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Summaries of Papers in this Issue

Nitrogen Heterocycle and Polynuclear Hydrocarbon Fluorescence and Adsorption Effects in the Presence of Silica Gel. Applications in High-pressure Liquid and Microcolumn Chromatography

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Microcolumns (90 × 1 mm) exhibit a ten-fold reduction in theoretical plate height compared with conventional high-pressure columns of the same adsorbent. Separations in such columns mounted on a microscope stage can be monitored by spectrofluorimetric microscopy of a silica gel particle located at the end of the column.

The techniques described are applied to heterocycles in creosotes, polynuclear hydrocarbons in gasolines and sump oil, and wood splinters, soot particles and trace amounts of pitch.

J. B. F. LLOYD

West Midland Forensic Science Laboratory, Gooch Street North, Birmingham, B5 6QQ.

Analyst, 1975, **100**, 529–539.

A Rapid Gas-chromatographic Method for the Determination of Acetaldehyde in the Vapour Phase of Cigarette Smoke

A simple and rapid procedure is described for the gas-chromatographic determination of acetaldehyde in the vapour phase of cigarette smoke. The acetaldehyde is efficiently extracted into cold water in Drechsel bottles and is determined by gas chromatography of an aliquot of the solution. Results are comparable with those obtained by more complicated techniques involving derivative formation or the direct injection of smoke samples into high-resolution columns. A relative standard deviation of ±3·4 per cent. was obtained in the absence of an internal standard. The method is particularly convenient for evaluating the effectiveness of cigarette filters in retaining acetaldehyde.

D. J. EVANS and R. J. MAYFIELD

C.S.I.R.O., Division of Textile Industry, P.O. Box 21, Belmont, Victoria 3216, Australia.

Analyst, 1975, **100**, 540–543.

Improvements in the Atomic-fluorescence Determination of Mercury by the Cold-vapour Technique

Relatively simple modifications to a cold-vapour, mercury-fluorescence detector have resulted in a large increase in sensitivity and have decreased the time required for each measurement. A 2σ detection limit of 0·02 ng was achieved.

K. C. THOMPSON and R. G. GODDEN

Shandon Southern Instruments Ltd., Frimley Road, Camberley, Surrey, GU16 5ET.

Analyst, 1975, **100**, 544–548.

Suppression of Iron(III) Interference in the Determination of Iron(II) in Water by the 1,10-Phenanthroline Method

A method for the determination of iron(II) in water in the presence of iron(III) with 1,10-phenanthroline is described, in which the interfering effect of iron(III) ions is suppressed by masking with complexones. In the absence of a chelating agent for iron(III) the colour intensity of samples being analysed is unstable, owing to redox processes induced by the effect of 1,10-phenanthroline on the iron(II) - iron(III) system, causing reduction of iron(III) and formation of the corresponding coloured iron(II) chelate compound in a stoicheiometrically proportional concentration. The advantages of complexones, especially nitrilotriacetic acid, over other chelating agents are discussed.

HUBERT FADRUS and JOSEF MALÝ

Water Management Board, Vodohospodářská Správa, Brno, Czechoslovakia.

Analyst, 1975, **100**, 549–554.

A Composite Scheme for the Analysis of Steels by Atomic-absorption Spectroscopy Using the Air - Acetylene Flame

A composite scheme is described for the determination of chromium, molybdenum, manganese, nickel and copper in all types of steel. The scheme is based on a single sample solution containing 1 g of steel per 100 ml. Recent developments in technique have enabled all these elements to be determined with the air - acetylene flame and the scheme can be extended as required to include other elements such as lead and cobalt.

W. R. NALL, D. BRUMHEAD and R. WHITHAM

Ministry of Defence, Materials Quality Assurance Directorate, Bragg Laboratory, Janson Street, Sheffield, S9 2LJ.

Analyst, 1975, **100**, 555–562.

A Rapid and Sensitive Spectrophotometric Procedure for the Determination of Diphenhydramine and Related Ethers

An oxidative procedure for the determination of diphenhydramine and related ethers is described. Its high sensitivity is a consequence of the oxidation of the drug to new products that exhibit high absorption in the ultraviolet region of the spectrum. The developed method is discussed with respect to precision, specificity, sensitivity and applicability to drug determination in biological samples.

B. CADDY, F. FISH and J. TRANTER

Division of Pharmacognosy and Forensic Science, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW.

Analyst, 1975, **100**, 563–566.

Simple Determination of the Coccidiostat Robenidine in Poultry Feed

1,3-Bis(4-chlorobenzylideneamino)guanidinium chloride (robenidine) is a new preventive coccidiostat used in poultry feed. A fast, accurate method of determination is proposed, which consists in extraction of the robenidine from the feed, purification of the extract by means of thin-layer chromatography and development of a yellow coloration by addition of sodium hydroxide to a solution of the robenidine in dimethylformamide. The absorbance is measured at 464 nm and is proportional to the concentration of robenidine between 0 and 3 μg ml^{-1}. No interference is observed during its determination in fatty or pigmented feeds.

G. F. BORIES

I.N.R.A., Laboratoire de Recherches sur les Additifs Alimentaires, 180 chemin de Tournefeuille, 31300 Toulouse, France.

Analyst, 1975, **100**, 567–569.

The Analyst

Nitrogen Heterocycle and Polynuclear Hydrocarbon Fluorescence and Adsorption Effects in the Presence of Silica Gel. Applications in High-pressure Liquid and Microcolumn Chromatography

J. B. F. Lloyd

West Midland Forensic Science Laboratory, Gooch Street North, Birmingham, B5 6QQ

A flow-through cell packed with silica gel is used for the spectrofluorimetric examination of adsorbed states and as a detector in high-pressure liquid chromatography. The intensified fluorescence of adsorbed benzo homologues of quinoline and acridine is found to be caused by protonation of the electronically excited states by the gel. Sub-nanogram amounts of such compounds can therefore be analysed by high-pressure liquid chromatography.

In the presence of silica gel the fluorescence of polynuclear hydrocarbons is enhanced proportionally to their radiative lifetimes. The effect is disfavoured by adsorbate formation, and can therefore be employed in both adsorption and reversed-phase liquid chromatography in order to improve selectivity and sensitivity towards readily quenched fluorescences.

Microcolumns (90 × 1 mm) exhibit a ten-fold reduction in theoretical plate height compared with conventional high-pressure columns of the same adsorbent. Separations in such columns mounted on a microscope stage can be monitored by spectrofluorimetric microscopy of a silica gel particle located at the end of the column.

The techniques described are applied to heterocycles in creosotes, polynuclear hydrocarbons in gasolines and sump oil, and wood splinters, soot particles and trace amounts of pitch.

Transfer from solution to an adsorbed state may extensively modify a fluorescence emission. The effect is, for instance, sometimes noted when fluorescent compounds are analysed by thin-layer chromatography.[1] Nicholls and Leermakers[2] have recently reviewed the subject, mainly with reference to the nature of adsorbate - adsorbent interactions.

Adsorbate fluorescence can readily be applied to enhance the sensitivity and selectivity of fluorescence detection in high-pressure liquid chromatography, and enables microcolumn separations to be monitored by spectrofluorimetric microscopy. Because the existence of previously undetected interactions is indicated, some of the results are significant, forensically as well as analytically: the detection of certain types of contact trace depends on the formation of fluorescent adsorbates.[3]

Experimental

Materials

Solvents used are commercial spectrophotometric grades, exhibiting no detectable extraneous fluorescence emission or ultraviolet absorption. Diethyl ether and ethyl acetate for chromatography are one third saturated with water.[4] Compounds, not below analytical-reagent grade initially, are purified by thin-layer or liquid column chromatography. The various stationary phases are: neutral aluminium oxide containing 4·5 per cent. *m/m* of water, 18–30-μm particle size (Woelm); Corasil C_{18}, 37–50 μm (Waters Associates Inc.); Porasil C, 37–75 μm (Waters Associates Inc.); silica gel CT, 11·5 μm (Reeve Angel Scientific Ltd.); and thin-layer chromatographic sheets of silica gel Sil G (Machery-Nagel and Co.). For the last named, scanning electron microscopy gives a mean particle diameter of 21 μm.

High-pressure Liquid Chromatography

Columns are dry-packed in stainless-steel tubes (2·15 mm i.d., 0·5–1·15 m in length) connected through a union T-coupling and a length of steel tubing (0·76 mm i.d., 3 m in length), which serves as a flow pulsation damper, to either a Micropump, Series 2 (F. A. Hughes Ltd.), or a Metripump, Type HM (Metering Pumps Ltd.). Samples are injected through the T-coupling. The column outlet is connected by PTFE tubing (0·25 mm i.d., made by stretching 0·5 mm diameter tubing) to a flow-through cell (Spectrosil tube of 1 mm i.d.) mounted in the microcell attachment of a Baird Atomic SF 100E spectrofluorimeter. Similar apparatus has been described previously.[5]

Solvents are de-aerated by a stream of "white spot" nitrogen (British Oxygen Ltd.). In order to eliminate oxygen from columns it is necessary to pump with de-aerated solvent for at least 2·5 h. This operation is complete when the fluorimetric response to pyrene peaks no longer increases.

Inlet joints of packed detectors are subject to pressures of the order of 100 lb in^{-2}; the joints are made as follows [Fig. 1(*a*)]. The wall of a flow-through cell (length 60 mm, o.d. 3·5 mm and i.d. 1 mm) is slightly constricted by heating it at a point 7 mm from one end; the constriction in internal diameter is approximately 0·1 mm. Through the other end of the cell is threaded a stretched piece of PTFE tubing, initially of 1 mm o.d., into the unstretched end of which has been pushed a segment (5 mm) of a size 15 hypodermic needle. This reinforced part of the tubing is pulled down hard into the constriction. No joint made in this way has failed with use over a period of 2 years with packings down to 10 μm in particle size and with solvent flow-rates of up to 2 ml min^{-1}. The outlet joint is made in a similar fashion, except that the cell walls are unconstricted. The cells are packed with dry adsorbent, which is retained between plugs of cotton- or glass-fibre held in place between the inlet and outlet fittings.

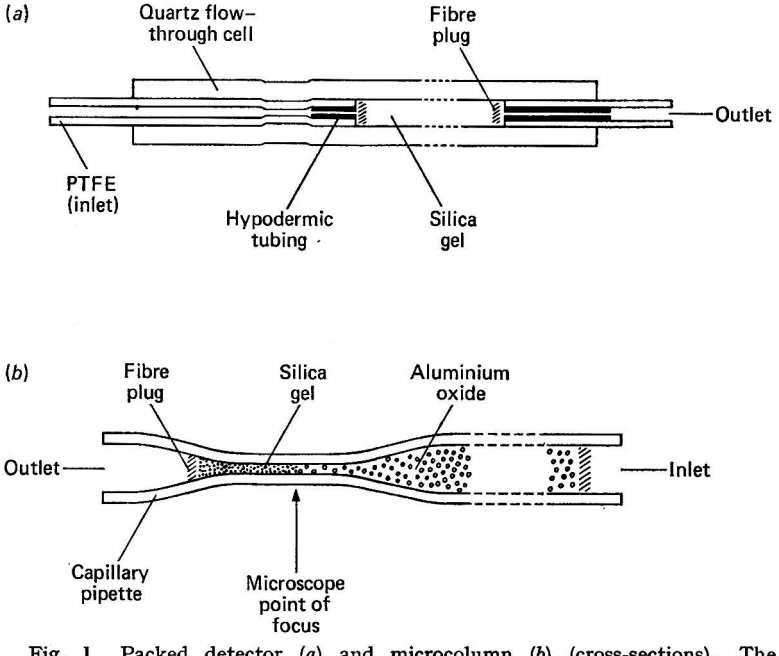

Fig. 1. Packed detector (*a*) and microcolumn (*b*) (cross-sections). The dimensions are given in the text.

Sample solutions are forced through the detector from a hypodermic syringe until the emitted fluorescence is constant. For chromatography, the detector is purged with solvent until a stable base-line is obtained. Detector lifetime is determined by the accumulation of strongly adsorbed fluorescent material, *i.e.*, by the nature of the samples and by photodecomposition products. Typically the packing is replaced after 1 week's use.

Microcolumn Liquid Chromatography

Columns [Fig. 1(*b*)] are made from Drummond Microcap pipettes (100 μl, length 116 mm and i.d. 1·05 mm). At a distance of 10 mm from the outlet the pipette is heated and pulled down to form a constriction that is 10 mm in length and of 0·2 mm i.d. Into the outlet end of the constriction is introduced a plug of cotton-fibre, and, from the column inlet, sufficient fragments of silica gel to occupy the lower half of the constriction. The remainder of the constriction and the column, to within 2 mm of the top, are filled with dry adsorbent. Typically, when the adsorbent is aluminium oxide, 85 mg are used.

The column is mounted on a microscopy stage illuminated through a dark-field condenser by a Leitz 200 W mercury lamp, from which the 366-nm emission is isolated by UG 1 filters. Optical contact between the restricted part of the column and the surface of the condenser is established by use of immersion oil. The microscope is focused on the junction of the silica gel and the column packing at a magnification such that the fluorescence emitted from a single particle of gel can be collected with a Microscope Spectrum Analyser (Farrand Optical Co. Inc.) mounted on the eyepiece and connected to a strip-chart recorder. Monitoring wavelengths are indicated in the text and in the figure captions.

Solvent is forced through the column either by an Agla micrometer syringe driven with a synchronous motor, or by compressed nitrogen. In the latter instance, which is the more suitable for prolonged running times, solvent is fed from a spiral of PTFE tubing connected to the column inlet. Flow-rates in the region of 20 μl min^{-1} require a pressure of 30 lb in^{-2}.

Liquid samples, *e.g.*, 10 nl, are transferred by capillary micropipette to the inlet plug of the disconnected column. Solid samples are pushed into the top of the capillary of a micropipette where they are extracted with 1–2 μl of solvent, usually ethyl acetate. The extract is discharged into the inlet plug where the solvent is evaporated in a stream of air from the pipette. All of these operations are conducted under a dissecting microscope.

Fluorescence Spectra

The spectra are recorded with a Baird Atomic SF 100E spectrofluorimeter at a half-band width usually of 5 nm; they are uncorrected and subject to variation when other instruments are used.

Results and Discussion

Heterocyclic Compounds

The weak fluorescence of acridine in neutral solution is strongly intensified by the addition of acids.[6,7] Thus, a dichloromethane solution [Fig. 2(*a*)] yields weak excitation and emission spectra (characteristic of the neutral molecule) that are transformed into the intensified (×500) spectra of the protonated form on the addition of trifluoroacetic acid.

When a neutral dichloromethane solution of acridine is injected into a flow-through cell packed with silica gel (thin-layer chromatographic material so as to permit the subsequent correlation with R_F data) the fluorescence is again intensified, but the excitation spectrum remains essentially that of the neutral molecule, whereas the emission is evidently from the protonated form [Fig. 2 (*a*)]. Hence, the intensification is due to protonation by the acidic gel surface of electronically excited acridine molecules. The effect is not observed on basic adsorbents such as aluminium oxide.

On the basis of fluorescence emission spectra, Aleksandrova *et al.*[8] reported that on hydrated silica vaporised acridine is adsorbed in the protonated state. However, in the absence of excitation spectra their conclusion is also explicable in the foregoing terms.

Benzo[*f*]quinoline behaves in the same way as acridine [Fig. 2(*b*)]. Excited states of benzo[*h*]quinoline and benz[*c*]acridine are protonated to a reduced extent by the gel so that emissions of both protonated and unprotonated forms are present in the spectra. Quinoline is protonated in the ground state. Carbazoles (carbazole, 13*H*-dibenzo[*a*,*i*]carbazole and 7*H*-dibenzo[*b*,*g*]carbazole), which are non-basic, exhibit unmodified spectra in the adsorbed state.

From the R_F value (0·037) of acridine in dichloromethane on the silica gel used for the cell packing, and from the ratios of gel to solvent in the cell and in the thin-layer chromatogram, it follows that adsorption increases the concentration of acridine in the irradiated part of the cell by a factor of 9·7 relative to an unpacked cell when both are equilibrated with the same solution. After making allowance for this effect, the observed increase relative to the

neutral and acidified solutions in unpacked detectors is $\times 590$ and $\times 1{\cdot}2$, respectively. Hence, the production of excited acridinium ions by the gel surface or in acidic solution occurs to comparable extents.

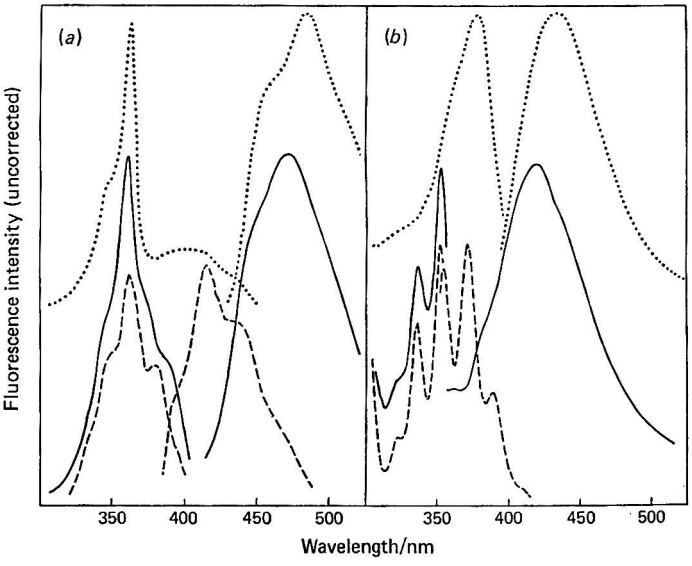

Fig. 2. Fluorescence excitation and emission spectra of (*a*) acridine and (*b*) benzo[*f*]quinoline, at 0·1 μg ml^{-1} concentration in dichloromethane. Broken lines are spectra from neutral solutions, dotted lines are from solutions acidified with 10 μl ml^{-1} of trifluoroacetic acid and full lines are from neutral solutions injected into silica gel contained in a flow-through cell.

Solvent effects, which do not modify the spectra of the adsorbed state qualitatively, show that adsorption occurs mostly prior to excitation. Thus, the fluorescence intensities from a packed cell, equilibrated with constant amounts of acridine in various solvents, increase according to R_M values[9] from thin-layer chromatograms [$R_M = \log (R_F^{-1} - 1)$] and hence according to logarithms of the corresponding distribution coefficient of the ground state. Some results are shown in Fig. 3. (Two, off-scale, results for n-hexane and carbon tetrachloride are excluded because, as follows from the corresponding R_F value of 0, equilibrium could not be established.) A linear least-squares analysis of the results obtained with n-hexane and ethyl acetate mixtures yields a gradient not significantly different from unity (5 per cent. level). However, increasingly less basic solvents lie increasingly above this line (no correction for the absorption of excitation by nitromethane has been made, hence the result underestimates the high fluorescence yield in the presence of this solvent) and proton-donating solvents lie below, presumably because of the varying extent to which the solvents compete with the gel surface for electronically excited acridine molecules.

Clearly, proton-donating solvents should not be used for chromatography if sensitivity is to be at a maximum. If broadened peaks are to be avoided: distribution coefficients of eluted compounds on cell packings should not exceed the corresponding values on column packings; other chromatographic parameters that characterise the cell should not be inferior to those of the column; and the length of the cell viewed by the spectrophotometer should be small relative to the column length occupied by separated compounds. Ideally, therefore, the detector should simply be an extension of the column.

These conditions are not difficult to meet in practice, even when cell and column packings differ. Thus, coupled to a 0·5-m column of aluminium oxide eluted with a dichloromethane and acetonitrile mixture (95 + 5) at the rate of 1 ml min^{-1}, a silica gel packed detector gives peak widths corresponding to a theoretical plate height of 1·28 mm (standard error, 0·067), which is not significantly different (5 per cent. level) from the value of 1·16 mm (0·037) obtained for anthracene and benz[*a*]anthracene on the same type of column in the absence

of cell packing. The comparison cannot be made with acridine detected in the unadsorbed state, because in the absence of cell packing no response is obtained.

Peak heights increase slightly less than linearly with chromatographed amounts in the range 0·6–1280 ng, but a graph of the data in double logarithmic co-ordinates is linear, and yields a linear least-squares analysis of $y = 0{\cdot}0417 + 0{\cdot}937x$, $s_{y/x} = 0{\cdot}0436$, $s_a = 0{\cdot}0258$ and $s_b = 0{\cdot}0132$, where y and x are logarithms of fluorescence intensities and amounts of acridine, s values are standard deviations, and a and b are the intercept and slope, respectively.

An application of the technique to the differentiation of microlitre amounts of two creosotes is shown in Fig. 4. With excitation and emission wavelengths of 358 and 475 nm (optimum for adsorbed acridine), both exhibit an initial peak mainly of unretained polynuclear hydrocarbons followed by heterocyclic components that differ in relative intensities, and in the presence of an additional, partly resolved, component in sample A. Also in Fig. 4 are shown chromatograms of (C) one of the samples monitored in the absence of cell packing, when only the polynuclear hydrocarbon peak can be seen; and (D) of 0·6 ng of acridine, which represents the practicable limit of detection under these particular conditions.

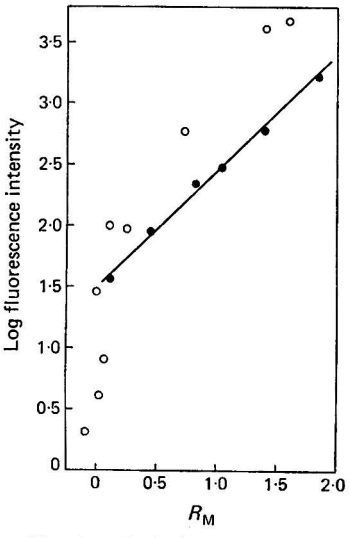

Fig. 3. Variation with solvent of fluorescence emitted at 475 nm (excitation, 358 nm) from a silica gel packed detector equilibrated with solutions of 0·1 μg ml^{-1} of acridine in various solvents. R_M values are from thin-layer chromatograms in each of the solvents on the same gel. Closed circles and the corresponding least-squares line represent mixtures of ethyl acetate and n-hexane (containing from 100 to 2 per cent. V/V of ethyl acetate). Open circles in order of increasing fluorescence represent 20, 10 and 5 per cent. V/V of methanol in ethyl acetate, acetone, diethyl ether, acetonitrile, nitromethane, dichloromethane and benzene.

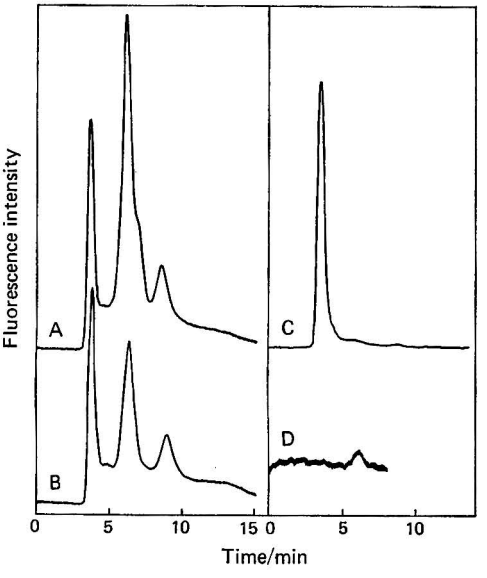

Fig. 4. Liquid chromatograms on 0·5 m × 2·15 mm columns of 18–30-μm aluminium oxide eluted with dichloromethane - acetonitrile (95 + 5) mixture at the rate of 1 ml min^{-1}. Fluorescence detection is at 358 nm (excitation) and 475 nm (emission). Samples A and B are of different creosotes (1 μl injected) monitored with a silica gel packed detector; C is sample A monitored with an unpacked detector; and D is 0·6 ng of acridine monitored with a packed detector.

Polynuclear Hydrocarbons

The enhanced fluorescence yields of heterocycles adsorbed on silica gel in the presence of generally strongly quenching solvents, *e.g.*, carbon tetrachloride and nitromethane, suggests that the technique might be used to potentiate fluorescence emission from readily quenched polynuclear hydrocarbons.

In the presence of silica gel the excitation and emission spectra of these compounds are not usually varied relative to their dissolved states. Pyrene is exceptional. When a cyclohexane solution is injected into a cell packed with silica gel, the relative intensities of the vibronic transitions are considerably modified (Fig. 5) to yield an emission spectrum that is closely comparable with spectra in polar solvents such as methanol. Excitation spectra are not affected. Leermakers and co-workers[2,10] reported that addition of silica gel to cyclohexane solutions leaves the spectrum of pyrene monomer unaltered. Under their conditions, however, vibrational fine structure is unresolved. (Effects involving excimers reported by Leermakers and co-workers are not observed at the low concentrations used here.)

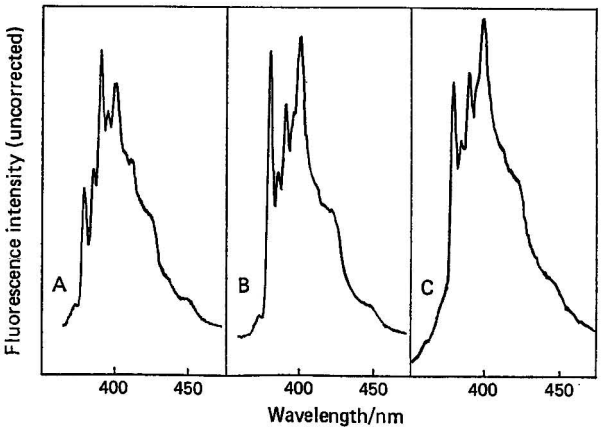

Fig. 5. Fluorescence emission spectra of pyrene (1 μg ml^{-1}) in de-aerated cyclohexane (A), methanol (B) and cyclohexane (C) injected into a silica gel packed detector.

