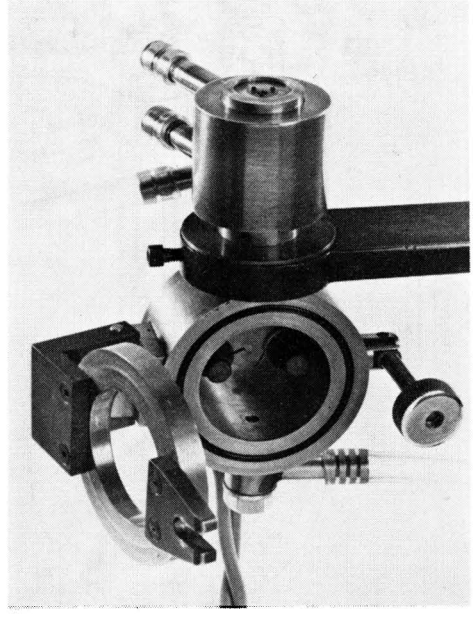

Fig. 1. Tungsten filament vaporiser and burner.

[to face page 228

Following the application of the sample to the filament, the door of the compartment is closed and the nitrogen to hydrogen change-over effected; after re-ignition of the main flame the pilot hydrogen supply is turned off. This switching on and off of the pilot hydrogen and the change-over and reversal of the nitrogen and main hydrogen supplies are effected by use of electromagnetic valves that are switched in sequence by microswitches actuated by motor driven cams. The same motor also drives an additional set of cams and microswitches, which, in turn, energise the coils of four separate five-pole relays. Each relay selects the variable transformer (contact A), timer resistance and capacity (contacts B and C) and energises a single-pole relay RL_2 (contact D) to trigger the timer. The timer then energises a three-pole relay, RL_3, which stops the cam drive motor for the duration of the timing interval, connects the mains supply (contact E) to the selected variable transformer and energises a single-pole change-over relay, RL_4, via contact F to switch the photomultiplier anode from earth to the input of the electrometer.

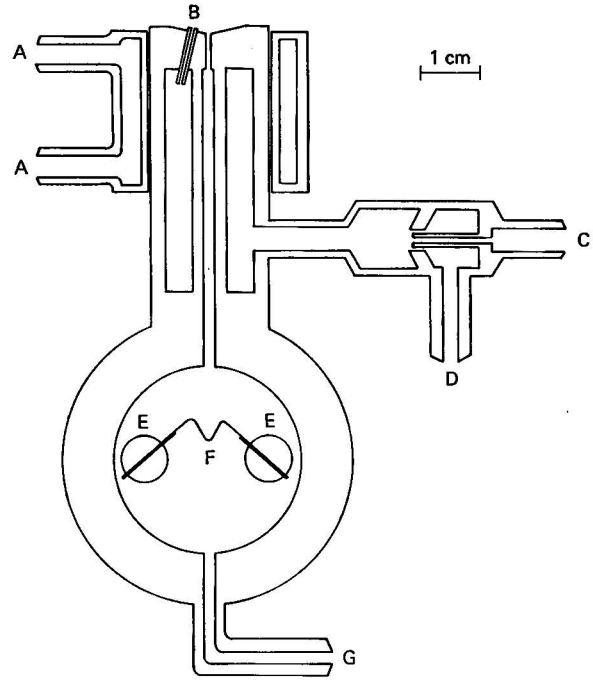

Fig. 2. Schematic cross-section of vaporiser and burner: A = cooling water, B = pilot flame jets, C = oxygen, D = hydrogen, E = filament terminals, F = filament, G = hydrogen or nitrogen.

The electrometer input, E_1, is normally earthed through the contacts of a single-pole relay, RL_5, but just prior to vaporisation of the sample (channel 3) this relay is de-energised by a cam-opening microswitch, MS, so that the flame emission is integrated when the sample is vaporised. At the expiration of the timing interval the filament current is switched off and the photomultiplier anode re-earthed by de-energising relay 4.

The integrated charge stored in the input capacitor of the electrometer corresponds to the total photomultiplier current for the period defined by the setting of the timer and consists of the dark current and the current due to flame background plus the sample emission. As the recorder response lags behind that of the electrometer a sufficient time interval, defined by the dwell angle of the lobes of the cam controlling relay 5, allows the recorder pen to reach the correct indication of the magnitude of the charge, after which the electrometer is discharged by re-earthing the input and the pen returns to zero. Twin lobes on

this cam and the cam actuating the microswitch of channel 3 repeat this channel and thus provide a recording of the flame background plus dark current. Following channel 4 and cleaning of the filament the gas solenoid valves are operated in order to re-light the pilot flame and change over the filament hydrogen supply to nitrogen. To limit the recorder chart drive to the appropriate part of the cycle, chart drive switches are incorporated in the five-pole relays and, together with over-ride switches, these allow the chart drive to be on or off in any channel.

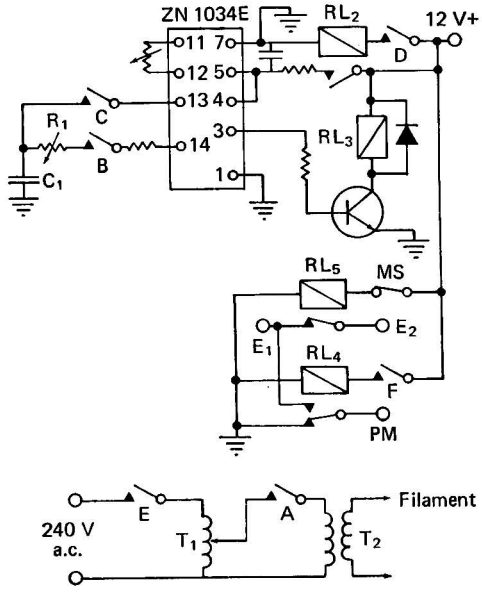

Fig. 3. Timer,* control circuit and filament supply: A, B, C, D = five-pole relay contacts; E, F = three-pole relay contacts; RL_2 = three-pole relay; RL_3 and RL_4 = one-pole relay; RL_5 = one-pole relay change-over; R_1 and C_1* = timer resistance and capacitor; ZN 1034E = I.C. timer*; T_1 = variable transformer, 0–250 V, 0.7 A; T_2 = 240 V/6 V, 100 VA transformer; MS = microswitch; E_1 = electrometer input; E_2 = electrometer earth; PM = photomultiplier anode. * R.S. Components Ltd. Data sheet R/2466 for component types, values and timing data.

The sample volume as applied to the filament is of the order of 0.3–4 μl and, provided this is identical for the standard and the sample, it is not necessary to know the actual volume. The sample is pipetted with a small Pasteur pipette drawn from approximately 2 mm o.d. silica tubing. For volumes below 1 μl it is drawn out to about 0.25 mm i.d. with the tip drawn to a diameter of 0.1 mm. Depending on the volume required a mark is made with waterporoof ink 10–20 mm from the tip. For volumes over 1 μl the pipette is drawn out so as to form a small bulb of such a volume as to contain the major portion of the sample, as in a conventional bulb pipette, and with a mark on the constricted portion of the neck above the bulb. Either type of pipette is attached to a small rubber teat and is filled to the mark mainly by capillary effect, pressure on the teat being used to stop the meniscus at the required level and later to expel the contents gently so that the liquid hangs as a single drop near the tip. It can then be transferred to the filament. The use of a magnifying glass is recommended when pipetting. Being made of a silica, these pipettes can be thoroughly cleaned in a Bunsen burner flame after rinsing with distilled water.

Drying of the sample requires a current of about 4.0 A to pass for about 45 s. In the

presence of protein this drying time may need to be extended. It is important that visible bubbling or boiling of the solution on the filament should be avoided, otherwise loss may occur. When required, the removal of sodium and potassium is achieved at a dull red heat, *i.e.*, at about 750 °C. This, or a slightly lower temperature, is also suitable for ashing of protein and, depending on the nature of the sample, the duration of treatment at this temperature is usually about 25 s, with a filament current of about 8 A.

The evaporation of elements such as calcium and magnesium requires a current of about 12 A, but this need only be applied for about 0.3 s. The optimum evaporating current varies for different elements and lies between 9.0 and 12 A for the elements listed in Table I below. The final clean-up of the filament requires the maximum power available, *i.e.*, about 16 A, which is applied for approximately 4 s.

The reproducibility of the timer - integrator part of the circuit was measured by means of a constant current source being substituted for the photomultiplier and integrated for a period of 0.35 s. The peak to peak difference for 24 separate integrations was less than 0.3% of the recorder full-scale deflection when this deflection was equal to 3×10^{-8} C.

The photometric precision was assessed from a series of integrations of the 404.64 nm line of a low pressure mercury lamp at a band width of 0.1 nm. The photomultiplier dynode voltage was 1200 and the integration period and electrometer sensitivity were as above. The level of illumination was set to produce a photomultiplier anode current of about 8×10^{-8} A; the dark current was 3×10^{-10} A. These conditions correspond closely to those employed in measuring the elements listed in Table I. The mean and relative standard deviation of the integrated charge were 2.24×10^{-8} C $\pm$ 1.6% ($n = 11$). This degree of variation is principally a result of photomultiplier noise, which can be reduced by increasing the light level and reducing either the dynode voltage or the input sensitivity of the electrometer. For example, with the band width increased to 1 nm and the dynode voltage reduced to 900, the relative standard deviation is reduced to 0.6%.

TABLE I

BACKGROUND EQUIVALENT CONCENTRATION, C_{Beq}, AND DETECTION LIMITS, C_L

Element	Analytical line/nm	Band width/ nm	(*a*) p.p.m.	(*a*) S_r, %	(*b*) C_{Beq}, p.p.m.	(*c*) C_L, p.p.m.
Calcium	422.67	0.11	0.04	5.4	0.0053	0.0008
Chromium	425.43	0.11	0.20	4.0	0.065	0.0085
Copper	327.39	0.04	0.30	4.4	0.035	0.009
Iron	371.99	0.08	1.00	4.4	0.100	0.020
Lead	405.78	0.10	2.50	3.5	0.600	0.040
Lithium	670.78	0.40	0.004	5.8	0.0005	0.0002
Magnesium	285.21	0.03	0.20	3.6	0.025	0.006
Manganese	403.08	0.10	0.05	5.6	0.0085	0.0014
Nickel	341.48	0.06	0.50	3.0	0.075	0.010
Silver	338.29	0.06	0.10	5.5	0.008	0.003
Strontium	460.73	0.15	0.05	6.0	0.008	0.001
Thallium	377.57	0.08	0.20	4.8	0.030	0.004

(*a*) Concentration of element used to determine C_{Beq} and calculate C_L. Relative standard deviation, S_r, calculated from 11 replicate determinations of each element.

(*b*) C_{Beq} = concentration of each element required to produce a signal level equal to the flame background.

(*c*) C_L = concentration of element required to give a signal equal to three times the standard deviation of the background noise.

Results and Discussion

The instrument has been applied to the measurement of calcium in adrenal cells by Mackie *et al.*,[5] and to the analysis of cochlear endolymph for calcium and magnesium by Bosher and Warren.[6] The elements so far investigated in addition to sodium and potassium are shown in Table I.

Fig. 4 is reproduced from recordings made during the compilation of the data recorded in Table I and illustrates the deflections obtained from eleven vaporisations of 3 μl of a 0.05 p.p.m. manganese solution. Adjacent to each peak is the corresponding background

deflection. The mean and standard deviation for the line and the background were 36.00 ± 2.00 and 6.2 ± 0.34, respectively, the units being divisions of the chart where 100 is equal to full-scale deflection. To the right are the deflections obtained from 1, 2, 3 and 4 drops of the same manganese solution applied sequentially to the filament, dried and vaporised. For these last recordings the input sensitivity for full scale was reduced from 3×10^{-8} to 10^{-7} C.

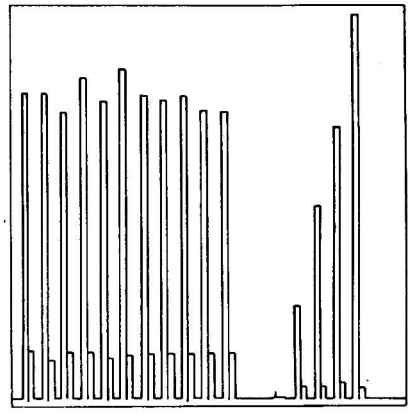

Fig. 4. Reproducibility and linearity for a 0.05 p.p.m. manganese solution (see text). Wavelength = 403.08 nm.

If the band widths given in Table I are increased a reduction in sensitivity occurs. With strontium, if the entrance and exit slits are increased from 0.020 mm to 0.20 mm the flame background signal increases by a factor of 100, but that from the line increases only about 16 times and the background equivalent concentration is increased from 0.008 to 0.045 p.p.m. When the element concentration is higher the need to differentiate between the spectrum line and the flame background is reduced, the band width transmitted by the monochromator can then be increased, and the precision improved by a reduction in the dynode voltage of the photomultiplier, as is shown above. By using a band width of 1 nm, a dynode voltage of 800 and an input sensitivity of the electrometer of 3×10^{-8} C, a manganese solution of 2 p.p.m. was analysed 11 times (22 evaporations), which gave a mean and standard deviation of 2.019 ± 0.030 p.p.m. At this level the flame background following evaporation was not discernible, nor could it be detected adjacent to the spectral line during evaporation. If the flame background does increase during evaporation of the sample it must be measured and the level subtracted from the sample signal. This measurement is made adjacent to the spectral line and requires the wavelength setting of the monochromator to be altered accordingly. The present monochromator can be set with the necessary precision to ensure that the wavelength reproducibility is within the required limits, *i.e.*, at 404.35 nm to within 0.004 nm.

When the concentration of the element is such that the flame background is not discernible, it is advantageous to increase the band width. This may not be practicable if it allows molecular bands or lines of other elements to overlap the analytical line, as was described by Warren[7] for the hydroxyl band overlapping magnesium and copper lines.

The analysis of magnesium at 285.21 nm at a concentration below 1 p.p.m. and with the band width greater than about 0.06 nm is complicated by the overlapping of the 285.27 nm hydroxyl band, and as the concentration increases the sensitivity is proportionally reduced as self-absorption increases. With copper the more intense persistent line at 324.75 nm is superimposed on the profile of a hydroxyl band and is therefore incapable of separation by increased instrument resolution, whereas the 327.40 nm line is usable down to at least 0.1 p.p.m., provided that the band width does not exceed about 0.03 nm.

It has been shown that when the element concentration is increased the band width can also be increased, allowing the dynode voltage or the input sensitivity of the electrometer to

be reduced. Alternatively, a smaller monochromator could be employed. By use of a Hilger Uvispec monochromator with a 55 × 86 mm, 30° glass prism and adapted to use a 13-stage photomultiplier, a 0.1 p.p.m. calcium solution was measured with a background equivalent concentration and a limit of detection of 0.028 and 0.004 p.p.m., respectively. This monochromator, equipped with a quartz prism, is less suitable for the analysis of magnesium and copper for the reasons already given.

The potential of electrothermal evaporation in flame emission spectrometry has been described and demonstrated. Normally one sample per minute can be measured as no cool-down period is required between samples. The burner is silent in operation and gas consumption is very low compared with other flame methods. The application of this method is not confined to the twelve elements listed here and probably extends to a further ten. The most notable feature of the method is the very small sample size, the repeated measurement of which does not appear to introduce an unacceptable error, as indicated by the relative standard deviation for the 2 p.p.m. manganese solution (1.5%) as opposed to the photometric precision (0.6%). The absolute amount of element applied to the filament and vaporised is between 12 pg (for lithium) and 7 ng (for lead). As with other emission methods it is also potentially capable of simultaneous multi-element analysis. Additionally, the prior removal and analysis of the alkali metals by thermal fractionation is possible where a sufficiently marked difference in evaporating temperature exists. This can be useful in reducing the flame background by eliminating the continuous emission of these elements. The technique has so far been applied in the analysis of calcium and magnesium.

The original construction of the monochromator was financed by a grant from the Paul Instrument Fund of the Royal Society. Construction of the timer and filament supply and development of the burner was financed by the Smith Kline and French Foundation.

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Received *August 23rd*, 1979
Accepted *September 17th*, 1979

Effect of Tetraphenylphosphonium Chloride on D.C. and Differential-pulse Polarograms of Synthetic Food Colouring Matters

A. G. Fogg and D. Bhanot

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU

Tetraphenylphosphonium chloride (TPPC) has a pronounced effect on the d.c. and differential-pulse (d.p.) polarograms of some synthetic food colouring matters. The d.c. half-wave potentials and d.p. peaks of two pyrazole azo colouring matters, tartrazine and Yellow 2G, and of the triphenylmethane colouring matter Brilliant Blue FCF, are shifted to more negative potentials on the addition of TPPC. The mean limiting diffusion currents and peak currents of these and certain other synthetic colouring matters are altered significantly on its addition, the changes being greater in d.p. polarography. For both d.c. and d.p. polarograms the changes occur at TPPC concentrations up to 100 μg ml^{-1}; above this concentration half-wave and peak potentials and limiting diffusion and peak currents change very little in most instances. The implication of these effects on the use of polarography for the determination of synthetic food colouring matters is discussed.

Keywords: *Tetraphenylphosphonium chloride*; *food colouring matters*; *differential-pulse polarography*

In previous papers,[1,2] procedures were given for the determination of tartrazine - Sunset Yellow FCF, tartrazine - Green S, amaranth - Green S and Chocolate Brown HT - tartrazine - Green S mixtures in soft drinks by differential-pulse (d.p.) polarography without prior separation of the colouring matters from the sample. Tartrazine and Sunset Yellow FCF are reduced at similar potentials in Britton - Robinson buffer and normally cannot be determined in admixtures. The addition of tetraphenylphosphonium chloride (TPPC), however, was shown to cause the peak potential of tartrazine to be shifted 70 mV to a more negative potential at pH 9, and to cause the peak current to be doubled. The peak potential of Sunset Yellow FCF, on the other hand, was unchanged on the addition of TPPC, although its peak current was halved. This difference in the behaviour of tartrazine and Sunset Yellow FCF was made the basis of a polarographic method for their determination in mixtures.[1] The capability of shifting the tartrazine peak and suppressing the peaks of Chocolate Brown HT and Green S by adding TPPC was made the basis of a method of determining mixtures of these three dyes.[2]

This work was carried out in order to investigate more fully the effect of TPPC on the polarograms of synthetic food colouring matters, and to investigate further the potentialities of using TPPC in the analysis of mixtures of food colouring matters.

Experimental

Polarographic measurements were made with a PAR 174 polarographic analyser (Princeton Applied Research). Three-electrode operation was employed using a dropping-mercury electrode, a platinum counter electrode and a saturated calomel reference electrode. Polarography was carried out at room temperature (approximately 22 °C). Solutions for polarography were deoxygenated with nitrogen that had previously been passed through a vanadium(II) scrubber. D.c. polarography was carried out with a natural drop time (2.80 s in Britton - Robinson buffer of pH 2 on open circuit), low pass filter 3 and a sweep rate of 5 mV s^{-1}. D.p. polarography was carried out with a forced drop time of 1 s, a scan rate of 5 mV s^{-1} and a pulse height of 50 mV.

Solutions for polarography were prepared with Britton - Robinson buffer (pH 1.9; 0.04 M in boric acid, acetic acid and orthophosphoric acid) in 25-ml calibrated flasks. Aliquots of solutions of the colouring matter and 10 ml of Britton - Robinson buffer (pH 1.9) were adjusted to the required pH with 2 or 4 M sodium hydroxide solution, diluted to 25 ml in

the calibrated flask, and transferred to the polarographic cell and deoxygenated for 10 min before polarography. To study the effect of increasing concentration of TPPC, aliquots of a 0.1 M (37.4 mg ml^{-1}) TPPC solution were added using a 10- or 100-μl syringe. Only brief deoxygenation (2 min) was required after each addition of TPPC. In the same way, the effect of pH was studied by adjusting the pH of the solution directly in the polarographic cell.

Results

The effect of pH on the d.p. peak potentials of the synthetic colouring matters in the absence of TPPC is summarised in Fig. 1. The position of the polarographic peak, taken in conjunction with the colour of the colouring matter, might be used in routine determinations as tentative confirmation of the identity of the colouring matter. With experience, even minor differences in peak shapes can sometimes be distinguished and used for identification purposes. Most colouring matters give excellent d.p. peaks over at least part of the pH range. The quality of the d.p. peak obtained for each colouring matter is indicated in Table I.

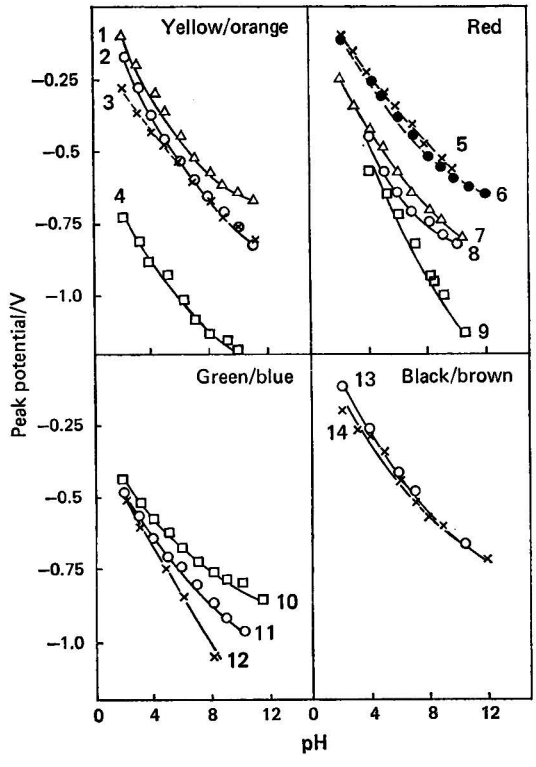

Fig. 1. Effect of pH on d.p. peak potentials: 1, Sunset Yellow FCF; 2, tartrazine; 3, Yellow 2G; 4, quinoline yellow; 5, carmoisine; 6, armaranth; 7, Red 2G; 8, Ponceau 4R; 9, Erythrosine BS; 10, Green S; 11, Patent Blue V; 12, Brilliant Blue FCF; 13, Black PN; and 14, Brown FK.

When the data given by Fogg and Yoo[1] for the effect of TPPC concentration on the d.p. peak of Sunset Yellow FCF at pH 9 are plotted, it can be seen that i_p falls sharply to a minimum value at about 50 μg ml^{-1} of TPPC and then rises again, reaching a steady value

TABLE I

DIFFERENTIAL-PULSE POLAROGRAPHY OF FOOD COLOURING MATTERS

Colouring matter	Optimum pH for determination in absence of TPPC	Remarks	Effect of TPPC (750 μg ml^{-1}) on d.p. polarography of 1 × 10^{-5} M colouring matter: Peak potential (ΔE_p quoted)	Peak current	Remarks
Tartrazine	7–8	No well defined peak at pH <4. Small pre-peak at pH >6	220 mV at pH 4 (new peak) 100 mV at pH 7 90 mV at pH 8 70 mV at pH 9	No change Slight enhancement Significant enhancement	Exceptional improvements on adding TPPC at pH 9
Quinoline yellow	2	i_p at pH 2 double that at higher pH	50 mV at pH 2	Halved at pH 2	Second wave appears with TPPC
Yellow 2G	2–8		50 mV at pH 4 190 mV at pH 9		Peak shape improved at pH 9
Sunset Yellow FCF	2 or >6	Peak splits at pH 3, i_p less at pH 3–5	0	Suppressed at all pH values	Base line better without TPPC
Carmoisine	2 or 7–8	Second peak appears at pH >2	0	Suppressed at pH 4	
Amaranth	2–6, 8–12	i_p greater at pH >11, better base line at high pH	0	General suppression	
Ponceau 4R	7	No peak at pH <4. Peaks at low pH small and broad. Distorted base line at high pH	Negligible	pH 3 peak present (absent if no TPPC). pH 7 peak suppressed and broader. pH 9 peak suppressed but improved base line	TPPC improves base line if suppression tolerated. Post-peak appears at higher TPPC concentrations
Erythrosine BS	4, 8.5	Precipitation at pH 2 at higher concentrations. Ill-defined peak at pH 5.5–7.5	~40 mV at pH 8.2 to more positive potential	pH 4 peak completely suppressed. pH 6–8 peak greatly suppressed	
Red 2G	2–11	i_p greatest at pH >8 and better base line	30 mV at pH 4	pH <7 peak, slight enhancement. pH 9 peak, extensive suppression	
Patent Blue V	2–3		0	Suppressed	
Indigo carmine	8–9		0	Suppressed at low TPPC concentrations but enhanced at higher concentrations	
Brilliant Blue FCF	3	pH >8 peak ill-defined and broad	220 mV at pH 3	i_p halved	Peak at pH 9 now well-defined
Green S	2–11	Better defined in acidic solution and i_p greater. i_p decreases as pH increases but some increase again at pH >9	0 at pH <6. pH >6, E_p constant at −0.68 V	General extensive depression. Reduced depression at pH >9	
Brown FK	10.6	Best shaped peak at pH 10.6. Acidic pH, broad peaks; pH 4–8, shoulders. i_p maximum at pH 7–8 but bad shape	0	Shoulders removed. Slight enhancement in acidic solution but bad shape. Depression in alkaline solution. pH 5–7 peak ill-defined	
Chocolate Brown HT	4–8	Double peak, i_p constant	0	Complete suppression	
Black PN	2, 10.5	E_p constant at pH >8. Peaks well defined at low and high pH, ill-defined at neutral pH	0	Less affected at pH 2. Greatly suppressed at pH 10.5	

of about 50% of the original current at TPPC concentrations above 100 μg ml^{-1}. Under the same conditions at pH 9, i_p for tartrazine rises sharply at about 50 μg ml^{-1} of TPPC to give a steady value about 2.5 times larger than the initial current. With tartrazine, E_p is shifted 70 mV to a more negative potential, whereas E_p for Sunset Yellow FCF is unchanged.

The effect of TPPC concentration on the peak potentials and peak currents of the d.p. peaks of other permitted synthetic food colouring matters (generally at the 1 × 10^{-5} and 1 × 10^{-4} M levels) was studied. In some instances the effect was studied at several pH values. The results are summarised in Table I. The effect of TPPC concentration on the peak currents is shown in Fig. 2. For three permitted synthetic food colouring matters (tartrazine, Yellow 2G and Brilliant Blue FCF) the value of the peak potential is affected

significantly by the addition of TPPC. The effect on tartrazine at pH 4 (not illustrated) differs to some extent from that at pH 9. At pH 4 the peak potential of the initial peak varies very slightly on the addition of TPPC, whereas the peak current decreases markedly with increasing TPPC concentration. For TPPC concentrations above 250 μg ml^{-1}, however, a second peak appears at a potential 170 mV more negative than the initial peak and this second peak rapidly increases in size and becomes the major peak.

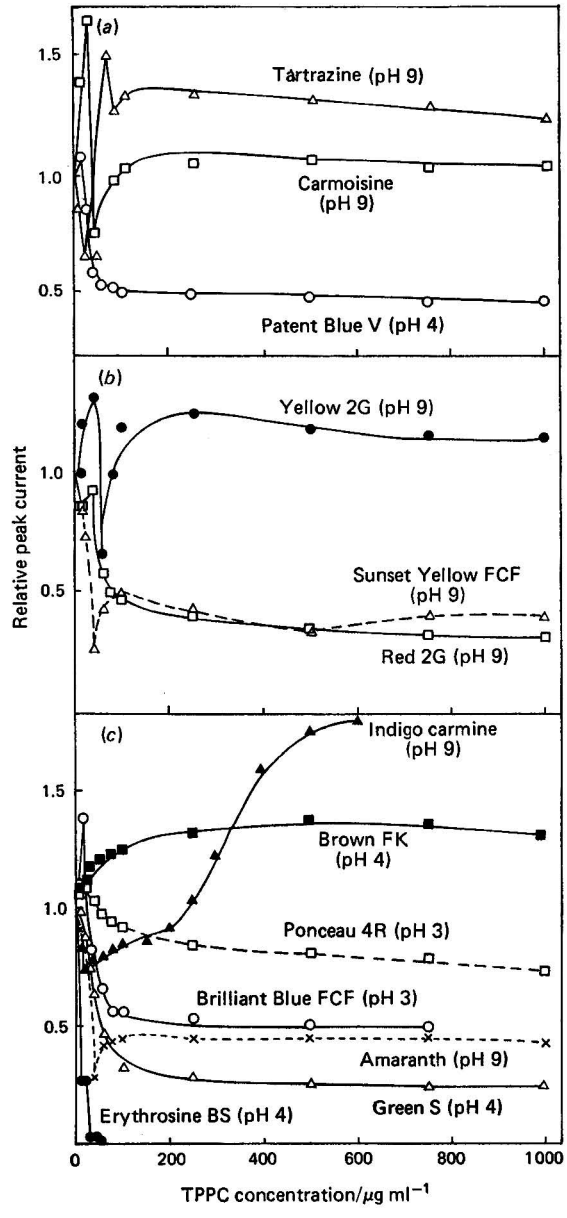

Fig. 2. Effect of TPPC concentration on d.p. peak currents. Colouring matter concentration, 10^{-4} M. Peak currents are shown relative to zero TPPC concentration.