Relative changes in fluorescence yields caused by silica gel can be determined by liquid chromatography. Anthracene, because of its relatively short fluorescence lifetime, and consequently reduced sensitivity to quenching under usual circumstances, is used as an internal standard. Anthracene and each hydrocarbon in solution are separated [using 1120 $\times$ 2·15 mm columns of 24-μm aluminium oxide, in a mixture of n-hexane and diethyl ether (100 + 6), at the rate of 0·6 ml min^{-1}] with fluorescence detection at wavelengths set in regions of spectral overlap of anthracene and the compound in question.

In aerated solvents, *i.e.*, under conditions of oxygen quenching, silica gel packed detectors give varyingly increased fluorescence yields relative to unpacked detectors. In the series fluorene, biphenyl, fluoranthene, benz[*a*]anthracene, chrysene, naphthalene, triphenylene, and pyrene, chromatographic peak heights relative to anthracene vary according to radiative lifetimes. Thus, peak height ratios increase from 1·19 to 4·16 as radiative lifetimes (from Birks[11]) increase from 15 to 690 ns to give a correlation coefficient of 0·951 (significance level, 0·1 per cent.).

Theoretical plate heights are unaffected by the detector packing. Ten peaks from five chromatograms of an anthracene and benz[*a*]anthracene mixture monitored with a packed detector give a value of 1·12 mm (standard error, 0·042), which is not significantly different (0·1 per cent. level) from the value previously quoted for an unpacked detector.

When the solvent is de-aerated, the response of an unpacked detector is similar to that of a packed detector in the presence of aerated solvent. Relative to these conditions, the response is further increased when a packed detector functions in de-aerated solvent. Examples are given below.

The extent to which the above hydrocarbons are adsorbed on silica gel varies only slightly between them [thin-layer chromatographic mobilities relative to anthracene (1·0) vary from 0·79 to 1·13] and shows no correlation with fluorescence enhancement. Fluorescence yields are reduced if the thin-layer chromatographic gel with which the detector is packed is replaced by Porasil C or by the micro-particulate silica gel CT, 11·5 μm. When a packed detector is de-activated by injection of water into the eluate stream, or similarly with bis(trimethylsilyl)-

acetamide, the fluorescence response is increased (as much as ×1·8). Hence, adsorption reduces fluorescence yields of polynuclear hydrocarbons, presumably by increasing quenching encounters between adsorbates, or by promoting internal conversion or inter-system crossing processes. The increased fluorescence of non-adsorbed molecules is attributed to a reduction in diffusion-controlled rates of quenching encounters in the fluid occupying the pores and interstices of the gel.[12] From this unexpected result it follows that packed detectors can enhance the specificity and sensitivity of reversed-phase (aqueous) as well as adsorption chromatographic systems.

Examples of the modified sensitivities towards polynuclear hydrocarbons induced by silica gel are shown in Figs. 6 and 7. Fig. 6 shows a 98-octane gasoline chromatographed in a mixture of n-hexane and diethyl ether on aluminium oxide. Monitored at 325 nm (excitation) and 390 nm (emission), intensities of peaks in the naphthalene and pyrene positions (the first and third peaks) are increased relative to that of anthracene (second peak) by factors of 2·3 and 8·0 when conditions are varied from aerated solvent and unpacked detector to de-aerated solvent and packed detector. De-aerated unpacked and aerated packed detectors give intermediate results. At 340 and 410 nm, at which wavelengths naphthalene is not detected, the same variation increases the pyrene peak intensity by a factor of 11·6. The variation in the pyrene result with monitoring wavelengths is attributed to the presence of other, unresolved, compounds at the anthracene and pyrene positions, and to the changes caused in the pyrene spectrum by silica gel. (Peaks due to pure pyrene relative to pure anthracene exhibit a similar dependence on wavelength, but the over-all increases in intensities are in the region of 20-fold.) The addition of up to 0·6 μg of pyrene to the gasoline injected yields peak intensities that increase linearly by factors of up to 7·6.

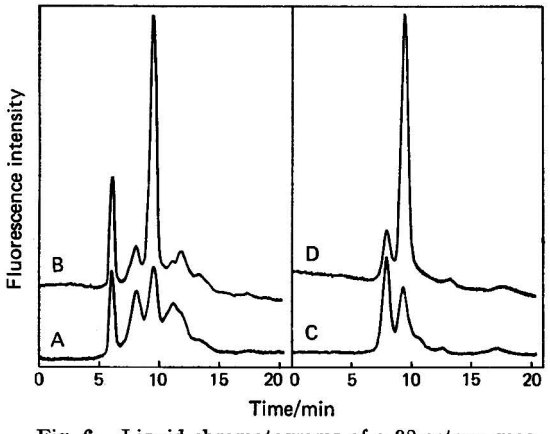

Fig. 6. Liquid chromatograms of a 98-octane gasoline (5-μl samples) on a 1·12 m × 2·15 mm column of 18–30-μm aluminium oxide eluted with n-hexane - diethyl ether (100 + 6) mixture at the rate of 0·6 ml min^{-1}. Chromatograms: A was obtained with aerated solvent and unpacked detector, and with excitation and emission set at 325 and 390 nm; B was obtained at the same wavelengths with de-aerated solvent and packed detector (silica gel); and C and D are a similar pair but monitored at 340 and 410 nm.

A reversed-phase separation of a used oil on Corasil C_{18}, essentially according to the conditions used by Vaughan, Wheals and Whitehouse,[13] is shown in Fig. 7. From the solvent used (aqueous methanol) silica gel does not adsorb ground states and presumably excited states of polynuclear hydrocarbons. However, in accordance with the foregoing, peaks due to pyrene (at 7·5 min) and chrysene (at 13 min) are increasingly intensified as the conditions are varied from unpacked aerated to packed de-aerated detectors. Relative to the anthracene peak (6·5 min), the over-all increases are by factors of 12·1 and 10·4.

Packed and unpacked detectors do not yield peak height ratios significantly different in variance. Although increased noise levels are associated with packed detectors, due to

increased levels of scattered radiation (the increase is approximately three-fold, depending on excitation and emission wavelengths), this effect is more than offset by the increased fluorescence yields obtained. Hence, with readily quenched fluorescences, the described conditions enable detection limits to be reduced by as much as one tenth.

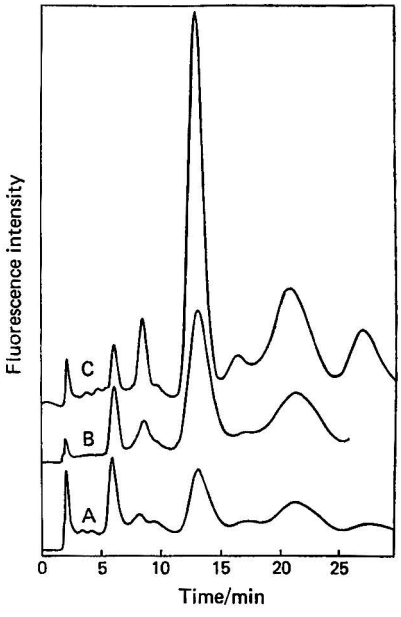

Fig. 7. Liquid chromatograms of a sump oil (1-mg samples) on a 1·12 m × 2·15 mm column of Corasil C_{18} eluted with methanol - water (80 + 20) mixture at the rate of 1 ml min^{-1}. Fluorescence detection is at 350 nm (excitation) and 380 nm (emission) with (A) aerated solvent and unpacked detector, (B) de-aerated solvent and unpacked detector and (C) de-aerated solvent and silica gel packed detector.

Microcolumn Liquid Chromatography

The application of spectrofluorimetric microscopy to thin-layer chromatography has been mentioned by Parker.[14] The technique is readily adapted to microcolumn chromatography, which can be conducted on a microscope stage as described under Experimental. Although fluorescence in the fluid effluent from a microcolumn can be monitored microscopically, signals of much improved intensity and stability result when emission from silica gel fragments is monitored.

That the technique is practicable depends on the considerable reduction in theoretical plate height exhibited by the microcolumns, which results in separation efficiencies comparable with conventional 1-m long high-pressure columns of the same adsorbent. Thus, anthracene and benz[*a*]anthracene separated in a microcolumn (88 × 1 mm) under conditions otherwise as previously described yield a value of 0·0941 mm (standard error, 0·0042) from 18 peaks in nine chromatograms, in contrast to the former values of 1·16 and 1·12 mm for aluminium oxide columns. Apart from the effect of the relatively low flow velocity in the microcolumn, the improved efficiency is probably due also to the reduction of diffusional broadening by reduced column dimensions, and to the complete elimination of the dead-volume effects between column and detector that tend to limit the resolution that can be obtained with conventional columns.

Because the column, and therefore, the point of focus, are disturbed during sample injection, quantitative measurements are best made against internal standards. In the presence of 10 ng of anthracene the relative response to between 10 and 120 ng of benz[*a*]anthracene monitored at 410 nm yields a linear least-squares analysis of $y = 0{\cdot}0210x - 0{\cdot}0156$, $s_{y/x} = 0{\cdot}0583$, $s_a = 0{\cdot}0367$ and $s_b = 0{\cdot}00056$, where y is the fluorescence intensity due to x ng of the benzanthracene relative to 10 ng of anthracene, and s, a and b have their previously indicated significance. Similarly, 50 pg to 2 ng of acridine relative to 500 ng of perylene chromatographed in a mixture of n-hexane and ethyl acetate (90 + 10) and monitored at 475 nm yields $y = 1{\cdot}140x - 0{\cdot}0403$, $s_{y/x} = 0{\cdot}0622$, $s_a = 0{\cdot}118$ and $s_b = 0{\cdot}0444$.

The separation of *N*-heterocyclic compounds from 10-nl volumes of samples of creosotes is shown in Fig. 8. These chromatograms should be compared with those of the same samples in Fig. 4. Although the sample size is reduced by one hundredth, the microcolumn reveals the presence of compounds that were previously undetected. The patterns are reproducible to the extent that relative to the most prominent heterocyclic peak the coefficient of variation of the remainder is 7·51 per cent. An initial, variable injection spike can be seen in some of the chromatograms. Also, the broad, rapidly eluted polynuclear hydrocarbon peak varies in resolution and intensity, probably because with these compounds the column is in an overload condition. When the microscope is focused on an aluminium oxide particle, the polynuclear hydrocarbon peak is the only peak detected.

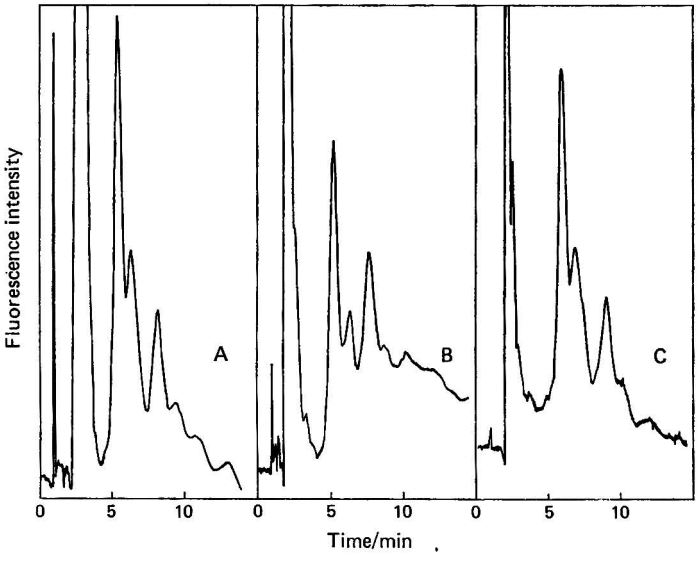

Fig. 8. Microcolumn (88 × 1 mm) chromatograms on 18–30-μm aluminium oxide in n-hexane - ethyl acetate (95 + 5) mixture, 15 μl min^{-1}, monitored by spectrofluorimetric microscopy of a silica gel fragment at 366 nm (excitation) and 475 nm (emission). Samples A and B (10 nl) are the same creosotes as in Fig. 4. Sample C is a splinter (76 μg) from fencing coated with A.

A chromatogram derived from a small splinter (76 μg) of wooden fencing that had been coated with one of the creosotes is included in Fig. 8. A variety of other splinters of the same source give the same pattern. Evidently, it should be possible to correlate items of this sort with likely points of origin.

In Fig. 9 are shown chromatograms of a soot fragment (about 1 μg) and a trace amount of pitch (about 3 μg). The patterns obtained reflect the varying polynuclear hydrocarbon compositions of the samples. Other materials rich in this class of compounds[15] can be similarly characterised.

Future Developments

The techniques already described offer a means, applicable on a microscopic scale, of analysing

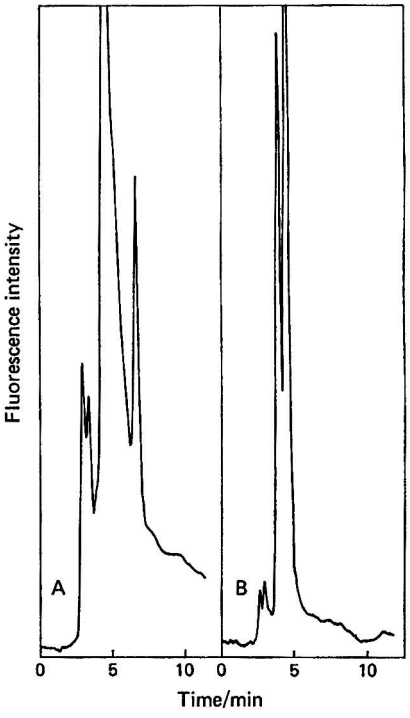

Fig. 9. Microcolumn (88 × 1 mm) chromatograms on 18–30-μm aluminium oxide in n-hexane - diethyl ether (100 + 6) mixture, 20 μl min^{-1}, monitored by spectrofluorimetric microscopy of a silica gel fragment excited at 366 nm, emission followed at 430 nm. Sample A is of pitch (about 3 μg) and B is a fragment of soot (about 1 μg).

minute amounts of potentially fluorescent compounds, and of studying the nature of electronically excited adsorbed states. However, many other types of detector packing are feasible. For instance, chemically bonded stationary phases might be used to exploit specific fluorescence effects. The phosphorescence observed at room temperatures in the adsorbed state by Schulman and Walling[16,17] and luminescence quenching, widely used in thin-layer chromatography and, very recently, in gas chromatography by Schulz and Vilceanu,[18] are of obvious significance. Again, micro-particulate adsorbents should enable microcolumns of even greater efficiency to be produced. Indeed, the full potentialities in other applications are unlikely to be fully realised until such packings are employed in conjunction with spectro-microscopy in order to eliminate entirely the problem of connecting high-resolution columns to detectors.

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A Rapid Gas-chromatographic Method for the Determination of Acetaldehyde in the Vapour Phase of Cigarette Smoke

D. J. Evans and R. J. Mayfield

C.S.I.R.O., Division of Textile Industry, P.O. Box 21, Belmont, Victoria 3216, Australia

A simple and rapid procedure is described for the gas-chromatographic determination of acetaldehyde in the vapour phase of cigarette smoke. The acetaldehyde is efficiently extracted into cold water in Drechsel bottles and is determined by gas chromatography of an aliquot of the solution. Results are comparable with those obtained by more complicated techniques involving derivative formation or the direct injection of smoke samples into high-resolution columns. A relative standard deviation of $\pm 3{\cdot}4$ per cent. was obtained in the absence of an internal standard. The method is particularly convenient for evaluating the effectiveness of cigarette filters in retaining acetaldehyde.

The ciliatoxicity of the gas phase of cigarette smoke has been the subject of several reports.[1–5] Much of this ciliatoxic effect has been attributed to the presence in this phase of volatile aldehydes[2,3,5] such as formaldehyde, acetaldehyde and acrolein. To facilitate the development of cigarette filters that selectively remove ciliatoxic components a simple and rapid procedure was required for the determination of acetaldehyde in the mainstream smoke of cigarettes.

Gas-chromatographic procedures have been reported previously for the quantitative determination of acetaldehyde and other volatile carbonyl compounds present in complex mixtures such as tobacco smoke,[6–9] car exhausts,[10] food flavours and aromas,[11] and atmospheric samples.[12] These methods have involved either prior derivative formation[10–12] or direct gas chromatography[6–9] of gas samples. Derivative formation is time consuming in that it often necessitates extractions or special clean-up procedures prior to the gas-chromatographic analysis. Direct injection of samples of tobacco smoke into a gas chromatograph involves the use of specialised sampling equipment, high-resolution columns and temperature programming in order to separate the large number of components.[6–7] A gas-sampling valve alone is insufficient as a smoke sample representative of the whole cigarette must first be collected. Neither of the foregoing techniques is convenient for routine applications because of the slow elution of the less volatile components of cigarette smoke.

In this paper, a simple and rapid procedure for the accurate determination of acetaldehyde in the mainstream smoke of cigarettes is described. Acetaldehyde in the vapour phase of the smoke is trapped in gas scrubbers containing cold water and is determined by gas-chromatographic analysis of an aliquot of the resulting solution. Special sampling equipment is unnecessary and interference from other smoke components is minimal as only water-soluble components are effectively collected. The precision of this method compares favourably with that of other methods,[6–8] but its main advantage is the ease with which it can be applied to multiple analyses for acetaldehyde.

Method

Materials

Cigarettes. Condition cigarettes at 60 per cent. relative humidity for at least 48 h.

Acetaldehyde. Re-distil analytical-reagent grade (99 per cent.) acetaldehyde immediately before use.

Standard acetaldehyde solution. Weigh accurately a stoppered 50-ml calibrated flask containing about 40 ml of distilled water. Introduce by means of a cooled syringe 230–270 mg of acetaldehyde, stopper the flask tightly and re-weigh. Swirl the contents and quickly dilute to 50 ml with distilled water. Dilute a 10-ml aliquot of the primary standard to 100 ml in a calibrated flask so as to give a solution containing 460–540 μg ml^{-1} of acetaldehyde, and stopper the flask tightly. Prepare working standards from this solution by removing 4-, 5-, 6-, 7- and 8-ml portions and diluting to 100 ml in calibrated flasks with distilled water. Keep the flasks tightly stoppered at all times and store in a refrigerator when not in use.

Apparatus

A Bendix, 2500 Series, gas chromatograph equipped with flame-ionisation detectors and a 1·9 m × 2 mm i.d. glass column packed with Chromosorb 101 (Johns-Manville) was employed isothermally at 110 °C. The flow-rate of the nitrogen carrier gas was 30 ml min^{-1} and the detector and injector temperatures were maintained at 125 °C. Samples (4·0 μl) were introduced into the column by means of a 5-μl syringe (SGE, Type A). Peak areas were determined by a DISC integrator.

Cigarettes were smoked with a CSM 100 4-channel smoking machine programmed to take 35-ml puffs of 2-s duration at intervals of 1 min. The smoke was drawn through a Cambridge filter assembly containing a glass-fibre pad to separate the particulate phase from the vapour phase. The trapping system consisted of two 50-ml test-tubes with B29 ground-glass joints fitted with adjustable Drechsel bottle heads. The tubes each contained distilled water (35 ml) cooled to 2 °C with an ice - water mixture and were placed in series between the smoking machine and the Cambridge filter holder. The puff volume of the smoking machine was adjusted with the traps and Cambridge filters in position.

Procedure

On the machine, smoke four cigarettes in succession to a constant butt length and pass the vapour phase of the smoke through the cooled traps; take a clearing puff at the end of each cigarette. Immediately on completion of smoking, transfer the cold smoke solutions to a 100-ml calibrated flask, rinsing the traps once with distilled water. Adjust the volume to 100 ml with distilled water, stopper the flask tightly and set it aside.

To analyse the smoke solution, inject a 4-μl sample into the gas chromatograph and record the peak area for acetaldehyde. Wait for approximately 4 min for acetone to elute before injecting another sample. Alternatively, inject a second sample immediately following elution of acetaldehyde from the first injection. After elution of acetaldehyde from the second injection allow 5 min to elapse for the elution of acetone from both injections before proceeding with another sample. Analyse each sample in triplicate, take the mean peak area and calculate the acetaldehyde concentration in micrograms per 100 ml direct from the calibration graph.

Standardise the procedure by injecting 4·0-μl samples of the acetaldehyde standards into the gas chromatograph. Repeat this procedure in triplicate, calculate the mean peak area for each standard and prepare a calibration graph by plotting peak area against concentration (micrograms per 100 ml).

Results

The efficiency of collection of acetaldehyde in the cold-water traps was determined by passing the vapour phase from four cigarettes through a series of four traps and measuring the acetaldehyde content of each trap. Table I shows that all of the acetaldehyde was collected in the first two traps and that 97 per cent. of the total was retained by the first trap. Thus, provided that the traps are cooled to 2 °C during the smoking procedure, a system of two traps collects virtually all of the acetaldehyde from four cigarettes smoked in succession.

TABLE I

EFFICIENCY OF COLLECTION OF ACETALDEHYDE

Collection system consisted of 4 traps, each containing 35 ml of water and cooled to 2 °C.

Trap	Acetaldehyde content/ μg per 100 ml	Total acetaldehyde, per cent.
1	2870	97
2	92	3
3	Not detectable	0
4	Not detectable	0

To assess the stability of the acetaldehyde collected in the cold-water traps, the contents were stored at 2 °C for 7 h and samples removed at regular intervals for gas-chromatographic analysis. No appreciable loss of acetaldehyde occurred under these conditions. Hence, analysis can be delayed for several hours following the smoking procedure, provided that the vapour-phase solution is stored at 2 °C in an air-tight vessel. The stability of acetaldehyde

over 7 h in cold aqueous smoke solution does not appear to have been appreciated previously and, consequently, it provides the basis for this method.

Table II shows the acetaldehyde content, as determined by the present method, of smoke from several commercial cigarettes and also gives results obtained by others[6–8] who employed similar smoking procedures in conjunction with gas-chromatographic procedures.

TABLE II

ACETALDEHYDE CONTENT OF SMOKE FROM SOME COMMERCIAL CIGARETTES

	Acetaldehyde content* by—				
	present method		other methods†/μg per puff		
Filter type‡	μg per cigarette	μg per puff	A[6]	B[7]	C[8]
Cellulose acetate (20 mm)	801	97	96		82
Cellulose acetate (17 mm)	823	100			
Cellulose acetate - charcoal (17 mm)	656	77	101	24	
Wool (20 mm)	788	90			
Treated wool (experimental, 20 mm)	593	71			
Unfiltered	625	85		92	81

* Owing to differences between cigarettes and filters strict comparisons are not significant.
† Methods of analysis by use of gas chromatography.
‡ Butt length of tobacco column in this method: unfiltered 12 mm; filtered 6 mm.

The cold-water traps employed here preferentially retain the water-soluble vapour-phase components. This substantially reduces the complexity of the vapour phase in subsequent analysis and facilitates resolution of acetaldehyde without cryothermal conditions, high-resolution columns or temperature programming. Fig. 1 illustrates the simple gas chromatogram obtained by chromatography of the aqueous smoke solution. Acetaldehyde is well separated from other, minor peaks, allowing accurate integration of the peak area. A second injection can be made immediately following the elution of the acetaldehyde peak from the first injection, as the retention time of acetone is more than twice that of acetaldehyde and no other peaks interfere in this region. By this means the analysis time required for several samples can be considerably shortened.

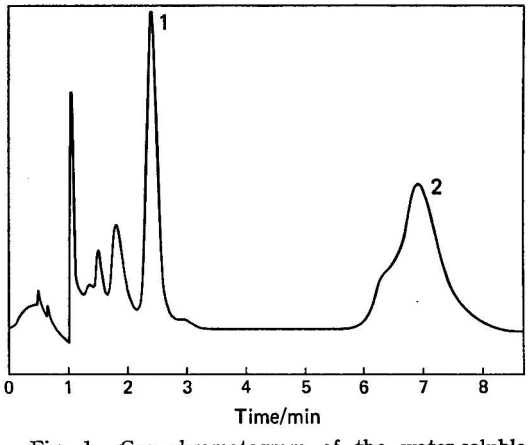

Fig. 1. Gas chromatogram of the water-soluble components of tobacco smoke. 1, Acetaldehyde; 2, acetone.

An internal reference standard was not employed and therefore aliquots of standards and samples for analysis by means of chromatography were accurately measured with a high-quality syringe. Analysis of each standard and sample was carried out three times and the mean acetaldehyde peak area was determined. The reproducibility of the method was established from the results of 12 determinations utilising 48 cigarettes, *i.e.*, the acetaldehyde

content was found to be 776 μg per cigarette $\pm$ 3·7 per cent. relative standard deviation (90·2 μg per puff $\pm$ 3·4 per cent. relative standard deviation). The calibration graph for the acetaldehyde standards was linear and the standards remained unchanged over 48 h if kept in a tightly stoppered flask and stored at 2 °C when not in use.

Conclusion

The present method is a simple and rapid one for the accurate determination of the acetaldehyde content of the vapour phase of cigarette smoke. The results are particularly useful in evaluating the effectiveness of cigarette filters in retaining acetaldehyde. The method could possibly be extended to the determination of other biologically active water-soluble components present in cigarette smoke.

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Improvements in the Atomic-fluorescence Determination of Mercury by the Cold-vapour Technique

K. C. Thompson and R. G. Godden

Shandon Southern Instruments Ltd., Frimley Road, Camberley, Surrey, GU16 5ET

Relatively simple modifications to a cold-vapour, mercury-fluorescence detector have resulted in a large increase in sensitivity and have decreased the time required for each measurement. A 2σ detection limit of 0·02 ng was achieved.

Recently a number of papers have appeared that describe various techniques for improving the sensitivity of determinations of mercury using the cold-vapour absorption technique. These techniques have included partitioning the mercury between the liquid phase and a fixed volume of air,[1] colloid flotation,[2] amalgamation with gold,[3] concentrating the mercury from the sample into a small volume of acidified potassium permanganate solution,[4] improved purging efficiency of the carrier gas through the reduced sample[5] and pre-concentration in a cold trap.[6] This last system, using a liquid nitrogen cold trap to isolate the mercury initially, gave the best absolute detection limit (0·2 ng of mercury).

West,[7] by using a theoretical treatment, has shown that the atomic-fluorescence determination of mercury, using a cold-vapour technique, should be more sensitive and produce considerably less interference from non-specific background absorption than the corresponding absorption technique. Previous reports on the cold-vapour, atomic-fluorescence determination of mercury have borne out these observations.[8–11] The detection limits reported vary from 0·5 to 3 ng of mercury. With minor modifications to an existing mercury cold-vapour fluorescence apparatus a detection limit of 0·02 ng of mercury could readily be obtained.

Experimental

The results were obtained by using a Shandon Southern Instruments A3600 atomic-absorption spectrophotometer, a modified Shandon Southern Instruments A3460 fluorescence mercury detector, and a Shandon Southern Instruments Autograph recorder.

The system is depicted in Fig. 1. Argon was bubbled through the tin(II) chloride reagent contained in cell A, and, on addition of the sample, mercury was released into the argon stream. The mercury then passed up through tube B and fluoresced in region C; a drying column was not required. A sheath unit, D, was constructed from acetyl resin and fitted around the top of tube B. Argon was fed into the sheath unit, which produced a laminar argon shield around the top of tube B. This device minimised entrainment of air into the sample - argon stream issuing from tube B, entrainment of air having been shown to result in severe quenching of the fluorescence radiation.[8,9]

The following modifications were made to the mercury lamp unit of the A3460 instrument: the size of the lamp aperture, E, was reduced from 22 × 10 mm to 15 × 6·5 mm by means of a clip-on mask; a 6 mm o.d. copper tube, F, was fitted on the rear of the lamp housing directly opposite to aperture E and a small flow of argon was passed into the lamp housing through this tube and issued from the lamp aperture E. The mercury lamp, a Philips OZ4W low-pressure lamp,[8] was run at an optimum current of 0·38 A.

Optimisation of Operating Parameters

Wavelength and spectral band pass

The 253·7 nm mercury line was used with the maximum spectral band pass of 6 nm.

Minimisation of the Constant Background Level

The constant background level was mainly attributed to direct specular reflection of light (at 253·7 nm) from the mercury lamp into the monochromator entry aperture. This back-

ground was carefully minimised by removing both hollow-cathode lamps from the lamp turret and removing the remaining lens.

Tube B was positioned just below the point at which direct specular reflection from the top of the tube commenced. For maximum sensitivity it was essential to minimise the background level.

Damping

The A3600 atomic-absorption spectrophotometer was operated at maximum damping (the time constant being 10 s). The recorder output of the spectrophotometer (20 mV for full-scale deflection) was set to twice that of the recorder (10 mV for full-scale deflection) in order to prevent overloading of the amplification system.

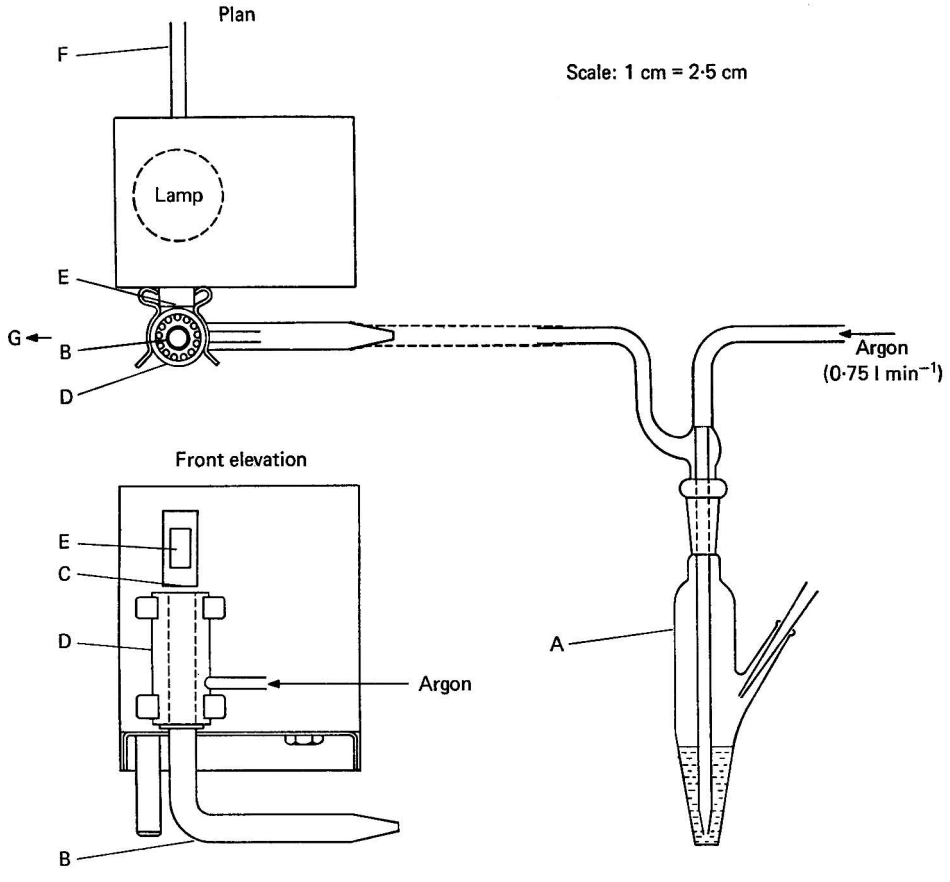

Fig. 1. Diagram of the improved mercury fluorescence detector: A, cell; B, 7 mm i.d. Pyrex tube; C, region where fluorescence occurs; D, sheath unit; E, lamp aperture; F, gas inlet pipe; and G, optical axis (orifice of tube B was 75 mm from monochromator entry slit).

Gas Flows

Cell A. Argon gas was used[8,9] and the optimum flow-rate was 0·75 l min^{-1}.

Sheath. The signal amplitude was doubled, and the noise level on the base-line halved, when the argon sheathing gas was flowing. This effect was attributed mainly to minimised air entrainment. The optimum sheath gas flow-rate was 1·6 l min^{-1}.

Lamp housing. Occasionally trace amounts of mercury would condense on the front face of the mercury lamp, especially with the sheath gas flowing. This condensation resulted in an increase in the constant background (specular reflection) level; it also considerably increased the base-line noise level and caused drifting of the base-line. However, if argon was directed

on to the rear face of the mercury lamp, through tube F, the mercury condensed on this rear face, resulting in a 30 per cent. increase in sensitivity and a stable base-line. The increase in the signal magnitude could be caused by various factors, *e.g.*, removal of any ozone present between the lamp and tube B (ozone absorbs strongly at 253·7 nm), reduced self-absorption or self-reversal of the 253·7 nm mercury line, or even stepwise line fluorescence at 253·7 nm following excitation by the 185·0 nm mercury line. The argon flow-rate through the lamp housing was not very critical; in fact, a flow-rate of 1 l min^{-1} was used. A small increase in the constant background level (approximately 10 per cent.) was observed under these conditions.

Reagents

Mercury solutions. The mercury solutions were prepared just before use from a 10 $\mu g\ ml^{-1}$ stock solution of mercury in 1 per cent. *V/V* nitric acid. This last solution was prepared daily from a similar 1000 $\mu g\ ml^{-1}$ stock solution. All of the calibrated flasks and pipettes used in this study were soaked in 50 per cent. *V/V* nitric acid for 1 week prior to use and all of the mercury and blank solutions contained 1 per cent. *V/V* of nitric acid.[12] In order to achieve good, long-term stability (up to 5 months) of dilute (less than 10 ng ml^{-1}) mercury solutions, the addition of 5 per cent. *V/V* of nitric acid and 0·01 per cent. *m/V* of dichromate ion has been recommended.[13]

Tin(II) chloride solution, 2 per cent. m/V. A 2-g amount of tin(II) chloride was dissolved in 20 ml of hydrochloric acid (36 per cent. *m/V*) and 80 ml of 1·5 M sulphuric acid were then added. Argon, at a flow-rate of 0·3 l min^{-1}, was continuously bubbled through this solution in order to remove any trace amounts of mercury and prevent oxidation of the tin(II) chloride by air.

Procedure

A 1-ml volume of the 2 per cent. *m/V* tin(II) chloride solution was introduced through the top of cell A. The cell was then connected to the cell head and the sample, contained in a 1-ml MLA pipette (Shandon Southern Instruments Ltd.), was inserted into the side-arm of cell A. After standing for 5 s, in order to allow for the removal of any entrained air, the sample was injected into the tin(II) chloride solution and the peak recorded. The cell was then emptied, washed and the procedure repeated. Typical peaks are shown in Fig. 2. The peak width was 1·3 min. The maximum volume of sample that could be added to cell A was found to be 10 ml. The calibration graph was linear over the range 0·02–200 ng of mercury.

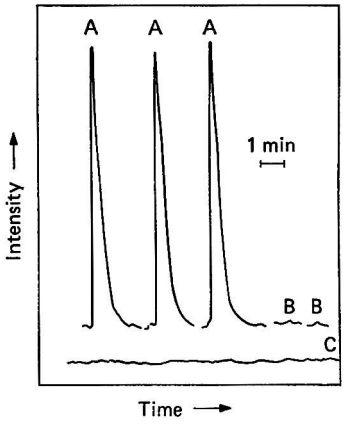

Fig. 2. Typical mercury trace. A, 1 ml of 0·001 $\mu g\ ml^{-1}$ mercury solution; B, 1 ml of blank solution; C, base-line stability. Voltage to photomultiplier = 770 V.

Results

The new system was compared with the standard A3460 mercury detector. (This unit has been described previously.[8]) Table I gives a comparison of the two systems and it can be seen that the performance of the new detector system was much better than that of the standard system. Another advantage of the new system was the smaller change in the base-line level on removal of the pipette from the side-arm of cell A, equivalent to 0·03 ng of mercury for the new system and 1 ng of mercury for the standard A3460 system. This change in level was attributed to a change in the partial pressure of water vapour in the argon stream on removal of the pipette.

TABLE I

COMPARISON OF THE IMPROVED AND STANDARD MERCURY DETECTORS

All absolute mercury masses are based on a 1-ml sample volume.

	New detector system	Standard A3460 detector system
Relative sensitivity (comparison of peak heights for 2 ng of mercury addition)	9	1
Constant background level (expressed as ng of mercury)	0·4	4·5
Time for complete measurement/min	1·5	4·5
2σ noise level on base-line (expressed as ng of mercury)	0·015	0·3

The relative standard deviation (17 measurements) for a 1-ml addition of a 1 ng ml^{-1} mercury solution was 4·5 per cent. During the course of this study the blank solution (1 per cent. V/V nitric acid) gave signals equivalent to a mercury level between 0·02 and 0·05 ng ml^{-1}. The standard deviation (13 measurements) on 1 ml of a blank solution giving a signal corresponding to 0·05 ng ml^{-1} of mercury was 0·01 ng ml^{-1}, which is equivalent to a 2σ detection limit of 0·02 ng.

The system has been tested on urine and blood samples. In the limited time available breakdown procedures for organically bound mercury were not studied. However, on adding 1 ml of urine to cell A, containing 1 ml of 2 per cent. m/V tin(II) chloride solution and 100 μl of 1 per cent. m/V silicone anti-foaming agent (BDH Chemicals Ltd.), reproducible signals were obtained for urine samples from non-exposed subjects. Mercury could also be detected in blood from non-exposed subjects by adding 1 ml of blood - water (1 + 9 V/V) (which had been subjected to ultrasonic agitation for 15 min) to cell A containing 5 ml of 5 per cent. m/V tin(II) chloride and 100 μl of 1 per cent. m/V silicone anti-foaming agent. The mercury signal from the blood (unlike aqueous standards) decreased markedly if the tin(II) chloride concentration was decreased below 4 per cent. m/V. This indicates a decreasing degree of breakdown of organically bound mercury compounds. For the complete breakdown of organically bound mercury compounds various procedures have been recommended.[14–17]

Conclusions

Improvements have been made to a previously described mercury-fluorescence detector system. An improvement in the detection limit of approximately 20 times has been achieved. This improvement results from various factors: decreasing the cell volume increased the sensitivity and decreased the time of measurement; an argon sheath minimised air entrainment and improved the base-line stability; a restricted lamp aperture resulted in a relative decrease in the constant background (specular reflection) level; and finally, cooling the rear face of the mercury lamp with a stream of argon improved both the sensitivity and the base-line stability and also prevented the formation of ozone (which absorbs strongly at 253·7 nm) between the lamp and the mercury vapour stream.

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Suppression of Iron(III) Interference in the Determination of Iron(II) in Water by the 1,10-Phenanthroline Method

Hubert Fadrus and Josef Malý

Water Management Board, Vodohospodářská Správa, Brno, Czechoslovakia

A method for the determination of iron(II) in water in the presence of iron(III) with 1,10-phenanthroline is described, in which the interfering effect of iron(III) ions is suppressed by masking with complexones. In the absence of a chelating agent for iron(III) the colour intensity of samples being analysed is unstable, owing to redox processes induced by the effect of 1,10-phenanthroline on the iron(II) - iron(III) system, causing reduction of iron(III) and formation of the corresponding coloured iron(II) chelate compound in a stoicheiometrically proportional concentration. The advantages of complexones, especially nitrilotriacetic acid, over other chelating agents are discussed.

The method for the photometric determination of iron, after converting it into iron(II) ions, with 1,10-phenanthroline has been thoroughly investigated and proved to be satisfactory.[1] If, however, it is required to differentiate between the two valency forms of iron by using this method, the omission from the basic procedure of the addition of reductant used in the determination of iron(II) in the presence of iron(III) ions is recommended.[2] The results thus obtained are, however, rather unsatisfactory, especially because of the instability of the resulting colour intensity with time.[3] This drawback also becomes apparent in the method in which iron(II) is determined indirectly by calculation from the difference between the absorbances of the iron(II) - iron(III) - 1,10-phenanthroline system at two specific wavelengths.[4]

The applicability of the methods for the determination of iron(II) with 1,10-phenanthroline in the presence of excess of iron(III) ions is restricted by the following requirements: the iron(II) concentration must be higher than 1 mg l^{-1}, an amount of reagent equivalent to at least 30 times the total iron content must be used and the absorbance read within 10 to 15 min; also, the sample to be analysed must be free from all foreign matter and protected against direct sunlight.[3] However, even when these conditions are satisfied, the results obtained are not reliable.[5] Similarly, the use of 2,9-dimethyl-1,10-phenanthroline instead of 1,10-phenanthroline does not give satisfactory results.

The cause of the poor reproducibility of the results for the determination of iron(II) with 1,10-phenanthroline in the presence of iron(III) ions is attributable to the different stabilities of the iron(II) and iron(III) chelate compounds formed with 1,10-phenanthroline,[6] the presence of which gives rise to a shift in the redox potential of the Fe^{2+} - Fe^{3+} system to more negative values in the reaction

$$Fe^{3+} + 3(phen) + e^- = [Fe(phen)_3]^{2+}$$

with reduction of iron(III) ions and formation of the corresponding coloured iron(II) chelate compound in stoicheiometrically proportional concentration. The reaction takes place slowly, but is more rapid in the presence of reductants as electron acceptors. In order to achieve accurate results in iron(II) determinations under such conditions it is necessary to shorten the time of contact of the iron(III) ions with the reagent to a minimum, so that errors that arise from the increase in concentration of the component being determined would be negligible. Compliance with this requirement, *e.g.*, by preliminary separation of iron(III) ions in the form of iron(III) hydroxide, may introduce further errors caused by interference with the balance between acid - base and redox conditions in the Fe^{2+} - Fe^{3+} system.[7] The masking of the iron(III) ions by the addition of chelate-forming compounds and this method of removing the interference from iron(III) ions in the determination of iron(II) with 1,10-phenanthroline have been further studied.

Experimental

The colour intensities of the 1,10-phenanthroline complexes with iron(II) and iron(III) ions in the presence of other compounds were measured in 2-cm cells on a Pulfrich photometer with ELPHO-2 supplementary equipment, using an S-51 filter (510 nm). The necessary concentrations of the components in the final solutions were obtained by adding the reagents in the order given in the figure captions.

The required concentration of iron(II) ions was obtained by diluting ammonium iron(II) sulphate standard solution (see Reagents). Trace amounts of iron(III) salts in this solution were reduced with a Jones reductor.

The required concentration of iron(III) was obtained by diluting ammonium iron(III) sulphate stock solution containing 500 mg l^{-1} of iron(III) and 2 ml l^{-1} of concentrated sulphuric acid. The exact iron(III) concentration was determined gravimetrically. In order to remove trace amounts of iron(II) the solution was always freshly prepared after evaporation to fumes with perchloric acid and hydrogen peroxide and re-dissolution.

Solutions were adjusted to the appropriate pH by use of the following buffers: glycocoll (glycine) for pH 2·5, acetate for pH 5·5 and borax for pH 8·0.

Results and Discussion

Under the conditions of the determination of iron(II), iron(III) ions react with 1,10-phenanthroline to form a labile yellow-coloured chelate compound, $[Fe(phen)_3]^{3+}$, which, in the presence of excess of reagent, changes slowly into a stable red chelate compound, $[Fe(phen)_3]^{2+}$. The rate of the reduction is dependent on the pH of the medium as well as on the reagent concentration (Fig. 1); however, contrary to a previous report,[3] we found that it is not affected by diffused light. A considerable acceleration in the rate of reduction of the $[Fe(phen)_3]^{3+}$ chelate is obtained in the presence of other substances that have reducing characteristics (Fig. 2).

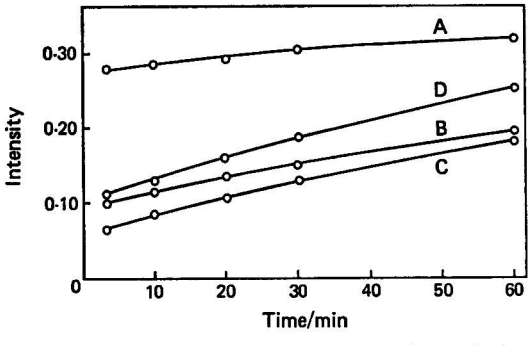

Fig. 1. Colour development in the solution containing 1,10-phenanthroline and Fe(III) ions. [Fe(III)], 12·5 mg l^{-1}. Concentration of 1,10-phenanthroline: A, B and C, $1{\cdot}25 \times 10^{-3}$ M; and D, $3{\cdot}75 \times 10^{-3}$ M. pH of solution: A, 5·5; B, 2·5; C, 1·95; D, 2·5.

By adding to the solution after the addition of 1,10-phenanthroline, or simultaneously with it, compounds that mask iron(III) ions, it is possible to suppress the course of the reduction reactions or to decrease their rate to a minimum. Neither tartaric nor citric acid proved satisfactory for this purpose, not only because of insufficient stability of the corresponding iron(III) chelate compounds, but especially because, in the presence of 1,10-phenanthroline, there occurs in the corresponding tartrate and citrate chelate compounds an intramolecular redox process in which the central iron(III) ion is reduced by its own chelate, forming a ligand to iron(II) ion, which changes to a stable complex $[Fe(phen)_3]^{2+}$ (Fig. 3, curves A and B).

Use of fluoride provides reliable results in an acidic medium, but because of the aggressive effects of hydrogen fluoride on the glass cells of the photometer, it has only a limited applicability. In a neutral medium the masking efficiency decreases and in an alkaline medium FeF_6^{3-} decomposes as a result of hydrolysis. Diammonium hydrogen orthophosphate, recom-

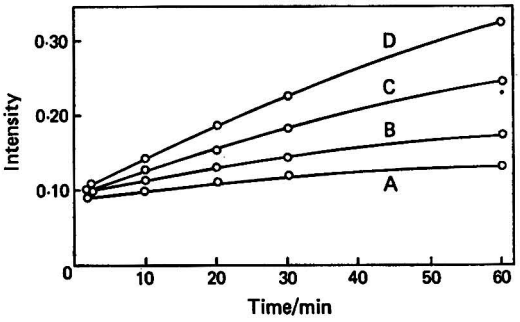

Fig. 2. Effect of reductants on the colour development in the solution containing 1,10-phenanthroline and Fe(III) ions: A, no reductant added; B, 250 mg l^{-1} of NH_4HS; C, 500 ml l^{-1} of sewage with $B.O.D._5$ = 82 mg l^{-1} of O_2; and D, 250 mg l^{-1} of sucrose. [Fe(III)], 12·5 mg ml^{-1}; [1,10-phenanthroline], 1·25 × 10^{-3} M. pH, 2·5.

mended in extraction processes in combination with 2,9-dimethyl-1,10-phenanthroline, did not prove satisfactory in direct photometry,[8] because the pH (about 2·2) cannot easily be controlled and the gradual formation of a turbidity occurs as iron(III) phosphate is only slightly soluble. By lowering the pH to 1·5 it is possible to remove the turbidity while maintaining the masking effect of the phosphate ions on iron(III) ions, but an undesirable retardation of the colour development also occurs.

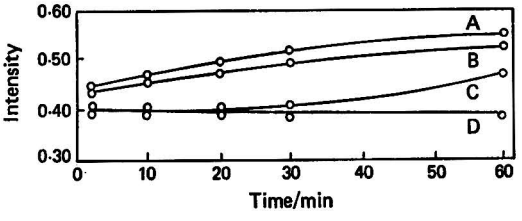

Fig. 3. Colour development in the solution containing Fe(III) ions, 1,10-phenanthroline and complexones. [Fe(II)], 0·5 mg l^{-1}; [Fe(III)], 12·5 mg l^{-1}; [1,10-phenanthroline], 1·25 × 10^{-3} M; and [citric acid], 1 g l^{-1}, added as a reductant, together with 1,10-phenanthroline. Complexones are added after the addition of 1,10-phenanthroline.

Curve	pH of solution	Complexone
A	2·5	—
B	8·2	—
C	2·5	0·01 M NTA
D	8·2	0·01 M NTA + 0·01 M DCTA

Reduction of the $[Fe(phen)_3]^{3+}$ complex with 1,10-phenanthroline can be retarded or stopped by the addition of complexones (Fig. 3, curves C and D). The complicated mechanism of the interaction of the components in this system is a function of the stability of the chelate compound formed by the iron with 1,10-phenanthroline and with the complexone, which is expressed by the corresponding stability constants. An unsuitable choice of the order of addition of the reagents leads therefore to non-reproducible and erroneous results, thus giving rise to criticism of the use of complexones.[8] This can be explained as follows: if to a solution containing both iron(II) and iron(III) ions, complexones are added together in a mixture with 1,10-phenanthroline, they have the effect shown in Fig. 4. By adding fluoride

or nitrilotriacetic acid (NTA) the same colour intensity is obtained as with the solution that contains an equivalent amount of iron(II) ions but no iron(III) ions, whereas on adding ethylenediaminetetraacetic acid (EDTA) or 1,2-diaminocyclohexane-*NNN'N'*-tetraacetic acid (DCTA) a marked decrease in the colour intensity, which is proportional to the difference between the stability constants of the chelate compounds of iron(III) ions with 1,10-phenanthroline and with EDTA or DCTA, respectively, is experienced.

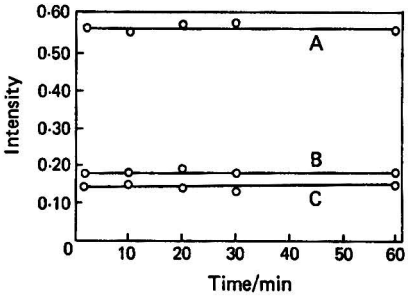

Fig. 4. Effect of complexones on the colour development in the solution containing 1,10-phenanthroline and Fe(II) ions: A, without complexone or with NTA; B, with EDTA; and C, with DCTA. [Fe(II)], 0·75 mg l^{-1}; [1,10-phenanthroline], $1{\cdot}25 \times 10^{-3}$ M; [complexone], 2×10^{-2} M. pH, 2·5. Complexones are added together with 1,10-phenanthroline.

The probable cause of the reduced absorbance values and, in turn, of the erroneous results of the iron(II) determination, is that with such an effective lowering of the potential of the Fe^{2+} - Fe^{3+} system by the masking of iron(III) ions with the complexone used, the formation of the $[Fe(phen)_3]^{2+}$ chelate compound evidently competes with the rapid and parallel oxidation of iron(II) ions by the oxygen dissolved in the given mixture:

$$[Fe(phen)_3]^{2+} + H_2Y^{2-} = FeY^- + 3(phen) + e^-$$

This assumption was verified by adding fluoride or complexone to the reaction mixture prior to the 1,10-phenanthroline, which, in the presence of a sufficient concentration of dissolved oxygen, caused an almost immediate oxidation of iron(II) ions to such an extent that the reaction between 1,10-phenanthroline and the component to be determined was completely negative. On the other hand, the $[Fe(phen)_3]^{2+}$ already produced, even in the presence of oxygen, is stable towards complexone inasmuch as the intensity of its colour changes only very slowly with time, according to the conditions in the reaction mixture.