At pH 9 the peak potential of Yellow 2G is shifted 190 mV to a more negative potential at TPPC concentrations above 50 μg ml^{-1}; the peak current follows a similar pattern to that of Sunset Yellow FCF, passing through a peaked minimum at 50 μg ml^{-1} of TPPC. The peak potential for 2×10^{-5} M Brilliant Blue FCF at pH 3 is displaced 220 mV to a more negative potential at TPPC concentrations above 50 μg ml^{-1} and i_p passes through a peaked minimum, giving a steady value of about half the original at TPPC concentrations above 100 μg ml^{-1}. With Brilliant Blue FCF, the effect of colouring matter concentration was studied. At the lower concentration of 2×10^{-6} M the effect of TPPC on E_p and i_p was essentially the same as at 2×10^{-5} M, whereas at 2×10^{-4} M the potential of the main peak remained unchanged, but a minor peak of constant i_p and E_p was present at a potential 100 mV more positive than the main peak at all concentrations of TPPC. With increasing TPPC concentration, i_p for the main peak reached a peaked maximum and was then reduced to about 30% of its original value.

Although the addition of TPPC to the other permitted colouring matters had only a slight effect on their peak potentials, the effect on i_p was usually just as marked as for the other three dyes. The effect of TPPC concentration on the d.p. peak currents of several colouring matters, excluding those giving ill-defined peaks, is illustrated in Fig. 2 and other details are given in Table I.

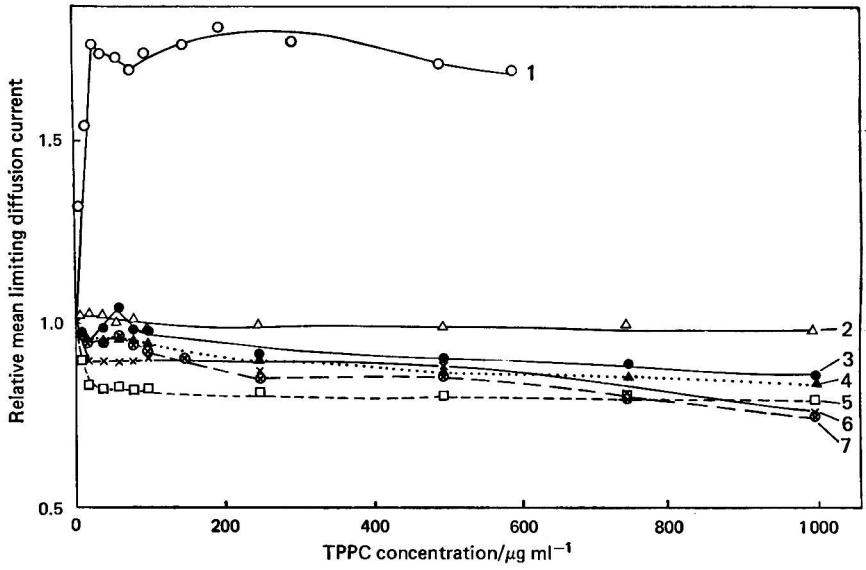

Fig. 3. Effect of TPPC concentration of d.c. limiting diffusion currents. Colouring matter concentration, 10^{-4} M. Mean limiting diffusion currents are shown relative to zero TPPC concentration. 1, Indigo carmine (pH 9); 2, Patent Blue V (pH 4); 3, amaranth (pH 9); 4, Green S (pH 4); 5, Sunset Yellow FCF (pH 9); 6, Brown FK (pH 4); and 7, Ponceau 4R (pH 3).

A study was also made of the effect of TPPC on d.c. polarograms of the colouring matters. D.c. limiting diffusion currents are independent of the irreversibility of the system and are less affected by changes in the composition of the supporting electrolyte than are d.p. peak currents.[3] Changes in half-wave potentials were found to follow the same pattern as the changes in peak potentials. The effect of TPPC concentration on the limiting diffusion currents of several colouring matters is illustrated in Fig. 3. Those which give polarographic maxima in the absence of TPPC have been excluded from Fig. 3, but details are given in Table II. In most instances the decrease in i_d on addition of TPPC is relatively small. With Red 2G at pH 9 some depression is apparent but a polarographic maximum is conveniently removed; some enhancement of i_d is apparent at pH 4. The limiting current for

Brilliant Blue FCF is doubled on the addition of more than 100 μg ml^{-1} of TPPC, although the wave does appear to be produced by a coalescing of two waves. This enhancement of the d.c. limiting current is in marked contrast to the halving of the d.p. peak current. D.c. waves for indigo carmine are enhanced considerably by the addition of TPPC, but a pre-wave is present; precipitation of the colouring matter occurs at TPPC concentrations above 600 μg ml^{-1}.

TABLE II

D.C. POLAROGRAPHY OF FOOD COLOURING MATTERS

Colouring matter	pH	Quality of d.c. wave in absence of TPPC	Effect of TPPC (750 μg ml^{-1}) addition on limiting diffusion current	Remarks
Tartrazine	9	Polarographic maximum	Wave suppressed even though d.p. peak enhanced	Maximum removed by 20 μg ml^{-1} of TPPC to give well defined wave
Quinoline yellow	4	No maximum	Some suppression	Ill-defined wave reflecting d.p. peaks
Yellow 2G	9	Polarographic maximum	Very little change	Maximum removed by 10 μg ml^{-1} of TPPC to give a well defined wave
Sunset Yellow FCF	9	No maximum	Some suppression	Well defined wave
Carmoisine	9	Polarographic maximum	i_d halved	Maximum removed by 20 μg ml^{-1} of TPPC but wave not well defined
Amaranth	9	No maximum	Very little change	Well defined wave
Ponceau 4R	3	No maximum	Very little change	Well defined wave; a second wave appears at TPPC concentrations >60 μg ml^{-1}
Erythrosine BS	4	Ill-defined wave	Almost totally suppressed (at TPPC concentrations >30 μg ml^{-1})	
Red 2G	9	Polarographic maximum	i_d halved	Maximum removed by 10 μg ml^{-1} of TPPC to give a well defined wave
Patent Blue V	4	No maximum	Very little change	Well defined wave
Indigo carmine	9	No maximum	i_d doubled (precipitation at >600 μg ml^{-1} of TPPC)	
Brilliant Blue FCF	3	Polarographic maximum	i_d doubled (at >10 μg ml^{-1} of TPPC)	Maximum removed by 10 μg ml^{-1} of TPPC to give well defined wave
Green S	4	No maximum	Very little change	Well defined wave
Brown FK	4	No maximum	Very little change	Reasonably well defined wave
Chocolate Brown HT	4	Double wave	Slight suppression	Not ideal for analytical use
	9	Ill-defined wave	Wave splits	
Black PN	9	No maximum	Slight suppression	Wave reflects characteristics of twin d.p. peaks

Discussion

Tetraphenylphosphonium chloride has been used previously[1,2] in the determination of mixtures of colouring matters. TPPC was shown to shift the peak potential of tartrazine to a more negative potential at pH 9 and to enhance its peak current: this allowed mixtures of tartrazine and Sunset Yellow FCF to be determined.[1] TPPC also has the property of suppressing extensively the peak currents of certain colouring matters; this is the case with Green S and Chocolate Brown HT at pH 9 and allows the determination of tartrazine in their presence. In this paper, data on the d.p. polarography of other synthetic colouring matters with and without the addition of TPPC are presented as an aid to the development of procedures for the determination of other mixtures of colouring matters.

Information is also presented on the d.c. polarography of these colouring matters. In general, d.c. polarograms are not altered as markedly as are d.p. polarograms by the addition of TPPC, although the polarographic maxima of several colouring matters are conveniently suppressed. D.c. polarography is carried out typically at a concentration of 10^{-4} M, and at this and higher concentrations some colouring matters show adsorption effects (basically the broadening and splitting of waves) at the dropping-mercury electrode. The d.c. wave of Patent Blue V, for example, appears to be made up of two waves that have coalesced, which makes it unsatisfactory for analytical use. This coalesced d.c. wave appears as a shoulder on the d.p. peak. The advantage of d.p. polarography, however, is that lower concentrations

can be polarographed and at a 1×10^{-5} M concentration of Patent Blue V a sharp d.p. peak is obtained without this shoulder.

For most of the colouring matters studied here any changes in peak current and peak potential that occur on the addition of TPPC appear in the range 0–100 μg ml^{-1}; with tartrazine at pH 4, however, marked changes occur at TPPC concentrations above 250 μg ml^{-1}. The changes must be caused by the adsorption of the tetraphenylphosphonium ion on the mercury surface, which means that the colouring matters are reduced through an adsorbed layer of tetraphenylphosphonium ion. Plots of drop time against potential for Britton - Robinson buffer containing different concentrations of TPPC show a marked decrease in drop time on the negative side of the electrocapillary maximum and confirm this adsorption. A limiting depression of the drop time is reached at about 100 μg ml^{-1} of TPPC, which presumably corresponds to the concentration of TPPC at which complete coverage of the mercury surface occurs.

Numerous studies have been made of the effect on d.c. polarograms of adsorption of inert electrolytes, and a particular study has been made of the effect of electrolytes and adsorbed species on the reduction of anions.[4,5] This is particularly relevant here as the colouring matters studied are acidic. Changes in half-wave potentials and limiting diffusion currents caused by changes in the structure of the double layer are to be expected. Pietrzyk and Rogers[6] studied the effect of dodecyltrimethylammonium chloride on the reduction of nitro compounds, and advocated the use of the shifts in half-wave potential observed in the analysis of mixtures. Our present work represents an application of this idea.

Further applications of the use of polarography in the determination of food colouring matters are being studied, including the use of TPPC in the paired-ion extraction of food colouring matters and in suppressing background currents arising from surface-active materials in real samples.

The authors thank Dr. N. T. Crosby of the Laboratory of the Government Chemist for helpful discussions and for providing samples of colouring matters.

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Received *July 27th*, 1979
Accepted *October 8th*, 1979.

Analyst, March, 1980, Vol. 105, pp. 241–245

Determination of Nitrate and Nitrite in Meat Products by Using a Nitrate Ion-selective Electrode

K. K. Choi and K. W. Fung

Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong

A procedure for the determination of nitrate and nitrite in meat products by a direct potentiometric method with a nitrate ion-selective electrode is presented. The soluble nitrate and nitrite were extracted from the meat products with a Soxhlet extractor using borax buffer solution (pH 9). The effect of pH on the recovery of nitrite and nitrate was studied. Results obtained by the proposed method were compared with those obtained by recommended spectrophotometric methods. Storage of samples before analysis was also studied.

Keywords: *Nitrate determination*; *nitrite determination*; *meat analysis*; *potentiometry*; *nitrate ion-selective electrode*

Nitrate and nitrite have been used in the curing of meat, poultry and fish for many years. Nitrite combines with myoglobin, a meat pigment, to form nitrosohaemoglobin, which is responsible for the characteristic red colour of meat products.[1,2] Nitrate and nitrite together with sodium chloride have been reported to have antimicrobial action,[1] and are common preservatives used in meat products.

Much concern has been shown recently about the levels of nitrate and nitrite in food products because of their possible reaction with amines to form toxic and carcinogenic nitrosoamines. Under UK regulations controlling the use of preservatives in foods, the levels of sodium nitrate and nitrite must not exceed 500 and 200 p.p.m., respectively.[3]

Nitrate and nitrite can be determined by a variety of methods.[1,2,4–6] Most commonly, nitrite is determined by the Saltzmann modification of the Griess method,[7] in which sulphanilic acid is diazotised by free nitrite ion in acidic medium and the diazonium ion is coupled with naphthylethylenediamine to give an azo dye. The intensity of the colour, corresponding to the concentration of nitrite in the sample solution, is determined spectrophotometrically. In a separate sample solution, nitrate is reduced to nitrite with a cadmium column[1,8,9] and the total nitrite in the resulting solution is determined. The concentration of nitrate is equivalent to the increase in the concentration of nitrite. Fine suspensions in the meat extract introduce errors into the absorption measurements, and a lengthy procedure is necessary in order to obtain a clear solution before measurement.[9–11] Also, the cadmium column must be regenerated frequently in order to preserve high efficiency. Fluorimetry[12,13] has also been used in nitrate and nitrite determinations. However, this method suffers from quenching effects due to the presence of some interferents. Differential-pulse polarography has recently been applied in the determination of nitrite.[14,15] This method is very sensitive but it is time consuming.

Coloration and cloudiness of the sample solution will not cause any interferences in a direct potentiometric method. Also, the method is simple and rapid. A nitrate ion-selective electrode has been used in the determination of nitrate in soil, natural water, waste water and food products,[16–18] but it has not been applied to the determination of nitrite in food products. In this paper, the applicability of the nitrate ion-selective electrode to the determination of nitrate and nitrite in meat products is described.

The soluble nitrate and nitrite in meat products were extracted continuously with borax buffer solution (pH 9). As nitrite is unstable in low pH, the effect of pH on the recovery of nitrite was studied. Storage of the samples before analysis was also studied. The results obtained by the proposed method were compared with those obtained by recommended spectrophotometric methods.

Experimental

Apparatus

An Orion nitrate ion-selective electrode (Model 93–07) and an Orion glass electrode (Model 91–01) were used in conjunction with an Orion double-junction reference electrode (Model 90–02) with ammonium sulphate (0.12 M) as the filling solution for nitrate and pH measurements. All potentials were measured with an Orion, Model 701A, digital pH/millivolt meter, which was coupled to an Orion electrode switch (Model 605), and were recorded on a Thomas printer (Model 80). An Oxford 25-μl syringe was used for the addition of standard nitrate solutions.

Reagents

All chemicals were of analytical-reagent grade. Sodium nitrate and sodium nitrite were dried under vacuum at about 100 °C overnight before use. Dilute standard solutions of nitrate and nitrite were freshly prepared by diluting stock standard solutions (1000 μg ml^{-1}).

Procedure

About 200 g of sample were chopped and homogenised into fine pieces in a blender for 5 min. An accurately weighed, homogenised sample (10 g) was transferred into the cellulose extraction thimble of a Soxhlet extractor. After continuous extraction for 3 h (3–4 cycles per hour) with 80 ml of buffer solution, the extract was transferred into a 100-ml calibrated flask. The extractor was rinsed with the same buffer solution, the washings were transferred into the calibrated flask and the volume was made up to the mark with water.

For potentiometric measurement of nitrate ion, 20 ml of extract were pipetted into a 100-ml beaker. While stirring, 5 ml of a solution of aluminium sulphate (0.03 M) in boric acid (0.06 M), 1 ml of sulphamic acid solution (0.01 M), 3 ml of silver sulphate (0.16 M) in 33% ammonia solution and 1 ml of ammonium sulphate solution (2.0 M) were added in the order given. A delay of 1–2 min was required between the addition of successive reagents. The pH was then adjusted to 3.3 $\pm$ 0.1 with concentrated sulphuric acid. The potential of the nitrate ion-selective electrode was measured with respect to the double-junction reference electrode at 25 °C and was recorded at 30-s intervals. The concentration of nitrate in the resulting solution was determined by the standard additions method[14] or the calibration graph method. The concentration of nitrate in the blank solution (2 μg ml^{-1}), which was low compared with that in the sample solution, was determined by the same procedure. The difference between the results for the sample solution and the blank was used to calculate the level of nitrate in the meat products.

Measurement of nitrite-ion concentration was carried out in a separate sample solution using the same procedure as that for nitrate except that 0.01 M sulphamic acid solution was replaced with 0.01 M potassium permanganate solution, which was used to oxidise nitrite (maximum level 600 p.p.m.) to nitrate. The excess of permanganate was reduced by the addition of 25 μl of hydrogen peroxide (35%). The increase in nitrate-ion concentration was used to calculate the concentration of nitrite ion in the sample solution.

The procedure for the spectrophotometric determination of nitrate and nitrite has been described elsewhere.[9]

Results and Discussion

Several methods have been proposed for extracting nitrate and nitrite from meat and meat products,[5,9,10] but Soxhlet extraction has not been used. It should have the advantage of combining all steps into a single process. The soluble nitrate and nitrite are continuously extracted from the meat products and the particles are retained in the cellulose thimble, so that the extract can be used directly for the determination. In acidic media nitrite is unstable, particularly at high temperatures, and it can also react with some functional groups, such as amines and sulphydryl, in food samples. Hence, the extract must be alkaline. Table I shows the effect of pH on the recovery of nitrate and nitrite in a meat product. The recovery was calculated on the basis that the meat product was spiked with 10 or 50 μg g^{-1} of nitrate and nitrite. The concentrations of nitrate and nitrite in the resulting solution were determined by the proposed method. The results show that the pH of the extracting

solution has no effect on the recovery of nitrate. However, quantitative recovery of nitrite requires an alkaline extracting solution. The pH of the extracting solution was therefore maintained at about 9 by using a borax buffer in subsequent studies.

The sensing module of the nitrate electrode was found to have a life span of about 6 months and a linear range from 5.6×10^{-5} to 10^{-2} M of nitrate, with a slope of -56 mV per decade increase in nitrate-ion concentration at pH 3.3 ± 0.1 in 4.0×10^{-2} M ammonium sulphate solution. The working pH range was found to be 2–6 at low nitrate-ion concentrations (1.6×10^{-4} M). Hence the pH of the resulting solution must be controlled in this range for the determination of low nitrate-ion concentrations. The pH of the sample solution was adjusted to 3.3 ± 0.1 with concentrated sulphuric acid before potentiometric measurement.

TABLE I

EFFECT OF pH IN EXTRACTING NITRATE AND NITRITE FROM MEAT SAMPLES

pH*	Buffer extracting system	Recovery, % Nitrate	Nitrite
3.1	H_3BO_3 (0.1 M) + H_2SO_4	108	69
5.4	H_3BO_3 (0.1 M)	100	75
6.5	KH_2PO_4 (0.025 M) + Na_2HPO_4 (0.025 M)	95	84
7.8	$Na_2B_4O_7$ (0.01 M) + H_2SO_4	97	103
9.0	$Na_2B_4O_7$ (0.01 M)	98	99
10.8	$Na_2B_4O_7$ (0.01 M) + NaOH	100	99

* The pH values were measured after extraction for 3 h.

The nitrate electrode suffers from interferences from carbonate, hydrogen carbonate, nitrite, halide, sulphide, phosphate, carboxylic anions, chlorate and perchlorate.[19,20] The interferences due to carbonate and hydrogen carbonate are eliminated at the low pH used and those due to halide, sulphide and phosphate can be eliminated by precipitating them with silver ion.[16,19] In the last instance the major interferent is chloride. The concentration of silver ion must be high enough to precipitate all the chloride in the sample solution. Ammonia solution, which readily complexes with silver sulphate,[4] was used to prepare the concentrated silver sulphate solution. Aluminium ion was used to complex carboxylic anions and other water-soluble organic anions,[16,17] while any nitrite present was complexed with sulphamic acid. Chlorate and perchlorate are rarely found in meat products. Nitrite was found to be oxidised quantitatively to nitrate with potassium permanganate, as reported.[18]

The results obtained by the standard additions method and the calibration graph method did not show any statistically significant difference. As the electrode drifted in the negative direction at a steady rate of 0.5 mV d^{-1}, re-calibration of the electrode with a standard solution each day was necessary in the calibration graph method.

The precision of the method was checked by determining nitrate and nitrite levels in a meat product using seven independent measurements. The standard deviations for nitrate and nitrite levels were 2.0 and 0.8 $\mu g\ g^{-1}$, with mean values of 205.4 and 20.3 $\mu g\ g^{-1}$, respectively. The coefficients of variation were 0.96 and 3.8% for nitrate and nitrite determinations, respectively. Hence the precision of the method is good.

TABLE II

NITRATE CONTENTS IN HAM DETERMINED POTENTIOMETRICALLY AND SPECTROPHOTOMETRICALLY

Sample No.	Nitrate level/$\mu g\ g^{-1}$ Potentiometric method	Spectrophotometric method
1	28	28
	30	29
2	36	34
	35	35
3	50	47

Results obtained by use of this method and recommended spectrophotometric methods are given in Tables II and III. The correlation coefficients were 0.9918 and 0.9954 for the nitrate and nitrite determinations, respectively. The proposed method therefore agrees well with the recommended methods.

The nitrate and nitrite levels in several meat products were determined by the proposed method and the results are given in Table IV. The nitrate and nitrite levels are much lower than the permitted values.

TABLE III

NITRITE CONTENTS DETERMINED POTENTIOMETRICALLY AND SPECTROPHOTOMETRICALLY

Sample	Number of determinations	Mean nitrite level/μg g^{-1}	
		Potentiometric method	Spectrophotometric method
Picnic ham	4	19	20
Leg ham (sliced)	4	5	5
Sausage Frances	3	3	4
White meat loaf	3	5	5
Ham	3	20	21
Ham	3	22	20
Canadian bacon	3	6	5
Canned beef	3	7	7
Beef	3	3	3
Ham	3	14	14

It was found that the concentration of the extractable nitrite in the meat products depended on the time elapsed between opening the can or the package and sampling. The nitrite level, but not the nitrate level, decreased drastically. Storing the samples under an atmosphere of nitrogen or even under vacuum at room temperature did not help. Hence, it is essential to measure the nitrite level as soon as possible after the can or the package has been opened. Alternatively, the sample must be kept at 0 °C, at which temperature the nitrite level remains the same for more than 180 h. This may be due to the fact that at this temperature the rate of conversion of nitrite into other substances, chemically or biologically, is significantly reduced.

TABLE IV

NITRATE AND NITRITE CONTENTS OF VARIOUS MEAT PRODUCTS DETERMINED BY THE PROPOSED METHOD

Sample	Nitrate level/μg g^{-1}	Nitrite level/μg g^{-1}
Picnic ham	179	19
Leg ham (sliced)	92	5
Sausage Frances	75	4
White meat loaf	250	5
Ham	135	22
Canadian bacon	203	6
Canned beef	35	7
Ham (frozen)	205	20
Luncheon meat	141	24

The proposed method does not require the addition of a clearing agent, filtration or the use of a cadmium reducing column. It reduces the time required for the determination of nitrate and nitrite to 30 min after extraction. However, the method is not suitable for samples that contain a high ratio of nitrate to nitrite, in which event the change in potential after oxidising the nitrite to nitrate will be very small and a large error will be obtained in the determination of nitrite concentration.

This research was supported by the Research Grants Committee of the University of Hong Kong.

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Received *August 2nd*, **1979**
Accepted *September 18th*, **1979**

Simple and Rapid Determination of Iodine in Milk by Radioactivation Analysis

S. Ohno

National Institute of Radiological Sciences, 9–1, 4-chome, Anagawa, Chiba-shi, Japan

Iodine has been determined in milk samples by radioactivation analysis by using a Van de Graaff accelerator. The iodine is separated from the irradiated sample with an iodine-loaded resin. The limits of detection of iodine by gamma-ray spectrometry and beta-counting are 10 and 5 μg, respectively. The precision of the proposed method is ±5% when the iodine content exceeds 0.01 p.p.m.

Keywords: *Iodine determination*; *milk*; *radioactivation analysis*

There have been many investigations of the determination of iodine in the environmental and medical fields, because this element plays an important role in life processes.[1] Many workers have studied the iodine content of environmental samples, because the radioactive iodine released from nuclear power plants or by nuclear emergencies may show the same behaviour as stable iodine.

The determination of iodine in biological materials has frequently been carried out by radioactivation analysis,[2–5] as it can be determined with high sensitivity by thermal neutron activation. For instance, Al-Shahristani and Abbass[6] determined iodine in some foodstuffs using resonance neutron activation analysis. Law[7] determined iodine in some sea weeds and Ohno[8] has also determined iodine in other biological materials by this technique.

In radiochemical methods for the separation of the radioactive iodine present in the samples, an anion-exchange method and solvent extraction have usually been used[9–11] and, as reported by Heurtebise and Ross,[12] iodine in biological fluids was determined by this technique. However, before irradiation the samples were treated with chemical reagents in order to prevent the oxidation of iodine during irradiation in the reactor.

In this work, a simple and rapid radioactivation analysis method is presented for the determination of iodine in milk by using a Van de Graaff accelerator.

Experimental

Apparatus

A Northern Scientific 1024-channel pulse-height analyser with an 80-cm^3 germanium (lithium) solid-state detector and an Aloka Geiger - Müller counter were used.

Reagents

All reagents used were of analytical-reagent grade.

Iodine standard solution. Dissolve 1.3119 g of potassium iodide in distilled water and dilute with water to give a solution containing 5 mg ml^{-1} of iodine. Transfer an aliquot of 200 μl of this solution with a micropipette into a polyethylene vial (inner diameter 0.5 cm and length 2 cm) and then heat-seal it.

Iodine-loaded resin. Wash 10 g of dried resin (Dowex 1-X8, 100–200 mesh) with 1 l of water and place it in a beaker. Add 350 ml of a solution prepared by dissolving 25 g of iodine in 1 l of 1 M potassium iodide solution and stir the mixture with a magnetic stirrer until the iodine is completely adsorbed on the resin, as shown by the colour of the solution becoming constant; this procedure is a slight modification of that reported by Heurtebise and Ross.[12]

Sample Collection

The samples for irradiation were collected from commercial stores of milk collected from near Chiba and Hokkaido.

Irradiation

The milk sample (300 ml) was transferred into a polyethylene bottle, in which the iodine standard solution in the polyethylene tube had been placed, as shown in Fig. 1, and then irradiated in a thermal neutron flux of about 1–5 $\times$ 10^8 neutrons cm^{-2} s^{-1} for 1 h.

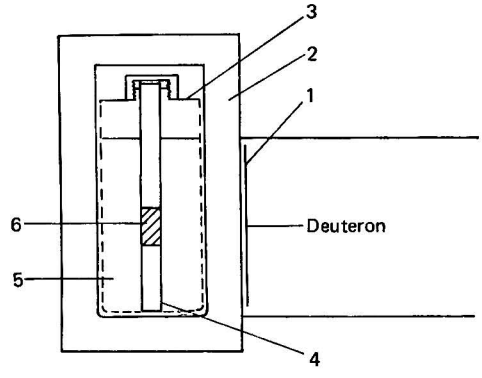

Fig. 1. Diagram of irradiation system: 1, Be target; 2, shielding with paraffin (thickness 5 cm); 3, polyethylene bottle for irradiation; 4, standard holder (polyethylene tube, radius 16 mm); 5, milk sample in the bottle; 6, iodine standard solution.

Radiochemical Procedures

Open the polyethylene bottle containing the irradiated milk sample and transfer it into a 1-l beaker. Add 200 mg of iodine-loaded resin and stir it vigorously with a magnetic stirrer for 5 min, then collect the resin with adsorbed iodine-128 on a filter-paper and transfer it into a polycarbonate counting disk for gamma-ray spectrometry or beta-counting with a Geiger - Müller counter.

Activity Measurement

The radiochemical purity of the separated iodine-128 was checked by gamma-ray spectrometry and by following the decay curve. For quantitative measurement, the main photopeak of this nuclide at 0.43 MeV was selected and counted for 100–600 s depending on the activity level. The time required for the separation, from the end of irradiation to counting, is less than 10 min. As iodine-128 is a short-lived radionuclide, counting of its radioactivity must be carried out without delay.