With the exception of fluoride and NTA, whose chelate compounds with iron(III) are less stable than the similar chelate compounds with EDTA and DCTA and which can, therefore, be applied in combination in a mixture with 1,10-phenanthroline, it is necessary to add EDTA and DCTA to the reaction mixture separately only after the development of the colour is completed. In such an instance, when EDTA or DCTA is added 1 min following the addition of 1,10-phenanthroline to the solution containing iron in both valency forms, the absorbance of the resulting mixture is identical with that of a similar sample containing only an equimolar concentration of iron(II) salt. Changes in the colour intensity of the investigated solutions brought about by the redox processes of the iron(II) and iron(III) compounds in 1 min are negligible. The significance of the order of addition of reagents in the determination of iron(II) has to be taken into consideration in all instances when the interfering components are masked by complexones, otherwise even procedures recommended in the literature may be valueless.

It is possible, however, to use reaction mixtures that are neutral or alkaline (*e.g.*, by use of a borax buffer of pH 8) when reducing substances that cause less interference, *e.g.*, sulphides, are present. The addition of chelating substances that give soluble complexes with iron(III) ions in this pH range is, under these conditions, essential in order to prevent the occurrence of turbidity due to the hydrolysis of the iron(III) salts present. Of these chelating agents,

however, only NTA can be used and can be added together with the reagent, because owing to the action of EDTA or DCTA, which is added after the development of the $[Fe(phen)_3]^{2+}$, the iron(III) hydrolysates formed dissolve only very slowly in the alkaline medium. As the stability of this complex ion is more marked in the presence of EDTA and DCTA, it is advantageous to combine all the complexones in such a way that NTA is added in admixture with 1,10-phenanthroline followed after 1 min by EDTA or even better by DCTA (Fig. 3, curves C and D).

Results for the determination of iron(II) in the presence of iron(III) in underground water from South Moravia obtained by using the recommended method and the procedure according to reference 2 are given in Tables I and II.

TABLE I

COMPARISON OF RESULTS FOR IRON(II) DETERMINATION BY USING THE RECOMMENDED METHOD AND PROCEDURE ACCORDING TO REFERENCE 2 ON PREPARED SOLUTIONS CONTAINING IRON(II) AND IRON(III) SALTS

All results are expressed in mg l^{-1}.

Iron(II) added	Iron(III) added	Iron(II) by recommended method*		Iron(II) by procedure in reference 2	
		Found	Difference	Found	Difference
1·06	10	1·10	+0·04	1·24	+0·18
	25	0·98	−0·08	1·28	+0·22
	50	1·12	+0·06	1·40	+0·34
	100	1·15	+0·09	1·51	+0·45
3·18	10	3·26	+0·08	3·30	+0·12
	25	3·23	+0·05	3·38	+0·20
	50	3·10	−0·08	3·56	+0·38
	100	3·32	+0·14	3·60	+0·42
5·30	10	5·32	+0·02	5·41	+0·11
	25	5·39	+0·09	5·55	+0·25
	50	5·42	+0·12	5·65	+0·35
	100	5·41	+0·11	5·82	+0·52

* Colour intensity measured 30 min after addition of reagents.

The differences between the values obtained by the two methods for colour intensity during the first 3 min provide evidence for interpreting the influence of iron(III) on the iron(II) determination as described above but cannot be explained theoretically because of lack of knowledge of the reaction mechanism involved in the development of the colour intensity of the complex salt of iron(II) with 1,10-phenanthroline during the interval 0–3 min.

TABLE II

COMPARISON OF RESULTS OBTAINED ON UNDERGROUND WATER FOR DETERMINATION OF IRON(II) IN THE PRESENCE OF IRON(III) BY RECOMMENDED METHOD AND PROCEDURE IN REFERENCE 2

Iron(III) content/ mg l^{-1}	Iron(II) content/mg l^{-1}, Procedure in reference 2*			Recommended method*		
	a	b	(b − a)	a	b	(b − a)
19·1	1·28	1·40	0·12	1·25	1·28	0·03
31·2	2·95	3·22	0·27	2·90	2·96	0·06
22·3	1·42	1·61	0·19	1·36	1·39	0·03
11·8	0·92	1·04	0·12	0·88	0·90	0·02
25·6	1·85	2·08	0·23	1·78	1·84	0·06
16·0	1·08	1·17	0·09	1·05	1·07	0·02

* Colour intensity measured a, 3 min and b, 30 min after addition of reagents.

Method

Reagents

Buffer mixture. Mix 5 volumes of 0·025 M 1,10-phenanthroline hydrochloride solution, 5 volumes of 0·5 M glycocoll solution adjusted to pH 2·9 with 0·5 N hydrochloric acid and 1 volume of a 0·1 M solution of the sodium salt of nitrilotriacetic acid (NTA) immediately before use.

Standard ammonium iron(II) solution. Dissolve 0·702 g of the reagent in distilled water containing 2 ml of concentrated sulphuric acid and make the volume up to 1 l; 1 ml of the solution contains 0·01 mg of iron(II). Its exact concentration is determined by titration with standard permanganate solution.

Apparatus

Pulfrich photometer with ELPHO-2.
S-51 filter (510 nm).
Measuring cells, 2 cm.

Procedure

Place a neutral or slightly acidic solution of the sample containing up to 125 μg of iron(II) and up to 2500 μg of iron(III) in a 50-ml calibrated flask, adjust the volume to about 25 ml and add 10·0 ml of buffer mixture. Agitate the mixture and dilute it to the mark. During the period 3 to 30 min after addition of the buffer mixture, measure the colour intensity of the solution at 510 nm against a sample prepared in the same way without the addition of 1,10-phenanthroline (in order to compensate for the colour of the iron(III) - complexone). The calibration graph, which is linear up to an iron(II) concentration of 5 mg l^{-1}, is plotted under identical working conditions.

Interferences

By using the procedure described for the determination of 0 to 5 mg l^{-1} of iron(II), the presence of up to 100 mg l^{-1} of iron(III) in the analysed solution is eliminated as well as the effect of all ions that form stable chelate compounds with NTA.

Tungstate, molybdate, iodide, rhodanide, perchlorate and cyanide precipitate the 1,10-phenanthroline - iron(II) chelate compound or form with it soluble ionic associates, which can be extracted into non-polar solvents. Vanadium(V) compounds interfere by forming a brown colour, phosphate retards the development of the colour and nitrite gives a yellow colour.

Conclusion

It has been proved that contrary to previous reports the determination of iron(II) ions with 1,10-phenanthroline in the presence of iron(III) ions cannot be achieved simply by omitting the addition of the reductant used in normal procedures because, owing to the lack of stability of iron(III) ions in the given reaction mixture, in which the action of redox processes is stimulated by the strong chelating effect of the reagent on the iron(II) ions, erroneous and insufficiently reproducible results are obtained as a consequence of the instability of the developed colour.

The interference of iron(III) ions can be suppressed by adding chelating substances, the most suitable of which is NTA. Knowledge of the reaction mechanism as well as of the velocities of the mutually competing processes between iron(II) and iron(III) ions, reagent, complexone used and redox active components that may be be present in the solution being analysed permits the introduction of the chosen complexone correctly into the order of addition of reagents, even in those photometric procedures in which complexones are used for masking other interfering components. When highly reducible compounds are present, the determination of iron(II) ions in the presence of iron(III) ions cannot be achieved by this method.

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A Composite Scheme for the Analysis of Steels by Atomic-absorption Spectroscopy Using the Air - Acetylene Flame

W. R. Nall, D. Brumhead and R. Whitham

Ministry of Defence, Materials Quality Assurance Directorate, Bragg Laboratory, Janson Street, Sheffield, S9 2LJ

A composite scheme is described for the determination of chromium, molybdenum, manganese, nickel and copper in all types of steel. The scheme is based on a single sample solution containing 1 g of steel per 100 ml. Recent developments in technique have enabled all these elements to be determined with the air - acetylene flame and the scheme can be extended as required to include other elements such as lead and cobalt.

Previous schemes for the determination of several elements on a single sample mass of alloy have involved the use of the nitrous oxide - acetylene flame[1] in order to overcome difficulties in the measurement of refractory elements or they have been concerned with relatively low contents of alloying elements.[2] A limited scheme[3] has been described for determining high contents of manganese, chromium and nickel in high-alloy steels by using an air - acetylene flame. In this last paper the use of the secondary resonance line of chromium was recommended but no reference was made to the precautions that are necessary[4] in the determination of this element in this type of flame.

The ability to apply the direct atomic-absorption technique to the determination of larger amounts of alloying elements has long been recognised to be desirable but the accuracy and reproducibility of the method have been such that elaborate schemes of averaging a number of readings have been required for this type of analysis. In recent years, however, there has been such a refinement of instrumentation that the required degree of accuracy should now be attainable directly and modern instruments should bring the method into line with other accepted techniques such as titrimetric analysis and molecular spectrophotometry.

If there is no requirement to determine elements that form refractory oxides, such as silicon, aluminium or titanium, it would be useful to have a composite scheme that includes the elements chromium and molybdenum that is based on the use of the air - acetylene flame alone. There are some laboratories in which the use of nitrous oxide is undesirable or prohibited on safety grounds and for these situations the proposed scheme would be applicable.

The problem of determining molybdenum in an air - acetylene flame[5] has been solved by the addition of ammonium chloride to the analyte and recently it has been shown[4] that chromium can be determined by incorporating quinolin-8-ol as a releasing agent.

Choice of Experimental Conditions

The usual working concentration of sample solution recommended for steel analysis[1] is 1 per cent. *m/V*, which is dilute enough to prevent deposition of salt in the burner jaws and yet provides a sufficient concentration of the minor elements in the sample for their accurate determination. In order to accommodate alloying elements that are present in larger amounts, dilutions have to be made to this initial sample solution as suggested by Thomerson and Price.[1] They recommended that the iron concentration should be restored to the initial 1 per cent. *m/V* in order to minimise interference and suppression and to equalise these effects between samples and standards.

While it was recognised that a constant iron concentration was required, the actual level does not seem to have been determined directly. This aspect has been examined and the results are given below. As part of the development of the scheme the linear working ranges for each element were also determined and the dilutions given below are such that the analytes

are always within these ranges. It was found that in all instances the best ranges were those which gave absorbances below 0·30.

The effect of various iron concentrations on the absorbance of the five elements chromium, molybdenum, manganese, nickel and copper in the proposed scheme was investigated (Fig. 1); the results given were obtained in the presence of the releasing agents described below (see

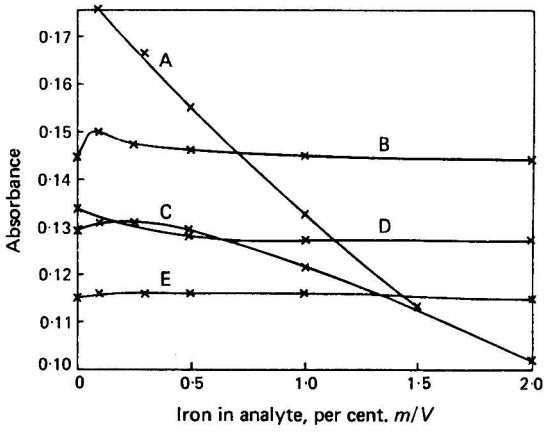

Fig. 1. Effect of various iron concentrations on absorbance. A, Chromium 10 p.p.m.; B, copper 2·5 p.p.m.; C, molybdenum 20 p.p.m.; D, nickel 10 p.p.m.; and E, manganese 2·5 p.p.m. Instrument conditions as in Table II.

Method). It was found that copper, nickel and manganese were virtually unaffected by varying the iron concentration but chromium and molybdenum were subject to serious interference, and it was decided to investigate the effect on these two elements more fully, by measuring the absorbance for chromium and molybdenum in the presence of their appropriate releasing agents and of increasing amounts of iron.

The results for chromium are shown in Fig. 2, from which it can be seen that at all concentrations of quinolin-8-ol, decreasing the iron content results in increased absorbance for a given amount of chromium. However, a practical limit is set to the minimum amount of sample (hence the iron content) that can be used in order to satisfy the sensitivity require-

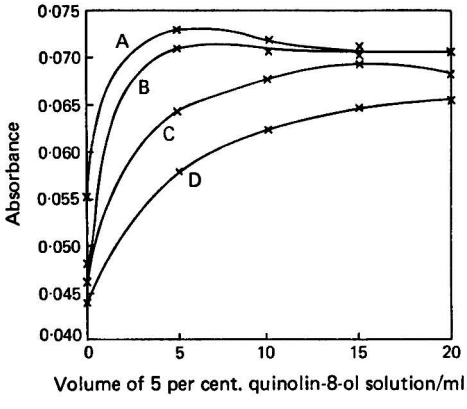

Fig. 2. Releasing effect on 5 p.p.m. of chromium of various amounts of 5 per cent. quinolin-8-ol solution in different concentrations of iron solution; final volume 100 ml. Iron solution: A, 0·1 per cent.; B, 0·2 per cent.; C, 0·5 per cent; and D, 1·0 per cent.

ments previously determined. The limiting element in the proposed sc heme is molybdenum, which, when present in its lowest range (0–1·0 per cent.), allows only a five-fold dilution in order to maintain the optimum concentration of 0–20 p.p.m. of molybdenum in the analyte (Table I). Consequently, with an initial solution of 1 per cent. *m/V*, the maximum dilution required to satisfy all conditions is to 0·2 per cent. *m/V*.

TABLE I

SAMPLE DILUTION

Element	Range in sample, per cent.	Concentration range in stock sample solution, p.p.m.	Dilution of initial to final volume* /ml	Dilution factor	Concentration range in analyte, p.p.m.	Stock iron solution added/ml
Nickel	0–1·0	0–100	20 to 100	5	0–20	0
	1·0–4·0	100–400	5 to 100	20	5–20	5·0
	2·5–10·0	250–1000	(*a*) 10 to 100			
			(*b*) 20 to 100	50	5–20	5·0
	5·0–20·0	500–2000	(*a*) 10 to 100			
			(*b*) 10 to 100	100	5–20	5·0
Manganese and copper	0–0·25	0–25	10 to 100	10	0–2·5	2·5
	0·10–1·0	10–100	(*a*) 25 to 100			
			(*b*) 10 to 100	40	0·25–2·5	5·0
	0·5–2·0	50–200	(*a*) 5 to 100			
			(*b*) 25 to 100	80	0·625–2·5	5·0
	1·0–5·0	100–500	(*a*) 5 to 100			
			(*b*) 10 to 100	200	0·5–2·5	5·0
Chromium	0–1·0	0–100	10 to 100	10	0–10	2·5
	1·0–5·0	100–500	(*a*) 10 to 100			
			(*b*) 20 to 100	50	2–10	5·0
	5·0–10·0	500–1000	(*a*) 10 to 100			
			(*b*) 10 to 100	100	5–10	5·0
	10–20	1000–2000	(*a*) 10 to 100			
			(*b*) 5 to 100	200	5–10	5·0
Molybdenum	0–1·0	0–100	20 to 100	5	0–20	0
	0·5–2·0	50–200	10 to 100	10	5–20	2·5
	1·0–4·0	100–400	5 to 100	20	5–20	5·0

* (*a*) and (*b*) denote two stages of a dilution.

The effect of varying the concentration of total solids in the analyte was determined and it was found that the analyte flow-rate through the nebuliser decreased in a linear manner from 3·8 to 3·2 ml min^{-1} as their concentration was increased from zero to 2·0 g per 100 ml. The result of this effect can be seen in Fig. 2, in which there is a significant suppression of absorbance as the iron content is increased from 0·1 to 1·0 per cent. *m/V* in the presence of 20 ml of 5 per cent. quinolin-8-ol solution.

The use of a composite scheme designed for as wide a range of alloys as possible requires that two conditions must be satisfied: the iron content of the analyte should be identical in samples and standards and the concentration of the total solids in the analyte should be constant so as to equalise nebulisation rates between samples and also between samples and standards.

Having fixed the concentration of the sample solution at 0·2 per cent. *m/V* in order to satisfy the above criteria, it can be seen from Fig. 2 that maximum absorbance for chromium is obtained with an addition of 5 ml of 5 per cent. *m/V* quinolin-8-ol solution and this amount is incorporated in the scheme.

Similar considerations applied to molybdenum (Fig. 3) indicated that, with a 0·2 per cent. *m/V* sample solution, satisfactory absorbance is obtained with 10 ml of 10 per cent. *m/V* ammonium chloride solution.

Ottaway and Pradhan[4] stated that the interference of iron in chromium determinations was strongly dependent on the flame height at which the absorbance measurements were made. In order to determine the correct burner height with the SP1900 instrument, absorbance measurements were made at various burner heights for two solutions: (a), 5 p.p.m. of chromium plus 5 ml of 5 per cent. *m/V* quinolin-8-ol and (b), as for (a) but with 0·2 per cent.

of iron added. The results of these determinations are given in Fig. 4, which shows that, at an observation height of 1·0 cm above the burner top, the presence of 5 ml of 5 per cent. *m/V* quinolin-8-ol completely removes the interference of iron with gas flow-rates of 1·2 l min^{-1} for acetylene and 4·8 l min^{-1} for air. This burner height setting is recommended in the method given below, as is the above flame composition, which is a compromise between complete removal of iron interference and maximum sensitivity.

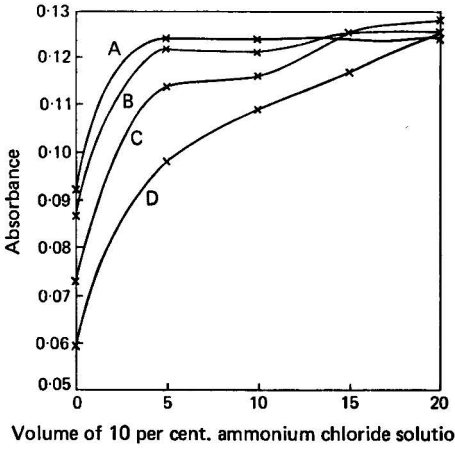

Fig. 3. Releasing effect on 20 p.p.m. of molybdenum of various amounts of 10 per cent. ammonium chloride solution in different concentrations of iron solution; final volume 100 ml. Iron solution: A, 0·1 per cent.; B, 0·2 per cent.; C, 0·5 per cent.; and D, 1·0 per cent.

A similar experiment was made in order to determine the optimum conditions for the molybdenum determination and the results are shown in Fig. 5, from which it can be seen that the interference of iron is completely removed at an observation height of 1·0 cm and, with a flame composition corresponding to 1·8 l min^{-1} for acetylene and 4·8 l min^{-1} for air, there is adequate sensitivity. This method of determining the optimum burner height setting for a given fuel to air ratio can be used for any type of instrument and is a necessary exercise if the information is not already established.

As a result of the above investigations it was decided to finalise the iron concentration at 0·2 per cent. in the method and to base the scheme on the following criteria: to restrict the scheme to the elements most commonly required to be determined in steel analysis; to use only the air - acetylene flame; and to dilute the sample solutions in order to obtain the optimum element concentration so that linear response could be obtained throughout each working range.

Method

Apparatus

All the determinations were made with the Pye Unicam SP1900 double-beam atomic-absorption spectrophotometer with digital read-out. The calibrated range of absorbance values is 0·0–1·999.

Solutions (Note 1)

Stock iron solution, 4 per cent. m/V. Dissolve 20 g of high-purity iron (BCS 260/4) in 100 ml of hydrochloric acid (sp. gr. 1·18) and cautiously oxidise it with the minimum amount of nitric acid (sp. gr. 1·42). Cool and dilute the solution to 500 ml with de-ionised water.

Quinolin-8-ol solution, 5 per cent. m/V. Dissolve 25 g of quinolin-8-ol in 25 ml of hydrochloric acid (sp. gr. 1·18). Cool and dilute the solution to 500 ml with de-ionised water.

Ammonium chloride solution, 10 per cent. m/V. Dissolve 25 g of ammonium chloride in de-ionised water and dilute the solution to 250 ml.

Primary standard metal solutions (nickel, copper, manganese, molybdenum, chromium). Dissolve

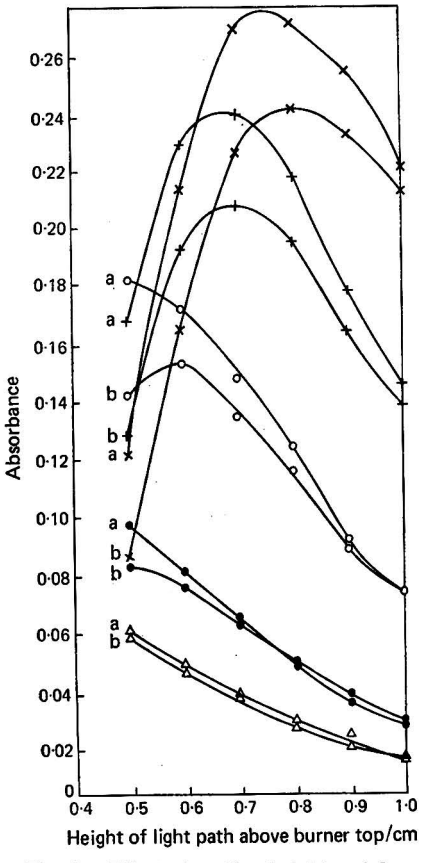

Fig. 4. Effect of reading height and flame composition on absorbance of 5 p.p.m. of chromium: a, 5 p.p.m. of Cr + 5 ml of 5 per cent. *m/V* quinolin-8-ol; b, 5 p.p.m. of Cr + 5 ml of 5 per cent. quinolin-8-ol + 0·2 per cent. of Fe. Air flow-rate: 4·8 l min^{-1}. Acetylene flow-rate: ×, 1·6; +, 1·4; ○, 1·2; ●, 1·0; and △, 0·8 l min^{-1}.

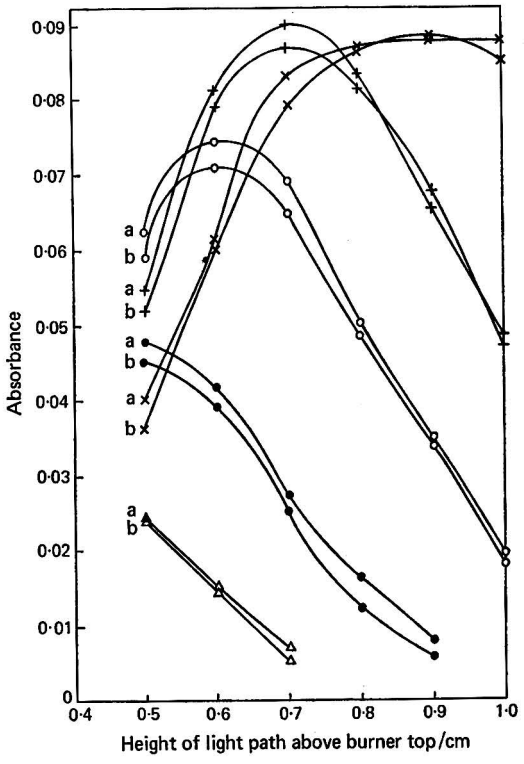

Fig. 5. Effect of reading height and flame composition on absorbance of 10 p.p.m. of molybdenum: a, 10 p.p.m. of Mo + 10 ml of 10 per cent. *m/V* NH_4Cl; b, 10 p.p.m. of Mo + 10 ml of 10 per cent. *m/V* NH_4Cl + 0·2 per cent. of Fe. Air flow-rate: 4·8 l min^{-1}. Acetylene flow-rate: ×, 2·0; +, 1·8; ○, 1·6; ●, 1·4; and △, 1·2 l min^{-1}.

0·5 g of the pure metal in 50 ml of hydrochloric acid (sp. gr. 1·18), warming gently. Cool and dilute the solutions to 500 ml (Note 2) (1 ml of solution ≡ 1 mg of metal).

Dilute standard nickel, copper and manganese solutions. Dilute 20 ml of the primary standard nickel solution to 100 ml (1 ml of solution ≡ 0·2 mg of nickel). Dilute 5 ml of the primary standard manganese and copper solutions to 200 ml (1 ml of solution ≡ 0·025 mg of metal).

Solution A. Transfer 25-ml aliquots of each of the dilute nickel, copper and manganese standard solutions into a 500-ml calibrated flask. Add 25 ml of stock iron solution (5 ml of solution ≡ 0·2 g of iron) and dilute to the mark. This solution contains 1·25 p.p.m. of manganese, 1·25 p.p.m. of copper and 10 p.p.m. of nickel.

Solution B. Transfer 50-ml aliquots of each of the dilute standard solutions into a 500-ml calibrated flask. Add 25 ml of stock iron solution and dilute to the mark. This solution contains 2·5 p.p.m. of manganese, 2·5 p.p.m. of copper and 20 p.p.m. of nickel.

For the blank solution, dilute 25 ml of the stock iron solution to 500 ml with de-ionised water. This solution is for use in determining the elements manganese, copper and nickel.

Dilute standard molybdenum solution. Dilute 20 ml of the primary standard molybdenum solution to 100 ml (1 ml of solution ≡ 0·2 mg of molybdenum).

Solution C. Transfer a 25-ml aliquot of the dilute standard molybdenum solution into a

500-ml calibrated flask. Add 25 ml of stock iron solution and 50 ml of 10 per cent. ammonium chloride solution. Dilute to the mark. This solution contains 10 p.p.m. of molybdenum.

Solution D. Transfer a 50-ml aliquot of the dilute standard molybdenum solution into a 500-ml calibrated flask. Add 25 ml of stock iron solution and 50 ml of 10 per cent. ammonium chloride solution. Dilute to the mark. This solution contains 20 p.p.m. of molybdenum.

For the blank solution, transfer 25 ml of the stock iron solution and 50 ml of 10 per cent. ammonium chloride solution into a 500-ml calibrated flask and dilute to the mark with de-ionised water. This solution is for use in determining the element molybdenum.

Dilute standard chromium solution. Dilute 25 ml of the primary standard chromium solution to 250 ml (1 ml of solution ≡ 0·1 mg of chromium).

Solution E. Transfer a 25-ml aliquot of the dilute standard chromium solution into a 500-ml calibrated flask. Add 25 ml of stock iron solution and 25 ml of 5 per cent. quinolin-8-ol solution. Dilute to the mark. This solution contains 5 p.p.m. of chromium.

Solution F. Transfer a 50-ml aliquot of the dilute standard chromium solution into a 500-ml calibrated flask. Add 25 ml of stock iron solution and 25 ml of 5 per cent. quinolin-8-ol solution. Dilute to the mark. This solution contains 10 p.p.m. of chromium.

For the blank solution, transfer 25 ml of the stock iron solution and 25 ml of 5 per cent. quinolin-8-ol solution into a 500-ml calibrated flask and dilute to the mark with de-ionised water. This solution is for use in determining the element chromium.

Preparation of Stock Sample Solution

Weigh 1 g of sample into a 250-ml conical beaker and dissolve it in 10 ml of hydrochloric acid (sp. gr. 1·18), warming gently. When solvent action ceases, add nitric acid (sp. gr. 1·42) dropwise until oxidation is complete and evaporate the solution just to dryness in order to remove the excess of nitric acid. Dissolve the residue in 10 ml of hydrochloric acid (sp. gr. 1·18), warming to obtain complete dissolution. Cool and dilute the solution with approximately 25 ml of water and note if any insoluble material remains. Filter the solution, if necessary, through a Whatman No. 40 filter-paper, washing the residue well with water. Dilute the filtrate to 100 ml with water. Certain types of high-silicon alloys may produce a further precipitate of silica at this stage, which must be filtered off before proceeding.

The above procedure may not give a complete solution of all types of alloy steel and insoluble portions would have to be filtered off, ignited and treated with hydrofluoric acid in a platinum vessel in order to ensure that all of the sample is brought into solution. In this event it is important to remove fluoride by evaporation with hydrochloric acid before combining the solution derived from the insoluble portion with that of the acid-soluble portion in the first filtrate.

Determination of Manganese, Nickel and Copper

Transfer a suitable aliquot (see Table I) of the stock sample solution into a 100-ml calibrated flask, add a suitable amount of the stock iron solution (see Table I) and dilute to the mark with de-ionised water. Aspirate the appropriate blank solution and zero the spectrophotometer, using the conditions specified in Table II. Continue as described under Absorption Measurement (see below).

TABLE II

INSTRUMENT CONDITIONS

Flame, air - acetylene, 10 cm; burner, in line; air flow-rate, 4·5–5·5 l min^{-1}; and integration period, 4 or 20 s.

	Manganese	Nickel	Copper	Chromium	Molybdenum
Wavelength/nm	279·5	341·5	324·8	357·9	313·3
Slit width/mm	0·10	0·10	0·20	0·20	0·10
Observation height/cm ..	0·8	1·0	1·0	1·0	0·8
Acetylene flow-rate/l min^{-1}..	1·4	1·0	1·0	1·2	1·8
Lamp current/mA	6	10	4	8	5

Determination of Molybdenum

Transfer a suitable aliquot of the stock sample solution (see Table I) into a 100-ml calibrated flask, add a suitable amount of the stock iron solution (see Table I) and 10 ml of 10 per cent.

m/V ammonium chloride solution, then dilute to the mark with de-ionised water. Continue as described under Determination of Manganese, Nickel and Copper.

Determination of Chromium (Note 3)

Transfer a suitable aliquot of the stock sample solution (see Table I) into a 100-ml calibrated flask. Add a suitable amount of the stock iron solution (see Table I) and 5 ml of 5 per cent. *m/V* quinolin-8-ol solution, then dilute to the mark with de-ionised water. Continue as described under Determination of Manganese, Nickel and Copper.

Absorption Measurement

In order to obtain the highest accuracy the value for the appropriate standard solution (A–F) should be determined before and after that for each sample and the average absorption value for the standard used for calculation of the result. Some degree of scale expansion can be used when the signal is sufficiently stable. Alternatively, the concentration read-out scale of the SP1900 instrument can be calibrated for a given element, using the high and low standard solutions as described in the instrument instructions, and the results read directly from the scale.

NOTES—

1. The stock iron and primary standard metal solutions can be stored in plastic bottles for future use. Similarly solutions A – F and the blank solutions prepared for the determination of each element are stable if stored in plastic bottles in a cool place.
2. In the preparation of the molybdenum solution the pure metal is dissolved in 100 ml of hydrochloric acid (sp. gr. 1·18) in order to prevent hydrolysis on dilution. The copper can be dissolved in a small volume of nitric acid prior to adding the hydrochloric acid.
3. It is preferable to make this determination after the instrument has been in use for at least 20 min so as to allow the burner to become thermally stable. This practice reduces any tendency for absorbance readings to drift and is simply arranged by determining the other elements before chromium.

TABLE III

RESULTS FOR ANALYSIS OF BRITISH CHEMICAL STANDARDS STEELS

Element	Steel type	BCS No.	Value found, per cent.	Certificate value, per cent.
Manganese	Low alloy	402	0·197, 0·197, 0·197, 0·196	0·19
	Low alloy	409	0·494, 0·488, 0·485, 0·494	0·48
	Ferritic stainless	342	0·89, 0·89, 0·89, 0·89	0·91
	Austenitic stainless	331	0·76, 0·76, 0·76, 0·76	0·78
	Austenitic stainless	336	0·81, 0·81, 0·81, 0·82	0·81
Chromium	Mild	274	0·191, 0·192, 0·185, 0·185	0·185
	Low alloy	402	0·56, 0·57, 0·56, 0·55	0·55
	Low alloy	409	1·26, 1·23, 1·23, 1·23	1·22
	Ferritic stainless	342	16·0, 16·2, 16·1, 16·0	16·15
	Austenitic stainless	331	15·3, 15·3, 15·3, 15·3	15·2
	Austenitic stainless	336	17·6, 17·6, 17·6, 17·6	17·6
Molybdenum	Mild	274	0·080, 0·066, 0·074, 0·080	0·070
	Low alloy	402	0·166, 0·159, 0·166, 0·153	0·16
	Low alloy	409	0·78, 0·77, 0·78, 0·79	0·77
	Ferritic stainless	342	0·69, 0·68, 0·70, 0·70	0·69
	Austenitic stainless	336	2·41, 2·44, 2·47, 2·44	2·43
Nickel	Mild	274	0·132, 0·132, 0·132, 0·132	0·125
	Low alloy	402	0·71, 0·71, 0·71, 0·72	0·71
	Low alloy	409	3·12, 3·12, 3·12, 3·12	3·14
	Ferritic stainless	342	2·15, 2·17, 2·18, 2·18	2·16
	Austenitic stainless	331	6·28, 6·31, 6·28, 6·22	6·26
	Austenitic stainless	336	9·44, 9·48, 9·44, 9·40	9·48
Copper	Mild	274	0·038, 0·038, 0·036, 0·036	0·040
	Low alloy	402	0·23, 0·23, 0·23, 0·23	0·23
	Low alloy	409	0·23, 0·23, 0·23, 0·23	0·23
	Austenitic stainless	336	0·118, 0·118, 0·117, 0·118	0·11

Results

British Chemical Standards steels were used to evaluate the analytical scheme and the results obtained, compared with the certified figures, are given in Table III. An integration time of 4 s was used except for high nickel, chromium and molybdenum contents, for which the time was increased to 20 s.

A further evaluation of the reproducibility of the instrument was made with standard solutions of each element. The results were expressed as a coefficient of variation and are based on ten replicate measurements at the concentrations shown in parentheses below.

	Manganese (2·5 p.p.m.)	Chromium (10 p.p.m.)	Molybdenum (20 p.p.m.)	Nickel (20 p.p.m.)	Copper (2·5 p.p.m.)
Coefficient of variation, per cent.	0·32	0·36	0·71	0·34	0·52

Discussion

Composite schemes of atomic-absorption analysis have been described for nickel base[6] and cobalt base alloys[7] based on the use of both air - acetylene and nitrous oxide - acetylene flames. The present work enables the analysis of complex steels to be made with the use of the air - acetylene flame only (except for the refractory oxide elements).

The effect of iron on the absorption of chromium and molybdenum has been studied and the findings of previous workers confirmed.[1,4,5] It has been shown, however, that in the presence of releasing agents for chromium[4] and molybdenum[5] the amount of iron can be reduced considerably, thus enabling the present scheme to be devised. Satisfactory results are given for a wide range of British Chemical Standards steels and this range can be extended, if desired, to include any element that can be determined in the air - acetylene flame.

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A Rapid and Sensitive Spectrophotometric Procedure for the Determination of Diphenhydramine and Related Ethers

B. Caddy, F. Fish and J. Tranter*

Division of Pharmacognosy and Forensic Science, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW

An oxidative procedure for the determination of diphenhydramine and related ethers is described. Its high sensitivity is a consequence of the oxidation of the drug to new products that exhibit high absorption in the ultraviolet region of the spectrum. The developed method is discussed with respect to precision, specificity, sensitivity and applicability to drug determination in biological samples.

The eight compounds examined in this work (Table I) all possess antihistaminic properties and are prescribed for the treatment of various allergic conditions. Orphenadrine, benztropine and chlorphenoxamine also have parasympatholytic activity and are mainly used as parasympatholytics. While an oxidation procedure for this group of drugs has previously been reported,[1] this paper reports a simpler and quicker oxidative - spectrophotometric method which, although less sensitive than the gas-chromatographic procedure of Vessman, Hartvig and Strömberg,[2] has been found to possess adequate sensitivity for the detection of diphenhydramine (and hence presumably the other ethers) in human urine following the ingestion of less than therapeutic doses.

Experimental

The reagents and apparatus, with the following exceptions, have been adequately described in an earlier paper.[1]

Apparatus

A Linson Instrument tilt-shaker, modified to accommodate six glass-stoppered tubes of approximately 40-ml capacity, and a roller extractor made in the laboratory following the design described by Moss,[3] were used.

Preparation of Standard Calibration Graphs

Dissolve a suitable amount of each drug (10 to 100 μg, as the salt specified in Table II) in approximately 4 ml of water contained in a 40-ml glass-stoppered test-tube. Add 5 ml of potassium dichromate solution (4 per cent.), 15 ml of sulphuric acid (66 per cent., prepared by mixing 6 volumes of concentrated sulphuric acid with 4 volumes of water) and 5 ml of spectroscopic grade hexane. Tilt-shake the tube for 30 min at room temperature, then remove an aliquot of the hexane layer and record its ultraviolet spectrum over the range 220–400 nm in a cell of 1-cm path length with hexane as reference.

Drug Administration

A subject was administered one 50-mg capsule of diphenhydramine hydrochloride twice daily for 2 d. The total urine voided over a period of 5 d, inclusive of those 2 d on which the capsules were administered, was collected. The urine collected over certain intervals (Table III) was bulked and aliquots from each bulked sample were assayed.

Extraction from Body Fluids

Place 10 ml of urine, 5 ml of 1 N sodium hydroxide solution and 100 ml of diethyl ether in a 500-ml reagent bottle. Roll the bottle for 20 min at approximately 40 rev min^{-1}, separate the ether layer, filter it through a Whatman No. 1 filter-paper, measure the volume and transfer it into a second reagent bottle together with 5 ml of 1 N sulphuric acid. Roll this

* Present address: Forensic Science Laboratory, 17th Floor, May House, Police Headquarters, Arsenal Street, Hong Kong.

second bottle for 20 min, separate the aqueous phase and evaporate 4 ml of it under reduced pressure at 50–60 °C for 3 min in order to remove the last traces of organic solvent. Oxidise the resulting acidic solution as described for the solutions of drug salts used in preparing calibration graphs.

Extractions from blood can be carried out in a similar manner, except that the blood is made alkaline by the addition of 2 or 3 ml of concentrated ammonia solution.

Results and Discussion

The oxidation products were identified by gas - liquid chromatography in conjunction with their ultraviolet spectral characteristics[1] (Table I).

TABLE I

GAS-CHROMATOGRAPHIC DATA AND $\lambda_{max.}$ VALUES IN HEXANE FOR THE OXIDATION PRODUCTS OF DIPHENHYDRAMINE AND RELATED ETHERS

Drug	Oxidation product	Relative retention time*	$\lambda_{max.}$/nm
Benztropine	Benzophenone	1·00 (*a*)	247
Bromodiphenhydramine	4-Bromobenzophenone	3·18 (*a*)	257
Chlorphenoxamine	4-Chlorobenzophenone	1·98 (*a*)	254
Deptropine	DiHDBCH†	0·97 (*c*)	264
Diphenhydramine	Benzophenone	1·00 (*a*)	247
Diphenylpyraline	Benzophenone	1·00 (*a*)	247
Embramine	4-Bromobenzophenone	3·08 (*a*)	257
Orphenadrine	2-Methylbenzophenone	1·10 (*b*)	247

*(*a*) 1 per cent. OV-25 at 160 °C with retention times relative to benzophenone: under these conditions benzophenone has a retention time relative to the solvent front of 2·4 min; (*b*) 10 per cent. Apiezon L, temperature programmed from 150 to 200 °C at 1 °C min^{-1}, retention time relative to benzophenone: under these conditions benzophenone has a retention time relative to the solvent front of 23·2 min; (*c*) 1 per cent. OV-25 at 190 °C with retention time relative to DiHDBCH: under these conditions DiHDBCH has a retention time relative to the solvent front of 3·3 min.

† 10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one.

Fifteen replicate assays were carried out on a 100-μg sample of diphenhydramine hydrochloride in order to assess the reproducibility. Under the experimental conditions employed no decomposition was apparent and the precision was found to be of a high order with a coefficient of variation of 1·7 per cent.

The specificity of the procedure for the determination of these drugs in biological samples is good within the limits described below. The extraction procedure eliminates interference from highly ultraviolet absorbing acidic and neutral drugs. As the oxidation is carried out under acidic conditions, highly absorbing basic drugs that are not affected by the oxidation do not partition into the hexane layer and, therefore, do not interfere. Interference from compounds that give rise to highly absorbing basic oxidation products (*e.g.*, basic benzophenones from drugs containing the benzodiazepine structure) does not occur as they also remain in the acidic phase. Acidic oxidation products from basic drugs (*e.g.*, benzoic acid from ephedrine), which partition into the hexane phase, can be eliminated by washing the latter with dilute sodium hydroxide solution.

The only unavoidable interference that may arise is from neutral oxidation products, with high $E^{1\%}_{1cm}$ values, formed from basic drugs. In this respect, all the drugs considered in this work must be considered as mutually interfering. Even though some of them will give rise to different products, the difference between their $\lambda_{max.}$ values is not sufficient to permit the determination of any two in admixture at the same time. Other drugs that may cause similar interference include amitriptyline and some of its derivatives. Obviously, it is necessary to establish qualitatively that only one of these ethers, and no amitriptyline, is present before the oxidative assay can be carried out. Other basic drugs that possess the diphenylmethylene group ($Ph_2C<$) and may be oxidised to benzophenones, but which do not interfere under the conditions used, include cyclizine and related amines and methadone and similar compounds.

Because of the high $E^{1\%}_{1cm}$ values for benzophenones (about 1000) the sensitivity is high and certainly higher than that obtained with direct ultraviolet spectrophotometry. The

minimum concentration in urine, which is easily measured, is approximately 1·5 μg ml^{-1} in a 10-ml sample volume. Absorbance values obtained for this concentration of different drugs range from about 0·07 for deptropine citrate to 0·14 for diphenhydramine hydrochloride, assuming that all the drugs have recoveries similar to that of diphenhydramine hydrochloride. By using the procedure on the scale described, background absorbance is low (0·02 absorbance unit or less), but if the ratio of urine to hexane is raised so as to increase sensitivity, appreciable background absorbance can arise. For example, with a 50-ml volume of urine sample and 2 ml of hexane and taking absorbance readings in cells of 2-cm path length, the background may be as high as 0·09 absorbance unit. However, it is anticipated that such an increase in sensitivity will not be required. When using the procedure on the scale proposed it is better to use a "blank urine" in order to determine the background rather than subtracting the absorbance at 300 nm of a test urine as recommended by Wallace,[4] as "blank urine" determinations usually exhibited a greater absorbance at 247 nm than at 300 nm.

Standard calibration graphs for all drugs studied in the range 0–20 μg ml^{-1} (equivalent drug concentration in the final hexane solution) were linear with little scatter (Table II). Assays for diphenhydramine hydrochloride added to blood and urine in the same concentration range also produced good linear absorbance - concentration relationships (Table II).

TABLE II

ABSORBANCE IN HEXANE OF VARIOUS CONCENTRATIONS OF DIPHENHYDRAMINE AND RELATED ETHERS FOLLOWING OXIDATION

Drug	$\lambda_{max.}$/nm	Final drug* concentration in hexane/μg ml^{-1} 20	15	10	5	2
		Absorbance† at $\lambda_{max.}$				
Benztropine	247	0·84	0·63	0·42	0·22	0·09
Bromodiphenhydramine	257	1·11	0·83	0·55	0·27	0·11
Chlorphenoxamine	254	1·15	0·88	0·58	0·29	0·12
Deptropine	264	0·60	0·45	0·31	0·14	0·07
Diphenhydramine	247	1·24	0·94	0·63	0·31	0·14
Diphenylpyraline	247	1·20	0·89	0·61	0·30	0·13
Embramine	257	0·86	0·64	0·43	0·22	0·09
Orphenadrine	247	0·91	0·67	0·45	0·22	0·09
Diphenhydramine extracted from blood‡	247	1·05	0·79	0·55	0·27	0·11
Diphenhydramine extracted from urine‡	247	1·12	0·80	0·55	0·27	0·11

* As the salt: benztropine mesylate, deptropine citrate, all others as hydrochloride.
† Reported absorbance values are the mean of at least two determinations.
‡ Corrected for ether and acid losses.

The results obtained for the assay of diphenhydramine in the urine of a subject given oral doses of this compound are given in Table III. Diphenhydramine was readily determined during the 2 d of its administration and for up to 30 h after the final capsule had been taken. The level determined between 30 and 54 h after final administration was low (0·5 μg ml^{-1}) and must be considered to be near to the minimum concentration detectable by the described procedure. No diphenhydramine was detected after 54 h.

TABLE III

CONCENTRATION IN URINE OF DIPHENHYDRAMINE HYDROCHLORIDE FOLLOWING ITS ORAL ADMINISTRATION

The first capsule taken at 0 h and the fourth (the last capsule) taken at 31 h. All determinations were carried out on 10-ml sample volumes.

Sample code	Time during which urine collected/h	Volume collected/ml	Drug concentration/μg ml^{-1}
D1	0–14	980	1·9
D2	14–37	1630	2·4
D3	37–61	1000	3·0
D4	61–85	1290	0·5
D5	85–103	1220	0·0

That diphenhydramine was determined by this procedure for up to 54 h after the final dose indicates that the method possesses adequate sensitivity for most purposes, especially when it is considered that the dosage was half that of the recommended drug regimen. From a normal therapeutic dose of diphenhydramine the peak level in blood is of the order of 1 μg ml^{-1} and thus from a 10-ml volume of blood sample the oxidation yield would be at the lower end (2 μg ml^{-1}) of the calibration graph. Although the method as applied to blood may not be sufficiently sensitive for routine use following therapeutic dosage, it should be adequate for detecting overdoses.

The drug values recorded are a measure of diphenhydramine and its demethylated metabolites. These latter may be expected to occur at low levels as they are suspected by Drach and Howell[5] to be intermediates in the formation of the major metabolite, diphenylmethoxyacetic acid, which exists in both free and bound forms. Diphenylmethoxyacetic acid is not determined by the proposed method because it is not extracted from urine under the conditions used. Similarly, another major metabolite, the *N*-oxide, reported by Drach, Howell, Borandy and Glazko,[6] is not determined.

Orphenadrine undergoes similar metabolic transformations to diphenhydramine[7] and hence it is possible that some of the other related ethers, particularly those in which the amine on the ether function is demethylated, also undergo similar degradation.

In conclusion, it is thought that the reported procedure is adequate for the routine determination of diphenhydramine and related ethers in urine following their therapeutic administration and also in blood in cases of drug overdosage.

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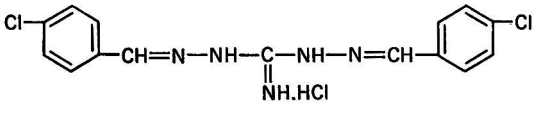
Simple Determination of the Coccidiostat Robenidine in Poultry Feed

G. F. Bories

I.N.R.A., Laboratoire de Recherches sur les Additifs Alimentaires, 180 chemin de Tournefeuille, 31300 Toulouse, France

1,3-Bis(4-chlorobenzylideneamino)guanidinium chloride (robenidine) is a new preventive coccidiostat used in poultry feed. A fast, accurate method of determination is proposed, which consists in extraction of the robenidine from the feed, purification of the extract by means of thin-layer chromatography and development of a yellow coloration by addition of sodium hydroxide to a solution of the robenidine in dimethylformamide. The absorbance is measured at 464 nm and is proportional to the concentration of robenidine between 0 and 3 μg ml^{-1}. No interference is observed during its determination in fatty or pigmented feeds.

Robenz,* robenidine or 1,3-bis(4-chlorobenzylideneamino)guanidinium chloride is a guanidine derivative containing a number of conjugated double bonds that undergo electronic reorganisation in an alkaline medium. This reorganisation results in a shift of the absorption



maximum to longer wavelengths. The absorption maximum of robenidine in dimethylformamide is about 373 nm; on addition of sodium or potassium hydroxide solution this value shifts to 464 nm and at the same time absorption by interfering substances is greatly diminished.

Experimental

Apparatus

A 1-l bottom-drive homogeniser, Desaga thin-layer chromatographic apparatus and a Jobin and Yvon spectrophotometer were used.

Reagents

Prepare chromatographic plates by coating 20 × 20 cm plates with a 0·5–0·75 mm thick layer of silica gel (Kieselgel G, Merck) and activating at 105 °C for 30 min.

Acetone - hydrochloric acid. Dissolve 1·66 ml of concentrated hydrochloric acid in 200 ml of acetone. The concentration of hydrochloric acid in this mixture is 0·1 M.

Dimethylformamide. Analytical-reagent grade.

Rhodamine B solution. Make up a 1 per cent. *m/V* solution of Rhodamine B (Eastman Kodak) in acetone.

Developing solvent, chloroform - methanol (95 + 5).

Sodium hydroxide solution, 1 M.

Procedure

Weigh 25 g of a feed containing 66 p.p.m. of robenidine and homogenise it for 3 min with 200 ml of acidified acetone. Filter the solution and place 50 ml of the filtrate in a round-bottomed flask. Evaporate the filtrate under vacuum to a volume of 1–2 ml, transfer this solution into a 5-ml calibrated flask and make up to the mark with acidified acetone.

* Cyanamid International registered trade name.

With a microsyringe, place 0·5 ml of the extract on to a chromatographic plate prepared as described above. Arrange margins on each side of the plate that are wide enough to enable a spot of the same extract plus 5 μl of a 10 μg ml^{-1} solution of robenidine in methanol to be placed on each margin so as to assist in locating the robenidine spot after chromatography. Place the plate in a tank lined with paper and containing the developing solvent. After complete development, dry the plate and render the two spots (R_F 0·75) in the margins visible by spraying with Rhodamine B solution. An ultraviolet lamp (wavelength 265 nm) can be used to detect with more precision the strip of robenidine corresponding to the feed extract. Outline and scrape off the area of silica gel containing the coccidiostat and transfer the powder into a 30-ml glass tube. Add 20 ml of dimethylformamide, close the tube and agitate the mixture well for several minutes. Filter the solution through a filter-paper into a 25-ml conical flask and add 0·1 ml of 1 M sodium hydroxide solution to the filtrate. During the next 15 min, measure the absorbance at 464 nm, using 1-cm cells, of the yellow coloration that develops and compare it with the absorbance of a reference solution of 0·1 ml of 1 M sodium hydroxide solution in 20 ml of dimethylformamide.

Solutions of concentrations 1, 1·5, 2 and 2·5 μg of robenidine per millilitre of dimethylformamide, to which 0·1 ml of 1 M sodium hydroxide solution has been added, are used as standards.

Results and Discussion

Colour Development and Measurement

When the robenidine is dissolved in dimethylformamide, addition of 1 M sodium hydroxide solution causes the immediate development of a yellow coloration having an absorption spectrum with a maximum at 464 nm. This coloration is stable for 20 min and for robenidine concentrations between 0 and 3 mg μl^{-1} Beer's law is obeyed. The effect of the volume of 1 M sodium hydroxide solution used has been studied (Fig. 1) and the results show that 0·1–0·2 ml of 1 M sodium hydroxide solution should be used for a solution of robenidine in 20 ml of dimethylformamide. The molar absorptivity under these conditions is 7·6 $\times$ 10^4 l mol^{-1} cm^{-1}.

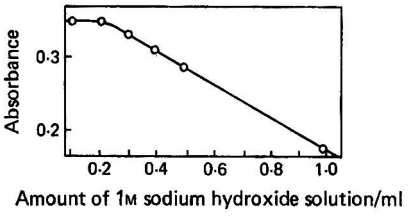


Fig. 1. Graph of absorbance *versus* volume of 1 M sodium hydroxide solution in 20 ml of dimethylformamide.