Results and Discussion

The amounts of iodine in some milk samples were determined by radioactivation analysis using the Van de Graaff accelerator and the results are shown in Table I. The accuracy of the procedure was checked by the standard-additions method as follows. Three analytical samples of each were prepared by introducing 300 ml of the milk sample alone or spiked with 50 and 100 μg of iodine as potassium iodate into irradiation bottles. After irradiation, they were submitted to the chemical separation and counting as described above. The results obtained in triplicate determinations on each sample are given in Table II, from which it can be seen that the average precision is about 5% at an iodine concentration of 0.045 p.p.m.

When the proposed method was used for the determination of iodine in biological samples such as milk and urine, other halogen ions, especially chloride, were also absorbed on the resin. Therefore, as a washing procedure was added at the final step in the radiochemical separation, the chloride could be removed by using a suitable amount of dilute sodium hydroxide solution as the wash liquid, as shown in Fig. 2. It seems that the proposed method is a simple and rapid technique for the determination of iodine in liquid samples of large volume.

TABLE I

IODINE CONTENT IN MILK

Sample No.	Location	Iodine/μg l^{-1} By gamma-ray spectrometry	Iodine/μg l^{-1} By beta-counting
1	Hokkaido (Obihiro)	56.7, 56.3, 55.3	54.7, 52.9, 50.8
2	Kanazawa (Fujisawa)	197.4, 202.5, 200.0	190.4, 191.8, 189.1
3	Chiba (Funabashi)	214.4, 206.3, 201.7	204.7, 200.0, 194.2
4	Hokkaido (Hidaka)	36.2, 32.0, 39.4	35.4, 32.8, 33.6
5*	Hokkaido	51.9, 54.6, 50.6	57.6, 56.1, 55.0

* Skim milk.

To establish the effect of pH on the retention characteristics of the resin, aqueous solutions that contained 100 μg of iodine either as potassium iodide or as potassium iodate with different pH values were prepared. The results obtained are given in Table III. The induced radioactivity of iodine-128 in the irradiated sample was measured with a Geiger - Müller counter. After the absorption of iodine-128 on the iodine-loaded resin, as described above, the resin was transferred into a beaker containing 5 ml of 20% saccharose solution and 95 ml of 1 M sodium hydroxide solution and the mixture was stirred vigorously for 5 min with a magnetic stirrer. Then, after removing the resin by filtration, the iodine-128 in the filtrate was precipitated as silver iodide and the precipitate collected on a filter-paper in order to measure the radioactivity of iodine-128 with a Geiger - Müller counter. The results obtained are given in Table I. The recovery of the precipitate was checked by using iodine-125 as a tracer, which was measured by gamma-ray spectrometry.

TABLE II

DETERMINATION OF IODINE IN MILK BY STANDARD ADDITIONS METHOD

Sample No.	Volume/ml	Amount of standard added/μg	Activity on the resin,* counts per 100 s	Iodine found/ μg l^{-1}
1	300	—	181.76	—
		50	329.65	197.4
		100	491.59	190.7
2	300	—	1456.0†	—
		50	2671.2†	199.6
		100	3745.6†	211.9

* The activity on the resin was calibrated at the end of the irradiation period.
† Values obtained by beta-counting using a Geiger - Müller counter.

The recovery of iodine-128 from organoiodine compounds such as thyroxine iodide in a sample solution was measured as follows. Iodine was removed from a milk sample by passing it through an anion-exchange resin column (Dowex 1-X8, 50–100 mesh; inner diameter 1.2 cm, length 10 cm) at a flow-rate of 5 ml min^{-1}, then a 300-ml volume of the de-iodinated sample was spiked with a known amount of iodine as thyroxine iodide. After irradiation, the induced radioactivity of iodine-128, which was produced by the Szilard - Chalmers reaction, was separated using the iodine-loaded resin. From the results obtained (Table III), it appears that the proposed method for the determination of organoiodine in milk samples is reliable. However, when the milk sample passed through an iodine-loaded resin column, before irradiation, of the size mentioned above, a small amount of iodine that was released from the resin was activated by radioactivation and the amount of this iodine released into the milk sample was determined by reactivation. In this instance, the iodine released from the iodine-loaded resin was about 1 mg, as shown in Fig. 2. Hence it can be seen that the

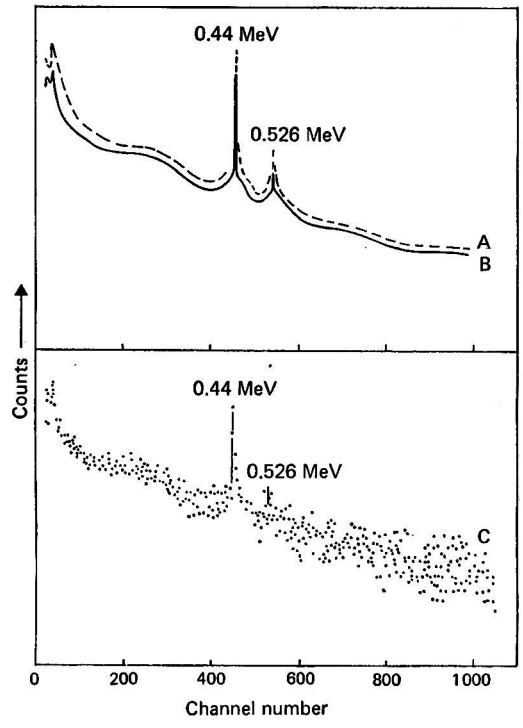

Fig. 2. Gamma-ray spectra of: A, released iodine-128 from iodine-loaded resin; B, iodine standard solution; and C, iodine-128 from irradiated sample.

released iodine in the milk sample solution originated from a reduction reaction between iodine bound to the resin and a carbohydrate such as lactose, and also that an isotopic exchange reaction between iodine in the iodine-loaded resin and iodide or iodate in the milk sample solution occurred, as reported by Ikeda and Takahashi.[13]

TABLE III

EFFECT OF pH ON THE ADSORPTION OF IODINE ON IODINE-LOADED RESIN

	Adsorption of iodine, %		
pH	Iodide added (10 μg)*	Iodate added (10 μg)*	Thyroxine iodide added (100 μg)†
1–6	99.2	99.8	99.5
7	99.4	99.6	99.2
8	98.0	98.3	99.2
9	84.4	86.3	84.7

* Adsorption determined by using iodine-125 as tracer.
† Adsorption determined by using radioactivation technique,

With the proposed method, the determination of trace amounts of iodine in samples of large volume, such as milk, could easily be achieved without any other chemical treatment, for instance, addition of chemicals to prevent the reduction of iodine during irradiation, as was reported by Heurtebise and Ross.[12] When the pH of a milk sample is adjusted to less than 5, coagulation occurs, so the determination of iodine was therefore carried out without the addition of any chemicals to the milk samples.

The radiochemical purities of the separated iodine-128 fractions from the irradiated milk samples were established from their gamma-ray spectra, as shown in Fig. 2. The sensitivity of detection was calculated on the basis of the minimum detectable photopeak area for iodine-128; the limit of detection of iodine was found to be 10 μg using a gamma-ray spectrometer and 5 μg using beta-counting with a Geiger - Müller counter, and the precision was about 5% for contents exceeding 40 $\mu g\ l^{-1}$.

The author thanks Messrs. H. Imaseki and T. Nakayama of the Technical Service Section of the National Institute of Radiological Sciences for operating the Van de Graaff accelerator.

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Received *August* 31*st*, 1979
Accepted *October* 1*st*, 1979

Analyst, March, 1980, Vol. 105, pp. 251–256

Determination of Residual Methyl Bromide in Fumigated Commodities Using Derivative Gas - Liquid Chromatography

R. J. Fairall and K. A. Scudamore

Ministry of Agriculture, Fisheries and Food, Slough Laboratory, London Road, Slough, Berkshire, SL3 7HJ

A sensitive method for the determination of residual methyl bromide in fumigated foodstuffs is described. Methyl bromide in extracts was converted stoicheiometrically into methyl iodide by reaction with sodium iodide and analysed using gas - liquid chromatography with electron-capture detection. Residual methyl bromide was readily detected at the 10 $\mu g\ kg^{-1}$ level in a variety of foodstuffs.

Keywords: *Methyl bromide determination*; *fumigation*; *residues analysis*; *derivative gas - liquid chromatography*

Current awareness of possible undesirable effects of low levels of contaminants in foodstuffs has increasingly necessitated the development of highly sensitive and specific analytical techniques for their detection and measurement. Methyl bromide (bromomethane), a potent alkylating agent, has been used for many years for pest control in food and other commodities. The Ministry of Agriculture, Fisheries and Food has estimated that at least 100000 tons of food commodities were treated with methyl bromide in the UK in 1976. The actual amount is likely to have been considerably higher.

It is known from previous studies[1] that most residual methyl bromide disappears fairly rapidly from fumigated commodities, although certain products, for example groundnuts, retain detectable amounts for some time after exposure, especially at low temperatures. However, measurement of residues below the milligrams per kilogram level has been difficult.

Denis *et al.*[2] detected methyl chloride formed through halogen exchange during a methyl bromide treatment, and we have induced partial replacement of chlorine atoms in 1,2-dichloroethane by iodine through reaction with sodium iodide in acetone. A similar halogen exchange reaction was utilised in the work described here for the rapid and quantitative conversion of methyl bromide into methyl iodide at room temperature.

The increased response of the electron-capture detector to iodine-containing compounds enables a considerable decrease in the minimum detectable amount of methyl bromide extracted from foodstuffs to be achieved.

Experimental

Apparatus

A gas chromatograph fitted with a pulsed electron-capture detector operated at 200 °C was employed.

A 4 m × 2 mm i.d. × 3.2 mm o.d. stainless-steel column packed with 15% Apiezon L on Chromosorb P, operated at 100 °C with a nitrogen carrier gas flow-rate of 25 $ml\ min^{-1}$, was primarily used during development of the method and is recommended for normal application.

A second 4 m × 3 mm i.d. × 6.4 mm o.d. glass column packed with 15% LB 550X on Chromosorb W (100–120 mesh), operated at 80 °C with a carrier flow-rate of 15 $ml\ min^{-1}$, was also used successfully during the experimental work.

Retention times for methyl bromide and methyl iodide were 3.0 and 4.0 min, respectively, for the Apiezon L column, and 2.5 and 3.5 min, respectively, for the LB 550X column.

A Rotamixer (Hook and Tucker Instruments) was employed for rapid mixing of immiscible solvents.

Reagents

Analytical-reagent grade compounds should be used where possible.
Pentane, 99.5%.
Acetone.
Methyl iodide.
Sodium iodide.
De-ionised or distilled water.

Procedure

Extract 10–20 g of unground commodity by soaking in 60 ml of acetone - water (5 + 1) for 24 h. Add 0.5 g of sodium iodide to 20 ml of the undried extract, dissolve by swirling and allow the solution to stand for 1 h at room temperature.

Transfer exactly 3 ml of reacted extract into a 25-ml graduated cylinder containing 5 ml of pentane. Add 15 ml of water, mix and allow to separate. Draw off or decant the top layer as completely as possible and transfer it into a 25-ml calibrated flask. Wash the acetone - water layer with two further 5-ml volumes of pentane, transfer the washings into the flask and dilute to 25 ml with pentane. Inject 1 μl of the pentane solution into the gas chromatograph, operated as indicated under Experimental. Prepare standard solutions of methyl iodide in pentane for calibration purposes.

Calculation of Results

$$\text{Methyl bromide (mg kg}^{-1}) = \frac{H_e}{H_s} \times \frac{P}{V} \times \frac{0.3345}{M} \times D$$

where H_e = peak height from sample; H_s = peak height from standard; P = mass of standard (pg); V = injection volume (μl); M = mass of commodity (g); and D = dilution.

Results and Discussion

Detector Calibration

The response of the electron-capture detector to methyl iodide was linear over the range 0–100 pg. The sensitivities of the detector for methyl bromide and methyl iodide were 100 and 0.25 pg, respectively, for 1% of full-scale deflection on the maximum usable range compatible with noise levels.

Reaction Temperature

Temperature has a considerable effect on the rate of reaction between methyl bromide and iodide ion. Fig. 1 presents results for the rate of conversion of methyl bromide into methyl

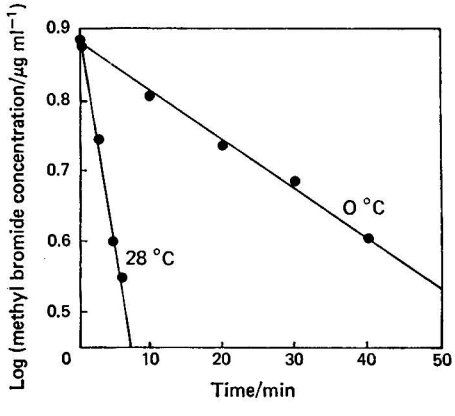

Fig. 1. Effect of temperature on the rate of reaction between methyl bromide and sodium iodide.

iodide at 28 and 0 °C. A 0.6-g amount of sodium iodide was used in 30 ml of acetone - water (5 + 1).

The relative rates of reaction indicate an approximate doubling of the rate per 10 °C increase in temperature, which suggests a regular reaction mechanism, which in this instance appears to be an S_N2 displacement process.

Iodide Concentration

The effect of varying the concentration of sodium iodide at constant temperature whilst maintaining a fixed methyl bromide concentration is shown in Fig. 2. A 30-ml volume of acetone - water (5 + 1) was used and logarithmic plots of the results against time were linear. As the iodide was present in considerable excess, the kinetic results are in agreement with an expected pseudo-first-order reaction. Potassium iodide was also used but the reaction was slower than that with the readily soluble sodium salt. A 0.5-g amount of sodium iodide per 20 ml was chosen for the standard reaction mixture. Reaction of methyl bromide with sodium iodide in dry acetone proceeded to completion in less than 3 min and the method could therefore be used with non-aqueous extraction systems if required.

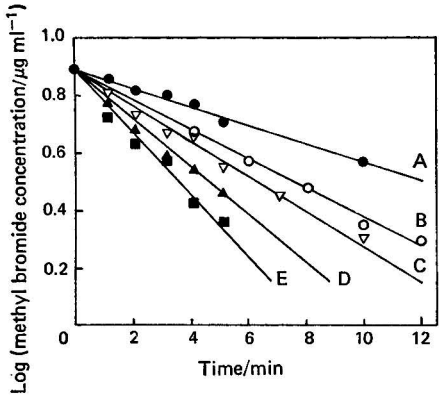

Fig. 2. Effect of iodide concentration on the rate of conversion of methyl bromide into methyl iodide at 28 °C. A, 0.2 g of NaI; B, 0.4 g of NaI; C, 0.6 g of NaI; D, 1.0 g of KI; and E, 1.0 g of NaI.

Conversion Efficiency

Table I shows the percentage conversion and recovery of methyl bromide as methyl iodide following reaction of aliquots of standard solutions containing between 0.002 and 4.0 μg ml^{-1} of methyl bromide with sodium iodide in acetone and water, using standard methyl iodide solutions for calibration. Recoveries were close to 100%, even with amounts that would correspond to residues in commodities of below 5 μg kg^{-1}.

Commodity Extracts

Acetone - water extracts of a variety of untreated commodities, including whole wheat, flour, groundnuts, rapeseed, dried milk powder and cocoa beans, were partitioned using pentane as described earlier and analysed by gas - liquid chromatography. With the exception of cocoa beans, from which the extracts when chromatographed showed a small peak with a retention time similar to that of methyl iodide, all of the samples gave extracts almost free of interfering peaks at a level corresponding to a residue of 0.01 mg kg^{-1} of methyl bromide.

No increased interference or modification of the chromatograms was observed when the same acetone - water extracts were treated with 0.5 g of sodium iodide 1 h prior to partitioning and analysis. However, with a further increase in sensitivity, interference became more troublesome and is the limiting factor in the method.

Secondary Reactions with Sodium Iodide

Extracts from a number of commodities previously fumigated with methyl bromide at about 25 times the normal level for pest control, and thoroughly aired on addition of sodium iodide, gave no peaks for methyl iodide or additional peaks under the conditions used for gas - liquid chromatographic analysis. This suggests that no secondary reactions between sodium iodide and previously methylated food constituents occurred.

No reaction was observed when standard solutions of a range of fumigants in acetone - water were treated with sodium iodide at room temperature. These included 1,2-dichloroethane, 1,1,1-trichloroethane, 1,2-dibromoethane, carbon disulphide, chloroform and trichloroethylene. Ethylene oxide reacted to give ethylene iodohydrin. With a boiling-point of 176 °C, this compound emerges as a peak with a long retention time and might cause some interference on the chromatogram. However, the likelihood of a commodity containing both methyl bromide and ethylene oxide residues is extremely remote. Concentrations of the fumigants used were in the range 1–10 μg ml^{-1}.

TABLE I

EFFICIENCY OF THE METHYL BROMIDE - SODIUM IODIDE HALOGEN EXCHANGE REACTION

Methyl bromide concentration/ ng ml^{-1}	Methyl iodide concentration after extraction and dilution/ ng ml^{-1}		Conversion, %
	Theoretical	Experimental	
2	1.5	1.51	101
8	6.0	5.64	94
20	15.0	16.2	108
40	30.0	29.5	98
400	60.0	60.8	101
800	60.0	57.0	95
4000	120.0	118.0	98

Comparison of Methods

Five commodities (whole wheat, cocoa beans, maize, oats and rice) were fumigated with methyl bromide at 15 °C at concentration–time products of 2800 mg l^{-1} h (140 mg l^{-1} for 20 h) for wheat and cocoa beans and 657 mg l^{-1} h (7.3 mg l^{-1} for 90 h) for maize, oats and rice, and residual methyl bromide was determined during airing by the method described under Procedure, and by a previously described method[3,4] for comparison.

TABLE II

COMPARISON OF DIRECT AND INDIRECT METHODS FOR THE DETERMINATION OF METHYL BROMIDE RESIDUES IN COMMODITIES

Results given are methyl bromide concentrations (mg kg^{-1}).

Commodity	Method	Time of airing/h						
		0	1	4	7	24	48	72
Wheat	Direct	N.A.*	21.5	16.0	13.9	8.7	5.3	3.3
	Indirect	27.6	19.7	14.9	13.9	8.6	4.7	3.0
Cocoa beans	Direct	23.8	15.0	15.0	16.2	10.3	10.4	7.7
	Indirect	22.9	13.3	12.5	12.7	8.7	8.7	4.4
White maize	Direct	18.7	10.8	8.3	8.8	2.9	2.5	
	Indirect	18.0	8.7	7.5	8.4	N.A.*	1.2	
Oats	Direct	20.1	15.3	11.3	10.5	3.9	2.2	
	Indirect	18.8	15.6	11.1	10.9	N.A.*	1.7	
Rice	Direct	8.4	6.3	3.4	2.4			
	Indirect	8.7	6.9	3.0	2.6			

* N.A. = not analysed.

Each sample was initially extracted by soaking in acetone - water (5 + 1). Aliquots were then taken for analysis by each of the two methods. The results are given in Table II.

Earlier work had shown that a 5 + 1 mixture of acetone and water gives a high extraction efficiency of known amounts of methyl bromide added to a variety of food commodities.[1,3] The inherent insensitivity of the direct method limits the possibilities of comparison with the new method at much below 3 mg kg^{-1}, because at this level only 10% full-scale deflection on the maximum usable range was obtained when methyl bromide was measured directly. However, as the indirect method gives comparable results at levels that can be measured with confidence by a proven method and the new method is capable of detecting 1% of the amount, detectable as methyl bromide, it can be inferred that determination at the lower levels is also satisfactory when the linear nature of the detector response is taken into account.

Although with acetone - water solutions of standards most of the methyl iodide was extracted into the pentane layer on a single partition, in the presence of certain commodity extracts more methyl iodide remained in the acetone - water layer. This was probably due to the complex mixture of co-extractives such as starches and oils producing a different partitioning effect. The amounts for wheat and cocoa beans shown in Table II include about 10% extracted and quantified separately by a second partioning of the aqueous layer against pentane, whereas those for maize, oats and rice are the results from a single partition.

In general good agreement was obtained between the two methods.

Field Samples

Table III compares results using the two methods obtained for residual methyl bromide in samples from a field fumigation of barley. Results are also included for wheat samples taken after a store fumigation.

TABLE III

ANALYSIS OF FIELD SAMPLES

Sample	Method	Methyl bromide/mg kg^{-1} A	B	C	D
Barley	Direct	48	49	1.8	4.4
	Indirect	45	54	2.0	4.0
Wheat	Indirect	0.03	0.018	0.036	0.025

The indirect method described was used to follow the rate of disappearance of methyl bromide from several commodities obtained after practical fumigations. Samples were analysed initially immediately on return to the laboratory, and then stored under controlled conditions with samples being taken at intervals for analysis. The results are given in Table IV.

No methyl bromide was detected in any commodity after storage for 1 month. These results indicate *inter alia* that no spurious formation of methyl iodide occurred either by reaction of sodium iodide with food constituents or with any of those modified by fumigation. The increased sensitivity of the indirect method is illustrated by the results in Tables III and IV. None of the residues below 0.1 mg kg^{-1} would have been detected using the direct method. Here this value represents at least 10 times the lowest level of detection. For commodities that do not give interfering peaks detection at even lower levels is possible.

It is known that methyl bromide normally disappears from commodities both by volatilisation and by various methylation reactions[5] with commodity constituents. It can be concluded that any such products in the commodities tested were resistant to treatment with sodium iodide at room temperature, at least in relation to the formation of methyl iodide.

Conclusion

A simple, highly sensitive and precise method for the determination of residual methyl bromide in foodstuffs by conversion into methyl iodide is described. Residues can be determined down to the 10 μg kg^{-1} level or below, whereas the response of electron-capture

TABLE IV

METHYL BROMIDE RESIDUE LEVELS (mg kg^{-1}) DURING STORAGE

Commodity	Days 0	0.04	0.25	1	2	4	7	11
Dried milk	0.5	0.15	0.03	0.006	—	—	—	—
Wheat	0.8	0.42	0.33	0.08	0.04	N.D.*	—	—
Flour	0.28	0.09	0.04	0.02	N.D.*	—	—	—
Rapeseed	4.2	3.0	1.5	—	0.95	0.5	0.10	0.08
Groundnuts	7.7	4.5	3.2	2.6	1.6	0.38	0.38	0.03

* N.D. = none detected.

detectors to methyl bromide is much less satisfactory. The method is suitable for application to a wide range of commodities and, with the exception of ethylene oxide, which reacts to give ethylene iodohydrin, would appear not to respond to residues of other fumigants that might be present in the samples. The occurrence of free ethylene oxide in samples not recently treated with that fumigant is unlikely. Elevation of the reaction temperature should be avoided, however, as 1,2-dichloroethane and 1,2-dibromoethane, for example, are known to react at temperatures in excess of 50 °C.

The method should be adaptable for use with many commodities and need not necessarily be limited to extractions using acetone or acetone - water because, for example, acetonitrile has been found to be an equally useful solvent for the conversion reaction.

Maximum guideline residue levels for unchanged methyl bromide in named commodities at various stages of handling suggested by the FAO/WHO Joint Meeting on Pesticide Residues at present range from 0.5–100 mg kg^{-1}.[6] For samples taken at the point of retail sale or when offered for consumption, the lower levels reflect the limits of detection imposed by the analytical techniques that were available at the time of evaluation. Results obtained by the method described here appear to be independent of commodity type. Use of the method would allow the extension of the guideline limits to below 0.5 mg kg^{-1} if desired.

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Received *November 9th*, 1978
Accepted *September 11th*, 1979

Analyst, March, 1980, Vol. 105, pp. 257–261

Assay of Carbaryl in Honey Bees (*Apis mellifera*) by High-performance Liquid Chromatography

K. A. Lord, G. R. Cayley, L. E. Smart and R. Manlove
Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ

A method for detecting and measuring carbaryl in poisoned honey bees (100 ng per bee) using high-performance liquid chromatography is described. Clean-up of extracts on a Florisil column removed all substances that interfered with fluorescence detection and most that affected ultraviolet detection at 215 nm.

Gas chromatography of carbaryl derivatives was not consistently useful because bee constituents interfered either with the formation of derivatives or with detection. Only the *N*-acetyl derivative was formed quantitatively in the presence of cleaned-up bee extracts, but the nitrogen-specific detector was sometimes, and the electron-capture detector always, subject to interference from bee constituents remaining after clean-up.

Keywords: Carbaryl residues; high-performance liquid chromatography; gas chromatography; honey bees

The possible dangers of pest control chemicals to beneficial insects have long been recognised. Of these insects, the honey bee is perhaps the most readily observed, being kept in hives by watchful beekeepers so that unusual mortality may be readily noted. The direct economic importance of bees as pollinators and as a source of food provides an incentive to investigate and eliminate adverse effects of pesticides. As part of this process, the detection of pesticides in bees is used to identify chemical pest-control practices harmful to bees and to develop safer control methods.[1]

The high wax content of bees makes the separation of pesticide residues difficult so that it is particularly important to examine extraction in conjunction with the final assay procedure. These two aspects are interdependent because the sensitivity and specificity of the method for identification and measurement determine the extent to which the chemical to be assayed must be separated from other material extracted from bees.

Numerous methods have been reported for the assay of carbaryl residues in a variety of soils and biological materials,[2] but few for carbaryl residues in bees.[3,4] A high-performance liquid chromatographic method for the determination of carbaryl and naphthol in honey bees has been reported,[4] but the levels of carbaryl residues were greater than the values for both acute contact and acute oral LD_{50} toxicity reported by Stevenson *et al.*[1] To measure the small levels of carbaryl expected from the LD_{50} values (oral, 0.14 μg per bee; contact, 1.3 μg per bee) using fewer bees (about ten), a more rapid and sensitive method of assay was required.

This paper describes the development of a method suitable for detecting carbaryl in groups of five individual bees suspected of being poisoned with carbaryl.

Experimental

The methods investigated for assaying carbaryl, either directly or as derivatives, were high-performance liquid chromatography, fluorescence spectroscopy and gas chromatography. All required at least partial separation of carbaryl from other materials extracted from bees.

Derivative Formation

The formation of carbaryl and naphth-1-ol derivatives, naphth-1-ol trichloroacetate,[5] *N*-acetylcarbaryl,[6] naphth-1-ol dinitrobenzyl ether,[7] carbaryl *N*-trifluoroacetyl ester[8] and dansylnaphth-1-ol,[9] was examined using published methods.

Conditions for the Assay of Carbaryl in Honey Bees by High-performance Liquid Chromatography

Extraction

The extraction method was essentially as described by Argauer *et al.*,[3] except that the procedures were scaled down to use five bees instead of 25 g of bees. Five bees were macerated for 1 min with 2.5 g of anhydrous sodium sulphate and 7 ml of dichloromethane. The macerate was filtered through a sintered-glass funnel and the cake was re-extracted with 5 ml of methanol and then 5 ml of dichloromethane. The filtrates were pooled, evaporated nearly to dryness on a rotary evaporator at room temperature and taken up with 5 ml of dichloromethane.

Clean-up

A 2.5-g amount of Florisil pre-equilibrated with 625 mg of water was packed into a glass column of diameter 15 mm and washed with 5 ml of dichloromethane equilibrated with water. The sample extract was percolated through the column and the carbaryl eluted with a further 5 ml of dichloromethane. The filtrate was then evaporated nearly to dryness using a rotary evaporator and taken up in 1.0 ml of methanol.[3]

High-performance liquid chromatography

A previously described[5] constant-pressure apparatus was used with a Cecil CE212 variable-wavelength ultraviolet monitor connected in series with a Perkin-Elmer 2000 fluorescence spectrophotometer. The column was a 50 cm × 2 mm i.d. stainless-steel column packed with Permaphase ODS or Co-pell ODS and the mobile phase was 1 + 4 methanol - 50 mM phosphate buffer (pH 7). Both carbaryl and naphth-1-ol were monitored at 215 nm using their non-specific absorption and naphth-1-ol was also monitored fluorimetrically using an excitation wavelength of 330 nm with emission at 467 nm.

Fluorescence Spectroscopy

Samples containing carbaryl in 1 ml of methanol were hydrolysed by adding 2.5 ml of 0.1 M sodium hydroxide solution and leaving the samples to stand at room temperature for 30 min. The fluorescence spectra of these samples were then recorded on an Aminco-Bowman spectrophotofluorimeter using an excitation wavelength of 320 nm with emission maximum at 460 nm.[3]

Gas Chromatography

Carbaryl derivatives were chromatographed on a Varian Series 1400 gas chromatograph with nitrogen-specific flame-ionisation detection or on a Pye 104 gas chromatograph with an electron-capture detector. A 60-cm column of 5.0% SE-30 on Chromosorb W was used at 170 °C for naphth-1-ol trichloroacetate and 220 °C for *N*-acetylcarbaryl and naphth-1-ol dinitrobenzyl ether. Nitrogen was used as the carrier gas at a flow-rate of 30 ml min^{-1}.

Results and Discussion

Measurement of trace amounts of pesticides in dead bees presents different problems from those encountered in the assay of residues in crops and soils. The more usual objective is to determine the mean residue or distribution in a large bulk of material where obtaining representative samples presents a considerable problem. Hence the constraints on the amount of material taken for analysis are mainly cost and convenience. In contrast, dead bees from colonies or hives have all received at least a lethal dose but the number of individuals available is limited. Therefore, the methods used must be applicable to amounts of chemicals generally smaller than are examined in the more usual residue assays, and the methods had to be checked before being applied to the assay of the small amounts of carbaryl expected in bees.

Spectrophotometry

Direct spectrophotometric and colorimetric methods for assay of carbaryl or naphth-1-ol are generally not sufficiently sensitive to be applied to bees.[2,10] The spectrofluorimetric

method described for use with bee extracts[3] did not detect with certainty just lethal doses of carbaryl as naphth-1-ol because some samples of bees contained interfering fluorescing substances. However, spectrofluorimetry provides useful supporting evidence for the presence of carbaryl when interfering materials render the evaluation of chromatograms doubtful and proved even more valuable as a detection method with high-performance liquid chromatography.

Formation of Carbaryl Derivatives and Gas Chromatography

Neither carbaryl, nor its major characteristic hydrolytic product naphth-1-ol, can be readily subjected to gas chromatography and the formation of a derivative is desirable or essential. Of the derivatives of carbaryl investigated only the *N*-acetyl derivative could be formed quantitatively on the microgram scale; this compound was amenable to gas chromatography and was strongly electron capturing. The reaction used to prepare *N*-trifluoracetylcarbaryl was not satisfactory, and only the corresponding *N*-acetyl derivative was formed and identified by nuclear magnetic resonance spectroscopy and gas chromatography. The other reactions were satisfactory on the milligram scale.

Carbaryl derivatives could not be prepared satisfactorily in the presence of crude bee extracts that contained materials that compete for the reagents. However, after preliminary partial separation of carbaryl from co-extracted bee constituents the *N*-acetyl derivative was readily formed, but other strongly electron-capturing substances were formed from most bee extracts. The nitrogen-specific flame-ionisation detector provided a more certain assay, although it was still subject to interference from some bee components.

High-performance Liquid Chromatography

This method was repeatedly calibrated using known amounts (1 μg per gram of bee) of carbaryl added to bees and half the added carbaryl was consistently measured either directly or as naphth-1-ol with a detection limit of 0.01 μg per bee. This was adequate for our

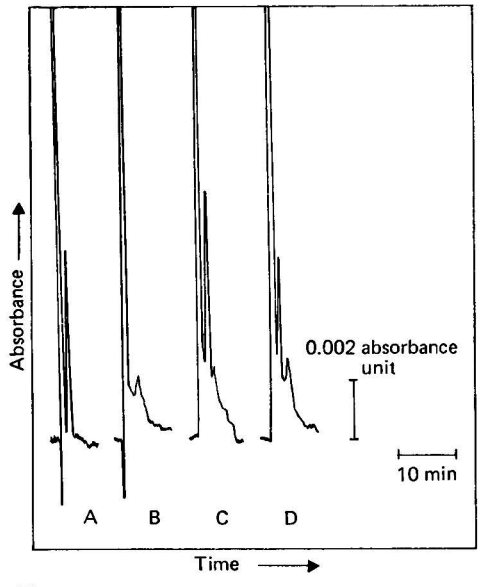

Fig. 1. Reversed-phase chromatography of carbaryl on 0.5-m Co-pell ODS with 25% methanol in 50 mM phosphate buffer (pH 7). Flow-rate, 0.9 ml min^{-1}; ultraviolet detection, 215 nm; injection volume, 5 μl. A, Carbaryl standard (11 ng); B, extracts of untreated bees after clean-up; cleaned-up extracts of bees treated with 3 μg of carbaryl per bee; C, killed; and D, survivors.

purposes and no advantage was obtained from using the dansylated naphth-1-ol derivative because bee extracts consumed much reagent and the products overloaded the chromatographic column, making separation difficult.

Typical chromatograms of carbaryl from poisoned bees are shown in Fig. 1, in which reversed-phase high-performance chromatography with ultraviolet detection was used. However, even after clean-up, some bee samples contain materials that give tailing peaks, which may interfere in the trace analysis of carbaryl. This can be overcome by hydrolysing the carbaryl to naphth-1-ol by the addition of an equal volume of 0.1 M sodium hydroxide solution and the use of ultraviolet and fluorescence detectors, in series, to detect the naphth-1-ol. The sample should be chromatographed immediately after hydrolysis because in alkaline solution naphth-1-ol is oxidised by air and sometimes bee materials found in the extract react slowly with the alkali to produce substances that interfere in the ultraviolet detection of naphth-1-ol. Typical chromatograms are shown in Fig. 2. The advantage of fluorescence detection is greater specificity and freedom from interference by substances from bees, although it is no more sensitive than detection by ultraviolet absorption at 215 nm. For the successful reversed-phase chromatography of naphth-1-ol, it is essential to use a buffer in the mobile phase in order to maintain a correct balance between fluorescence and retention on the column, because although the anion is not retained by the column it fluoresces more strongly than the neutral molecule. The mobile phase should have a sufficient buffering capacity to maintain a pH between 7 and 8 (about 50 mM), even on injection of solutions that contain sodium hydroxide to hydrolyse the carbaryl.

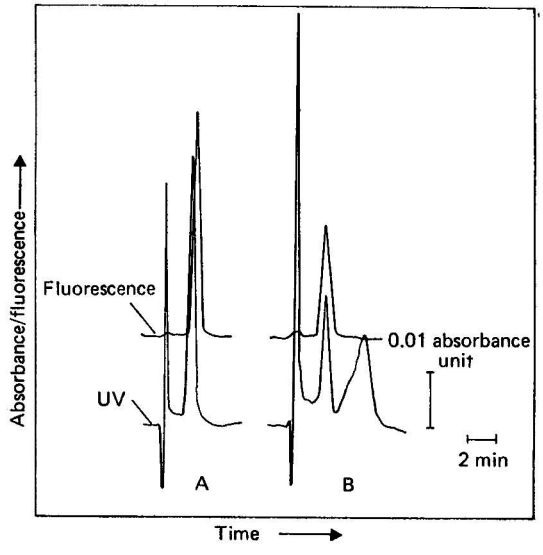

Fig. 2. Comparison of detectors in series for assay of carbaryl as 1-naphthol. Chromatography on 0.5-m Co-pell ODS with 25% methanol in 50 mM phosphate buffer (pH 7). Flow-rate, 1.05 ml min^{-1}; ultraviolet detection, 215 nm; fluorescence detection, excitation 313 nm and emission 467 nm. A, Carbaryl standard (56 ng); and B, cleaned-up extract of bees and carbaryl.

For separating carbaryl and naphth-1-ol the choice of a reversed-phase packing material is critical as the separation of the two compounds varies with the stationary phase used. For example, naphth-1-ol and carbaryl co-chromatographed on Co-pell ODS using 1 + 4 methanol - buffer as the mobile phase but were well resolved on Permaphase ODS with the same solvents.

Conclusion

Without the development of more extensive clean-up procedures, the gas-chromatographic assay of bee extracts containing trace amounts of carbaryl is liable to interference from bee components. The direct high-performance liquid chromatography of carbaryl and its spectrofluorimetric assay as naphth-1-ol are equally liable to interference, but combination of high-performance liquid chromatography and fluorimetric detection of naphth-1-ol can be used easily and reliably to assess the carbaryl taken up by bees.

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Received *June 18th*, 1979
Accepted *October 8th*, 1979

Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part VII.* Fingerprinting of Essential Oils by Temperature-programmed Gas - Liquid Chromatography Using a Carbowax 20M Stationary Phase

Problems of obtaining reproducible results in the "fingerprinting" of essential oils by temperature-programmed gas - liquid chromatography have been examined; in particular an absorption-coating technique for the column packing is described. The requirements for standardisation of column efficiency, selectivity and reproducibility have been worked out using as a basis the method of column characterisation described by van den Dool. This column characterisation is referred to as the "*g*-pack" value and is determined from experimentally determined relative retention indices for a set of test substances using the even carbon numbered n-alkanes from C_8 to C_{24}.

A collaborative study with Carbowax 20M as stationary phase, and a specification of *g*-pack values for the column packing, has resulted in the production of a method that yields reproducible relative retention indices for the test substances limonene, linalol, linalyl acetate, acetophenone, naphthalene and cinnamyl alcohol, and has been applied with satisfactory results to oils of bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg. A recommended method is given for the reproducible temperature-programmed gas - liquid chromatographic fingerprinting of essential oils when Carbowax 20M is used as a stationary phase.

Keywords: *Essential oils analysis*; *temperature-programmed gas - liquid chromatography*; *Carbowax 20M stationary phase*

The Analytical Methods Committee has received and approved for publication the following Report from its Essential Oils Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. A. M. Humphrey (Chairman), Mr. M. E. Arnold (to November 1978), Mr. C. J. Brett, Dr. D. Farley, Mr. J. H. Greaves (to November 1978), Mr. B. Hine (from November 1978), Mr. W. S. Matthews, Mr. P. Metson (from November 1978), Miss D. M. Michalkiewicz (from November 1978), Mr. D. A. Moyler, Mr. A. Osbiston (from December 1978), Mr. R. G. Perry, Mr. J. Ridlington, Mr. R. A. Stocks, Mr. G. Watson and Mr. D. A. Waugh (to November 1978), with Mr. P. W. Shallis (to September 1977) and (the late) Dr. N. W. Hanson (from September 1977) as Secretaries.

Introduction

By the turn of this century the major components of most of the known essential oils, and a large number of the minor components, had been isolated and identified. The methods used were mostly chemical and various chemical assays were introduced in order to assess the types, origins and purities of oils. Many of these methods became highly refined and, until the advent of gas chromatography in the early 1950s, remained the only routine methods of assay. The new technique of gas - liquid chromatography (GLC) was immediately applied

* For details of Part VI of this series, see reference list, p. 273.

to the examination of essential oils and, within a very short space of time, essential oil analysis moved into a new era. The new method gave improved results over the older methods, many of which we now know gave precise but inaccurate results. Subsequent developments in the technique of GLC brought about more accurate analyses for specific components[1–6] but the concept of using the technique for "fingerprinting" oils had not been satisfactorily realised.

The chemical nature of essential oils makes them particularly suitable for analysis by GLC. If temperature-programmed operation is used, a very high proportion of the total number of components present can be resolved.[7,8] This fact has led many workers to attempt to establish libraries of temperature-programmed gas-chromatographic tracings. The advantage of such libraries would be that sample tracings could be compared with reference tracings in a similar manner to that which is used for comparing fingerprints, and the authenticity and other details of the sample could then be established. It was quickly found that the conditions for the GLC analyses had to be strictly controlled but even under the most careful supervision results were poorly reproducible, particularly among different laboratories. It was apparent that the temperature programming and the nature of the column itself caused the largest variation in the results and, as a consequence, many individual libraries of tracings were built up. Many of the tracings were based on separations at several different isothermal temperatures but few of them could be directly related to one another.

The problems of achieving reproducible results using temperature-programmed GLC have been examined at several times in the past by the Essential Oils Sub-Committee and, apart from the study reported here, have not been entirely satisfactory. Lack of reproducibility was attributed to the lack of reproducibility of the columns themselves, which in turn was attributed to the two problems of coating the support and the subsequent ageing of the packing with use. There was a requirement for the standardisation of the column efficiency and of its selectivity without using one as a factor for the other.

Early in 1975 the attention of the Sub-Committee was drawn to a paper by van den Dool[9] describing a method for the characterisation of GLC columns using the relative retention indices[10] of a group of six test compounds. By applying a series of calculations to the observed relative retention indices, van den Dool obtained a figure described as the "*g*-pack," which depends on the type of stationary phase and any modifications made to it during its previous use.

During subsequent studies by the Sub-Committee using Carbowax 20M, it was found that the *g*-pack value of a column was unaffected by changes in any of the operating parameters of the gas chromatograph such as carrier-gas flow-rate, temperature-programming rate, initial temperature and initial temperature hold. However, the value could be decreased by loss of stationary phase over a period of time due to column bleeding and could be increased by modification of the stationary phase due to oxidation. In either of these instances the selectivity of the column would alter, which would affect any fingerprint obtained.

A collaborative fingerprinting study using Carbowax 20M as a stationary phase, with a specification of the *g*-pack value, gave more reproducible results than had been obtained before, but it was found that certain other difficulties arose concerning resolution and the elution temperatures of the individual components. However, these factors had not apparently affected the measured *g*-pack values and during the following 3 years all of the subsequent studies relied heavily on the findings of van den Dool.

The full details of the mathematical derivation of the *g*-pack value of a column were described in the paper by van den Dool[9] and a summary of it together with the details of all of the work of the Essential Oils Sub-Committee on the subject will be published in a monograph that is in the course of preparation. For the purposes of this recommendation, a brief outline only will be given here.

Van den Dool's paper describes work with only two stationary phases: SE-30 as an example of a non-polar type and Carbowax 20M as an example of a moderately polar type, although the *g*-pack concept can be applied to any stationary phase. The method uses six test compounds, which were chosen to exhibit a range of properties similar to those found in essential oils. These compounds are limonene, linalol, linalyl acetate, acetophenone, naphthalene and cinnamyl alcohol, and a mixture of them is referred to as the N.C. (Netherlands Committee) mixture.

It was considered that the polar stationary phases were more difficult to use in a reproducible manner than the non-polar phases; the investigations were therefore concentrated on the use of Carbowax 20M. The recommendations in the Appendix apply only to this stationary phase, although a later paper will give recommendations on the use of non-polar stationary phases.

Experimental

The difficulties, referred to above, in applying the *g*-pack calculations particularly concerned the ability of the chromatographic system to elute all of the test compounds within the limits of the temperature programme and it was necessary to make reference to the parameter of elution temperatures for the test compounds. In practice, the N.C. mixture is itself mixed with a series of n-alkanes in order to be able to determine the relative retention indices and the highest n-alkane used is C_{24}. A satisfactory upper temperature for Carbowax 20M was found to be 225 °C and the conditions of gas chromatography were adjusted to give an elution temperature of 225 °C for the C_{24} n-alkane. This parameter can be expressed in a different form in which the relative retention index corresponding to any given temperature point in the programme is determined. Thus, a relative retention index at 225 °C (R.R.I.225) of 2400 is satisfactory and all of the test compounds and n-alkanes up to C_{24} elute within the programme. It was found that the biggest effect on the R.R.I.225 value was caused by the programming rate, the carrier-gas flow-rate had only a minor effect and the starting temperature and initial isothermal period had no significant effect at all. An increase in the programming rate had the effect of lowering the R.R.I.225 value, *i.e.*, the elution temperatures of the compounds were increased.

The effect of stationary phase loading was investigated and was found to have a small measurable effect on the *g*-pack value of the column. Further, it was found that the loading on different supports to give the same *g*-pack value was different and this was attributed to the different absorptive capacities and surface areas of the different supports. Only two supports were examined in detail, Chromosorb W HP and Gas-Chrom Q. The effects on column efficiency of particle size and evenness of distribution as well as methods of packing the column were also examined.

Van den Dool[9] concluded that the most satisfactory method of determining column efficiency was to measure the separation of a closely eluting pair of compounds using the peak/valley ratio method of expression. The N.C. mixture was chosen by van den Dool so that linalol and linalyl acetate formed such a pair when run on Carbowax 20M; the degree of separation of these two components was used as a measure of column efficiency in all of the Sub-Committee's investigations.

A major factor in the success of the investigations was the use of an improved method of coating the support with stationary phase. In all of the Sub-Committee's previous collaborative work packings had been prepared by the slurry coating technique; reproducibility even within the same laboratory was poor and column efficiencies could not be guaranteed even after extensive conditioning periods. The new method, in contrast, gave very reproducible results with high efficiencies, and column conditioning was reduced to the minimum. Such columns were examined for their bleed rates and changes in performance with time and were found to be superior to those prepared from slurry coated packings. The new method involved dissolving the stationary phase in the minimum volume of solvent and absorbing this solution on the support in such a way that the damp packing remained particulate and free-flowing. Following an equilibration time of at least 12 h the solvent was allowed to evaporate from the packing in a gentle current of air and, after sieving, the packing was ready for use. This new method is referred to as the "absorption coating technique" and forms one of the important aspects of the recommended method in the Appendix. Its use allowed the preparation of large numbers of different packings, the characteristics of which could then be reliably compared. The absorption coating technique was applied successfully to the coating with OV-210 and it was as a result of this that the technique was included in the recommended procedure given in Part V of this series.[5]

As it is known that the retention indices of some compounds on some stationary phases are not independent of temperature, it is necessary to standardise the elution temperatures of the test compounds. A study was therefore made of the effect of stationary phase loading

on elution temperatures and on the R.R.I.225 value. It was found that graphs of the elution temperatures against stationary phase loading were sinusoidal curves for all of the compounds and that the points of minimum inflection occurred at a loading of about 15% Carbowax 20M on Chromosorb W HP and 12% Carbowax 20M on Gas-Chrom Q.

The effects of carrier-gas flow-rate, column length and inner diameter, programming rate and starting temperature were also studied in detail and, on the basis of the results, a full collaborative trial was arranged in which the operating conditions were specified in a manner very similar to those given in the Appendix for the standardisation of the chromatographic system.

Results

The results of the collaborative study using the N.C. mixture with the proposed standard procedure (Appendix I) are given in Table I. The mean values, standard deviations and relative standard deviations for the retention indices are given in Table II. These results were considered by the Sub-Committee to be satisfactory.

TABLE I

RESULTS OF COLLABORATIVE STUDY OF RECOMMENDED METHOD (APPENDIX I)

	Laboratory						
	1	2	3	4	5	6	7
g-pack	1.277	1.278	1.273	1.276	1.278	1.279	1.273
R.R.I.225	2392	2408	2399	2400	2390	2392	2397
Resolution, %	96.6	95	97.6	95	98	91	100
Time/min	77	77.5	75	77.5	74.2	74.2	75
Column inner diameter/mm	4	4	2	4	3.5	2	3.8
Flow-rate/ml min^{-1}	66	77.5	75	50	60	67	50
Test compounds: relative retention indices—							
Limonene	1212	1213	1209	1212	1214	1215	1209
Linalol	1542	1544	1535	1540	1542	1543	1536
Linalyl acetate	1563	1562	1556	1560	1561	1563	1558
Acetophenone	1655	1658	1645	1654	1658	1650	1649
Naphthalene	1748	1751	1739	1748	1753	1742	1742
Cinnamyl alcohol	2268	2270	2259	2262	2270	2252	2256
n-Alkanes: elution temperatures/°C—							
C_{10}	84	84	85	84	84	85	84
C_{12}	97	98	99	98	98	99	99
C_{14}	118	119	120	119	119	122	121
C_{16}	141	142	144	143	142	145	145
C_{18}	164	165	166	166	164	167	167
C_{20}	186	186	188	186	185	188	188
C_{22}	206	206	207	206	204	208	208
C_{24}	224	224	225	225	226	226	226
Test compounds: elution temperatures/°C—							
Limonene	98	99	100	99	100	101	100
Linalol	135	136	136	136	135	138	137
Linalyl acetate	137	138	139	139	137	140	139
Acetophenone	148	149	149	149	148	151	150
Naphthalene	158	160	160	160	159	161	160
Cinnamyl alcohol	212	212	212	211	210	212	213

A collaborative trial using five essential oils (bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg) produced a series of chromatograms, which, when reduced photographically to similar dimensions, were compared subjectively by the Sub-Committee. Each set of chromatograms for each oil was considered to show minimum variations and the Sub-Committee was satisfied that the chromatographic procedure was satisfactory.

Discussion

Throughout all of the different collaborative and research exercises, the Sub-Committee was conscious of the fact that many of the parameters examined were chosen within arbitrary limits and that some justification for the choices should be made. In particular this applies to the nature of the test compounds in the N.C. mixture and the use of the relative retention index system. This particular system, although not ideal, is widely used and most gas chromatographers are acquainted with it. Its particular merit lies in its simplicity and, although it is a relative rather than an absolute system, it allows for a choice of reference compounds that can be made according to other requirements. For the purpose of the present investigation it was felt that the homologous series of n-alkanes were the most suitable reference compounds. They are readily available in pure form, are extremely stable and are the least likely compounds to exhibit chromatographic anomalies. In addition, they formed the basis of the work of van den Dool. It was noticed, however, that the elution of a variety of homologous series under temperature-programmed conditions was not linear and that the departure from linearity increased as the programming rates decreased.[11] It was also noticed that this departure was greater for homologous series of polar compounds than for the n-alkanes and this gave another reason for the choice of the latter.

TABLE II

SUMMARY OF RESULTS IN TABLE I: MEAN RELATIVE RETENTION INDICES AND STANDARD DEVIATIONS

Test compound	Mean relative retention index	Standard deviation	Relative standard deviation, %
Limonene	1212	2.31	0.19
Linalol	1540	3.50	0.23
Linalyl acetate	1560	2.64	0.17
Acetophenone	1653	4.89	0.30
Naphthalene	1746	5.21	0.30
Cinnamyl alcohol	2262	7.16	0.32

This non-linearity of elution raised the question of the method of application of the system, *i.e.*, whether an assumption of linearity between successive n-alkanes would introduce undue errors. Van den Dool recommends the use of both even and odd carbon numbered n-alkanes to reduce any error, whereas all of our collaborative work was carried out with the even carbon numbered compounds only, making the assumption of linearity between each pair. Comparisons have been made of the effect of the calculated relative retention indices of the six N.C. test compounds when using all of the n-alkanes against only the even carbon numbered compounds and also by calculating on the assumption of linearity as well as by a graphical method to obtain more accurate figures. The differences that were found were so small (in some instances zero) as to be insignificant when compared with the standard deviations shown in Table II. Therefore, our recommended procedure uses only the even carbon numbered n-alkanes and assumes linearity among them.

The six compounds in the N.C. test mixture were selected by van den Dool on the basis of their similarity to the types of compounds found in essential oil analyses and their choice could be criticised if used in conjunction with other sample types. However, the N.C. mixture is used for calibration of the column irrespective of its ultimate use and, as the mathematical treatment of the results for the N.C. mixture has already been worked out by van den Dool, there seems little point in changing. In addition, the mixture was chosen so that resolutions and catalytic activity of the column could be studied. In applications other than essential oil analyses an additional test for catalytic activity could be included to simulate the type of sensitivity pertaining to the analysis being done.

The choice of six compounds rather than any other number appears to be a reasonable compromise between having an excessive number of possibly interfering peaks and a *pro rata* increase in the mathematical calculations with a reduced amount of experimental data leading to a less accurate result.

The choice of limonene as the first emerging peak seems satisfactory as only a few terpene hydrocarbons normally elute before it while the cinnamyl alcohol elutes near the very end

of the temperature programme when using Carbowax 20M. Indeed, there was some difficulty in the earlier stages of the work in arranging conditions such that it eluted within the temperature programme at all. The N.C. mixture therefore fulfils a number of simultaneously desirable functions and, although its choice appears at first sight to be arbitrary, the Sub-Committee could find no justification for any change. The quantitative composition of the N.C. mixture has been adjusted such that each of the components gives a similar peak height.

It was not the aim of the Sub-Committee to prepare a treatise on the technique of GLC and it has always been assumed that good chromatographic practices would be followed. However, some particular points of technique have been examined and are included in the discussion. It was found that mass flow controllers should be used under temperature-programmed conditions but their performances can be unreliable in respect of their return to a reproducible setting after the completion of a programme. The newer laminar flow type is much more reliable than the older diaphragm type in this respect. However, with either type great care must be taken to prevent any leakage of gas between the flow controller and the column. The two most likely points of leakage are the septum and the column seal to the injector block. Chromatographs that are fitted with pressure gauges between the flow controllers and the columns give an indication of any leakage at these points.

As reproducibility of the results is particularly dependent on the starting and finishing temperatures and the time taken between them, it is important that the temperature calibrations of the chromatograph should be checked carefully and adjusted if necessary. Adjustment of the programming rate may not be possible on older instruments but, if not exactly correct, the R.R.I.225 value can be adjusted by changing the carrier-gas flow-rate. The choice of 2 °C min^{-1} for the programming rate was arrived at by the desire to achieve an R.R.I.225 value of 2400 and, although the analysis time of 75 min may be considered to be unduly long in some circumstances, there is further justification for using this particular rate. The effect of programming rate on column efficiency and resolution has been studied by Scott,[12] who concluded that 2 °C min^{-1} is the optimum. It was found in our experiments that a reduction in the programming rate below 2 °C min^{-1} gave a lower resolution for linalol and linalyl acetate.

The actual temperature limits specified in the recommendations give the widest practical range for the stationary phase being used, in this instance Carbowax 20M. Many chromatographs do not have reliably stable ovens at temperatures much below 75 °C and, in any event, Carbowax 20M begins to solidify when the temperature is reduced below 65 °C and is not then suitable for GLC. The upper temperature limit is set by the thermal stability and vapour pressure of the Carbowax 20M, although it has been shown that these vary according to the method of preparation of the packing. Recent publications have shown that even more stable coatings than those obtained by the absorption method may be prepared at low loadings.[13–16] However, it has been demonstrated that the use of the absorption coating method with Carbowax 20M gives packings that are acceptably stable after long periods at 240 °C and that therefore an upper temperature of 225 °C can be confidently recommended with the occasional use of a higher temperature in order to obtain a stable base line.

The use of an initial isothermal hold was considered but in practice it was found not to give any additional resolution when examining the monoterpene hydrocarbon contents of a range of essential oils; as it would introduce a further complication to the operating parameters, its inclusion was rejected.

The subject of resolution and its measurement is discussed extensively by van den Dool and his recommendations were accepted by the Sub-Committee. Difficulties in discussion of this subject arise because there is no absolute measure of resolution and it can have significance only in relation to a given separation. It may occur that a satisfactory separation of two compounds will be achieved with a column having a very low efficiency, but the same column would be unpredictable when separating an unknown mixture and it is therefore desirable to use columns with high efficiencies. Efficiency in this connection may be described as the ability of the column to pass a sample of a single compound throughout its length with a minimum of band spreading; any variation from the theoretical Gaussian spread due to diffusion effects can be used as a measure of efficiency. This approach is satisfactory in fractional distillation theory where stationary phase effects do not interfere, but in GLC the nature and size of the test sample and its interaction with the stationary phase affect the result. In addition, the isothermal distillation analogy is not applicable under conditions of

temperature-programmed operation. It then seems reasonable to accept van den Dool's approach and use two test compounds with a degree of separation significant to the type of analyses being carried out. It then remains to choose a pair whose individual behaviours are unlikely to be different if other operating parameters are changed. So far as can be ascertained from results of the collaborative work, the pair linalol and linalyl acetate are satisfactory in the present application, although moderate reductions in the polarity of the column will make a measurable increase in their resolution even though other evidence suggests that this is not due to any inherent increase in the efficiency of the column. More suitable pairs could possibly be found amongst isomeric pairs of esters but it was not considered that the small advantage to be gained would be justified in view of all of the previous work.