Recovery of Robenidine from Feeds

Robenidine can be extracted from feeds by grinding the feed in acidified acetone. A satisfactory extraction (95 per cent. recovery) can be achieved by using a 0·1 M solution of hydrochloric acid in acetone. Dilute solutions of robenidine are sensitive to light and undue exposure of extracts to bright light should be avoided.

The efficiency of the chromatographic purification and of the recovery from the chromatogram was tested by measuring known amounts (40 μg) of pure robenidine spotted on to the plates, either by themselves or with an extract of fatty (10 per cent. fat) or pigmented (4 per cent. lucerne) poultry feed that is composed of 65 per cent. of cereals (maize and wheat) and 20 per cent. of soya as well as fishmeals, vitamins and minerals. The results obtained are shown in Table I. Extracts of the feed with no robenidine, measured under the same conditions, showed a negligible blank value, thus proving the efficiency of the purification stage; the recovery rate is therefore high. The same table gives results of the determination of known amounts of robenidine added to a control feed before extraction, as well as the results of

TABLE I

RECOVERY OF ROBENIDINE FROM DIFFERENT SAMPLES

Recovery (per cent.) of 40 μg of pure robenidine deposited on chromatographic plates with—		Recovery (per cent.) of robenidine added to a feed to a level of 66 p.p.m. prior to extraction	Recovery (per cent.) of robenidine from a standard chicken feed containing 66 p.p.m. of robenidine
pigmented feed extract	fatty feed extract		
95	98·5	95·5	97
99·5	103	98·5	97
102	101	97	106
96	95	96·5	97
98	99	100	92·5
			95·5
			98·5
		Mean	.. .. 97·6
		Standard deviation	.. .. 4·2

determinations carried out on a chicken feed prepared from a pre-mix and containing 66 p.p.m. of Robenz. It should be noted that the presence of fat or pigments does not affect the percentage recovery.

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Use of Hydroxypropylmethylcellulose in the Determination of Chloride, Bromide or Iodide

M. Kapel, J. C. Fry* and D. R. Shelton

Procter Department of Food and Leather Science, University of Leeds, Leeds, LS2 9JT

A number of surface-active substances have been added to solutions of chloride, and the latter has been titrated with silver nitrate solution in the presence of an adsorption indicator. Although the use of agar was found to offer some advantages, that of hydroxypropylmethylcellulose gave rise to end-points that were much superior. The same additive also greatly facilitated the determinations of bromide and iodide. Attempts to determine these halides in admixture were, however, unsuccessful.

During the course of a different investigation, it was found that the presence of certain surface-active materials exerted a profound influence on the colloidal properties of silver chloride.[1] As the determination of halides by means of adsorption indicators is intimately connected with the colloidal properties of the silver halide produced, it seemed appropriate to examine the effects that surface-active polysaccharides might exert on this type of titration. There had been previous mention in the literature of the use of dextrin[2–4] and of some other materials[3–6] to facilitate the procedure, but many other potentially applicable substances did not appear to have been investigated in this connection.

In the present investigation, a number of surface-active materials were examined and, although agar was found to give greatly improved end-points in the determination of chloride, the use of hydroxypropylmethylcellulose was even more advantageous. The latter substance also gave excellent end-points with bromide and iodide. In each instance, the precipitate remained dispersed throughout the titration mixture and the end-point could be found by means of a colour change that seemed to involve the entire contents of the flask rather than simply a coagulated precipitate at the bottom. This fact added greatly to the convenience of the titration.

It was hoped that, by judicious choice of indicators used in conjunction with hydroxypropylmethylcellulose, the halides could be determined in admixture. Attempts in this direction, however, were unsuccessful.

Experimental

Reagents

Silver nitrate solution, 0·1 N.
Sodium chloride solution, approximately 0·03 N.
Potassium bromide solution, approximately 0·07 N.
Potassium iodide solution, approximately 0·1 N.
Fluorescein. Dissolve 0·2 g of fluorescein sodium in 100 ml of water.
Eosin. Dissolve 0·1 g of eosin in 100 ml of water.
Rose Bengal. Dissolve 0·15 g of Rose Bengal in 100 ml of water.
Diiodomethylfluorescein. Dissolve 1 g of diiododimethylfluorescein in 100 ml of 30 per cent. *V/V* aqueous ethanol.
Ammonium carbonate solution. Dissolve 5 g of ammonium carbonate in water and dilute to 100 ml.
Acetic acid, 6 N.
Hydroxypropylmethylcellulose. Treat 2 g of hydroxypropylmethylcellulose with 150 ml of hot water, allow to cool, stirring occasionally, and dilute to 200 ml with water.
Agar.

Procedure for the Determination of Halides

The halide solutions mentioned above were titrated with silver nitrate solution in the

* Present address: Consumer Products Development, The Boots Company Ltd., Nottingham, NG2 3AA.

presence of the adsorption indicators in accordance with the usual procedures. The determinations were then repeated in the presence of hydroxypropylmethylcellulose as described below.

A 20-ml aliquot of sodium chloride solution was treated with 8 drops of fluorescein and 2 ml of hydroxypropylmethylcellulose. The mixture was titrated with silver nitrate solution. At the end-point, the silver chloride precipitate remained dispersed and turned pink.

The same procedure was used for the determination of bromide, except that the amount of hydroxypropylmethylcellulose was increased to 5 ml. Iodide could be determined in a similar manner with the use of 3 ml of hydroxypropylmethylcellulose and the addition of 10 ml of water.

Bromide was also determined as follows. A 20-ml aliquot of potassium bromide solution was treated with 8 drops of eosin, 3 ml of hydroxypropylmethylcellulose and 1 ml of acetic acid. The mixture was titrated with silver nitrate solution. At the end-point, the dispersed silver bromide turned purple.

The procedure involving eosin also served for the determination of iodide provided that the mixture was diluted with 10 ml of water and the amount of hydroxypropylmethylcellulose increased to 5 ml.

Determination of iodide was attempted with two other indicators, diiododimethylfluorescein and Rose Bengal. Attempts with the former demonstrated that the presence of hydroxypropylmethylcellulose offered no advantage. When this surface-active material was used in conjunction with Rose Bengal, however, a suitable end-point was obtained in the presence of ammonium carbonate.

A 20-ml aliquot of potassium iodide solution was treated with 10 ml of water, 0·5 ml of Rose Bengal, 5 ml of hydroxypropylmethylcellulose and 0·7 ml of ammonium carbonate solution. The mixture was titrated with silver nitrate solution.

An attempt was made to use agar instead of hydroxypropylmethylcellulose in the determination of chloride. It was found that this substance also improved the end-point obtained but, owing to its lower solubility, it offered less advantage than hydroxypropylmethylcellulose.

Results

Table I gives the results of the titration of sodium chloride with silver nitrate solution in the presence and absence of hydroxypropylmethylcellulose. Fluorescein was used as indicator.

TABLE I

DETERMINATION OF CHLORIDE WITH SILVER NITRATE SOLUTION

Volume of 0·1 N silver nitrate solution required/ml	
In the presence of HPMC	In the absence of HPMC
6·75	6·65
6·75	6·65
6·75	6·65
6·78	6·70
6·80	6·70
6·80	6·70
6·80	6·75
6·80	6·75
6·80	6·80

From these results the value for the concentration of chloride obtained in the presence of hydroxypropylmethylcellulose was 1·20 g l^{-1} and in the absence of hydroxypropylmethylcellulose was 1·19 g l^{-1}. These values can be compared with the value of 1·20 g l^{-1} obtained by use of the Volhard titration.

When the methods for determining bromide and iodide were tested, there also appeared to be a satisfactory degree of correlation between the results found by the procedures recommended and the Volhard method. The relevant figures are given in Table II.

The range of concentrations over which the method is applicable is limited on the one hand by the detectability of the end-point and, on the other, by the capacity of the hydroxypropylmethylcellulose to keep the silver halide in a dispersed form. Although the limits of the range cannot be defined sharply, Table III gives figures recommended for use under the

conditions described above. It is possible, of course, that the use of different amounts of surface-active material might serve to provide an extension of the range.

TABLE II

DETERMINATION OF BROMIDE AND IODIDE WITH SILVER NITRATE SOLUTION

Halide	Indicator	Concentration of halide/g l^{-1}: Adsorption-indicator method	Volhard method
Bromide	Fluorescein and HPMC	5·06	5·04
	Eosin and HPMC	5·02	
Iodide	Fluorescein and HPMC	12·2	12·3
	Eosin and HPMC	12·2	
	Rose Bengal and HPMC	12·2	
	Rose Bengal	12·2	
	Diiododimethylfluorescein	12·2	

TABLE III

RANGE OF CONCENTRATIONS TO WHICH THE METHOD IS APPLICABLE

Halide	Indicator	Concentration range/N
Chloride	Fluorescein and HPMC	0·02–0·06
Bromide	Eosin and HPMC	0·01–0·07
Iodide	Rose Bengal and HPMC	0·02–0·06

Conclusions

The use of hydroxypropylmethylcellulose in the titration of halides with silver nitrate solution in the presence of adsorption indicators greatly facilitates the detection of the end-point. In this way, fluorescein can be used for the determination of chloride, bromide or iodide, eosin for bromide or iodide and Rose Bengal for iodide.

Our thanks are due to Professor A. G. Ward for his interest and encouragement and to British Celanese Limited for a gift of hydroxypropylmethylcellulose. Our thanks are also due to J. Sainsbury Limited for their generous financial support, which included a maintenance grant to one of the authors (J.C.F.). Another of the authors (D.R.S.) is indebted to the Science Research Council.

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Polarographic Studies on Some Organic Compounds of Arsenic

Part II.* Phenyl Arsenoxide

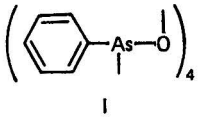
A. Watson† and G. Svehla

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

A study has been made of the polarographic behaviour of phenyl arsenoxide. Below 1×10^{-4} M concentration this compound gives rise to two well defined cathodic waves in acidic solutions below pH 2. The wave heights are diffusion controlled and proportional to concentration in the range 1×10^{-5} to 1×10^{-4} M. The current - potential relationships and the effect of pH, the electrode parameters and other factors have been investigated. With these and microcoulometric data the reaction path for both waves has been elucidated. The splitting of both waves at concentrations above 1×10^{-4} M by inhibition effects has been examined. The use of polarography has been proposed for the quantitative specific determination of phenyl arsenoxide and phenylarsonic acid in mutual mixtures.

For reasons similar to those given in Part I of this series for the arsonic acids[1] it was decided to carry out a comprehensive study on phenyl arsenoxide, the polarographic behaviour of which is discussed in the present paper.

Phenyl arsenoxide (arsenosobenzene) exists in the solid state as an oligomeric oxide (**I**) and in aqueous solutions as a monomeric dihydroxyl compound (**II**) (also known as phenylarsonous acid).



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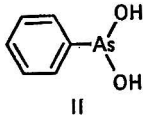


II

The phenyl arsenoxides are believed to have been the active agents produced by oxidation of the now obsolete drugs based on arsenobenzene, such as salvarsan.[2] The phenyl arsenoxides are the lower oxidation state of the phenylarsonic acids. The arsenoxides, however, are considerably more toxic and the maximum permitted levels are one or two orders lower.[3] In this paper a polarographic method is proposed for the specific determination of phenylarsonic acid and phenyl arsenoxide in mutual mixtures.

Although the arsenoxide was one of the earliest organic compounds to be examined polarographically, only one paper had appeared in the literature, that by Brdička[4] in 1933, which was concerned with the polarographic determination of the product (3-amino-4-hydroxyphenyl arsenoxide) of the oxidation in air of samples of salvarsan (3-amino-4-hydroxyarsenobenzene). Unfortunately, the work was carried out with unbuffered lithium chloride solutions and resulted in poorly formed waves, broad maxima and poor reproducibility. Phenyl arsenoxide was detected as an intermediate in a study of the electrolytic reduction of phenylarsonic acid by the present authors.[5] It was therefore considered to be of value to undertake a detailed study of the polarographic behaviour of phenyl arsenoxide.

Experimental

Apparatus

The apparatus used was identical with that described in Part I.[1]

* For details of Part I of this series, see reference list, p. 583.

† Present address: Max Planck Institut für Metallforschung, Institut für Werkstoffwissenschaften, Laboratorium für Reinstoffe, D-7070 Schwäbisch Gmünd, West Germany.

Reagents

Stock 5×10^{-3} M solutions of phenyl arsenoxide were prepared by shaking 5×10^{-4} mol with about 50 ml of 0·05 M potassium hydroxide solution until the solid had dissolved and then making the volume up to 100 ml with distilled water. It was found to be inadvisable to use this solution after it had been kept for more than 24 h. Acidic solutions prepared by dilution of this stock solution with hydrochloric acid were found to be stable for several weeks.

In most of the work 0·1 M hydrochloric acid was used as the supporting electrolyte. At other pH values the following buffer solutions were used: pH 1–3, hydrochloric acid - potassium chloride; pH 1·7–5·5, orthophosphoric acid - potassium dihydrogen orthophosphate (0·067 M); pH 3·5–5·5, acetic acid - sodium acetate (0·1 M); pH 5·5–8·5, potassium dihydrogen orthophosphate - disodium hydrogen orthophosphate (0·067 M); pH 7·5–13, boric acid (0·1 M) - sodium hydroxide; and pH 2–12, a standard Britton - Robinson buffer. In each instance analytical-reagent grade reagents were used.

Experimental Techniques

These were described in detail in Part I.[1]

For the macro-scale reduction 150 ml of a 5×10^{-3} M solution of phenyl arsenoxide in 0·1 M hydrochloric acid was placed above a large mercury-pool cathode (100 cm^2). The solution was de-aerated and a potential of −0·8 V set between the mercury-pool cathode and the reference electrode by passing a suitable current between the cathode and the silver-wire anode. The precipitate formed after several hours of electrolysis was filtered off and examined by mass spectroscopy.

Results and Discussion

The polarographic behaviour of phenyl arsenoxide was examined throughout the pH range 1–12 by using hydrochloric acid - potassium chloride, Britton - Robinson, acetate, phosphate and borax buffer solutions at a phenyl arsenoxide concentration of 5×10^{-5} M. Phenyl arsenoxide shows two main reduction phenomena i_1 and i_2 throughout this pH range. However, in the less acidic range above pH 2–3 the waves are very poorly formed and exhibit broad maxima. This finding is similar to the results obtained by Brdička.[4] A change of buffer at the same pH value did not alter or improve this situation. For this reason most of the work described in this paper has been carried out in 0·1 M hydrochloric acid, in which the waves obtained at concentrations below 1×10^{-4} M are well defined, reproducible and exhibit no maxima (Fig. 1).

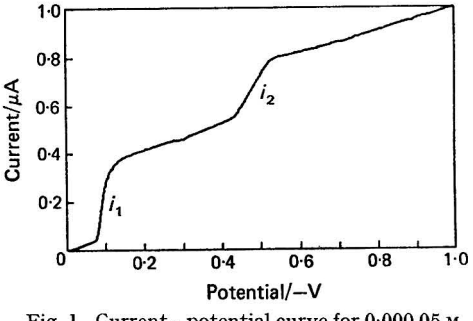


Fig. 1. Current - potential curve for 0·000 05 M solution of phenyl arsenoxide in 0·1 M HCl.

Characterisation of the Limiting Currents below 1×10^{-4} M Concentration

For each of several solid samples of phenyl arsenoxide a series of solutions in 0·1 M hydrochloric acid were prepared in which the concentration increased in steps of 1×10^{-5} M from 1×10^{-5} to 1×10^{-4} M. The results of linear regression analysis[6] show that a proportional relationship exists between the wave height of both waves and the concentration, behaviour characteristic of diffusion or kinetically controlled processes. The standard error of the

estimate is about 2 per cent. of the average current for both waves, an accuracy more consistent with diffusion control. Within experimental error the slopes of the graphs do not vary from sample to sample of the solid, confirming that solid phenyl arsenoxide yields a reproducible concentration of the electro-active form. The ratio of the two wave heights i_1 to i_2 is approximately 3:2.

The relationship between the wave height and the height of the mercury column was determined by linear regression analysis of the wave height *versus* the height and the square root of the height of the column as independent variables for both waves at 2·5 and 7·5 × 10^{-5} M. High positive values of the coefficients of correlation were found,[6] which indicate that neither wave is a kinetic or catalytic hydrogen wave, while the higher coefficients against the square root of the height of the column suggest diffusion rather than adsorption control. However, by themselves, the coefficients of correlation do not offer sufficient proof and so the intercepts of the regression line on both the axis for the height and the square root of the height of the column were compared with the predicted intercepts for diffusion and adsorption control and for the set of eight heights of the mercury column used. For both waves at these two concentrations the predicted intercepts for diffusion control fell within the tolerance intervals of the experimentally obtained intercepts while those for adsorption control did not.

For 5 × 10^{-5}, 7 × 10^{-5} and 1 × 10^{-4} M solutions of phenyl arsenoxide the instantaneous current during the drop lifetime was recorded oscillographically as a function of time at four potentials on the rising part of both waves from their base to the upper plateaux. Graphs were made of the logarithm of the instantaneous current *versus* the logarithm of the time.[6] These graphs were found to be curved owing to residual depletion effects carried on from the previous drop. As this effect is more marked at the beginning of the drop lifetime, the slope, corresponding to the exponent x in the instantaneous current - time relationship $i = kt^x$, was measured over the last 2 s of the drop lifetime. For the wave i_1 the exponent x lies in the region of the theoretical value 0·19 for a diffusion controlled process[7] at each potential from the base of the wave to the upper plateau, which also suggests that the reduction is reversible. For the wave i_2 the exponent x lies close to the theoretical value 0·19 only at the upper plateau and increases to some extent towards the base of the wave, thus confirming that the wave i_2 is diffusion controlled but suggesting that it is somewhat less reversible.

Effect of pH

The half-wave potential and wave height of the waves i_1 and i_2 were measured for 5 × 10^{-5} M phenyl arsenoxide solution at 25 pH values in the range pH 1–12 in a variety of buffers. The wave height of both waves was found to be independent of pH but increasingly less reproducible as the waves became more ill-defined with increasing pH. The graphs of half-wave potential *versus* pH showed a strong curvature as the processes i_1 and i_2 become increasingly irreversible with increasing pH. Change of buffer composition at the same pH had no effect.

The acidic region in which the waves are well formed and reproducible was examined in more detail with 5 × 10^{-5} and 1 × 10^{-4} M solutions of phenyl arsenoxide, prepared in hydrochloric acid - potassium chloride mixtures that differed by 0·2 pH unit from pH 1·0–2·2. Again both wave heights were found to be independent of pH. A linear relationship was found between the half-wave potential of each wave and the pH in this range. The slope calculated by linear regression analysis[6] for the wave i_1 lies close to the theoretical value of −0·059 V per pH unit for a reversible reduction involving an equal number of protons and electrons,[8] while the value for the wave i_2 is higher, thus suggesting that this process shows a lower degree of reversibility.

The Wave i_1

The most common way of investigating the reversibility of a process is by a logarithmic analysis of the shape of the wave. To this end five functions of current were plotted against the potential, corresponding to six current - potential relationships and six types of process, over the rising part of the wave i_1 for a series of different concentrations of phenyl arsenoxide. The same functions were used as in Part I. The coefficients of correlation indicate[6] that the function log $(i_d - i)$ *versus* potential has the most linear relationship (Fig. 2) and suggest that the wave i_1 corresponds to a reversible reduction to an insoluble product. The wave

(Fig. 1) has the characteristic shape, without an inflection point, for processes obeying this current - potential relationship.

However, the value of the reciprocal slope of this plot,[6] which one would expect to be 2·303 RT/nF, corresponds except at the lowest concentrations to a value of 1·25–1·35 electrons per molecule of phenyl arsenoxide, not an integral number; and yet both the instantaneous current - time curves and the half-wave potential *versus* pH graphs indicate that the wave is due to a reversible process. Further, the current - potential relationship[9] corresponding to the function log ($i_d - i$) applies only to a reversible process.

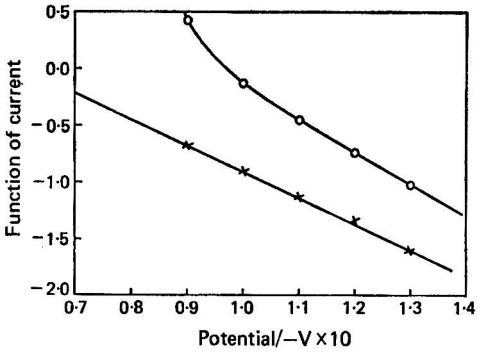


Fig. 2. Logarithmic analysis of the shape of the wave for 0·000 04 M phenyl arsenoxide solution. ○, Log $[(i_d - i)/i]$; and ×, log $(i_d - i)$.

By themselves, the coefficients of correlation do not offer sufficient proof. For each of the above functions of current and the corresponding current - potential relationship there exists an equivalent relationship between the half-wave potential and the logarithm of the concentration.[9,10] For processes in which log $[(i_d - i)/i]$ is linearly dependent on potential, the half-wave potential should be independent of concentration. For processes with log $[(i_d - i)/i^2]$ linearly dependent on potential, the half-wave potential should shift to more negative potentials at a rate of 2·303 RT/nF V for an increase in concentration of one decade, while for the remaining processes, including those with log $(i_d - i)$ linearly dependent on potential, the shift is to more positive potentials, also at a rate of 2·303 RT/nF per decade. Except at the very lowest concentrations a linear relationship was found to exist between the half-wave potential and the logarithm of the concentration (Fig. 3), with a slope of 0·043 ± 0·004 V per decade. This finding is in good agreement only with the reciprocal slope of the plots of log $(i_d - i)$ *versus* potential, confirming both the linear dependency of this function on potential and the value of 2·303 RT/nF.

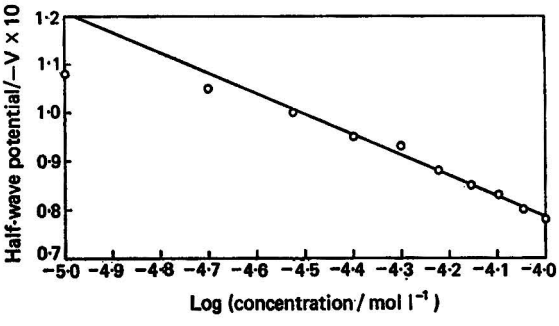


Fig. 3. Half-wave potential *versus* logarithm of the concentration of phenyl arsenoxide in 0·1 M HCl.

The half-wave potential was also found to be independent of the drop lifetime, a characteristic of most reversible processes.

The final conclusive evidence as to the reversibility is offered by direct measurement of the total number of electrons consumed per molecule by microcoulometry, that is, by electrolysis over a prolonged period on a small volume. From the decrease in the concentration and the total current passed the number of electrons per molecule was calculated for the wave i_1. The value for four 5×10^{-5} and four 1×10^{-4} M solutions of phenol arsenoxide in 0·1 M hydrochloric acid was indeed found to be $1{\cdot}30 \pm 0{\cdot}06$, as predicted by the logarithmic analysis of the wave shape, confirming the apparent reversibility of the process. As the molarity of the phenyl arsenoxide has been given in terms of a monomeric form, this value is the average number of electrons consumed per monomeric unit of phenyl arsenoxide.

As a possible explanation of the above behaviour the reaction path of the type

$$A + ne + nH^+ \rightleftharpoons Z$$

$$Z + (N-1)\,A \rightleftharpoons B\downarrow$$

can be proposed, in which an integral number of electrons is shared among several molecules of depolariser. If all the reactions were reversible the Nernst equation would take the form

$$E = E^\circ - \frac{RT}{nF} \ln \frac{[A]_e[A]_e^{N-1}[H^+]^n}{[B]_e}$$

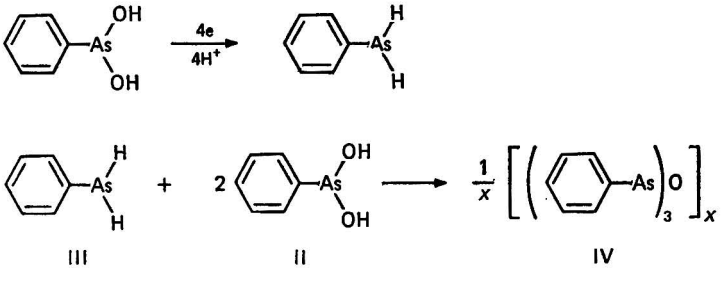
The activity, $[B]_e$, at the electrode of an insoluble product is a constant, and so by redefining the constant potential term to include this constant activity and substituting $(i_d - i)$, from the Ilkovič equation, for the activity $[A]_e$ of the depolariser at the electrode, the following current - potential relationship can be obtained

$$E = E^{\circ\prime} + \frac{NRT}{nF} \ln \frac{1}{KD_A^{\frac{1}{2}}} + \frac{NRT}{nF} \ln (i_d - i) + \frac{RT}{F} \ln [H^+]$$

This would give a linear graph of $\log (i_d - i)$ *versus* the potential, in which the reciprocal slope need not correspond to an integral number of electrons, and the slope of the half-wave potential - pH graph should be −0·059 V per pH unit, as was in fact observed.

The graphs of $\log (i_d - i)$ *versus* potential yield a value of 1·25–1·35 electrons per molecule of the electro-active form present, while microcoulometry gives a value of $1{\cdot}30 \pm 0{\cdot}06$ electrons per monomeric unit of phenyl arsenoxide. It can therefore be concluded with confidence that the phenyl arsenoxide is reduced from its monomeric form (**II**).

A reaction path similar to the above has already been proposed for the reduction of the phenyl arsenoxide intermediate in the macro-scale reduction of phenylarsonic acid.[5] In this reaction the intermediate corresponding to Z was identified as phenylarsine (**III**). The distinct odour of phenylarsine was also noticed during a macro-scale reduction of phenyl arsenoxide at a large mercury pool. This identification would give n the value of 4 and in order to yield a value of about 1·3 electrons consumed per molecule of phenyl arsenoxide, the value of N must on average lie close to 3, suggesting that the reaction path of the wave i_1 is as follows.



III II IV

The solid product formed in the macro-scale reduction of phenyl arsenoxide had a mass spectrum similar to that of the reduction products of phenylarsonic acid.[5] Computer analysis of this mass spectrum showed it to be the fragmentation pattern of a polymer such as **IV** containing three arsenic atoms to one of oxygen.

The Wave i_1 and Inhibition Effects

The build-up of an insoluble product at the electrode can in certain circumstances create an additional energy barrier for the depolariser to cross. Eventually, with increasing concentration, reduction at the potential of the original wave can occur only at the freshly created surface area of the growing drop and so the wave height becomes constant with respect to concentration and adopts characteristics similar to those of an adsorption wave. Further reduction must cross the inhibiting energy barrier and so a new wave will be formed at a higher potential. This new wave is frequently poorly formed and distorted. The original wave is often referred to as a pseudo-pre-wave.[11]

Above 1×10^{-4} M concentration the wave i_1 of phenyl arsenoxide begins to split and a second more negative, more poorly formed wave i_{1b} appears (Fig. 4), which rapidly moves to more negative potentials with increasing concentration. The wave height of the original wave i_1 becomes constant with respect to concentration, as can be seen in Figs. 4 and 5. The wave also develops a small maximum of the first kind. The total wave height of both processes i_1 and i_{1b} was found to have a proportional relationship with concentration up to 5×10^{-4} M, as the remaining depolariser is reduced at the higher potential of the wave i_{1b}.

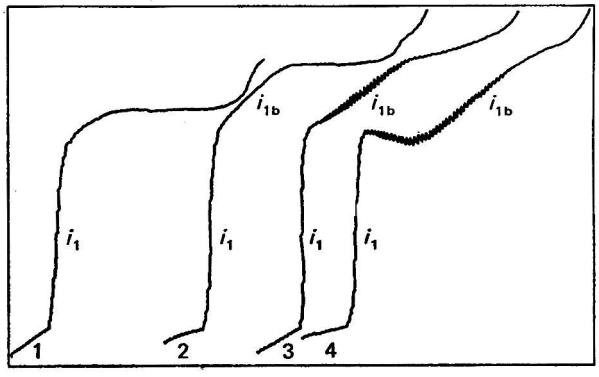


Fig. 4. Waves i_1 and i_{1b} at four concentrations in 0·1 M HCl: 1, 0·000 175; 2, 000 200; 3, 0·000 225; and 4, 0·000 250 M.

The relationship between the wave height and the height of the mercury column, for the wave i_1 at 5×10^{-4} and $7{\cdot}5 \times 10^{-4}$ M concentration of phenyl arsenoxide, was again determined by a statistical comparison of the graphs of wave height *versus* the height and the square root of the height of the mercury column. At these concentrations, however, the coefficients of correlation against the height of the column are the greater, suggesting an adsorption type of control.[6] The predicted intercepts, for adsorption control, of the regression line on the axis for the height and the square root of the height of the column fell within the tolerance intervals of the experimentally obtained intercepts, while those for diffusion control did not.[6]

Instantaneous current - time curves were recorded at −0·150 V on the upper plateau of the wave i_1 for solutions of $2{\cdot}5 \times 10^{-4}$, 5×10^{-4}, $7{\cdot}5 \times 10^{-4}$ and 1×10^{-3} M concentration, and were found to possess the same typical shape as curves due to an adsorption process, that is with a sharp increase to a maximum near the birth of the drop followed by a slower decrease in the current (Fig. 6). The graphs of the logarithm of the current *versus* the logarithm of the time were found to be strongly curved near the beginning of the drop lifetime. The slope, measured over the last 2 s of the drop lifetime, corresponding to the exponent x in the instantaneous current - time relationship $i = kt^x$, was found to lie close to the theoretical value of −0·33 for an adsorption controlled process.[6]

While a pseudo-pre-wave, limited by the build-up of an insoluble product at the electrode, has dependences on the concentration and the electrode parameters similar to those of an adsorption controlled process, it has different properties in other respects, which can be used to differentiate between the two processes. Raising the temperature encourages desorption of materials adsorbed on the electrode and so decreases the wave height of an adsorption

controlled process. For $2{\cdot}5 \times 10^{-4}$ and $7{\cdot}5 \times 10^{-4}$ M solutions the wave height of the wave i_1 was found to be independent of temperature between 20 and 70 °C. The addition of a strongly surface-active agent, such as Triton X-100, to the solution should eject the adsorbed material from the electrode, thus destroying the adsorption controlled wave. The current - potential curves of phenyl arsenoxide solutions containing 0·01 per cent. of Triton X-100 show that it is the wave i_{1b} that is eliminated, not the wave i_1, which remains unaffected. If the wave i_1 is a pseudo-pre-wave due to the uninhibited reduction of phenyl arsenoxide and the wave i_{1b} is due to reduction inhibited by a layer of insoluble product, the adsorption of Triton X-100 is unlikely to have a major effect on the wave i_1, but the creation of yet another energy barrier for the already unfavourable process i_{1b} to cross is likely to eliminate this process.

Above 4×10^{-4} M concentration the inhibition effects become more complex and a third wave i_{1c} appears between i_1 and i_{1b} (Fig. 5). The height of this wave i_{1c} changes little with concentration, while the wave i_{1b} and the over-all height of the three processes i_1, i_{1b} and i_{1c} become suppressed and actually decrease in height with increasing concentration.[6] The wave i_1 remains unaffected.

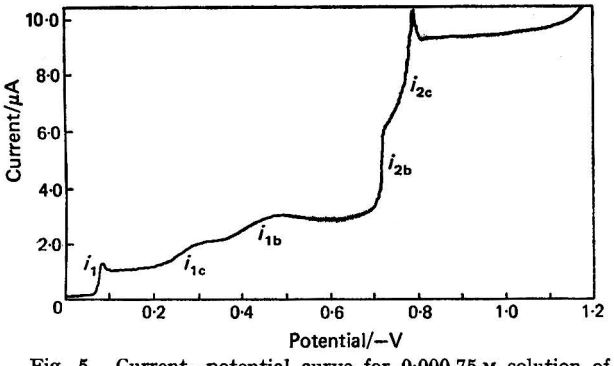


Fig. 5. Current - potential curve for 0·000 75 M solution of phenyl arsenoxide in 0·1 M HCl.

The instantaneous current - time curves for the waves i_{1b} and i_{1c} possess the complex shapes characteristic of inhibited processes (Fig. 6).

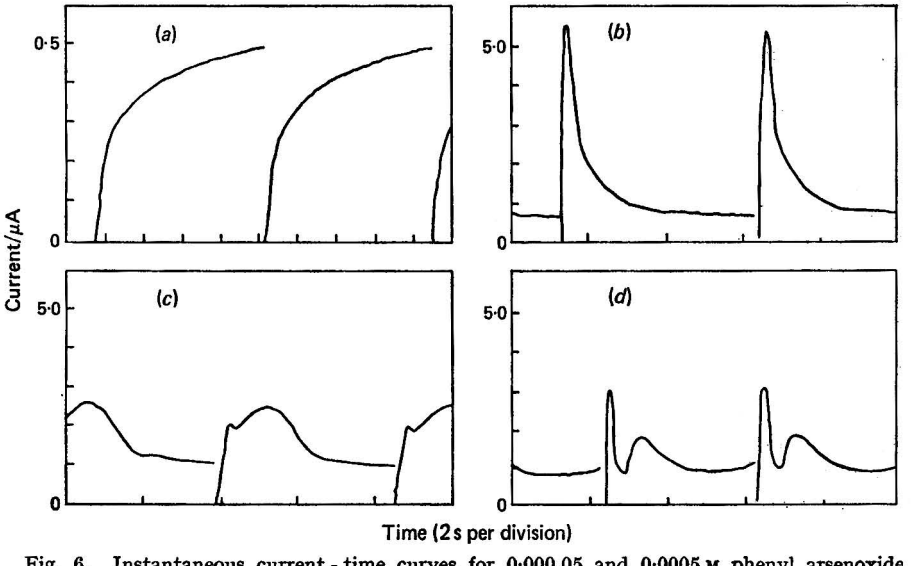


Fig. 6. Instantaneous current - time curves for 0·000 05 and 0·0005 M phenyl arsenoxide solution. (*a*), Wave i_1 at 0·000 05 M; and (*b*), (*c*) and (*d*), waves i_1, i_{1b} and i_{1c}, respectively, at 0·0005 M.

The Wave i_2

It has already been shown that the wave i_2 is diffusion controlled below 1×10^{-4} M concentration and has a proportional relationship between wave height and concentration. Above 1×10^{-4} M the wave i_2 begins to split into two waves, i_{2a} and i_{2b} (Fig. 7). At still higher concentrations a third wave appears, i_{2c} (Fig. 5).

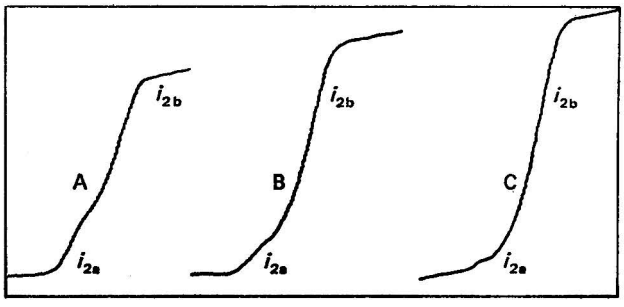


Fig. 7. Waves i_{2a} and i_{2b} at three concentrations in 0·1 M HCl: A, 0·000 175 M; B, 0·000 200 M; and C, 0·000 225 M.

The total current for all the waves i_1, i_2 and their components, measured at a series of concentrations from $2{\cdot}5 \times 10^{-5}$ to $2{\cdot}5 \times 10^{-4}$ M in steps of $2{\cdot}5 \times 10^{-5}$ M and from 1×10^{-4} to 1×10^{-3} M in steps of 1×10^{-4} M, was found to have a proportional relationship with concentration.[6]

The relationship between the total wave height of all the processes and the height of the mercury column was investigated as before for 5×10^{-4} and $7{\cdot}5 \times 10^{-4}$ M phenyl arsenoxide. The coefficients of correlation against the square root of the height of the column are the greater, suggesting diffusion control. The predicted intercepts, for diffusion control, of the regression line on the axis for the height and the square root of the height of the column fell within the tolerance intervals of the experimentally obtained intercepts, while those for adsorption control did not.[6]

Instantaneous current - time curves were recorded on the final upper plateau of the wave i_2 at two potentials for each of four concentrations. The slopes of the graphs of the logarithm of the current *versus* the logarithm of the time were measured over the last 2 s of the drop lifetime, when the curvature of the line can be neglected. This slope, x, corresponding to the exponent x in the instantaneous current - time relationship, $i = kt^x$, lies close to the theoretical value of 0·19 for a diffusion-controlled process for each concentration.[6]

The total number of electrons consumed per molecule was measured by microcoulometry at the upper plateau of the wave i_2. The value for four 5×10^{-5} and four 1×10^{-4} M solutions of phenyl arsenoxide in 0·1 M hydrochloric acid was found to be $1{\cdot}98 \pm 0{\cdot}04$.

When the over-all wave height of the processes i_1, i_{1b} and i_{1c} becomes limited with concentration, the total wave height of the over-all process i_2 continues to increase with the total current, for all the processes, in a manner proportional to concentration. Thus the wave i_2 cannot be the reduction of the product of the first wave i_1 as in this instance the wave height of the second wave would be controlled by the wave height of the first.

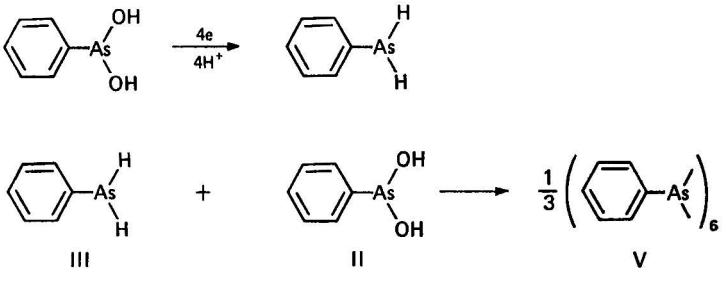
Below 1×10^{-4} M concentration both waves i_1 and i_2 are diffusion controlled and the possibility that the one wave is simply an adsorption pre-wave or post-wave of the other is eliminated. After prolonged electrolysis at the potential of the upper plateau of either wave the ratio of the two wave heights remains unaltered, which indicates that the two processes must involve either the reduction of the same species by different paths or separate species in equilibrium. It has been shown that both waves i_1 and i_2 remain unaltered in wave height by changes in pH, indicating that they are not due to differently protonated forms of phenyl arsenoxide. This inference is further supported by the fact that the pK_1 value is approximately 11.

Between 1×10^{-5} and 1×10^{-4} M the ratio of the two wave heights of the waves i_1 and i_2 remains constant (3:2), which is inconsistent with the fact that the waves i_1 and i_2 are the

reductions of monomeric and polymeric forms, respectively, of phenyl arsenoxide in equilibrium in the solution.

Considering the reaction path already proposed for the wave i_1, the most probable explanation consistent with the above facts is that the wave i_2 is due to an increase in the fraction of phenyl arsenoxide molecules that undergoes reduction to the arsine (**III**) and a decrease in the fraction of phenyl arsenoxide molecules that reacts with the arsine, the increase in the average number of electrons consumed per molecule forming the new wave i_2.

At the electrode there is competition for each molecule of phenyl arsenoxide between the electrochemical step and the "chemical" reaction with phenylarsine. One molecule of phenylarsine can react with several molecules of phenyl arsenoxide, but each successive reaction will probably become more difficult until the electrochemical step becomes more favourable and reduction to more phenylarsine occurs, thus controlling the stoicheiometry of the over-all reaction. For the wave i_1 it has been proposed that one molecule of phenylarsine reacts with two molecules of phenyl arsenoxide. On increasing the potential, the electrochemical step becomes more favourable while the "chemical" steps remain unaltered. At a certain potential one would therefore expect the reaction of the second phenyl arsenoxide molecule with phenylarsine to become less favourable than the electrochemical reduction, decreasing the total number of molecules consumed for one electrochemical step by one and increasing the total current flowing, that is, the formation of a new wave. The reaction path would then become



III II V

This reaction path consumes an average of two electrons per molecule to form arsenobenzene (**V**), the value obtained from the microcoulometric experiments for electrolysis at the upper plateau of the second wave.

The ratio of the two waves below 1×10^{-4} M concentration is fixed by the simple stoicheiometric ratios and this fact explains why it is unaltered by changes in pH, concentration (below 1×10^{-4} M) and prolonged electrolysis. When the height of the wave i_1 becomes limited with concentration owing to the build-up of its insoluble product at the electrode, this limitation need have no effect on the total current flowing at the potential of the wave i_2, as this insoluble product is not formed at these potentials. Arsenobenzene is equally insoluble but one need not expect it to have properties, as an inhibitor, identical with those of the product of the wave i_1.

Inhibition and the Wave i_2

A different type of inhibition effect is observed on the wave i_2. Above 1×10^{-4} M concentration the wave i_2 begins to split into two waves i_{2a} and i_{2b}. As can be seen from Fig. 7 the more positive wave i_{2a} decreases in wave height with increasing concentration until it is completely suppressed at 3×10^{-4} M, while the remaining current is carried by the wave i_{2b}. At still higher concentrations above 4×10^{-4} M the wave i_{2b} begins to split again into two waves, the original i_{2b} and a third wave i_{2c} (Fig. 5). The shape of both waves strongly suggests that they involve the ejection or rearrangement of an adsorbed inhibiting layer. The ratio of the current at the base of the wave i_{2c} to the total current has a constant value, 0·67.

The half-wave potentials of these three waves have a strong linear relationship (Fig. 8) with the logarithm of the concentration, with a slope of approximately 0·00, −0·16 and −0·32 V per decade for the waves i_{2a}, i_{2b} and i_{2c}, respectively.[6] It has already been shown

that the slopes of the half-wave potential *versus* pH graphs for the wave i_2 at concentrations below 1×10^{-4} M have a value of approximately −0·08 V per pH unit, which suggests that the half-wave potential of these waves can be expressed by the following relationship

$$E_{\frac{1}{2}} = E_k - \frac{2{\cdot}303\,RTXp}{\alpha nF} \log C_0 - \frac{2{\cdot}303\,RTp}{\alpha nF} \text{ pH}$$

where C_0 is the concentration of phenyl arsenoxide, p the number of protons concerned in the reduction preceding the rate-determining step, E_k a constant potential term and X has the value 0, 2 or 4 for the waves i_{2a}, i_{2b} and i_{2c}, respectively. This in turn could suggest that the wave i_{2a} is the uninhibited wave while the waves i_{2b} and i_{2c} are the same process but inhibited by the adsorption of oligomeric species produced by the reduction of two and four molecules of phenyl arsenoxide monomer, respectively, such as $H(C_6H_5As)_2OH$ and $H(C_6H_5As)_4OH$, two likely intermediates in the formation of the hexamer arsenobenzene (V) from phenylarsine and phenyl arsenoxide. The addition of 0·01 per cent. of Triton X-100 removes these inhibition effects and only one wave appears for the process i_2.

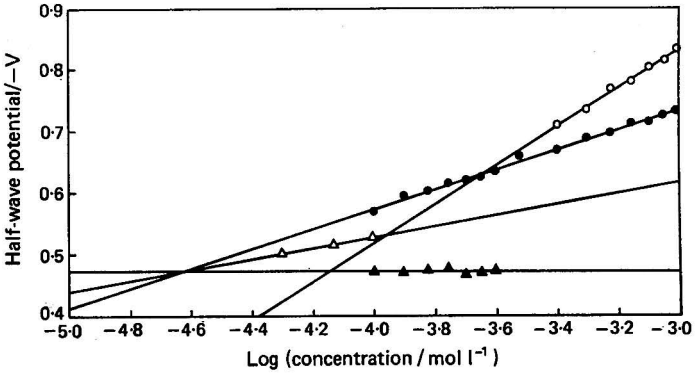


Fig. 8. Half-wave potential of waves i_{2a}, i_{2b} and i_{2c} *versus* logarithm of the concentration of phenyl arsenoxide. ▲, i_{2a}; ●, i_{2b}; ○, i_{2c}; and △, unresolved i_{2a} and i_{2b}.

Analytical Applications

Phenyl arsenoxide at concentrations below 1×10^{-4} M in 0·1 M hydrochloric acid gives rise to two well defined diffusion controlled waves. The wave heights are reproducible, proportional to concentration in the range 1×10^{-5} to 1×10^{-4} M and are independent of pH. These are suitable conditions for analytical application.

In analyses of known solutions of phenyl arsenoxide by the standard addition technique, with five additions of standard solution, errors of about 2 per cent. were obtained. Chloride, sulphate, nitrate, phosphate and acetate and constant amounts of ethanol, methanol and acetone were found not to interfere.

In a separate study,[1] phenylarsonic acid (the higher oxidation state) was found to give rise to a single well formed diffusion-controlled wave in 0·1 M hydrochloric acid at more negative potentials (−0·8 to −1·0 V) than the waves due to phenyl arsenoxide. As all three waves are separated by 300–400 mV below 1×10^{-4} M concentration and were found mutually not to interfere, polarography offers a simple, rapid and reliable method for the specific determination of the two compounds in mutual mixtures in the range 1×10^{-5} to 1×10^{-4} M of phenyl arsenoxide and 1×10^{-5} to 1×10^{-3} M of phenylarsonic acid. Above 1×10^{-4} M concentration of phenyl arsenoxide inhibition interferes with the "arsenoxide" waves and the shift of the wave i_2 to more negative potentials interferes with the wave of phenylarsonic acid. At higher concentrations of phenylarsonic acid the base of the "arsonic" wave interferes with the wave i_2 of phenylarsonic acid but not with the wave i_1. The use of the standard addition technique with five additions was found to give an error for both compounds of about 2 per cent. The phenylarsonic acids have extensive applications in agriculture[3] and

most of the available methods of analysis do not differentiate between the two oxidation states, which differ greatly in their toxicity.

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NOTE—Reference 1 is to Part I of this series.

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Polarographic Studies on Some Organic Compounds of Arsenic

Part III.* Triphenylarsine Oxide

A. Watson† and G. Svehla

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

A study has been made of the polarographic behaviour of triphenylarsine oxide. It gives rise to a single cathodic wave in 0·1 M hydrochloric acid, which is considerably complicated by inhibition effects. These effects are removed by the addition of a surface-active agent, thus yielding a well formed diffusion controlled wave the height of which is proportional to concentration (up to 1×10^{-3} M) and independent of pH. The current - potential relationships are discussed. On the basis of microcoulometric data and thin-layer chromatography the product of the electrode reaction has been shown to be triphenylarsine. Triphenylarsine oxide can be determined quantitatively in the presence of triphenylarsine by polarography.

In the reductions described in Parts I[1] and II[2] of this series phenylarsine reacted with phenyl arsenoxide, its own higher oxidation state, with the formation and loss of water by the reaction between the arsine hydrogen atom and the hydroxyl group of phenyl arsenoxide. Such a reaction is not possible for triphenylarsine, and so it was considered that a study of the polarographic behaviour of its oxidation product, triphenylarsine oxide, would make an interesting comparison with the investigations already undertaken on the phenylarsonic acids and phenyl arsenoxide.

Considerable interest has been shown in triphenylarsine oxide[3] and triphenylarsine[4] as ligands in co-ordination chemistry. Owing to the ease of oxidation of the arsine to the oxide, a method of analysis which differentiates between these two ligands would be useful. A suitable polarographic method is described in this paper.

A survey of the literature seemed to indicate that triphenylarsine oxide had not been studied from the electrochemical view-point. For these reasons it was decided to undertake the following study of the polarographic behaviour of triphenylarsine oxide.

Experimental

Apparatus

The apparatus used was identical with that described in Part I.[1]

Reagents

Stock 5×10^{-3} M solutions of triphenylarsine oxide were prepared in distilled water. These solutions proved entirely stable over a period of at least several weeks. In most of the work 0·1 M hydrochloric acid was used as the supporting electrolyte. At other pH values mixtures of hydrochloric acid and potassium chloride were used. The supporting electrolytes were of analytical-reagent grade.

In the chromatographic work the following solvents: methanol, ethanol, n-propanol, acetone, ethyl acetate, diethyl ether, chloroform, methylene chloride, toluene, carbon tetrachloride and cyclohexane, were tried singly or in mixtures. When possible analytical-reagent or spectroscopic grades were used.

Experimental Techniques

These were described in detail in Part I.[1]

* For Part II of this series, see p. 573.

† Present address: Max Planck Institut für Metallforschung, Institut für Werkstoffwissenschaften, Laboratorium für Reinstoffe, D-7070 Schwäbisch Gmünd, West Germany.

Results and Discussion

The polarographic behaviour of solutions of triphenylarsine oxide in 0·1 M hydrochloric acid was examined over the concentration range $2{\cdot}5 \times 10^{-5}$ to 1×10^{-4} M, and it was found that one main wave occurred at −0·75 to −0·85 V, which became much more complicated as a result of adsorption effects.

Below 1×10^{-4} M concentration some very poorly formed adsorption pre-waves can be observed (Fig. 1), the wave heights of which do not increase with increasing concentration.

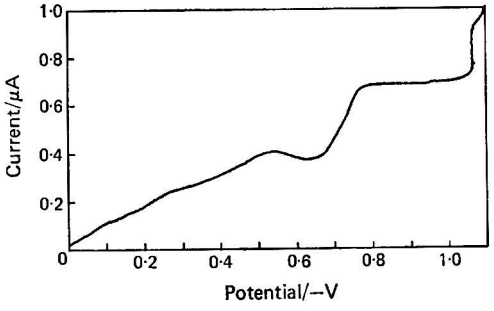


Fig. 1. Current - potential curve for 0·0001 M solution of triphenylarsine oxide.

Above 1×10^{-4} M these waves become so indistinct in shape that they can be observed only as a general increase in the residual current, and the upper plateau of the wave beyond a maximum of the first kind is divided into several distinct sections that are separated by sharp discontinuities in which the current either suddenly increases or decreases. The most important of these discontinuities occur at from −0·95 to −0·97 V and −1·06 to −1·10 V. For a given concentration these potentials are highly reproducible. Below 5×10^{-4} M concentration these sections increase in current stepwise (Fig. 2), forming two new waves, while above 5×10^{-4} M the current between −0·95 and −1·06 V is higher than the following section and is followed by a sharp decrease in current (Fig. 3). At still higher concentrations the pattern becomes even more complex (Fig. 4).

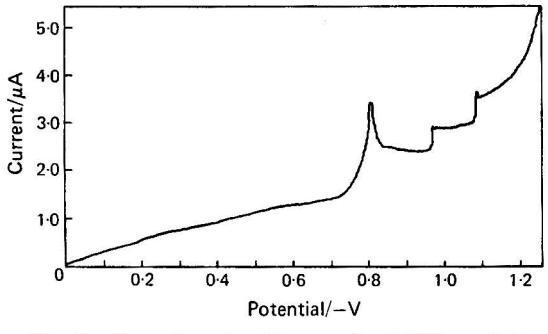


Fig. 2. Current - potential curve for 0·0003 M solution of triphenylarsine oxide.