The heart of any chromatographic system is the column, and much of the work of the Sub-Committee was concerned with its preparation. The choice of the support has an influence on performance and it was always found that deactivated supports gave better resolutions and lower degradation of linalyl acetate than untreated supports; it is therefore recommended that whichever support is chosen it should be the least active of its type even if it is not necessarily required for every analysis. The same argument applies to the choice of column material, and although stainless steel may be satisfactory for most applications, glass is recommended to cover those instances where stainless steel is not satisfactory. The mesh size of the support plays a small part in determining the resolutions obtained from the range of column sizes used; either 80–100 or 100–120 mesh is recommended, although 100–120 mesh is slightly better and is preferred for columns of inner diameter 2 mm and lower. Even smaller mesh sizes are available but the increase in carrier-gas back-pressure that is required in order to maintain the necessary carrier-gas flow-rate offsets the small increase in resolution. Larger mesh sizes give a noticeable reduction in resolution but a more important factor is the evenness of the particle-size distribution. The packing in the column must be as uniform as possible and it is therefore necessary to sieve the packing *after* coating and to pack the column with the minimum of attrition of the particles; the use of electric vibrators is therefore not recommended. It has also been found that the material used for the end plugs of the columns has a small but measurable effect. Deactivated glass-wool is recommended for essential oil applications, although its use for some other analyses may not be recommended.

The most significant single recommendation to be made concerns the use of the absorption coating method for the preparation of the packing. Its importance cannot be overstressed and its simplicity enables very reproducible results to be achieved. The recommended stationary phase loading was chosen by consideration of the experimental results, which showed that variations in elution temperatures and retention indices with changes of stationary phase loading were at a minimum with a 15% loading of Carbowax 20M on Chromosorb W HP. This figure must be related to the type of support being used, and it appears to be related to its surface area and bulk density. Thus, it was found that the equivalent loading for Gas-Chrom Q was 12% of Carbowax 20M and this was confirmed by experiments showing that identical results were obtained from these two supports with the appropriate loadings. The Sub-Committee did not examine other proprietary brands or types of support.

Conclusions

The Sub-Committee recommends the procedure given in Appendix I for the reproducible fingerprinting of essential oils by temperature-programmed GLC using a Carbowax 20M stationary phase.

Although the procedures have been developed and investigated for the analyses of essential oils it is felt that they have a much wider application and should find use in many other fields of GLC analysis.

APPENDIX I

Recommended Method for the Reproducible Fingerprinting of Essential Oils by Temperature-programmed Gas Chromatography Using a Carbowax 20M Stationary Phase

Preparation of the Packing

The packing will be coated with Carbowax 20M at a loading that depends on the type of support. A high-quality, acid-washed and silanised support with a mesh size of 80–100 or preferably 100–120 should be chosen. Proprietary products such as Chromosorb W HP (see Note) and Gas-Chrom Q have been found to be satisfactory. These two supports must be coated with 15% and 12% Carbowax 20M, respectively.

NOTE—
A range of proprietary calcined Celite supports is available under the description Chromosorb, and if the same volume of Carbowax 20M solution were used for coating, a number of the impregnated supports might not be sufficiently free flowing to give a satisfactory packing.

Weigh the support (17 g of Chromosorb W HP or 17.6 g of Gas-Chrom Q) into a 100-ml beaker. Weigh the Carbowax 20M (3 g for the Chromosorb W HP or 2.4 g for the Gas-Chrom Q) into a 250-ml conical flask and add 12 ml of chloroform. Stopper the flask and swirl it until solution is complete. Add the appropriate support from the beaker using a powder funnel. Re-stopper and rotate the flask gently until an even distribution is obtained. The packing at this stage should be particulate and free flowing. If the packing appears to be damp and the particles coalesce to any extent, this is indicative of a low-absorbing support and the preparation must be repeated using a smaller volume of solvent. Allow the flask and contents to stand overnight and then transfer the damp but free-flowing packing into a flat open dish in a fume cupboard. Allow the solvent to evaporate whilst gently turning over the packing with a spatula, avoiding vigorous action, which may break down the particles. When the solvent has evaporated sieve the packing and reject any "fines" or coarse lumps, then store the packing ready for use.

Smaller or larger amounts of packing may be prepared using *pro rata* amounts, and different supports can be used with the appropriate stationary phase loading and volumes of solvent as determined by experiment.

Packing the Columns

For the most reliable results the column must be made of glass; for some purposes stainless steel will be satisfactory. The column length must be 2 m; the inner diameter is not critical but should be known so that the optimum carrier-gas flow-rate can be estimated. Clean the column with solvent and dry it in a current of air. Plug one end with silanised glass-wool and attach it to a vacuum line (water-pump or similar). Fit the open end of the column with a funnel and add small amounts of the packing at intervals. Between each addition, tap the column gently with a metal spatula until no further settling takes place. When the column is full plug the open end with silanised glass-wool. Pack and operate the columns in pairs.

Conditioning the Columns

Connect the columns to the gas chromatograph at the injector end only and adjust the carrier-gas flow-rate to the appropriate value. Programme the temperature of the oven from 75 to 230 °C at the lowest rate available and leave the columns at 230 °C overnight with the carrier gas flowing. Cool the oven and examine the packing for signs of settling; if this has been severe (greater than 10 mm) unplug the column and top it up with fresh packing. Repeat the conditioning. The columns are then ready for use.

Gas-chromatographic Conditions

It is very important that the oven conditions and settings are checked before use. It is not uncommon to find that the temperature calibrations require adjustment and these should be set at 75 and 225 °C using a reliable thermometer as a reference. Any variations at temperatures between these limits are likely to be small and can be ignored. The

programming rate is also very important and should be adjusted to 2 °C min^{-1} using a stopwatch to time the complete programme from 75 to 225 °C. A tolerance of 75 $\pm$ 2 min for the run is permissible.

The carrier-gas flow-rate must be controlled by a mass flow controller; pressure control is not suitable. The actual flow-rate will depend on the inner diameter of the column.

The septum must be changed regularly and the maintenance of the correct carrier-gas flow-rate must also be checked at intervals. Some types of flow controllers drift with time and others do not re-set accurately after a temperature-programmed cycle. If a pressure gauge is fitted between the flow controller and the injection port it will give an indication of any change in the flow.

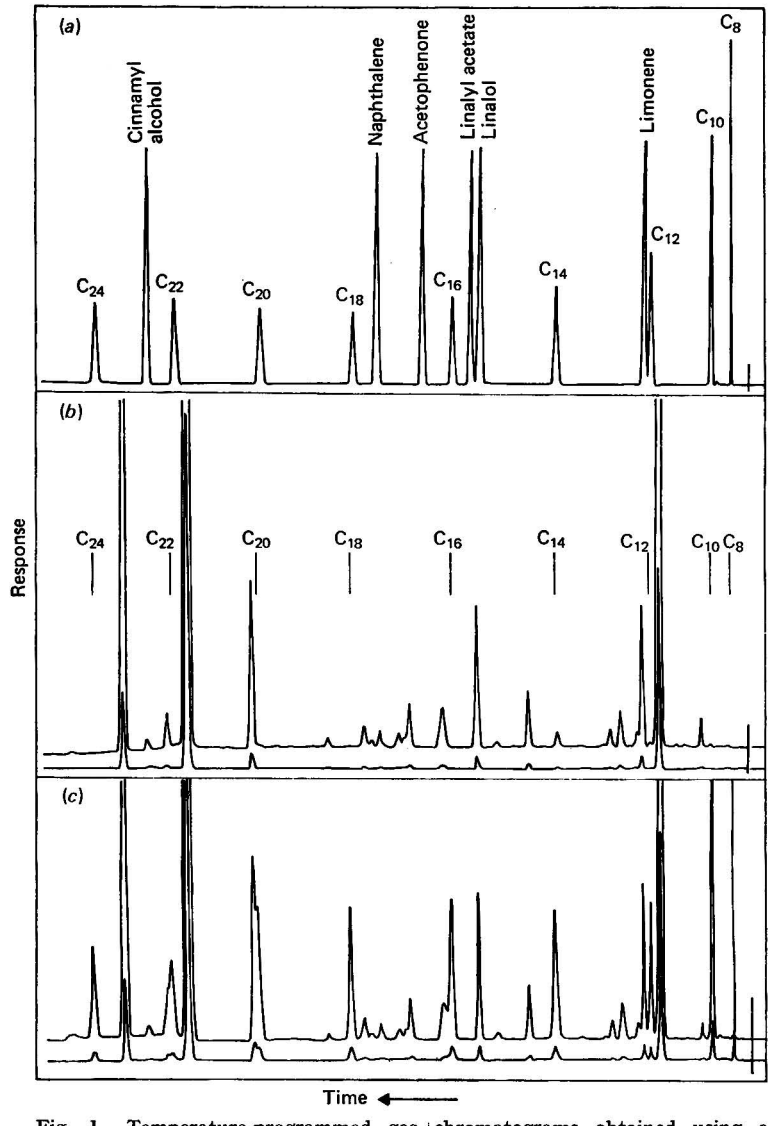

Fig. 1. Temperature-programmed gas chromatograms obtained using a Carbowax 20M stationary phase: (*a*) typical trace with the test mixture; (*b*) bay oil; and (*c*) bay oil + n-alkanes.

Preparation of Test Mixtures

Prepare a mixture of equal masses of the even carbon numbered n-alkanes from C_8 to C_{24}.

Prepare a mixture of 1.00 part of limonene, 1.37 parts of linalol, 1.60 parts of linalyl acetate, 1.40 parts of acetophenone, 1.13 parts of naphthalene and 1.80 parts of cinnamyl alcohol (N.C. mixture).

Prepare a mixture of 55% *m/m* of the N.C. mixture and 45% *m/m* of the hydrocarbon mixture. Each n-alkane then comprises 5% and each N.C. component approximately 10% of the total mixture.

Test Chromatogram

Set up the gas chromatograph, with the prepared columns, for temperature-programmed operation between 75 and 230 °C at 2 °C min^{-1}. Inject 0.2 μl of the combined test mixture and immediately start the programme and recorder chart. Mark the position of the chart at the 225 °C point and continue the isothermal heating at 230 °C until a stable base line is obtained. Cool the oven and re-stabilise at 75 °C. Repeat the run if necessary, adjusting the attenuation and flow-rate to bring all of the peaks on-scale and the elution temperature of the C_{24} hydrocarbon between 223 and 227 °C. Small alterations in the sample size may also be made. Examine the chromatogram to establish the absence of catalytic decomposition of the linalyl acetate; this is indicated by the presence of early emerging spurious peaks and a reduced peak height for the linalyl acetate. A typical tracing is shown in Fig. 1(*a*).

Calculation of Results

When a satisfactory tracing has been obtained, calculate the relative retention indices of the N.C. components and the R.R.I.225 point, assuming a linear span between adjacent hydrocarbon peaks. Using the values obtained for the N.C. components tabulate the results as in Table III and calculate the *g*-pack value for the column following the given worked example.

The *g*-pack value is given by $f_2\Sigma X - f_1\Sigma Y - f_3\Sigma Z$, where f_1, f_2 and f_3 are the appropriate multiplying factors.

Calculate also the elution temperatures of the N.C. components and the n-alkanes from the tracing, assuming a linear increase in the temperature from the start (75 °C) to the 225 °C point. Calculate the resolution between the linalol and the linalyl acetate using the peak/valley separation method (Fig. 2). The operating conditions and chromatographic system can be considered to be satisfactory if the results lie within the following specifications:

g-pack	1.272–1.279
R.R.I.225	2390–2410
Resolution	95–100%
Time for programme	73.5–77.5 min
R.R.I. values—	
Limonene	1205–1215
Linalol	1535–1545
Linalyl acetate	1555–1565
Acetophenone	1645–1660
Naphthalene	1735–1755
Cinnamyl alcohol	2250–2275

Elution temperatures—	
C_{10}	83–86 °C
C_{12}	96–100 °C
C_{14}	117–122 °C
C_{16}	140–146 °C
C_{18}	162–168 °C
C_{20}	183–189 °C
C_{22}	203–209 °C
C_{24}	223–227 °C
Limonene	95–102 °C
Linalol	134–139 °C
Linalyl acetate	136–141 °C
Acetophenone	147–152 °C
Naphthalene	157–162 °C
Cinnamyl alcohol	209–214 °C

Standardised Chromatograms of Essential Oils

As soon as the performance of a column within a chromatographic system has been satisfactorily established according to the procedures given above, it can be used under similar conditions for the analyses of essential oils. The performance of the system should be checked at least once a week.

A sample of the essential oil should be injected and run under the conditions established as above. The sample size and attenuation should be adjusted according to the sensitivity required for any subsequent examination. On completion of the run a mixture of the essential oil with the n-alkane mixture should be chromatographed using similar conditions. Comparisons of the two chromatograms should show identical retention times and comparisons of the peak heights should be consistent with any alterations due to dilution with the n-alkane mixture, or changes of the injected volume and attenuation.

TABLE III

METHOD OF TABULATING RESULTS FOR GLC COLUMN CHARACTERISATION USING CARBOWAX 20M COLUMNS

LABORATORY: A.M.C./E.O. COLUMN CODE: 15% Carbowax 20M DATE:

Chromatographic parameters:

g-pack	1.273
R.R.I.225	2400
Resolution	99%
Support	Chromosorb W HP
Particle size	100–120
Loading	15%
Length	2 m
I.D.	3 mm
Material	Glass
Injection temperature	150 °C
Starting temperature	75 °C
Finishing temperature	230 °C
Programming rate	2 °C min^{-1}
Programming time	75 min
Carrier-gas flow-rate	30 ml min^{-1}

Elution temperatures:

C_8	79 °C
C_{10}	84 °C
C_{12}	99 °C
Limonene	100 °C
C_{14}	121 °C
Linalol	137 °C
Linalyl acetate	139 °C
C_{16}	144 °C
Acetophenone	150 °C
Naphthalene	160 °C
C_{18}	167 °C
C_{20}	186 °C
C_{22}	207 °C
Cinnamyl alcohol	213 °C
C_{24}	226 °C

Calculation of *g*-pack value:

Test compound	Relative retention index	*Y* factor	*X* factor	*Z* factor
Limonene ..	1209	$\frac{\text{R.I.} \times 0.14 + 2}{136.23} = 1.2571$	$1.0584 \times Y = 1.3305$	$1.0584^2 \times Y = 1.4082$
Linalol ..	1536	$\frac{\text{R.I.} \times 0.14 + 2}{154.24} = 1.4072$	$1.0218 \times Y = 1.4378$	$1.0218^2 \times Y = 1.4692$
Linalyl acetate ..	1558	$\frac{\text{R.I.} \times 0.14 + 2}{196.28} = 1.1215$	$0.8797 \times Y = 0.9866$	$0.8797^2 \times Y = 0.8679$
Acetophenone	1649	$\frac{\text{R.I.} \times 0.14 + 2}{120.14} = 1.9382$	$1.3350 \times Y = 2.5875$	$1.3350^2 \times Y = 3.4543$
Naphthalene ..	1742	$\frac{\text{R.I.} \times 0.14 + 2}{128.16} = 1.9185$	$1.3361 \times Y = 2.5633$	$1.3361^2 \times Y = 3.4248$
Cinnamyl alcohol ..	2256	$\frac{\text{R.I.} \times 0.14 + 2}{134.17} = 2.3689$	$1.4993 \times Y = 3.5517$	$1.4993^2 \times Y = 5.3251$
		$\Sigma Y = 10.0114$ $\times 1.07977 = 10.8100$ Minus	$\Sigma X = 12.4574$ $\times 2.88734 = 35.9687$ Plus	$\Sigma Z = 15.9495$ $\times 1.49758 = 23.8857$ Minus

g-pack value = $f_2\Sigma X - f_1\Sigma Y - f_3\Sigma Z =$ **1.273**

These comparisons allow for the check on the reproducibility of the system and also enable the positions of the n-alkanes to be transferred from the second chromatogram [Fig. 1(*c*)] to the first [Fig. 1(*b*)] in such a manner that visual interference is avoided. In instances where a component of the essential oil overlaps or obscures an n-alkane peak, its position

can be determined by comparison with the chromatogram obtained for the *g*-pack calculation [Fig. 1(*a*)]. Thus, it is possible to determine relative retention indices for any of the peaks of interest in the essential oil chromatogram.

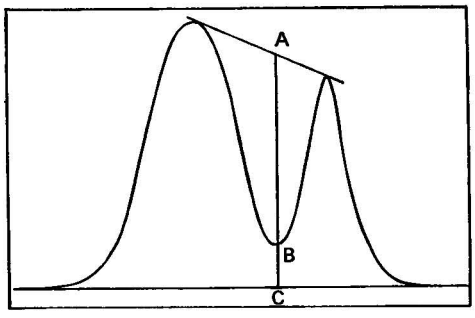

Fig. 2. Resolution by peak/valley separation method. Resolution (%) = (AB/AC) × 100.

The sensitivity of the system can be adjusted electronically to suit the purposes of the analyses but it is recommended that for general purposes two chromatograms should be obtained such that in one a peak representing 50% of the total gives about full-scale deflection and in the other a peak representing 5% of the total gives about full-scale deflection. This does not take into account variations in response factors; sensitivity settings should be adjusted on the basis of the average peak heights found for the n-alkanes in the standardisation test run where each n-alkane represents 5% of the total mixture. Small changes in the injection volume can also be used to adjust the peak heights obtained. Ideally a two-pen recorder should be used, allowing the chromatograms at different sensitivities to be obtained simultaneously and on the same chart as shown in the examples [Fig. 1(*b*) and (*c*)].

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NOTE—References 1–6 are to Parts I–VI of this series, respectively.

SHORT PAPERS

Determination of Trace Concentrations of Selenium in Soils and Sediments by the Introduction of Hydrogen Selenide into an Inductively Coupled Plasma Source for Emission Spectrometry

B. Pahlavanpour, J. H. Pullen and M. Thompson

Applied Geochemistry Research Group, Department of Geology, Imperial College, London, SW7 2BP

Keywords: Selenium determination; soils and sediments; hydride generation; inductively coupled plasma emission spectrometry

At present the most widely used methods for the routine determination of selenium in soils and sediments are based on the reaction between selenium and 2,3'-diaminonaphthalene (DAN) to form a fluorescent piazselenol compound.[1–6] While these methods give satisfactory results, they require very careful technique with strict attention to detail, especially in the sample dissolution stage. Complete destruction of organic matter is necessary in order to avoid the possibility of fluorescent interference from trace amounts of residual material, and is achieved by treatment of the sample with hot acidic oxidising mixtures. However, excessive temperatures or prolonged heating bring about the loss of selenium by volatilisation and considerable ingenuity is required to devise methods that will satisfy these conflicting requirements.

In addition to the difficulties of sample digestion, the use of DAN has itself a number of disadvantages: DAN needs to be purified, which is a lengthy process involving low temperatures and low light levels[6]; because of the unstable nature of the piazselenol, analysis must be carried out promptly; and DAN is chemically related to highly carcinogenic compounds.

In this paper we describe a method for the determination of selenium that gives results comparable to those obtained with the fluorescent methods, but is adaptable to the rapid analysis of large batches of samples. The solution containing the selenium is reduced with sodium tetrahydroborate(III) and the hydrogen selenide formed is swept into an inductively coupled plasma (ICP) source for determination by emission spectrometry. Calibrations are linear from the detection limit (1 ng ml^{-1}) to about 1000 ng ml^{-1} of selenium. The detection limit obtained is comparable to most of the values reported for selenium by atomic absorption - hydride systems, and the linear calibration range is much greater.[7–10]

Although the method is not subject to interference from most constituents of soils, small traces of copper inhibit the release of hydrogen selenide,[11] so the selenium is separated by co-precipitation with lanthanum hydroxide according to the method of Bedard and Kerbyson.[12] Treatment of the sample with hot oxidising acids at a controlled temperature is still required, but need not be prolonged as trace amounts of unoxidised organic matter do not interfere as they do in the fluorimetric method. In the final solution selenium must be present as selenium(IV), as the efficiency of the sodium tetrahydroborate(III) reduction depends on the oxidation state. This is achieved by adding 4% of potassium bromide to the final solution and heating at 50 °C.

Experimental

Instrumental

The hydride generator - ICP - spectrometer system used was as previously described by Thompson *et al.*[13,14]

Hydride generator

This consisted of a separation cell and a peristaltic pump. The glass cell was designed to provide mixing of the sample and the sodium tetrahydroborate solution, separation of the

hydrogen selenide plus hydrogen from the spent liquids and mixing of the gaseous products with the argon carrier gas. The pump was a Watson-Marlow Type MHRE200 (*i.e.*, a fast pump) fitted with narrow-bore tubing to obtain fast linear flow-rates, and hence rapid sample transfer and low dead space.

Plasma generator

A Radyne, Model R50, cavity-controlled oscillator with a maximum forward power of 8 kW, fitted with a plasma torch of the type described by Scott and Kokot,[15] was used.

Spectrometer

An Applied Research Laboratories 29000B Quantometer fitted with 40 channels was used. Light from the tail flame of the plasma was focused on to the slit of the spectrometer in such a way that the effective viewing height of the plasma could be varied.

Operating conditions

The operating conditions selected were similar to those previously described, except that the gas flow-rates and plasma viewing height could be optimised for selenium alone, rather than the use of compromise conditions for a multi-element analysis: forward power, 2.7 kW; viewing height, 6·mm above load coil; coolant-gas flow-rate, 17 l min^{-1} of argon; integration time, 20 s; pre-integration time, 15 s; selenium wavelength, 196.10 nm; sample acidity, 5 M in hydrochloric acid; sample flow-rate, 9.2 ml min^{-1}; reagent composition, 1% *m*/*V* sodium tetrahydroborate(III) in 0.1 M sodium hydroxide solution; reagent flow-rate, 4.5 ml min^{-1}; and carrier-gas flow-rate, 0.8 l min^{-1} of argon.

Heating block

A drilled-out, thermostatically controlled aluminium block made by Scienco-Western Ltd., which could accommodate 252 test-tubes (150 × 19 mm) to a depth of 120 mm, was used. The temperature could be controlled to within 1 °C.

Centrifuge

An MSE centrifuge with four buckets capable of accommodating 20 test-tubes (120 × 16 mm) and speeds of 6000 rev min^{-1} was employed.

Reagents

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

Nitric acid, sp. gr. 1.42.

Perchloric acid, 72% *m*/*m*.

Ammonia solution, sp. gr. 0.88.

Hydrochloric acid, 5 M. Dilute 431 ml of concentrated hydrochloric acid to 1 l with water.

Potassium bromide solution. Dissolve 400 g of potassium bromide in 1 l of water.

Lanthanum nitrate solution. Dissolve 50 g of lanthanum nitrate in 1 l of water.

Sodium tetrahydroborate(III) solution. Dissolve 10 g of the compound in 1 l of 0.1 M sodium hydroxide solution.

Procedure

Digestion of soil

Weigh 0.500 g of air-dried sample, ground to pass through a 200-μm sieve, into a 19 × 150 mm borosilicate test-tube. Add 2 ml of nitric acid and some crushed glass, gently swirl the mixture and leave it overnight at 50 °C in a hot-block. Remove the test-tubes and, when cool, add 1 ml of perchloric acid, re-mix and replace in the hot-block. Raise the temperature of the hot-block to 100 °C until the brown fumes cease to be evolved. Raise the temperature to 170 °C and remove the test-tubes when the samples are bleached but not completely dry. Add four drops of 5 M hydrochloric acid to the cooled test-tubes and transfer the contents with about 5 ml of water into centrifuge tubes. Spin the test-tubes at 4000 rev min^{-1} for 30 s, and decant the centrifugate into a graduated 10-ml centrifuge tube.

Precipitation with lanthanum nitrate solution

To each tube add 0.5 ml of lanthanum nitrate solution and 2 ml of ammonia solution, swirl the resulting suspension and centrifuge at 4000 rev min^{-1} for 30 s. Discard the centrifugate and wash the residue with 1 ml of ammonia solution and 3 ml of water. Centrifuge the tube again and discard the washings. Dissolve the residue in 5 ml of 5 M hydrochloric acid, add 1 ml of potassium bromide solution, heat in a water-bath at 50 °C for 50 min and dilute to 10 ml with 5 M hydrochloric acid. (The precipitation of ammonium perchlorate during this procedure has no adverse effect.)

Determination of selenium

Reduce the samples sequentially with sodium tetrahydroborate(III) solution, using the procedure and instrumental conditions described above. Use 5 M hydrochloric acid to establish the base line and a solution of sodium selenate (0.1 $\mu g\ ml^{-1}$ of selenium) in 5 M hydrochloric acid as a calibrator, checking the readings after every ten samples.

Reduction of selenium(VI) with bromide

The oxidative attack leaves selenium in the form of selenium(VI), which must be reduced to selenium(IV) before formation of the hydride is attempted. A very mild reducing agent is required in order to avoid the formation of selenium(0), which is lost by precipitation. Hydrochloric acid is commonly used for this purpose. Although this reduction is satisfactory, the bromide method is used in preference because of the more convenient temperature of reaction and because, under the experimental conditions given, arsenic(V) and antimony(V) are also reduced to the +3 oxidation state, which is useful if these elements are being determined simultaneously.

Results and Discussion

Selection of Method of Sample Attack

Various acids and acid mixtures for the dissolution of selenium in soils have been described[16]: nitric acid, nitric acid - perchloric acid, nitric acid - hydrochloric acid, hydrochloric acid and hydrochloric acid - hydrofluoric acid. It was found that the nitric acid - perchloric acid mixture was the most suitable for the determination of selenium, consistently giving the highest results. This is because selenium needs strongly oxidising conditions during the acid attack, not only to destroy organic matter, but also to prevent its reduction to selenium(0) by organic matter or iron(II) in the sample. The relatively small volume of nitric acid used allowed the duration of the attack to be shortened, provided that the heating sequence described was adhered to. Sufficient time must be allowed for the nitric acid to oxidise the organic matter before it is evaporated off at the higher temperatures used later in the heating sequence.

The temperature used during the final stage of the acid attack is of great importance. If the temperature exceeded 180 °C then selenium was lost by volatilisation (Table I), confirming the findings of Stanton and McDonald.[17] To prevent this loss occurring, an aluminium heating block was used to give very precise temperature control, and enabled large numbers of samples to be processed simultaneously. A batch of 150 samples required about 12 man-hours for analysis, this time being divided into weighing (2 h), sample digestion (5 h, of which only 1 h requires close supervision), precipitation and centrifugation (3 h) and instrumental analysis (2 h).

TABLE I

EFFECTS OF HIGH TEMPERATURES ON THE RECOVERY OF SELENIUM

Sample	Temperature/°C	Selenium recovered/ $\mu g\ g^{-1}$
Weald loam	170	0.21
Weald loam	>180	0.11

Comparison with Other Methods of Analysis

The method has been in regular use for many months in the Applied Geochemistry Research Group for the determination of selenium in soils for agricultural and mineral exploration studies. In each batch of analyses standard reference samples and samples analysed by other laboratories were included, and the results obtained are shown in Tables II and III. With few exceptions the results given by fluorimetry, chromatography and neutron activation analysis compare well with those obtained by the proposed method. The precisions obtained for the various samples were very good for between- and within-batch samples. The main factor limiting the precision that could be obtained was probably the instrumental system; the use of a modern integral plasma system would no doubt improve the precision.

TABLE II

COMPARISON OF MEAN VALUES ($\mu g\ g^{-1}$) OBTAINED USING HYDRIDE GENERATION - ICP WITH THOSE OBTAINED FROM OTHER LABORATORIES

Sample	ADAS*	ADAS*	Analysed by CCRMP†	AGRG‡		
				m	*s*	*n*
SO-1			0.10	0.08	0.01	7
SO-2			0.30	0.36	0.03	6
SO-3			0.05	0.18	0.02	7
SO-4			0.40	0.52	0.03	7
S778		0.20		0.30	0.04	6
S779		0.40		0.43	0.08	7
S783		0.50		0.27	0.03	5
S784		0.90		0.92	0.07	6
S788		1.10		1.08	0.08	6
S789		1.20		1.08	0.09	6
63/35	0.30			0.26	—	1
95/16	0.75			0.63	—	1
63/38	1.52			1.37	—	1

* Mr. R. J. Hall, Ministry of Agriculture, Fisheries and Food, Newcastle upon Tyne (method: fluorimetry).
† Canadian Certified Reference Materials Project (method: fluorimetry).
‡ Applied Geochemistry Research Group, Imperial College, London (method: this work). *m* = arithmetic mean value; *s* = between-batch standard deviation; *n* = number of observations.

Conclusion

A simple and rapid method for the determination of selenium in soils and sediments has been described, in which the selenium is extracted with nitric acid - perchloric acid, reduced to hydrogen selenide and determined by its emission at 196.10 nm in an inductively coupled plasma.

TABLE III

COMPARISON OF SELENIUM CONCENTRATIONS ($\mu g\ g^{-1}$) IN TWO STANDARD SOILS OBTAINED USING GAS - LIQUID CHROMATOGRAPHY (GLC), HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC), NEUTRON-ACTIVATION ANALYSIS (NAA) AND AN INDUCTIVELY COUPLED PLASMA SOURCE (ICP)

Sample	GLC*	HPLC*	NAA†	ICP‡		
				m	*s*	*n*
Weald loam	0.22	0.17	0.56	0.22	0.04	14
Lower lias	0.59	0.70	0.76	0.84	0.05	14

* Dr. G. Nickless, Department of Inorganic Chemistry, University of Bristol.
† Universities Reactor Centre, Warrington.
‡ Applied Geochemistry Research Group, Imperial College, London (this work). *m* = arithmetic mean value; *s* = between-batch standard deviation; *n* = number or observations.

Although the procedure is complicated by a separation stage using co-precipitation on lanthanum hydroxide, it is capable of adaptation to large batches of samples and is consequently a rapid method on a per-sample basis. It has the advantage over fluorimetric methods that it does not depend entirely on complete destruction of organic matter or require special purification of reagents. In addition, it has been found that the method can be used unchanged for soils with a high organic matter content.

We are indebted to Dr. G. Nickless, Department of Inorganic Chemistry, University of Bristol for analysis carried out and also to Mr. R. J. Hall of the Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, Newcastle upon Tyne, for helpful discussions on selenium analysis at low trace concentrations, to various laboratories of the Ministry of Agriculture, Fisheries and Food for permission to publish comparison analyses, to the Ministry of Agriculture, Fisheries and Food for financial support and to the Natural Environmental Research Council for financial support for Dr. B. Pahlavanpour.

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Received *October 3rd*, 1979
Accepted *October 18th*, 1979

Determination of Rhenium in Molybdenum Concentrates

Bret W. Budesinsky
Phelps Dodge Corporation, Morenci, Ariz. 85540, *USA*

Keywords: *Rhenium determination*; *molybdenum concentrates*; *solvent extraction - spectrophotometry*

Rhenium is a regular trace component of molybdenum concentrates and as such was discovered in 1925.[1] During recent decades there has been an increasing interest in rhenium because of its excellent properties as a metal. Analytically, the main problem has been the great similarity between molybdenum and rhenium, which also had been the reason for the late discovery of rhenium. Promising methods for the determination of rhenium are inductively coupled plasma emission spectrometry[2,3] and atomic-absorption spectrometry.[4] However, these methods have not been applied sufficiently for them to be considered reliable for this purpose. The sensitivity of 12 p.p.m. (1% absorption) at 346.05 nm in atomic-absorption spectrometry is low.[4]

There are many spectrophotometric methods for the determination of rhenium,[5] but most of them require a good preliminary separation of rhenium and molybdenum. The method

based on the formation of a colour complex between rhenium and 8-mercaptoquinoline is very selective (5000-fold excess of molybdenum does not interfere).[6] The main drawback of this method is the need for an expensive and unstable reagent so that only its disulphide form is recommended for storage[7]; moreover, the reaction with rhenium takes place in 8–10 M hydrochloric acid, which is an inconvenient combination for rhenium introduced into aqueous solution by alkaline fusion.

Rhenium and molybdenum can be separated by means of extraction, precipitation, distillation and ion exchange.[5] With regard to the time, instruments and chemicals expenditure, solvent extraction seems to be the most convenient method of separation. For that reason we investigated that method and developed a modification for the determination of rhenium that is simple, selective and accurate.

Experimental

Instruments

A Universal X-ray vacuum spectrometer (Norelco Corp., Mount Vernon, N.Y., USA) with a chromium target tube was used for the measurement of metal distribution after the solvent extraction. The conditions are given in Table I.

A Coleman 124D double-beam spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn., USA) with a 1-cm glass flow cell was used for spectrophotometric measurement and a wrist-action mechanical shaker (Burell Corp., Pittsburg, Pa., USA) for extraction.

TABLE I

CONDITIONS FOR X-RAY MEASUREMENT

X-ray tube voltage, 45 kV; current, 18 mA; lithium fluoride crystal; air path medium; scintillation detector; detector voltage, 0.8 kV; measurement time, 50 s; linear amplifier attenuation, 5; coarse collimation used throughout. All of the measured intensities were corrected for the counting dead-time and the background.

Parameter	Rhenium	Molybdenum
Pulse-height analyser base line/V	0	28
Pulse-height analyser window/V	57	64
Spectrometer angle, °2θ	41.43	20.49

Reagents

Fusion mixture. Mix well 900 g of sodium peroxide with 300 g of pulverised sodium hydroxide.

Tetraoctylammonium chloride (TOAC) solution. Dissolve 80 g of tetraoctylammonium chloride (Adogen 464, Aldrich) in chloroform and dilute to 1 l with the same solvent.

Furil α-dioxime solution. Dissolve 0.35 g of furil α-dioxime (Eastman Kodak) in acetone and dilute to 100 ml with the same solvent. Prepare fresh daily.

Tin(II) chloride solution. Dissolve 10 g of tin(II) chloride dihydrate in 10 ml of 12 M hydrochloric acid plus 20 ml of water and dilute to 100 ml with water. Prepare fresh daily.

Procedure

Place 8.0–8.5 g of the fusion mixture into a 20–25-ml iron or zirconium crucible, add a weighed amount of sample (usually 0.4–0.6 g) corresponding to 0.2–2.0 mg of rhenium, mix well with a glass rod and place in an electrical furnace pre-heated at 660 °C for 8–10 min (this method of fusion has been used for many years without any effects of a violent reaction). Cool the crucible by dipping its lower part into cold water and then place the crucible in a 200-ml beaker covered with a watch-glass. Wash the material out of the crucible by addition of three approximately 15-ml portions of water. When all of the material in the crucible has been removed, rinse the crucible thoroughly with water and remove it from the beaker. To decompose the purple ferrate ion, add about 1 ml of ethanol to the still warm leach mixture, mix well and cool to room temperature. Then, transfer the mixture quantitatively into a 100-ml calibrated flask, dilute to the mark with water and mix. Filter the solution through a dry Whatman No. 30 filter-paper into a dry 125-ml conical flask, collecting the first about 10 ml of filtrate separately and discarding it.

Pipette exactly 10 ml of the filtrate into a 60-ml separating funnel, add 10 ml of TOAC solution and shake for 5 min. Collect the lower organic phase in another 60-ml separating funnel and repeat the extraction with a further 10-ml portion of TOAC solution, collecting the organic phase in the same funnel. Wash the combined organic phase with two 10-ml portions of 0.2 M sodium hydroxide solution (shaking for 5 min in each instance) and then re-extract the perrhenate ion into the aqueous phase with two 8-ml portions of 2.5 M perchloric acid (again shaking for 5 min in each instance). Collect the aqueous phase in a 50-ml calibrated flask, add 2 drops of phenolphthalein indicator (0.1% ethanolic solution) and neutralise with 5 M sodium hydroxide solution. Add 4.0 ml of 12 M hydrochloric acid, 12 ml of furil α-dioxime solution, mix well, add 5.0 ml of tin(II) chloride solution, dilute to the mark with water and mix. After 45 min measure the absorbance at 530 nm against a reagent blank solution. At 22–26 °C the colour is stable for at least 60 min.

Construct a calibration graph using 0.02, 0.05, 0.10, 0.15 and 0.20 mg of rhenium in 16 ml of 2.5 M perchloric acid. The molar absorptivity is 41 300 l mol^{-1} cm^{-1}.

Results and Discussion

Molybdenum(VI) reacts at pH <1.5 as MoO_2^{2+} and at pH >5 as MoO_4^{2-}. The species H_2MoO_4 and $HMoO_4^-$ exist at molybdenum(VI) concentrations below 10^{-3} M and polymolybdates above that concentration in the pH range 1.5–5.0.[8] As perrhenic acid is a much stronger acid than H_2MoO_4 (the respective pK_a values are −1.25 and 3.89),[9] the existence of ReO_2^{3+} and ReO_4^- is shifted to the more acidic side of the acidity scale. This makes it possible that the pH 0–1.4 is suitable for the extraction of molybdenum(VI) whereas the alkaline range is more suitable for the extraction of ReO_4^- as an ionic associate of a stronger acid. Tungsten(VI) reacts in a similar manner to molybdenum(VI),[8] 1.5–2.0% being extracted with 8% TOAC (in chloroform) from 0.2 M sodium hydroxide solution.

The conditions and yields for the rhenium and molybdenum extraction are given in Table II, and show that separation by means of extraction with TOAC and TBAC* is the most efficient. It is advantageous to use this extraction because the alkaline leach solution obtained after the oxidative fusion can be used directly as the aqueous phase. The separation of aqueous and organic phases is rapid and sharp. There is only a brief mention of rhenium(VII) extraction with quaternary ammonium salts in the paper by Maeck *et al.*[10] On the other hand, the results in Table II disprove the suitability of cupferron[11] and potassium ethyl xanthate[12] as extractants. The latter reagent is also unstable and of disagreeable odour. A relatively wide range of yields in the extraction of rhenium is the result of that instability.

Table II

Distribution of rhenium and molybdenum in various solvent extraction systems

The aqueous phase contained either 1% *m*/*V* Mo or 0.1% *m*/*V* Re. Equal volumes of both phases were used; the time of shaking was 10 min. The extractions were performed at 22–25 °C.

Organic phase*	Aqueous phase	Concentration in organic phase, %	
		Rhenium	Molybdenum
1% cupferron in $CHCl_3$	1 M H_2SO_4	10.5–11.5	99.0–99.5
8% TOAC in $CHCl_3$	0.2 M NaOH	99.5–99.9	0.1–0.5
8% TOAC in $CHCl_3$	0.2 M Na_2CO_3	99.0–99.5	0.1–0.5
$CHCl_3$	50% K-EX,† 4.5 M HCl	15–20	99.5–99.9
1% TPAC in $CHCl_3$	0.2 M Na_2CO_3 to pH 8.2	98.0–99.0	0.3–0.6
1% TPAC in $CHCl_3$	0.2 M Na_2CO_3	98.1–99.1	0.4–0.8
1% HDPC in $CHCl_3$	0.2 M Na_2CO_3	97.0–98.0	0.5–1.5
1% TBAC in $CHCl_3$	0.2 M NaOH	99.4–99.9	0.1–0.6

* TOAC = tetraoctylammonium chloride; TPAC = tetraphenylarsonium chloride; HDPC = *N*-hexadecylpyridinium chloride (separation of phases with HDPC takes 2 h); and TBAC = tetrabutylammonium chloride.

† K-EX = potassium ethyl xanthate.

The results of the determination of rhenium in some standards and molybdenum concentrates are shown in Table III. There is reasonable agreement with the results of other methods. The precision of the proposed method is ±1.62% (relative).

TABLE III

DETERMINATION OF RHENIUM

Material*	Rhenium concentration, %	
	Present method	Other method
Potassium perrhenate + 0.3 g of ammonium molybdate	64.4, 65.5, 64.6, 64.7, 63.9	64.4†
Molybdenum concentrate NBS ..	0.089, 0.088	0.087‡
Molybdenum concentrate A	0.046, 0.047	0.048, 0.048§
Molybdenum concentrate B	0.047, 0.049	0.051, 0.048§
Molybdenum concentrate C	0.049, 0.051	0.051, 0.052§
Molybdenum concentrate X	0.095, 0.098	0.094, 0.096§
Molybdenum concentrate Y	0.036, 0.037	0.035, 0.038§
Molybdenum concentrate Z	0.021, 0.022	0.020, 0.022§

* The composition of concentrates A, B and C was 33–37% Mo, 5–9% Cu, 3–5% Fe, 12–32% SiO_2 and 23–26% S. The composition of concentrates X, Y and Z was 30–35% Mo, 1–5% W, 4–6% Cu, 2–4% Fe, 12–30% SiO_2 and 22–27% S.

† The amount of used rhenium was varied between 0.2 and 2.0 mg.

‡ Standard Reference Material No. 333 (National Bureau of Standards, Washington, D.C.). Result obtained by isotopic dilution mass spectrometry and thermal neutron-activation analysis. The content of other elements was 1.038% Cu, 55.3% Mo, 8.9 p.p.m. Au and 25.0 p.p.m. Ag.

§ Results obtained by inductively coupled plasma emission spectrometry at 221.4 nm with a Spectraspan III instrument (SpectraMetrics, Burlington, Mass.).

The fusion with sodium peroxide and hydroxide regularly oxidises the present iron to the purple ferrate, which passses through the filter and decomposes to iron(III) hydroxide within 2–6 h. Ferrate is very conveniently reduced with ethanol. At this point in the procedure various other workers suggest more or less complicated operations (without realising the problem of the existence of ferrate) in order to obtain the clean solution of perrhenate and molybdate.

Many tetraalkylammonium salts can be used for the rhenium extraction. However, TOAC (in fact containing a mixture of C_8–C_{10} alkyl groups with C_8 predominating) is the cheapest reagent. Thorough shaking of the chloroform phase with sodium hydroxide solution is important for removing the co-extracted trace amounts of molybdate. Final spectrophotometry has been performed with the rhenium complex of furil α-dioxime.[13] This reagent is preferred to thiocyanate as the spectrophotometry can be performed directly with the acetone - water solution, the coloured complex is far more stable and the sensitivity is about 50% higher than that of the rhenium - thiocyanate complex. The composition of the coloured complex is still the subject of speculation because of many factors involved [concentrations of the reagents, acetone, hydrochloric acid, tin(II) chloride and perchlorate and temperature].

The author is grateful to Dr. D. E. Jordan of the Extractive Metallurgy Department for the performance of inductively coupled plasma emission spectrometry and to Mr. D. R. Grady for technical assistance.

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Received *May 30th*, 1979
Accepted *August 30th*, 1979

Fungicide Residues

Part VII.* Determination of Residues of Fentin in Vegetables and Cocoa Products by Spectrofluorimetry

P. G. Baker, D. S. Farrington and R. A. Hoodless

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

Keywords: *Fentin residues*; *spectrofluorimetry*; *vegetables*; *cocoa*

Fentin (triphenyltin), available as the hydroxide or acetate, is a non-systemic fungicide and is included in the list of Approved Products for Farmers and Growers[1] for use in the control of potato blight and *Ramularia* leaf spot on sugar beet seed crops. Fentin compounds are also used on celery, celeriac, carrots, hops, groundnuts, pecans, coffee and cocoa in various parts of the world to control fungal diseases and the Codex Alimentarius Commission have put forward recommended maximum residue limits for fentin on a number of crops.[2] These compounds gradually degrade. Fentin acetate is first converted into the hydroxide, which eventually degrades to di- and monophenyltin hydroxide and finally to inorganic tin salts.

Methods for the determination of organotin compounds have been reviewed by Kumpulainen and Koivistoinen.[3] Thomas and Tann[4] developed a method for determining residues of fentin in potatoes based on oxidation to inorganic tin followed by spectrophotometric determination of the tin. Vernon[5] developed a method for potatoes that was more specific as it involves the formation of a complex between the triphenyltin moiety and 3-hydroxyflavone and measurement of the fluorescence of the complex. Blunden and Chapman[6] applied Vernon's procedure to the determination of triphenyltin compounds in water and showed that there was little interference from di- and monophenyltin compounds and inorganic tin. Soderquist and Crosby[7] determined triphenyltin hydroxide in water, after conversion into its hydride, by gas chromatography using an electron-capture detector. This paper presents a procedure based on Vernon's fluorimetric determination that is applicable to a wider range of substrates.

Experimental

Initial attempts to determine fentin directly by high-performance liquid chromatography using an "ion-pair" system failed because of lack of sensitivity and interference from co-extractives. Fentin hydroxide is not amenable to direct gas - liquid chromatography but Freitag[8] has proposed a gas - liquid chromatographic method for the determination of fentin chloride on a 5% DC 550 column. Fentin hydroxide is easily converted into the chloride by shaking with hydrochloric acid. However, it was found that residues could not be detected at the required levels owing to "on-column" breakdown of the fentin chloride.

* For Part VI of this series, see *Analyst*, 1977, **102**, 752.

Therefore, it was decided to use Vernon's fluorimetric procedure[5] but to carry out the complex formation in toluene instead of benzene. Attempts to use Vernon's method on potatoes were unsuccessful owing to the large amount of starch that was extracted, and in order to overcome this difficulty the extraction procedure had to be modified. By drying the grated potato with anhydrous sodium sulphate and extracting with dichloromethane in a Soxhlet apparatus, an extract containing very little starch was obtained. This procedure was suitable for other vegetables but could not be applied to cocoa products because too much fat was extracted. Therefore, a simple shaking procedure with acetonitrile was devised for cocoa products, which extracted only a small amount of fatty material. The extract obtained from vegetables or cocoa products was then cleaned up on an alumina column to remove co-extractives that quenched the fluorescence of the fentin - 3-hydroxyflavone complex and thus gave rise to apparently low recoveries.

Method

Apparatus

Spectrofluorimeter. Baird-Atomic, Model FP100, spectrofluorimeter.
Chromatographic columns. Length 300 mm, i.d. 7 mm, fitted with a stop-cock.
Soxhlet extraction apparatus.
Extraction thimbles. These were of single thickness, 28 mm i.d. × 80 mm long.
Rotary evaporator.

Reagents

Analytical-reagent grade materials should be used unless otherwise indicated.
Aluminium oxide, Woelm neutral, Brockmann grade II.
Sodium sulphate, anhydrous, granular.
Acetonitrile.
Dichloromethane.
Methanol.
Toluene. Glass distilled (available from Rathburn Chemicals Ltd.).
Sodium acetate solution, saturated.
3-Hydroxyflavone solution. Dissolve 20 mg of 3-hydroxyflavone (available from Koch-Light) in toluene and dilute to 200 ml with toluene.
Fentin standard solution. Dissolve 10.0 mg of fentin hydroxide (of at least 95% purity) in dichloromethane, warming the mixture if necessary, and dilute to 100 ml with dichloromethane. This solution is stable for at least 1 month if stored at 4 °C. Dilute the solution to give a standard solution containing 0.4 μg ml^{-1} of fentin hydroxide in dichloromethane. This solution should be prepared freshly each week.

Procedure

Extraction of vegetables

Transfer 20 g of grated potato, 10 g of grated celery or 10 g of grated sugar beet into an extraction thimble containing 20 g of anhydrous sodium sulphate and extract the mixture in a Soxhlet apparatus with 100 ml of dichloromethane, allowing about 20 solvent cycles. Cool the solution slightly and reduce the volume of dichloromethane to about 5 ml, using a rotary evaporator with a water-bath at 40 °C. Add 10 ml of methanol and reduce the volume of the solution to 3–4 ml. With celery, reduce the initial dichloromethane extract to dryness and take up the residue in 10 ml of methanol.

Extraction of cocoa and chocolate

Transfer 10 g of ground cocoa beans or grated chocolate into a 150-ml conical flask. Add 50 ml of acetonitrile and stopper and shake the flask on a mechanical shaker for 15 min. Filter the mixture through a Büchner funnel under suction using a Whatman No. 3 filter-paper or equivalent. Wash the flask with two 10-ml portions of acetonitrile and use the washings to wash the residue in the Büchner funnel. Transfer the filtrate into a 150-ml flat-bottomed flask and wash out the Büchner flask with a further 5 ml of acetonitrile. Remove the solvent from the combined extract and washings using a rotary evaporator with a water-bath at 40 °C. Take up the residue in 3–4 ml of methanol.

Analysis

Using a Pasteur pipette, transfer the methanol extract (for celery a 2-ml aliquot) on to a 3-g alumina column that has been slurry packed in methanol. Allow the solution to pass through the column until the liquid level reaches the top of the column. Wash the flask with 5 ml of methanol and transfer the washings to the column. Repeat the process with a further 10 ml of methanol and collect all of the eluate in a 25-ml flask. Remove the methanol using a rotary evaporator with a water-bath at 40 °C (take care to evaporate only just to dryness.) Add 5 ml of 3-hydroxyflavone solution to the flask, then add 1 ml of saturated sodium acetate solution, stopper the flask and shake it on a mechanical shaker for 10 min. Transfer the mixture into a centrifuge tube and centrifuge for 1 min. Record the fluorescence emission spectrum of an aliquot of the toluene layer in a 1-cm cell between 420 and 600 nm using an excitation wavelength of 415 nm. Measure the peak height at 495 nm and ascertain the amount of fentin hydroxide present in the sample solution by reference to a calibration graph.

Preparation of Calibration Graph

Transfer 1-, 2-, 5-, 8- and 10-ml portions of the dilute fentin standard solution, equivalent to 0.4, 0.8, 2.0, 3.2 and 4.0 μg, respectively, of fentin hydroxide, into a series of 10-ml centrifuge tubes and evaporate to dryness on a rotary evaporator. Add 5 ml of 3-hydroxyflavone solution and 1 ml of saturated sodium acetate solution to each tube and shake for 10 min. Centrifuge and record the fluorescence emission spectrum of the toluene layer in a 1-cm cell between 420 and 600 nm using an excitation wavelength of 415 nm. Measure the peak height at 495 nm.

TABLE I

RECOVERY OF FENTIN FROM FORTIFIED SAMPLES

Sample	Codex Alimentarius maximum residue limit*/mg kg^{-1}	Fentin added/ mg kg^{-1}	Fentin found/mg kg^{-1}
Potatoes (Maris Piper)	0.1	0	0.005
		0.01	0.010, 0.011, 0.009
		0.10	0.10, 0.10, 0.09
		1.00	1.0, 1.0, 0.98
Potatoes (Red Desirée)	0.1	0	0.006
		0.01	0.013, 0.011, 0.012
		0.10	0.09, 0.09, 0.09
		1.00	0.98, 1.0, 1.0
Potatoes (King Edward)	0.1	0	0.003
		0.10	0.09, 0.09, 0.09
		1.00	1.0, 1.0, 1.0
Celery	1	0	0.04
		1.00	0.86, 0.80, 0.97
Sugar beet (root)	0.2	0.20	0.20, 0.19, 0.19
Cocoa beans (sample 1)	0.1†	0	0.01
		0.10	0.08, 0.07, 0.07
		0.50	0.39, 0.39
Cocoa beans (sample 2)	0.1†	0	0.004
		0.10	0.08, 0.07, 0.07
		0.50	0.41, 0.41
Cocoa beans (sample 3)	0.1†	0	0.006
		0.10	0.10, 0.07, 0.07
Chocolate (manufacturer 1)	—	0	0.02
		0.10	0.10, 0.10
Chocolate (manufacturer 2)	—	0	0.05
		0.10	0.09
Cocoa powder	—	0	0.05
		0.10	0.11

* Expressed as fentin hydroxide, excluding inorganic tin and di- and monophenyltin.
† At or about the limit of determination.

Results

The recovery of fentin hydroxide from samples of vegetables and cocoa products was checked by adding known volumes of a standard solution to portions of the sample and allowing the solvent to evaporate. The samples were then treated as described under Procedure. The results obtained are shown in Table I. No allowance for the blank values obtained on the various substrates has been made. As the blank values become increasingly more important at levels below 0.05 mg kg^{-1}, this is taken as the practical limit of determination, which is an improvement on that previously quoted for cocoa beans.[9]

In general, the recoveries from cocoa products were lower than those from vegetables. It was found that most of these losses were due to quenching of the fluorescence of the fentin complex by co-extracted material. The cocoa powder and chocolate gave high blank values, which were due to a co-extractive that fluoresced with maximum emission at 475 nm. This compound did not react with 3-hydroxyflavone and therefore it would be possible to carry out a blank determination by taking up the final residue in 5 ml of toluene instead of the complexing reagent.

For celeriac and sugar-beet tops the final extracts contained much more interfering material that quenched the fluorescence of the fentin complex, and the recoveries at the 0.1 mg kg^{-1} level were of the order of 50%, although the blank values corresponded to about 0.006 mg kg^{-1} of fentin hydroxide.

There is little interference from cyhexatin because, although it forms a complex with 3-hydroxyflavone, the intensity of the fluorescence is about 100 times less than that of the fentin complex.

The authors thank the Government Chemist for permission to publish this paper.

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Received *August 24th*, 1979
Accepted *October 5th*, 1979

Spectrophotometric Analysis of Interferences due to Polyols in the Assay of Protein by Lowry's Method

P. Ninfali, M. Magnani, V. Stocchi and M. Dachà

Istituto di Chimica Biologica, Università degli Studi di Urbino, Via Saffi 2, Urbino, Italy

Keywords: *Protein assay*; *spectrophotometric analysis*; *polyol - copper complexes*; *glycerol, erythritol, ribitol and* D(−)*-sorbitol*

Frequent reports on the interferences due to non-protein substances in the Lowry method[1] have been published. The interfering substances concerned are organic compounds such as carbohydrates,[2–8] chelators,[9] buffer solutions,[9,10] thiol compounds[11] and inorganic salts such as manganese(II) and cobalt(II) chloride.[12]

The availability of data on the interferences is useful for investigators seeking to avoid spuriously high results, especially in samples from enzyme purifications where it is necessary to protect enzyme activity with stabilising agents. Glycerol is the most commonly used stabilising agent and its effect on protein determination has already been pointed out.[4] Zishka and Nishimura,[4] however, speculating on the mechanism of the interference, excluded the formation of copper complexes with glycerol, although such complexes have been implicated in interference studies of sucrose[5–7] and ethylene glycol.[8]

This paper describes results for visible spectrophotometric measurements carried out to obtain calibration graphs of glycerol, erythritol, ribitol and sorbitol interference in the Lowry method, and to demonstrate the relationship between absorbance and concentration of interferent in the concentration range considered. Furthermore, our studies with ultraviolet absorption spectra provide evidence of the formation of copper complexes as the source of interference of glycerol and its homologous compounds.

Experimental

Reagents

Polyol solutions. Standard stock solutions are prepared by diluting 87% glycerol (obtained from Merck) and dissolving vacuum-dried erythritol, ribitol and D(−)-sorbitol (from Sigma Chemical Co.) in glass-distilled water. These stock solutions are diluted to varying extents to provide the working solutions.

Copper tartrate reagent. On the day of use, dissolve 0.2 g of potassium sodium tartrate (from Carlo Erba) in 100 ml of 5 mM copper sulphate solution ($CuSO_4.5H_2O$ from Carlo Erba).

Alkaline buffer solution. Sodium carbonate solution (1 M) plus sodium hydroxide solution (0.25 M), pH 12, for adjustment of the pH.

Folin-Ciocalteau reagent. Previously diluted 1 + 3 with water (Merck).

Bovine serum albumin (BSA). Lyophilised fraction V (from Sigma Chemical Co.), used as a protein standard, prepared fresh every week in water and determined spectrophotometrically using an $A^{1\%}_{280nm}$ value of 5.8.

Apparatus

Absorbance measurements and absorption spectra were made on a Beckman 25 spectrophotometer.

Calibration Graphs

Lowry method

Remove a 0.5-ml volume from each polyol working solution and dilute to 1 ml with water. To this volume add 1 ml of alkaline buffer solution and 0.4 ml of copper tartrate reagent. After 10 min, add 0.75 ml of diluted Folin-Ciocalteau phenol reagent. After mixing and allowing to stand for 30 min at room temperature in order for the colour to develop, measure the absorbance value at 700 nm against a reagent blank containing 1 ml of water, plus appropriate amounts of the Lowry reagents.

Ultraviolet spectrophotometric analysis

To different aliquots of a 0.5 M standard solution of each polyol add 1 ml of alkaline buffer solution and 0.4 ml of copper tartrate reagent. Make each solution up to 10 ml with water. Read the absorbance values at 250 nm against a reference solution, prepared from the same volume of alkaline buffer solution and copper tartrate reagent as in the sample and diluted to 10 ml with water.

Results

From our experiments it is apparent that increasing concentrations of polyol cause a marked increase in absorbance in the Lowry analysis. The interferences are illustrated in Fig. 1. The slopes of the lines are glycerol 0.215, erythritol and ribitol 0.315 and sorbitol 2.3.

The range of concentrations over which there is a linear increase in absorbance differs for the compounds analysed.

A typical standard graph obtained for BSA at a concentration of 0–100 μg ml^{-1} is compared in Fig. 2 with the standard graphs obtained using the identical increasing concentration of protein in the presence of 50 and 100 mM of sorbitol, respectively. Fig. 2 also shows that the higher the concentration of sorbitol, the earlier the deviation begins. The same behaviour is also exhibited by the spectral data of the other three compounds. Therefore, the relationship between absorbance and concentration of interferent is linear in increasingly restricted intervals (*i.e.*, up to 45 μg ml^{-1} of BSA at 50 mM of sorbitol and up to 25 μg ml^{-1} of BSA at 100 mM of sorbitol).

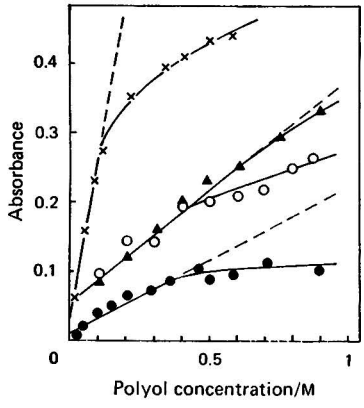

Fig. 1. Absorbance, at 700 nm, of colour developed by polyols with the Lowry reagent. Concentrations were expressed as molarity in the sample volume of 1.0 ml: ●, glycerol; ▲, erythritol; ○, ribitol; and ×, D(−)-sorbitol.

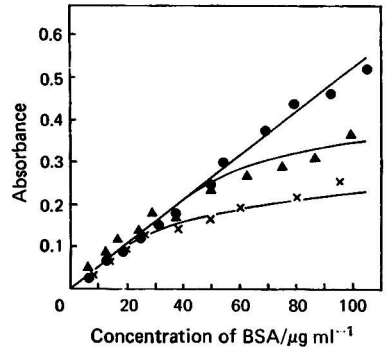

Fig. 2. Effect of sorbitol on protein determination. A 1-ml sample containing 10–100 μg of BSA; ●, no sorbitol present: ▲, 50 mM of sorbitol present: and ×, 100 mM of sorbitol. The samples were assayed for protein using the Lowry procedure. Absorbance values were read at 700 nm, subtracting a blank value with sorbitol at the respective concentrations.

The ultraviolet spectrophotometric analysis reveals that without the addition of the copper tartrate reagent none of the compounds exhibit absorption in the wavelength region considered. However, with the copper reagent present, at the same pH and temperature as in the above experiments, the polyol - copper complexes show a characteristic absorption band near 250 nm, which we assign to the charge-transfer transition involving ligand to metal ion. The absorbance variation of the complexes is shown in Fig. 3(*a*) as a function of the polyol to copper ratio. Fig. 3(*b*) illustrates the ultraviolet absorption band of the copper - sorbitol complex using a single sorbitol concentration.

Discussion

The presence of polyols in protein samples seriously interferes with protein determinations. Removal of these compounds by dialysis eliminates the interference, but this process is time consuming and may cause a loss of activity with enzymes. The use of an appropriate blank corrects the response for interferences only in a restricted concentration range. At higher concentrations, we observed a negative deviation from linearity and the interference could be corrected for over a wide range, by the use of a calibration graph, by plotting the data points on logarithmic graph paper, as suggested by Stauffer.[13]

In the experiments using the Lowry method we found that the blue colour is completely developed after 30 min for sorbitol and ribitol, while a shorter time is needed for glycerol and erythritol. The presence of copper seems to be necessary for the interference of these polyols in the Lowry method. In fact, no interference occurs with the Folin-Ciocalteau phenol reagent.[14]

Ultraviolet absorption spectra show identical absorption bands near 250 nm for all compounds analysed in this work and we interpret this band in terms of charge-transfer transition involving ligand to metal ion. We think, therefore, differently from Zishka and Nishimura,[4]

that the described interference is caused by copper - polyol complex formation. The complex reduces phosphotungstic and phosphomolybdic acids to molybdenum blue and tungsten, respectively,[1] and the exact stoicheiometric composition of the complex is unknown.

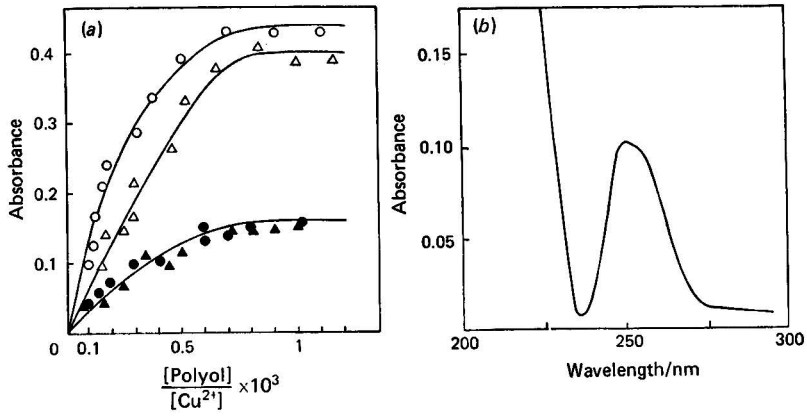

Fig. 3. (*a*) Effect of the increasing concentration of polyol - copper complexes on the absorbance at 250 nm. Copper tartrate concentration is maintained at 0.2 mM and polyol concentration ranges from 10 to 250 mM: ●, glycerol; ▲, erythritol; △, sorbitol; and ○, ribitol. (*b*) Absorption band of copper - sorbitol complex.

The height of the ultraviolet absorption band, which is due to the amount of the complex formed, reaches a maximum with ribitol. Therefore, it does not seem that the amount of the complex is proportional to the interference level in the Lowry method, where the slope of the sorbitol line greatly exceeds that of the other compounds (see Fig. 1). This effect is probably due to the action of molybdate, present in the Folin-Ciocalteau phenol reagent, on the chemical structure of the polyols, which are in equilibrium with their complexes. It is commonly known that sorbitol has a specific rotation of $-2.0°$ in water, which is reversed and increased to $+56°$ in the presence of molybdate. The incongruity of the absorbances in the ultraviolet and visible analyses could be attributed to this phenomenon.

In the ultraviolet analysis a plateau is reached for all compounds when the polyol to copper(II) ratio is 800:1, which is approximately the relative equilibrium concentration ratio.

With this comparison, we have shown that the mechanism of the interference is identical for these four compounds, and analogous to that of ethylene glycol[8] and sucrose.[5–7]

Further study with different techniques is needed before we can make an assumption about the coordination number and the stability constants of the complexes. We are of the opinion that these characteristics are similar for glycerol and erythritol and for ribitol and sorbitol.

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Received *June 20th*, 1979
Accepted *September 11th*, 1979

Determination of Halothane in Operating Theatre Air by Using a Passive Organic Vapour Dosimeter

M. M. Halliday and J. Anderson

University Department of Anaesthesia, Royal Infirmary, Glasgow, G4 0SF

Keywords: Operating theatre pollution; halothane; personal pollution dosimeter; gas - liquid chromatography

Because of the possible health risks involved,[1] it is important to establish the concentrations of anaesthetic waste gases, especially halothane and dinitrogen oxide, to which operating theatre staff are exposed. Several methods of measuring personal time-weighted average exposure levels have been used in operating theatres, including chemical adsorption tubes,[2,3] evacuated bottles[4] and plastic bags,[5] and glass syringes.[5] None of these methods meets all of the requirements of theatre pollution monitoring, and they are usually used in conjunction with background pollution monitors such as portable infrared spectrometers.[5,6]

A new type of personal pollution monitor is a passive organic vapour dosimeter, which samples organic pollutants in air by allowing them to diffuse on to, and be adsorbed by, an activated charcoal collection element. Pollutant concentrations are determined by subsequent desorption of the organic materials into a suitable solvent, followed by an analytical procedure, usually gas chromatography. This dosimeter has proved satisfactory for monitoring many of the pollutants present in industrial environments.[7,8] The purpose of the present paper is to describe the development of a gas-chromatographic analytical procedure for the dosimeter that will enable it to be used in hospital operating theatres.

Experimental

Dosimeter

The organic vapour dosimeter (Gasbadge, Abcor Inc., USA) was a small, light, personal sampler (51 × 65 × 16 mm, 25 g), which was clipped on to the wearer's lapel. Relying on diffusion in order to collect pollutants, there was no external power source. Before use an unused charcoal collection element was placed in the dosimeter, which was then sealed with a tightly fitting lid. The sampling period commenced when the lid was removed in the operating theatre and ended when it was replaced. The element was used once, analysed and then discarded.

Desorption Procedure

After use, the charcoal element was placed in a 4-ml glass vial with a metal sealed cap. Two millilitres of carbon disulphide (Aldrich Chemicals Ltd.) were added and the vial and contents roller-mixed for 30 min; 1-μl aliquots of the extraction solvent were removed for analysis by gas chromatography. This procedure was carried out immediately the used dosimeter was returned to the laboratory.

Gas-chromatographic Conditions

Previous studies[2,6] have shown the three most abundant organic pollutants in theatre air to be ethanol, propan-2-ol and halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). In choosing the chromatographic conditions for the present analysis it was therefore necessary to ensure that these three compounds were separated from each other. The column packing material chosen was 15% FFAP on Diatomite C, 80–100 mesh, used in a 2 m × 4 mm glass column. The chromatograph was a Pye 104 series, equipped with a flame-ionisation detector; other features were a column temperature of 343 K, a detector temperature of 453 K, a carrier gas (nitrogen) flow-rate of 60 ml min^{-1}, a hydrogen flow-rate of 50 ml min^{-1} and an air flow-rate of 500 ml min^{-1}.

Toluene, at a concentration of 5 mmol l^{-1}, was added to the bulk carbon disulphide extractant and it acted as an internal standard in the analytical procedure. Fig. 1 shows a typical chromatogram.

Calibration

Standard solutions in the concentration range 0–5 mmol l^{-1} were prepared by dilution of a stock solution of 50 mmol l^{-1} of halothane in carbon disulphide. All of the solutions were stored at 4 °C. A calibration graph of the ratio of peak heights given by halothane and toluene, plotted against halothane concentration, was obtained by injecting 1 μl of each standard solution into the chromatograph. Chromatograms of standard solutions showed no measurable deterioration over a period of 7 d (see Fig. 2) but thereafter the halothane peak height decreased. Standard solutions were therefore renewed weekly; the stock solution was renewed every 2 months.

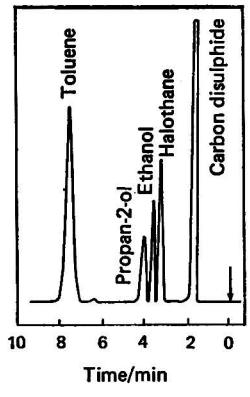

Fig. 1. Chromatogram of halothane, ethanol and propanol-2-ol extracted from a dosimeter collection element.

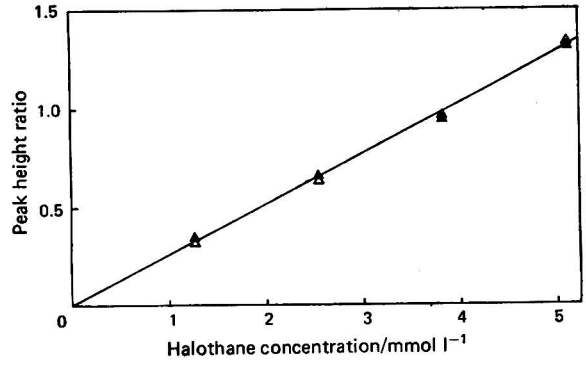

Fig. 2. Calibration graph for halothane: △, fresh standard solutions; ▲, 5-day old standard solutions.

The amount of halothane adsorbed on each collection element was calculated from the concentration of halothane in the extraction solvent and converted to the corresponding atmospheric halothane concentration by use of the following equation:

$$\text{Halothane concentration, p.p.m. (ideal, } V/V) = \frac{M}{DtE} \times 3360$$

where M is the amount of halothane adsorbed on the element (μmol), D the diffusion coefficient of halothane in air (0.0711 cm^2 s^{-1}), t the collection period (s), E the desorption efficiency for halothane and 3360 a constant depending on molar volume, dosimeter dimensions and temperature and pressure corrections. A full derivation of the calculation is given by Bamberger *et al.*[8]

Desorption Efficiency

The efficiency of desorption of halothane from the collection element was measured by placing an unused charcoal element in a glass vial and adding a known amount of liquid halothane, followed by 2 ml of carbon disulphide. The solvent was analysed after a 30-min equilibration period and peak-height ratios from extracted samples were compared with those from standard solutions (Table I). Over the concentration range of interest the desorption efficiency was found to be 1.0.

Dosimeter Performance

The accuracy of the technique was investigated by exposing dosimeters to known concentrations of halothane in a dynamic test chamber for periods of up to 3 h. Concentrations of halothane in the range 1–10 p.p.m. (ideal, V/V) were prepared by using a dynamic gas mixing rig in which gas from a Draeger halothane vaporiser was leaked at a known rate into a 10 l min^{-1} flow of air. The nominal concentrations from this apparatus were checked using a standard atmosphere generator.[9] The results are shown in Table II.

TABLE I

EFFICIENCY OF DESORPTION OF HALOTHANE FROM CHARCOAL ELEMENTS INTO CARBON DISULPHIDE

Halothane concentration/ mmol l^{-1}	Peak-height ratio	
	Standard	Extract
0	Not detected	Not detected
1.27	0.34	0.34
2.54	0.63	0.68
3.81	0.96	0.96
5.08	1.33	1.32

Discussion

The analytical procedure described here can detect 0.1 mmol l^{-1} of halothane, corresponding to an average atmospheric concentration of 0.2 p.p.m. (ideal, *V/V*) sampled over a 3-h period. In a recent study[6] of operating theatre pollution within a National Health Service District, the anaesthetists' personal exposure to halothane, measured by a chemical adsorption technique, lay in the range 0.5–20 p.p.m. (ideal, *V/V*). The 3-h sampling period used in this study corresponds to the normal length of an operating session, but the dosimeter can be used for up to 8 h in an atmospheric concentration of up to 150 p.p.m.

Halothane concentrations measured by the dosimeter fall within $\pm 10\%$ of the actual concentrations (Table II). This accuracy is comparable[8] to that achieved with chemical adsorption tubes and also with the performance of the dosimeter in measuring industrial pollutants.

TABLE II

PERFORMANCE OF DOSIMETER IN STANDARD HALOTHANE ATMOSPHERES

Concentration, p.p.m. (ideal, *V/V*)		
Atmospheric	Measured by dosimeter	Difference, %
5.0	5.5	+10
5.6	6.0	+7
3.6	3.3	−9
8.1	8.0	−1
6.9	6.6	−4
5.0	4.7*	−6

* All other dosimeter analyses were carried out immediately after exposure; with these the element was extracted and analysed 48 h after exposure.

The present work forms part of a wider study in which several methods of monitoring personal pollution levels are being investigated with respect to their suitability for use in operating theatres. It is important that operating staff are unhindered when wearing a personal sampler, and in this respect the dosimeter, having no external power source, is very acceptable, whereas samplers that incorporate air pumps may be noisy and cause inconvenience to the wearer.

Conclusions

The method described here offers a simple and reliable means of analysing halothane in hospital operating theatre air. The accuracy of the dosimeter is comparable to those of other pollution monitoring techniques. The value of the measurement is that it indicates the average personal exposure of theatre staff to halothane during a routine operating session.

The authors are grateful to Professor D. Campbell for his encouragement in this work and also to Dr. B. I. Brookes, Strathclyde Regional Chemist's Department, for access to the standard atmosphere generator.

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Received *September 10th*, 1979
Accepted *October 1st*, 1979

Rapid Determination of Chromium(VI) by Flow Injection Analysis

S. Storgaard Jørgensen and Marisa A. B. Regitano

Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Copenhagen V, Denmark

Keywords: *Chromium determination*; *water analysis*; *soil analysis*; *flow injection analysis*

The photometric determination of chromium(VI) in natural waters and soil extracts as the 1,5-diphenylcarbazide complex is well established as a standard method.[1] The complex is formed rapidly, has a high absorptivity at 540 nm and the method is highly selective when the pH is kept at 1–2. For rapid analysis of small sample volumes this method appears to be ideally suited for adaptation to the continuous flow injection technique of Růžička and co-workers.[2–4] The present investigation was undertaken when chromium(VI) had to be determined in a considerable number of soil extracts during a study of the behaviour of chromium in soils.[5]

Experimental

Reagents

All chemicals were of analytical-reagent grade.

Sulphuric acid, 0.040 M.

1,5-*Diphenylcarbazide reagent*. A 0.250-g amount of 1,5-diphenylcarbazide is dissolved in 50 ml of acetone. When a clear solution is obtained, 450 ml of water are added.

Standard chromium(VI) solution. A stock solution containing 1000 μg ml^{-1} of chromium(VI) is prepared by dissolving 3.735 g of potassium chromate in water and diluting to 1000 ml. Working solutions containing chromium(VI) in the range 0.02–20 μg ml^{-1} are prepared by suitable dilution of the stock solution.

Apparatus and Procedure

The reagent streams are pumped by an Ismatec Mini-S 840 peristaltic pump using Ismatec Tygon pumping tubes. The samples are introduced into the combined reagent streams through an injection port consisting of a precisely made rotary valve with a bore of volume 30 μl and furnished with a by-pass of higher flow resistance.[4] An injection valve with a bore of volume 20 μl was also used. The mixing manifold is made from polyethylene tubing (1.0 mm i.d.) and Lego building blocks.[6] The absorbance is measured by means of a simple

precision photometer especially designed for flow injection analysis. The photometer is equipped with a 540-nm optical filter, a Hellma flow-through cuvette, Type OS 178.12 (volume 18 μl, light path 10 mm), and connected to a Radiometer Servograph REC 51 recorder equipped with an REA 112 high-sensitivity unit.

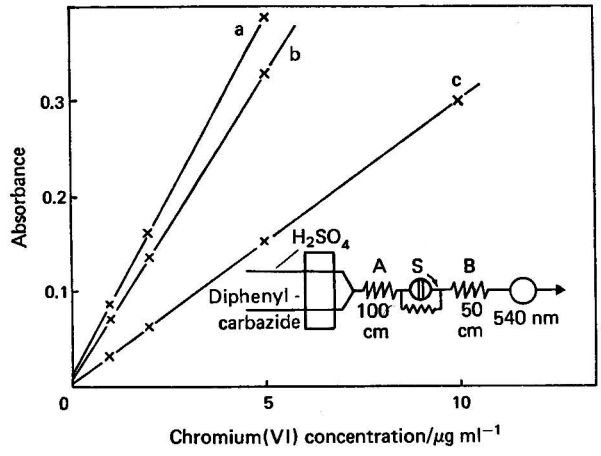

Fig. 1. Flow diagram and calibration graphs. Sulphuric acid and 1,5-diphenylcarbazide are pumped jointly to form a stream into which the sample is injected at S and carried through the mixing coil B towards the photometer flow cell. Sample volume, 30 μl. Calibration graphs are obtained at the following flow-rates of sulphuric acid and 1,5-diphenylcarbazide resulting in the dispersion values (D) given:

	Flow-rate/ml min^{-1}		
Graph	Sulphuric acid	1,5-Diphenylcarbazide	D
a	0.95	0.95	9.7
b	1.4	1.4	11.5
c	3.0	3.0	25.2

Results and Discussion

The manifold adapted for routine analyses is shown in Fig. 1, together with typical calibration graphs. It can be seen that there is a strictly linear relationship between measured absorbance and chromium concentration and that when extrapolated the lines very nearly pass through the origin. It can also be seen that the sensitivity of the method can be altered by altering the reagent flow-rates. A variation in the length of mixing coil B in the range from 30 to 100 cm had only a small effect on the sensitivity, indicating very rapid formation of the coloured complex.

TABLE I

COMPARISON OF CHROMIUM(VI) CONCENTRATIONS IN SOIL EXTRACTS AS DETERMINED BY FLOW INJECTION ANALYSIS (FIA) AND A STANDARD ATOMIC-ABSORPTION SPECTROPHOTOMETRIC (AAS) PROCEDURE

	Chromium(VI)/μg ml^{-1}	
Sample No.	FIA	AAS
1	1.0	1.0
2	2.0	1.8
3	3.0	3.0
4	3.6	3.3
5	8.0	8.2
6	8.5	9.1

In flow injection analysis, sensitivity is closely related to the sample dispersion, D, which is defined as the original analyte concentration divided by the analyte concentration at the peak maximum.[4] In analyses involving only rapid chemical reactions, one should attempt to obtain medium dispersion ($D \approx 10$) in order to give optimum sensitivity. The dispersion was determined by comparison of absorbances at the peak maxima with the absorbances of chromium - reagent mixtures of known composition. The results are presented in Fig. 1.

The rate of analysis (about 70 samples per hour) and the precision are illustrated in Fig. 2. The lower sensitivity ($D = 29.1$) than that demonstrated in Fig. 1 is due to the injection of smaller sample volumes.

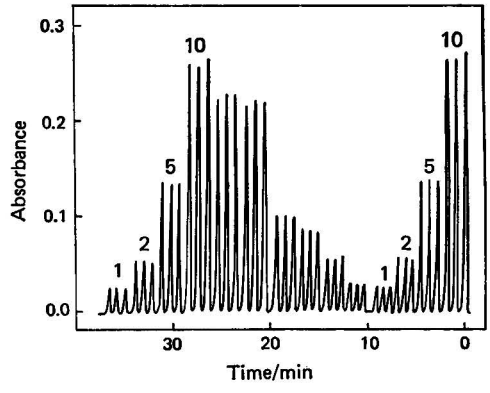

Fig. 2. Routine determination of chromium(VI) in soil extracts using the manifold configuration shown in Fig. 1. Reagent flow-rate, 1.4 ml min^{-1} each for sulphuric acid and 1,5-diphenylcarbazide; sample volume, 20 μl. From right to left: 10, 5, 2 and 1 p.p.m. standard solutions followed by six samples and the standard solutions repeated. Each solution is injected in triplicate. Average standard deviation, 1.26%.

In Table I some results obtained by the proposed method are compared with those obtained by flame atomic-absorption spectrophotometry. The samples contained only chromium in oxidation state VI, and in the latter measurements the flame composition recommended by Thompson[7] was employed.

Conclusion

The determination of chromium(VI) in natural waters and soil extracts can be performed by flow injection analysis in the range 0.1–20 μg ml^{-1} with a standard deviation of about 1.3% and at a rate of at least 70 samples per hour. The results obtained agree satisfactorily with those obtained by a standard flame atomic-absorption spectrophotometric procedure.

We thank Mr. C. E. Foverskov, Chemistry Department A, The Technical University of Denmark, for the design and construction of the photometer employed. We also thank the Danish International Development Agency (DANIDA) for a stipend to M.A.B.R.

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Received *August 13th*, 1979
Accepted *September 10th*, 1979

Communications

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.

Determination of Cadmium in Blood after Destruction of Organic Material by Low-temperature Ashing

Keywords: *Cadmium determination*; *blood analysis*; *atomic-absorption spectroscopy*; *low-temperature ashing*; *carbon tetrafluoride*

Increasing interest in exposure to cadmium, occupationally,[1,2] from cigarette smoking,[3] and its suspected connections with hypertension,[4] is resulting in more requests for blood cadmium determinations. Numerous techniques for this assay have been published.[5–7]

Cernik[5] adapted the Delves' cup technique[8] after pre-treatment of the sample by heating to 425 °C on a hot-plate. This reduces the non-atomic signal when the sample is subsequently analysed by atomic-absorption spectroscopy. With this method, as with proposed methods that use electrothermal atomisation,[6,7] problems occur in obtaining the correct temperature and timing to minimise the non-atomic signal without atomising any of the cadmium prematurely.

Low-temperature ashing utilising plasma-excited oxygen has been described previously[6] as a method for the destruction of organic material prior to atomic-absorption spectroscopy. The long preparation time required to achieve complete ashing has, however, limited the suitability of this technique for routine analysis.

This paper describes a convenient and rapid method for destroying organic material prior to atomic-absorption spectroscopy using low-temperature ashing with the introduction of carbon tetrafluoride into the gas flow. This modification was found to decrease the time necessary for ashing without any presently noticeable disadvantages.

Experimental

A 10-μl aliquot of whole blood was added to 50 μl of de-ionised, distilled water in a small nickel (Delves) cup. This was then dried on a hot-plate at 110 °C for 2 min (the addition of 50 μl of water prevented the formation of a thick band of dried blood around the inner rim and ensured an even layer of blood across the bottom of the cup). The nickel cups, supported in an aluminium rack, were then transferred to a Nanotech Plasmaprep P100. When a vacuum of 1 mmHg or

less had been produced, a gas mixture of oxygen and carbon tetrafluoride was allowed to bleed into the system at a rate of 30 ml min^{-1}. The vacuum was noted still to have a pressure of less than 1 mmHg. The radiofrequency power was then turned on and tuned to give maximum forward power (100 W) and minimum reflected power (less than 5 W). A plasma was seen to be produced.

After low-temperature ashing the samples were analysed for cadmium concentration by atomic-absorption spectroscopy using an Instrumentation Laboratories 353 instrument, with the following settings:

Lamp	Cadmium hollow cathode
Lamp current	3 mA
Wavelength	228.8 nm
Damping	Low
Mode	A
Band pass	1 nm
Flame	Air - acetylene
Sample introduction	Microsampling accessory

For calibration, 10 μl each of aqueous solutions of 5, 10 and 20 ng ml^{-1} of cadmium were added to 10 μl of whole human blood in nickel cups and a further 50 μl of de-ionised, distilled water were added to each cup. Various mixtures of oxygen and carbon tetrafluoride were used to discover the optimum ashing conditions.

Results

Table I shows the absorbances obtained after low-temperature ashing at various time intervals and using various mixtures of oxygen and carbon tetrafluoride. It should be noted that the percentages given make allowance for the comparative densities of the two gases, *e.g.*, a 1 + 1 mixture of the two gases corresponds to relative volumes of 5 + 3, respectively.

TABLE I

ABSORBANCES OBTAINED BY ATOMIC-ABSORPTION SPECTROSCOPY AFTER LOW-TEMPERATURE ASHING USING VARIOUS MIXTURES OF OXYGEN AND CARBON TETRAFLUORIDE

The results are means of 10 analyses. For all mixtures the total gas flow-rate was 30 ml min^{-1}.

Time of ashing/ min	Percentages of oxygen + carbon tetrafluoride							
	100 + 0	84 + 16	64 + 36	50 + 50	38 + 62	27 + 73	17 + 83	0 + 100
5.0	>0.90	>0.90	>0.90	>0.90	>0.90	>0.90	>0.90	>0.90
7.5	>0.90	>0.90	>0.90	0.25	0.83	>0.90	>0.90	>0.90
10.0	>0.90	>0.90	0.75	0.02	0.46	0.50	>0.90	>0.90
12.5	>0.90	>0.90	0.10	0.01	0.05	0.18	0.70	>0.90
15.0	0.66	0.51	0.05	0.01	0.01	0.01	0.50	>0.90

From these results, it was concluded that a 1 + 1 mixture of the two gases provided the optimum atmosphere for low-temperature ashing, and that the suggested time for ashing was 12.5 min.

Table II shows the absorbances obtained in three experiments:

(*a*) aqueous solutions dried at 110 °C without exposure to low-temperature ashing;

(*b*) the same aqueous solutions dried at 110 °C and subjected to low-temperature ashing by the proposed method;

(*c*) aqueous solutions added to whole blood and treated as in (*b*) above.

TABLE II

ABSORBANCES OBTAINED UNDER DIFFERENT CONDITIONS

Series	Nature of sample	Low-temperature ashing	Absorbance	
			Mean of 5 results	Standard deviation
(*a*)	Aqueous blank	No	0.004	0.001
	Aqueous + 5 ng ml^{-1} of Cd	No	0.055	0.002
	Aqueous + 10 ng ml^{-1} of Cd	No	0.100	0.004
	Aqueous + 20 ng ml^{-1} of Cd	No	0.193	0.006
(*b*)	Aqueous blank	Yes	0.010	0.001
	Aqueous + 5 ng ml^{-1} of Cd	Yes	0.060	0.003
	Aqueous + 10 ng ml^{-1} of Cd	Yes	0.105	0.004
	Aqueous + 20 ng ml^{-1} of Cd	Yes	0.198	0.006
(*c*)	Whole blood	Yes	0.012	0.002
	Whole blood + 5 ng ml^{-1} of Cd	Yes	0.052	0.002
	Whole blood + 10 ng ml^{-1} of Cd	Yes	0.098	0.004
	Whole blood + 20 ng ml^{-1} of Cd	Yes	0.188	0.006

The results of experiments (*a*) and (*b*) show little significant difference, indicating no apparent loss of cadmium during the low-temperature ashing procedure.

Although there is a slight depression in the absorbances obtained when aqueous solutions are added to whole blood, this does not appear to invalidate this assay in practice using the method of standard additions. By this method it is possible to achieve determinations of cadmium in blood within the range 0.2–20 ng ml^{-1}. The calculated limit of detection, using blood + 5 ng ml^{-1} of cadmium, is 0.33 ng ml^{-1}.

There does not appear to be any increase in the rate of deterioration of the nickel cups due to exposure to low-temperature ashing.

Discussion

Previous attempts to measure blood cadmium concentrations by the hot-plate method of Cernik[5] have met with only limited success. This was mainly due to the difficulty of eliminating the non-atomic signal at the time of atomisation of cadmium. Conventional low-temperature ashing as a practical alternative was unattractive because of the extended time required to destroy completely the organic matrix. This problem now appears to have been overcome by using a small volume of blood and a 1 + 1 mixture of oxygen and carbon tetrafluoride as the reactive gas in the low-temperature ashing chamber.

It is proposed to submit a full report of this work and further applications of this approach for the pre-treatment of samples for other trace metal determinations by atomic-absorption spectroscopy.

We thank Mr. Andrew Goode of Nanotech (Thin Films) Ltd. for his advice and technical assistance, and for the loan of the Plasmaprep P100.

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Received *December 20th*, 1979

Garry F. Carter
Walter B. Yeoman

Regional Toxicology Laboratory,
Dudley Road Hospital,
P.O. Box 293,
*Birmingham, B*18 7*QH*

Proton-induced Prompt Gamma-ray Spectrometry for Boron Analysis Coupled with the Potassium Bromide Disc Method

Keywords: Proton-induced prompt gamma-ray spectrometry; boron analysis; potassium bromide disc method

Production of gamma-rays following bombardment by low-energy particles has been widely studied in nuclear physics. The reactions are well known: nuclear reactions and inelastic scattering in light nuclides and Coulomb excitation in heavy nuclides. These reactions have been used for many years for analytical purposes,[1–5] and prompt gamma-ray spectrometry has proved to be a very convenient method.

In this paper, we describe the possibility of using prompt gamma-ray spectrometry with protons from a Van de Graaff accelerator and a high-resolution lithium-drifted germanium detector coupled to the method of forming a sample disc mixed with potassium bromide. The technique provides a rapid and non-destructive analysis for boron in solid samples.

Boron is commercially important as a metallurgical additive, is agriculturally significant as a component of fertilisers and is commonly used for home laundry and cleaning purposes.

Our results indicate that this method will be superior as a reliable, rapid and non-destructive determination of boron concentrations as low as 100 p.p.m. in solid samples.

Experimental

The sample preparation is illustrated in Fig. 1. Boron oxide and carbon were mixed with potassium bromide in an agate mortar for 10 min. The mass ratio of potassium bromide to added carbon and boron oxide is about 64. The powder obtained was placed in casting equipment for infrared absorption spectrometry and compressed at 800 kg cm^{-2} to form a disc of diameter

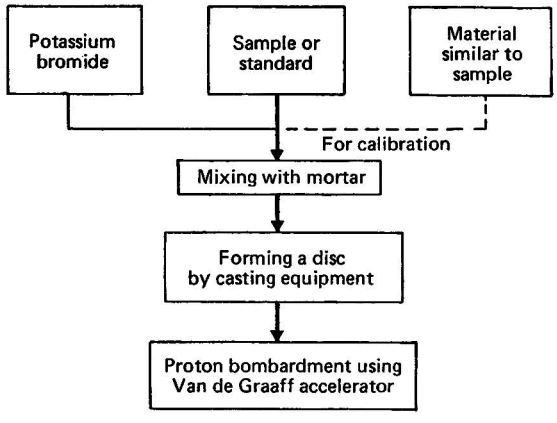

Fig. 1. Schematic diagram of potassium bromide disc method.

20 mm and thickness 0.8 mm. The disc was fixed to the sample holder, which was set in the irradiation chamber. The responses were not affected by the thickness of the discs under the experimental conditions.

The discs were irradiated for 500 s with a proton beam produced by a 2-MV high-voltage Van de Graaff accelerator. The prompt gamma-ray radiation was measured with a 23-cm^3 shielded lithium-drifted germanium detector positioned 5 cm away from the sample holder set in the irradiation chamber. A 4096-channel pulse-height analyser was coupled to the detector.

The analytical capabilities of this method were investigated by measuring the boron content of discs with standard additions.

Results and Discussion

Fig. 2 shows the prompt gamma-ray spectrum of a typical sample disc containing boron oxide, carbon and potassium bromide. It can be observed that irradiation for 500 s yields sufficient

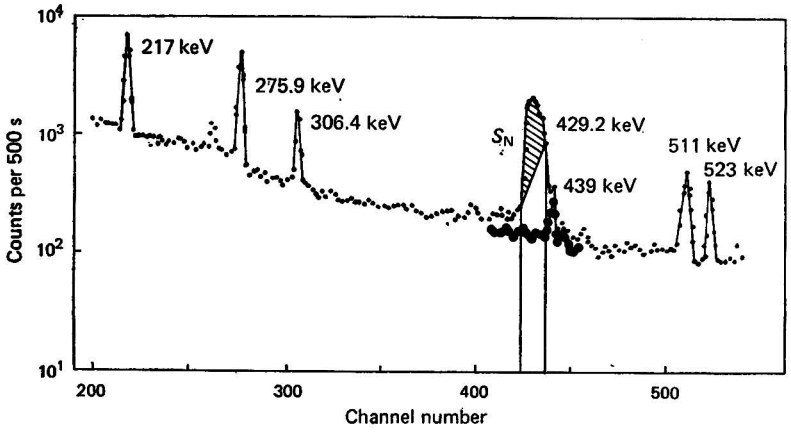

Fig. 2. Prompt gamma-ray spectrum of sample and potassium bromide. Small points, sample disc; and large points, potassium bromide disc.

counts for the statistical analysis to be satisfactory. The gamma-ray peaks were assigned as shown in Table I.

TABLE I

ASSIGNMENT OF THE GAMMA-RAYS DETECTED BY THE PROPOSED METHOD

Prompt gamma-ray energy/keV	Nuclide	Reaction
217	^{79}Br	^{79}Br(p,p′γ)^{79}Br
275.9	^{81}Br	^{81}Br(p,p′γ)^{81}Br
306.4	^{79}Br	^{79}Br(p,p′γ)^{79}Br
429.2	^{7}Be	^{10}B(p,$\alpha\gamma$)^{7}Be
439	^{23}Na	^{23}Na(p,p′γ)^{23}Na
511	—	Annihilation
523	^{79}Br	^{79}Br(p,p′γ)^{79}Br

The 429.2-keV peak was broader than the other peaks due to the Doppler effect of the recoiling nucleus, beryllium-7 and alpha particles.

From the spectrum, the 429.2-keV gamma-ray is well suited to the analysis of boron. To avoid interference from the 439-keV gamma-ray from the sodium-23 present as an impurity in potassium bromide shown in Fig. 2, the peak counts (S_N) were estimated as the net counts between channels 424 and 437.

The S_N estimated as above (per microcoulomb) was calculated from the gamma-ray spectra of five samples, and plotted against the boron concentration of the discs. The results showed good linearity. The data obtained are shown in Table II.

TABLE II

ANALYTICAL DATA OBTAINED BY THE PROPOSED METHOD

			Boron content			
Sample No.	S_N	S_N/μC	Added, p.p.m.	Determined, p.p.m.	Relative standard deviation, %	Relative error, %
1	1933	493	327	333	10.7	+1.8
2	5400	970	590	601	4.0	+1.9
3	4726	1304	805	789	4.5	−2.0
4	6411	1603	972	957	3.0	−1.5
5	9786	2234	1297	1312	2.3	+1.2

From Table II, it is apparent that the deviation from the calibration data is smaller than the relative standard deviation caused by the statistics of the system.

As is clearly demonstrated in Table II, the ^{10}B(p,$\alpha\gamma$)^{7}Be reaction coupled with the potassium

bromide disc method yields a rapid and non-destructive technique for the determination of boron in solid samples.

We are now studying the direct combination of micro high-performance liquid chromatography and Fourier transform infrared absorption spectrometry using potassium bromide discs as the chromatogram's memory material.[6] In the near future, we will demonstrate the metal profiling determined by proton induced X-ray and prompt gamma-ray spectrometry in biological compounds, which are separated and identified by liquid chromatography and infrared absorption spectrometry using potassium bromide discs.

It is proposed to test this method for the determination of sodium in solid samples.

The authors offer their sincere thanks to N. Ohshima and T. Kobayashi for the sample preparation. They are also grateful to the members of the Van de Graaff group of Nagoya University.

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Received *January 21st*, **1980**

Kiyokatsu Jinno

School of Materials Science,
Toyohashi University of Technology,
Toyohashi **440**, *Japan*

Susumu Amemiya
Toshio Katoh

Department of Nuclear Engineering,
Faculty of Engineering,
Nagoya University,
Nagoya **460**, *Japan*

Book Reviews

COULOMETRIC ANALYSIS. Edited by E. PUNGOR and I. BUZÁS. *Conference held at Mátrafüred, Hungary, 17–19 October,* 1978. Pp. x + 302. Budapest: Akadémiai Kiadó. 1979. Price $27.

This book contains the text of the eight plenary and 14 discussion papers presented at this conference. Comments and questions concerning each paper are also included. All the contributors, with the exception of Professors Bishop and Meites, are from Eastern Europe, primarily Hungary, Czechoslovakia and Poland.

Several of the plenary lectures consist of authoritative, although necessarily rather condensed, reviews of important aspects of coulometry. Professor Agasyan (Moscow) considers the wide range of coulometric titrants that may be generated. Professor Galus (Warsaw) reviews the theory and application of chronocoulometry. Professor Hulanicki (Warsaw) reviews titrations in non-aqueous media. Professor Meites (New York) describes the investigation of electrode processes by use of controlled-potential electrolytic techniques and also the application of modern data processing methods.

The other plenary lectures are, in effect, extended research papers in which the authors describe work carried out in their laboratories over several years, which is more extensive than can be covered in a discussion paper. Professor Bishop (Exeter) returns to the topic of "Q-functions" for the theoretical appraisal of coulometric titrations. Dr. Farsang (Budapest) describes an elegant application of controlled potential coulometry to the elucidation of reaction mechanisms. Finally, Dr. Nagy, on behalf of Professor Pungor's group (Budapest), describes methods for carrying out coulometric titrations and standard additions in flow systems.

The topics of the discussion papers range widely, within the over-all limitations of coulometry; they include work on titration end-point indication systems, reaction studies, non-aqueous titrations and pharmaceutical applications.

The book unwittingly demonstrates the relatively slow movement in this field and will probably interest only coulometric enthusiasts. P. L. BAILEY

L'ANALYSE DE L'EAU. EAUX NATURELLES, EAUX RÉSIDUAIRES, EAU DE MER. CHIMIE, PHYSICO-CHIMIE, BACTÉRIOLOGIE, BIOLOGIE. Sixth Edition. By J. RODIER. Pp. xxii + 1136. Paris: Dunod. 1978. Price F520.

A review (by the present reviewer) of the first English translation of the Fifth Edition of Rodier's comprehensive "L'Analyse de l'Eau" appeared in the *Analyst,* 1976, **101**, 405–406. The reviewer's general comments on the scope and role of the book apply equally to the Sixth Edition, and need not be repeated here.

A large proportion of the content of the book remains the same, and the main additions are as follows: (*i*) analytical methods for a number of substances, *e.g.*, chlorinated hydrocarbons of low relative molecular mass, organic mercury, mercaptans, chlorophenols, trihalomethanes, vinyl chloride and total oxygen demand, have been added; (*ii*) analytical methods for a number of substances have been revised or replaced; (*iii*) the chapter on pathogenic micro-organisms has been expanded and a new chapter added on virological analysis. A. L. WILSON

ORGANIC REAGENTS FOR COPPER. By FRANK J. WELCHER and ERWIN BOSCHMANN. Pp. xviii + 614. Huntington, New York: Robert E. Krieger Publishing Company. 1979. Price $34.50.

Analytical chemists have much for which to thank Professor Welcher, particularly his four-volume compendium on organic reagents and his book on EDTA, but perhaps his greatest service has been to produce, with his co-author, this exhaustive compilation of organic reagents for copper. Armed with this book, the research analyst need no longer comb the literature for appropriate reagents for copper, the long-suffering referees of papers describing yet more "new" reagents for copper will have information regarding any novelty (usually there isn't any) at their fingertips and those who still undertake research into the development of organic reagents for copper should be directed to those areas still requiring investigation.

The book deals with organic reagents in classes (chelons, carboxylic acids, oximes, etc.) and describes how each reagent has been used for the determination of copper, using spectrophotometry, gravimetry, titrimetry, polarography and many other instrumental techniques. A reasonable amount of detail is included, with occasional experimental procedures, and applications to specific samples are given. The book has been produced with meticulous care; the presentation is very clear and high-quality paper is used. Very few errors were detected. There are a few minor criticisms. The book is non-critical, so analytical chemists will have to exercise their own judgement about particular reagents and procedures, and as to those areas (if any) in which further research is necessary. Nor is there any discussion of the mechanisms of reaction of the reagents. However, these comments are overwhelmed by the magnitude of the compilation and the excellence of its production.

A. TOWNSHEND

BLOOD DRUGS AND OTHER ANALYTICAL CHALLENGES. Edited by E. REID. *Methodological Surveys in Biochemistry, Volume 7*. Pp. xii + 356. Chichester: Ellis Horwood. Distributed by John Wiley, Chichester. 1978. Price £19.50.

Meetings on bioanalytical topics are held every two years at the University of Surrey and this book is an account of the 1977 forum on the theme of blood drugs and other analytical challenges. As its title suggests, it is mainly concerned with the analysis of drugs in blood and as such will be of direct interest both to clinical biochemists and pharmacologists and to forensic drugs analysts. However, there is much in the book that can be read with profit by other analysts.

There are five sections. The first of these is devoted to the general approach to analytical problems, and includes some stimulating chapters on topics such as the compromise between sensitivity and specificity in analysis, statistics of drug analysis and the role of internal standards, quality control and sources of error and the evaluation of analytical methods. All of these should be required reading for prospective analysts.

There then follow sections on subtle gas chromatographic, mass spectrometric approaches, and high-performance liquid chromatography, thin-layer chromatography and non-chromatographic approaches. The final section is devoted to notes and comments on the preceding sections plus a number of analytical case histories. All of these sections have chapters containing a good deal of practical information and indeed the whole bias of the book is towards active laboratory workers who have to cope with the multitude of problems that continually come their way.

This book can be thoroughly recommended on the basis of its contents. However, there is a criticism. The book has been produced directly from typscript, which no doubt facilitates rapid production and reduces costs. The printed page, however, is very ugly with a right-hand margin as indented as the Norwegian coastline. Words are broken up with such infelicities as ana-lyzed, sulphanila-mide and so on. There is a generous sprinkling of typographical errors, tables and figures are jammed into any available space and the line quality of many of the diagrams is very poor.

R. L. WILLIAMS

COMPUTER ASSISTED STUDIES OF CHEMICAL STRUCTURE AND BIOLOGICAL FUNCTION. By ANDREW J. STUPER, WILLIAM E. BRÜGGER and PETER C. JURS. Pp. xii + 220. New York, Chichester, Brisbane and Toronto: John Wiley. 1979. Price £14.50.

This book deals with recent techniques for investigating the relationship between chemical structure and biological activity and with attempts to predict the activity of new compounds. Although it has a strong bias towards pharmacological problems, and in fact discusses many techniques currently used to develop new drugs, it does in fact touch on a fundamental problem of biochemistry, namely to what extent is the biological effect of a substance rationally predictable?

The authors start with an introductory treatment of the problems involved in studying structure - activity relationships and describe various methods of analysis by which the properties of compounds (so called parameters) can be related to their activity; this includes Hansch analysis, the Free - Wilson additivity model and various quantum mechanical methods. The authors then discuss in detail the principles of pattern recognition and give the theoretical basis of multidimensional scaling, non-linear mapping, parametric classification and clustering theory. The methodologies for processing information on the chemical structures of large numbers of compounds are then described, with an account of the various "descriptors" used to define structural features of molecules. The next chapter is a rather theoretical treatment of pattern recognition

and linear discriminant functions, including an account of the "Linear learning machine." There then follows a description of an interactive modular computer system, called ADAPT (Automatic Data Analysis using Pattern recognition Techniques), which is used in studying the relationship between activity and structure. The application of such computer techniques to the study of the activity of various groups of drugs, including psychotropic agents and barbiturates, is then discussed and the book finishes with a case study on structure - activity relationships in olfactory stimulants.

In general the book is not easy going. The authors have a tendency to use jargon or present arguments in an abstract, theoretical manner, which the lay reader could well find difficult. However, with effort the basic argument can be discerned. One has the impression that the authors are too close to their subject to appreciate the conceptual difficulties of an outsider. Certainly the last chapter is to all intents and purposes a highly specialised paper much more suited to a technical journal than a book. The fact that three authors were involved is all too obvious, even though the actual author of each chapter is not named. Nevertheless, there is much good meat in the soup and the interested reader should find some of the theoretical sections of considerable use.

In summary "Computer Assisted Studies of Chemical Structure and Biological Function" is an interesting, but somewhat uneven book dealing with an area on the interface between biology and chemistry that is currently attracting increasing attention. The application of computer techniques makes this field of study even more promising. DONALD B. ROODYN

ANALYTICAL LASER SPECTROSCOPY. Edited by NICOLÒ OMENETTO. *Chemical Analysis, Volume* 50. Pp. xiv + 550. New York, Chichester, Brisbane and Toronto: John Wiley. 1979. Price £24.50; $45.

This addition to the literature should be welcomed by all those analytical scientists currently using, or considering using, lasers in their work. Although the book is described as a monograph it is really a series of monographs covering such topics as the principles of lasers, the various forms of laser spectroscopy, remote sensing applications, signals and noise and the use of lasers as atomisers. Each author provides extensive references to the literature, however, the book is more than a selection of reviews as a considerable amount of background information is included rendering it a useful reference work.

The opening chapter by Sacchi and Svelto will be particularly welcomed by those seeking an introduction to laser physics. Those familiar with Svelto's book "Principles of Lasers," (Heyden, 1976) will recognise the direct explanatory style of the text aimed at providing the reader with a clear picture of the physical model as an aid to comprehending the mathematical formulation. Perhaps one of the most attractive uses for lasers is in the field of remote sensing. This subject is dealt with extensively in an excellent chapter by R. M. Measures. Considerable background detail is given and the relationships between the various techniques clearly expounded. Signals, noise and measurements are discussed in a chapter by Cova and Longoni. Some of the material in this section might prove rather advanced for those not already possessed of some background in this subject but the authors have attempted the difficult task of describing the means by which signals and noise may be given a mathematical representation and, more importantly, how the information conveyed is modified by the measurement system. As one might expect emphasis is given to those devices such as filters, integrators and correlators of various kinds that are employed to improve signal to noise ratios.

It is not possible to review each chapter individually, but there is much of interest and value in all sections of this book and I would highly recommend it. B. L. SHARP

MOLECULAR SPECTRA AND MOLECULAR STRUCTURE. IV. CONSTANTS OF DIATOMIC MOLECULES. By K. P. HUBER and G. HERZBERG. Pp. xvi + 716. New York, Cincinnati, Atlanta, Dallas, San Francisco, London, Toronto and Melbourne: Van Nostrand Reinhold. 1979. Price £20.65.

Volume I of this series, "Spectra of Diatomic Molecules," published in 1950, contained an appendix giving, for diatomic molecules, the fundamental spectroscopic constants and spectral data available at that time. Since then, the number of molecules for which such information has been measured and/or calculated has dramatically increased, and much more extensive and,

in many instances, more accurate information is available for those molecules included in the earlier work. The present compilation of data has been amassed over the past ten years and the results of this mammoth task are set out clearly in tables covering 680 pp. and over 900 separate diatomic molecules and ions.

The information given includes electronic energies, vibrational and rotational constants and observed transitions, with much additional information on potential energy curves, spin - coupling constants, hyperfine structure, dipole moments, oscillator strengths, etc., given in the form of footnotes. With the large number of species considered, the authors obviously could not ensure equal status in terms of literature coverage for each molecule and have sensibly given in each table the date of their final revision. This varies between late 1974 and 1977. An appendix includes relevant available publications up to 1977, and occasionally 1978, that could not be included in the original tables. This volume represents essential reference material for all chemists, physicists and astronomers interested in the measurement, interpretation or calculation of the spectra of diatomic molecules. The authors have provided a remarkable service for their colleagues and the availability of this material in this clear and comprehensive format will surely stimulate and assist current research in this area. J. M. OTTAWAY

METAL IONS IN SOLUTION. By JOHN BURGESS. *Ellis Horwood Series in Chemical Science.* Pp. 484. Chichester: Ellis Horwood. Distributed by John Wiley, Chichester. 1978. Price £25; $55 (hard back), £8.50; $20.75 (paper back).

This is an excellent and comprehensive review of the nature, physical properties and reactions of metal ions in solution. The author has aimed to provide background information for use by the inorganic and physical chemists studying reactions of metal ions, but many aspects of his book will be of value to analytical chemists. His treatment is concerned with solvated metal species alone and discussion or data on metal complexes and even metal oxo anions are excluded. The literature of even this restricted area is vast and widespread, and a comprehensive treatise of this type will be of considerable importance and value to many research workers.

The first half of the book is concerned with metal-ion solvation and includes chapters on techniques used to obtain information on solvation numbers, a review and comparison of data obtained from the various sources and the effect of mixed solvents in producing mixed solvates and selective solvation. The importance of NMR in the study of metal-ion solvation is given full recognition in a separate chapter. The second half of the book covers redox potentials, hydrolysis, polymerisation and the kinetics and mechanisms of simple metal ions undergoing solvent exchange, complex formation and redox reactions.

In all instances, the style is clear and concise, numerous references are given and the coverage is up to the end of 1975, with some references included from 1976. There are few typographical errors but MMR is an interesting misnomer in the Table of Contents. Dr. Burgess has provided a valuable service to his colleagues and it is predictable that he will be encouraged to produce a second edition in a few years' time. J. M. OTTAWAY

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part VII. Fingerprinting of Essential Oils by Temperature-programmed Gas Chromatography Using a Carbowax 20M Stationary Phase

Report prepared by the Essential Oils Sub-Committee

Problems of obtaining reproducible results in the "fingerprinting" of essential oils by temperature-programmed gas - liquid chromatography have been examined; in particular an absorption-coating technique for the column packing is described. The requirements for standardisation of column efficiency, selectivity and reproducibility have been worked out using as a basis the method of column characterisation described by van den Dool. This column characterisation is referred to as the "*g*-pack" value and is determined from experimentally determined relative retention indices for a set of test substances using the even carbon numbered n-alkanes from C_8 to C_{24}.

A collaborative study with Carbowax 20M as stationary phase, and a specification of *g*-pack values for the column packing, has resulted in the production of a method that yields reproducible relative retention indices for the test substances limonene, linalol, linalyl acetate, acetophenone, naphthalene and cinnamyl alcohol, and has been applied with satisfactory results to oils of bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg. A recommended method is given for the reproducible temperature-programmed gas - liquid chromatographic fingerprinting of essential oils when Carbowax 20M is used as a stationary phase.

Keywords: *Essential oils analysis*; *temperature-programmed gas - liquid chromatography*; *Carbowax 20M stationary phase*

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Analyst, 1980, **105**, 262–273.

Determination of Trace Concentrations of Selenium in Soils and Sediments by the Introduction of Hydrogen Selenide into an Inductively Coupled Plasma Source for Emission Spectrometry

Short Paper

Keywords: *Selenium determination*; *soils and sediments*; *hydride generation*; *inductively coupled plasma emission spectrometry*

B. PAHLAVANPOUR, J. H. PULLEN and M. THOMPSON

Applied Geochemistry Research Group, Department of Geology, Imperial College, London, SW7 2BP.

Analyst, 1980, **105**, 274–278.

Determination of Rhenium in Molybdenum Concentrates

Short Paper

Keywords: *Rhenium determination*; *molybdenum concentrates*; *solvent extraction - spectrophotometry*

BRET W. BUDESINSKY

Phelps Dodge Corporation, Morenci, Ariz. 85540, USA.

Analyst, 1980, **105**, 278–282.

Fungicide Residues. Part VII. Determination of Residues of Fentin in Vegetables and Cocoa Products by Spectrofluorimetry

Short Paper

Keywords: *Fentin residues*; *spectrofluorimetry*; *vegetables*; *cocoa*

P. G. BAKER, D. S. FARRINGTON and R. A. HOODLESS
Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1980, **105**, 282–285.

Spectrophotometric Analysis of Interferences due to Polyols in the Assay of Protein by Lowry's Method

Short Paper

Keywords: *Protein assay*; *spectrophotometric analysis*; *polyol - copper complexes*; *glycerol, erythritol, ribitol and* D(—)*-sorbitol*

P. NINFALI, M. MAGNANI, V. STOCCHI and M. DACHÀ
Istituto di Chimica Biologica, Università degli Studi di Urbino, Via Saffi 2, Urbino, Italy.

Analyst, 1980, **105**, 285–288.

Determination of Halothane in Operating Theatre Air by Using a Passive Organic Vapour Dosimeter

Short Paper

Keywords: *Operating theatre pollution*; *halothane*; *personal pollution dosimeter*; *gas - liquid chromatography*

M. M. HALLIDAY and J. ANDERSON
University Department of Anaesthesia, Royal Infirmary, Glasgow, G4 0SF.

Analyst, 1980, **105**, 289–292.

Rapid Determination of Chromium(VI) by Flow Injection Analysis

Short Paper

Keywords: *Chromium determination*; *water analysis*; *soil analysis*; *flow injection analysis*

S. STORGAARD JØRGENSEN and MARISA A. B. REGITANO
Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Copenhagen V, Denmark.

Analyst, 1980, **105**, 292–295.

Determination of Cadmium in Blood after Destruction of Organic Material by Low-temperature Ashing

Communication

Keywords: *Cadmium determination*; *blood analysis*; *atomic-absorption spectroscopy*; *low-temperature ashing*; *carbon tetrafluoride*

GARRY F. CARTER and WALTER B. YEOMAN

Regional Toxicology Laboratory, Dudley Road Hospital, P.O. Box 293, Birmingham, B18 7QH.

Analyst, 1980, **105**, 295–297.

Proton-induced Prompt Gamma-ray Spectrometry for Boron Analysis Coupled with the Potassium Bromide Disc Method

Communication

Keywords: *Proton-induced prompt gamma-ray spectrometry*; *boron analysis*; *potassium bromide disc method*

KIYOKATSU JINNO

School of Materials Science, Toyohashi University of Technology, Toyohashi 440, Japan.

SUSUMU AMEMIYA and TOSHIO KATOH

Department of Nuclear Engineering, Faculty of Engineering, Nagoya University, Nagoya 460, Japan.

Analyst, 1980, **105**, 298-300.

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ANALAO 105 (1248) 193–304 (1980) March 1980
ISSN 0003–2654

THE ANALYST

THE ANALYTICAL JOURNAL OF THE CHEMICAL SOCIETY

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