Although the reproducibility is rather poor, the height of the central section of the plateau from −0·95 to −1·06 V has a proportional relationship with concentration, while the height of the two other main sections of the upper plateau would seem to be tending to a maximum value, thus causing the discontinuity at −1·06 V to change from a sharp increase to a sharp decrease above 5×10^{-4} M concentration.

The instantaneous current - time curves were recorded for several concentrations of tri phenylarsine oxide at 0·050-V intervals from −0·500 to −1·200 V. Each curve is suggestive of a diffusion controlled process with strong interference from inhibition effects. The complexity of each curve indicates the complexity of the different adsorption processes responsible

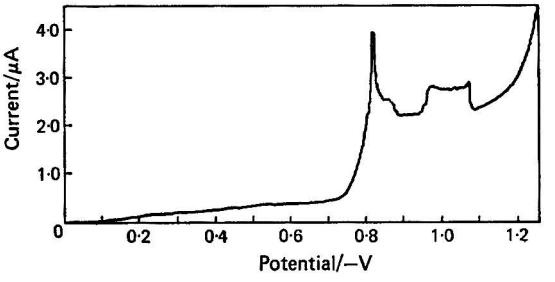


Fig. 3. Current - potential curve for 0·0005 M solution of triphenylarsine oxide.

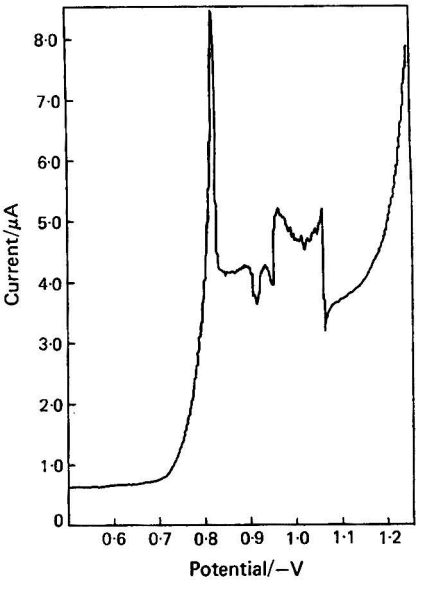


Fig. 4. Current - potential curve for 0·001 M solution of triphenylarsine oxide.

for the inhibition (Fig. 5). On the upper plateau the curves can be classified into three types, corresponding exactly to the potentials at which the sharp discontinuities in current occur. The complexity of the curves increases with increasing concentration.

Triphenylarsine oxide is a large bulky non-planar molecule and so there are many different possible adsorbed states of the molecule on the electrode. The adsorbed state that is stable at a particular potential may not be favourable to the electrochemical reduction. Further, a stable adsorbed layer of orientated molecules can create an energy barrier for the depolariser to cross, and so the wave becomes inhibited and the wave height decreases. As the potential is increased, the stability of the particular adsorbed layer decreases and at a certain potential rapid re-orientation of the layer can occur and so the inhibition vanishes, resulting in a sharp

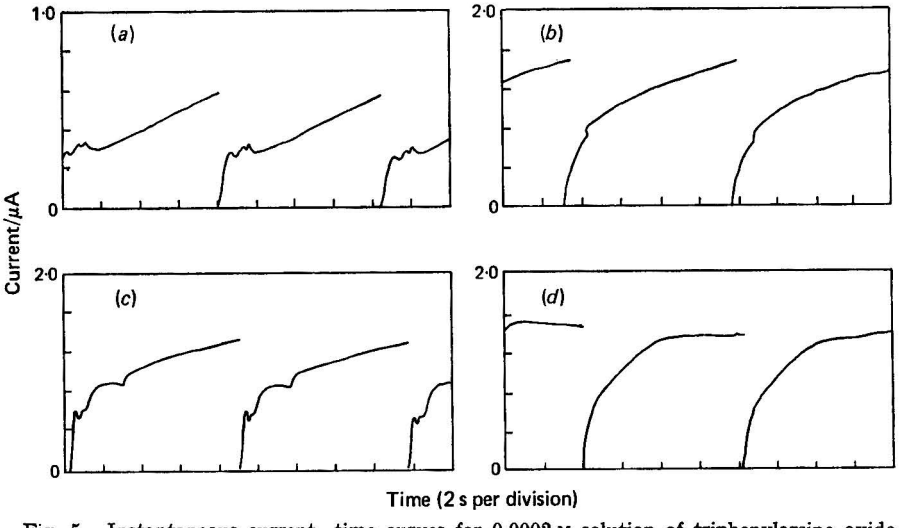


Fig. 5. Instantaneous current - time curves for 0·0002 M solution of triphenylarsine oxide: (*a*), at −0·775 V; (*b*), at −1·050 V; (*c*), at −0·900 V; and (*d*), at −1·150 V.

increase in current as was observed with triphenylarsine oxide. As several different adsorbed states can exist, several sharp discontinuities can occur. The situation will be even more complicated by the numerous adsorbed states of the probably equally bulky reduction product.

Clearly, measurements on the wave due to triphenylarsine oxide are not practical in the presence of these inhibition effects as the wave does not possess a clearly defined upper or lower plateau. Before any progress could be made in the investigation these effects had to be eliminated. To this end, a 20·0-ml volume of a 2×10^{-4} M solution of triphenylarsine oxide in 0·1 M hydrochloric acid was titrated with a 0·2 per cent. solution of the surface-active agent Triton X-100. The current - potential curve and instantaneous current - time curves were recorded after each addition.

The current beyond the second discontinuity at −1·1 V decreases rapidly and reaches the value preceding the discontinuity when the solution contains 0·0025 per cent. of Triton X-100, while the discontinuity itself remains at the same potential. The discontinuity initially at −0·960 V moves rapidly to more positive potentials until it reaches the rising part of the wave at a Triton X-100 concentration of 0·005 per cent., at which point the wave assumes a normal shape with well defined upper and lower plateaux (Figs. 6 and 7).

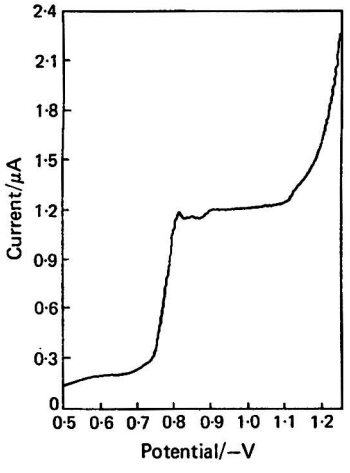


Fig. 6. Wave for 0·0002 M solution of triphenylarsine oxide in 0·1 M HCl containing 0·001 25 per cent. of Triton X-100.

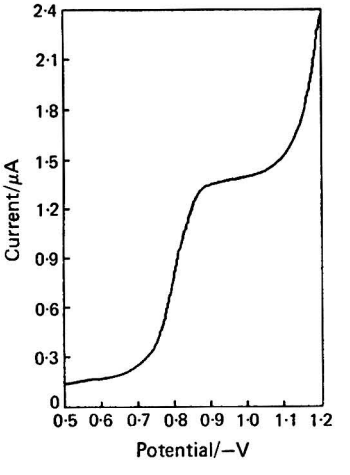


Fig. 7. Wave for 0·0002 M solution of triphenylarsine oxide in 0·1 M HCl containing 0·005 per-cent. of Triton X-100.

The initial addition of Triton X-100 greatly simplifies the shape of the instantaneous current - time curves. Further addition initially distorts the curves again but, finally, the distortion on the curves on the upper plateau of the wave disappears when the solution contains 0·005 per cent. of Triton X-100, and the curves [Fig. 8 (*a*)] become typical of an uninhibited diffusion controlled process. Inhibition effects unfortunately continue on the rising part of the wave [Fig. 8 (*b*)].

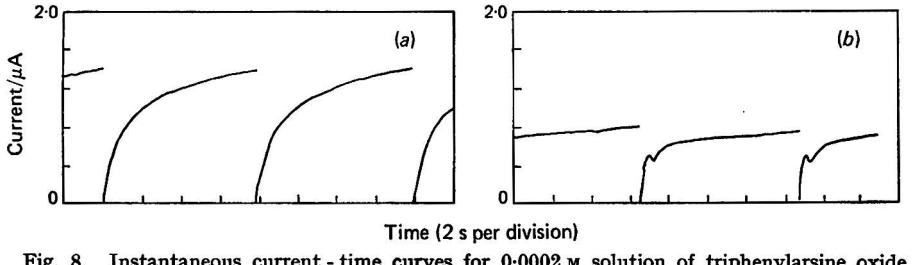


Fig. 8. Instantaneous current - time curves for 0·0002 M solution of triphenylarsine oxide in 0·005 per cent. Triton X-100: (*a*), −0·900 V; and (*b*), −0·775 V.

Further addition of Triton X-100 beyond 0·005 per cent. does not have a visible effect on the current - potential curve or the instantaneous current - time curves, probably because of the complete coverage of the electrode by Triton X-100 and the complete expulsion of the inhibiting layers at this concentration of Triton X-100. The remaining work was carried out on solutions containing 0·01 per cent. of Triton X-100.

Characterisation of the Limiting Current

For two solid samples of triphenylarsine oxide a series of solutions in 0·1 M hydrochloric acid containing 0·01 per cent. of Triton X-100 were prepared in which the concentration was increased in steps of 1×10^{-4} M from 1×10^{-4} to 1×10^{-3} M. Linear regression analysis showed[5] that a proportional relationship exists between the wave height and the concentration of triphenylarsine oxide, behaviour that is characteristic of a diffusion controlled process. The standard error of the estimate is of the order of 2–3 per cent. of the average wave height, which is slightly higher than the value that we obtained for the phenylarsonic acids[1] and phenyl arsenoxide.[2] The presence of a surface-active agent such as Triton X-100 frequently does lower the reproducibility. Within experimental error, the slopes of the graphs for the two solid samples are equal, confirming that solid triphenylarsine oxide yields a reproducible concentration of the electro-active form.

The relationship between the wave height and the height of the mercury column was determined by linear regression analysis of the wave height *versus* the height and the square root of the height of the column as independent variables for 2×10^{-4} and 7×10^{-4} M solutions of triphenylarsine oxide in 0·1 M hydrochloric acid containing 0·01 per cent. of Triton X-100. The high positive values of the coefficients of correlation indicated[5] that the wave is not a kinetic or catalytic hydrogen wave, while the higher coefficients against the square root of the height of the column suggest diffusion rather than adsorption control. However, by themselves, the coefficients of correlation do not offer sufficient proof and so the intercepts of the regression line on both the axis for the height and the square root of the height of the column were compared with the predicted intercepts for diffusion and adsorption control (see under Experimental Techniques) and for the set of eight heights of the mercury column used. The predicted intercepts for diffusion control fell within the tolerance intervals of the experimentally obtained intercepts while those for adsorption control did not.

Instantaneous current - time curves were recorded at four potentials on the upper plateau for four concentrations of triphenylarsine oxide in the presence of 0·01 per cent. of Triton X-100. The graphs of the logarithm of the current *versus* the logarithm of the time were found to be curved near the beginning of the drop lifetime. The slope, measured over the last 2 s of the drop lifetime, corresponding to the exponent x in the instantaneous current - time relationship $i = kt^x$, was found[5] to lie close to the theoretical value of 0·19 for a diffusion controlled process.[6]

Current - Potential Relationship and the Effect of pH

Current - potential curves were recorded for a series of 2×10^{-4} M solutions of triphenylarsine oxide in a mixed hydrochloric acid - potassium chloride supporting electrolyte, in which each solution differed by 0·2 pH unit from pH 1·0 to 2·2. The wave height was found to be independent of pH, as could be expected because triphenylarsine oxide does not possess any acidic protons. Linear regression analysis, excluding discordant data, on the graph of half-wave potential *versus* the pH of the solution gave a slope of $-0{\cdot}033 \pm 0{\cdot}004$ V per pH unit. However, the half-wave potentials for two pH values deviate considerably from the line (Fig. 9). This deviation was accompanied by a significant change in the slope of the rising part of the wave.

For this reason the work was repeated with a fresh series of solutions. Within experimental error the wave heights were equal to those of the first series of solutions. The graph of the half-wave potential *versus* the pH gave a slope of $-0{\cdot}045 \pm 0{\cdot}006$ V per pH unit, which is very different from that for the previous series, and yet the solutions were all prepared from the same original stock solutions. Once again it was observed that the slope of the rising part of the wave was very different for the waves corresponding to the two lines.

Further interpretation of the half-wave potential *versus* pH data clearly requires an analysis of the shape of the wave. To this end, various logarithmic functions of current were plotted

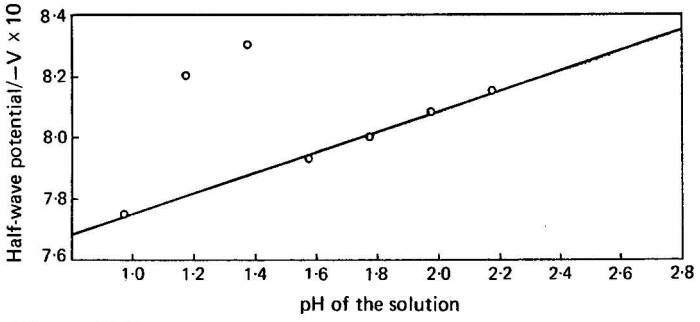


Fig. 9. Half-wave potential *versus* pH for series 1 of 0·0002 M solution of triphenylarsine oxide.

against potential on the rising part of the wave for each of the waves in the two series. The functions of current used were similar to those quoted in Part I.[1] Linear regression analysis gave consistently the highest coefficients of correlation for the graphs of log $[(i_d - i)/i^2]$ *versus* potential, indicating that this function has the best linear relationship with potential (Fig. 10). The apparent value of 2·303 $RT/\alpha nF$, given by the reciprocal slope of these graphs, is shown in Table I. Although there is a considerable variation in the value, those waves which fall on the same line in the half-wave potential *versus* pH graphs also share, within experimental error, the same apparent value of 2·303 $RT/\alpha nF$, while the divergent waves have different apparent values. The value for series 1 lies close to the theoretical value of 0·0296 V for a reversible reduction with two electrons, while for series 2 the value is somewhat higher, indicating a decrease in reversibility.

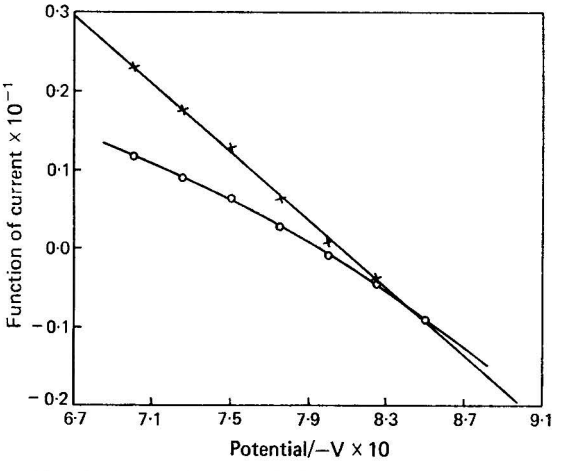
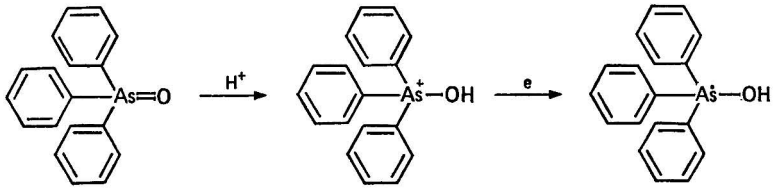


Fig. 10. Logarithmic analysis of the shape of the wave of 0·0002 M solution of triphenylarsine oxide at pH 1·175 (series 2). ○, Log $[(i_d - i)/i]$; and ×, log $[(i_d - i)/i^2]$.

The slope of the half-wave potential *versus* pH graph should be given by the product of 2·303 $RT/\alpha nF$ and an integral number corresponding to the number of protons involved in the reduction prior to the potential-determining step. We found that the slope of each line is equal, within experimental error,[5] to the corresponding apparent value of 2·303 $RT/\alpha nF$, indicating that one proton is involved prior to the potential-determining step. If, as is true for most irreversible processes, the addition of the first electron is the potential-determining step, then this would suggest that initially a rapid protonation of triphenylarsine oxide occurs, followed by the reduction of the resulting cation by the first electron, as shown below.



It has been shown that inhibition effects occur on the rising part of the wave but not at the limiting current, and this fact could be responsible for the lack of reproducibility in the value of $2{\cdot}303\ RT/\alpha nF$. Inhibition will cause a shifting of the potential for a given current to more negative values, thus altering the current - potential relationship. The linear dependence of log $[(i_d - i)/i^2)]$ on potential, obtained within experimental error, suggests that the potential term in the usual current - potential relationship (in which log $[(i_d - i)/i]$ is linearly dependent on potential) must be corrected by a further term that involves some function of the reciprocal of the current. This requirement is consistent with the decrease in the inhibition effects towards the upper plateau of the wave.

TABLE I

PREDICTED VALUE OF $2{\cdot}303\ RT/\alpha nF$ FROM GRAPHS OF LOG $[(i_d - i)/i^2]$ *versus* POTENTIAL FOR TWO SERIES OF SOLUTIONS OF TRIPHENYLARSINE OXIDE AT VARIOUS pH VALUES

Series 1		Series 2	
pH	$2{\cdot}303\ RT/\alpha nF$	pH	$2{\cdot}303\ RT/\alpha nF$
0·975	0·034	0·975*	0·033
1·175*	0·047	1·175	0·046
1·375*	0·046	1·375	0·046
1·575	0·029	1·575	0·047
1·775	0·034	1·775	0·046
1·975	0·033	1·975*	0·034
2·175	0·028	2·175	0·047

* The discordant values in the half-wave potential - pH graph.

Reaction Path

The number of electrons consumed per molecule in the reduction was directly determined for several solutions of triphenylarsine oxide, initially at 2×10^{-4} or 1×10^{-3} M concentration, by microcoulometry, that is, by means of prolonged electrolysis on a small volume at the potential of the upper plateau of the wave. From the decrease in the concentration and the current passed the number of electrons per molecule was calculated and found to be in the range $2{\cdot}03 \pm 0{\cdot}15$, which would suggest that triphenylarsine is the final product of the reduction.

However, positive "chemical" identification of the product is required before a reaction path can be proposed. To this end, 1-ml volumes of 1×10^{-3} M solution of triphenylarsine oxide were reduced so as to give a 50 per cent. decrease in wave height under the conditions for the microcoulometry and the products were examined by thin-layer chromatography.

As almost all organic compounds of arsenic can be extracted with diethyl ether from solutions with this acidity, the electrolysed solution was run out of the cell into a 1-ml glass-stoppered calibrated flask and shaken with six 1-ml volumes of ether. The layers were separated by careful use of a syringe fitted with a flat-tipped "canula." This technique of extraction was preferred, firstly, because of the small volumes involved and secondly, with the small amount of actual material involved (less than 0·1 mg) it was thought best to avoid possible contamination from the tap grease involved in the use of standard separating funnels.

The ether layers were successively poured into a small tube and the ether, and any trace amounts of water contained in it, were evaporated off by passing a stream of inert nitrogen through the tube. In order to ensure complete evaporation to dryness the nitrogen was passed through the tube for several hours after the addition of the last ether layer. This treatment was necessary as any trace amounts of water were found to alter completely

the subsequent chromatographic behaviour. The dry extract, which was small in amount but visible, was finally re-dissolved in 1 drop of ether (about 20 μl) and samples of it were drawn by capillary action into a 10-μl pipette and then spotted on to the silica gel chromatographic layer. Volumes (1 ml) of 1×10^{-3} M solution of triphenylarsine oxide and of 5×10^{-4} M solution of triphenylarsine were extracted and treated in a similar fashion in order to supply reference data.

A variety of eluting solvents were tried. Triphenylarsine oxide moves with the solvent front with methanol, ethanol, n-propanol and acetone, and remains stationary with the remaining single-solvent systems tried. Finally, a 50 + 50 mixture of n-propanol - ethyl acetate was found to give a well defined spot at R_F 0·7. Triphenylarsine was found to move with the solvent front with all the solvent systems tried except cyclohexane, with which it gives a well defined spot at R_F 0·6.

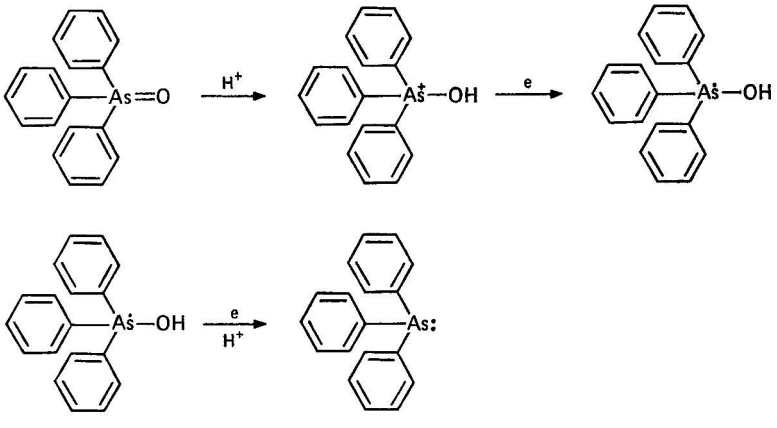
Two plates were spotted with the extract from the electrolysed solution and two plates each spotted with triphenylarsine oxide and triphenylarsine. The six plates were eluted first with cyclohexane and the R_F values obtained were noted. The solvent was allowed to evaporate and the plates were further eluted with the 50 + 50 mixture of n-propanol - ethyl acetate and the R_F values again noted (Table II). All the spots, except those at the solvent front in the second elution, were well defined when viewed under ultraviolet light.

TABLE II

R_F VALUES IN TWO SOLVENTS OF TRIPHENYLARSINE AND TRIPHENYLARSINE OXIDE AND THE EXTRACTS FROM THE PROLONGED ELECTROLYSIS OF TRIPHENYLARSINE OXIDE

		n-Propanol - ethyl acetate		Cyclohexane	
Compound	Plate	Spot 1	Spot 2	Spot 1	Spot 2
Triphenylarsine oxide	1	0·68	—	0·0	—
	2	0·72	—	0·0	—
Triphenylarsine	1	—	1·0	—	0·56
	2	—	1·0	—	0·58
Extract from the reduction ..	1	—	—	0·0	0·61
	2	0·71	1·0	0·0	0·58

These chromatographic results clearly show that after electrolysis the solution contains both unreduced triphenylarsine oxide and triphenylarsine. Other extractable organoarsenic compounds were not detected as no other spots were obtained at other R_F values in either solvent. From these and the previous results it is possible to propose the following reaction path for the polarographic reduction of triphenylarsine oxide.



The electrochemical step in the reduction of phenylarsonic acid[1] and phenyl arsenoxide[2] also yielded the arsine, which then reacted with a higher oxidation state by the elimination of water. Triphenylarsine, unlike the monophenylarsine, does not possess hydrogen atoms

bonded to the arsenic atom and so this reaction cannot occur and triphenylarsine remains the final product of the reduction.

Analytical Applications

Triphenylarsine oxide gives rise to a single well defined diffusion controlled wave in 0·1 M hydrochloric acid containing 0·01 per cent. of Triton X-100. The wave height is reproducible, proportional to concentration in the range 2×10^{-5} to 1×10^{-3} M and is independent of pH. Triphenylarsine is not electro-active. These conditions are suitable for the determination of triphenylarsine oxide in the presence of triphenylarsine by polarography. An error of 3–5 per cent. could be expected.

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NOTE—References 1 and 2 are to Parts I and II of this series, respectively.

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Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

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

Part IV.* Determination of Eugenol in Oil of Bay (*Pimenta racemosa, Miller*)

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

Report

The constitution of the Essential Oils Sub-Committee responsible for the preparation of this Report was: Mr. A. M. Humphrey (Chairman), Mr. J. H. Greaves, Mr. B. E. Kent, Mr. W. S. Matthews, Mr. D. A. Moyler, Mr. R. G. Perry, Mr. J. Ridlington, Mr. R. A. Stocks and Mr. G. Watson, with Mr. P. W. Shallis as Secretary and Mr. J. J. Wilson as Assistant Secretary.

Introduction

Phenols have traditionally been determined in essential oils by absorption into aqueous potassium hydroxide solution, as was originally proposed by Gildemeister and Hoffman.[1] Variations of this procedure have appeared in several national pharmacopoeias and official methods, including British Standard 2073.[2] The procedure has a number of disadvantages, particularly as several classes of substances, other than phenols, are absorbed by aqueous solutions of alkalis. This disadvantage applies particularly to oils that contain acetates of phenols and other acidic organic substances. Even if the method was specific for phenols, it would give only the total phenol content and would not distinguish between different phenols. This factor is important in the instance of oil of bay, which contains two phenols in large amounts, namely eugenol and chavicol. Furthermore, difficulty is often experienced in the determination of total phenols by the alkali-absorption method; for further details of this last method see British Standard 2073[2] and Garratt *et al.*[3]

For the above reasons the Essential Oils Sub-Committee investigated the application of gas - liquid chromatography to oil of bay. It was found necessary to confine attention to the determination of eugenol because this substance was available commercially in a high state of purity. No commercial source of chavicol, of sufficient purity for calibration purposes, was found.

Experimental

Earlier publications by the Essential Oils Sub-Committee[4–6] have established the general principles appropriate to quantitative gas-chromatographic methods for the analysis of essential oils. These principles have also been followed during the investigations on oil of bay *P. racemosa*).

An early problem to arise was the selection of a suitable internal standard. The first to be proposed was pentadecan-1-ol and the initial collaborative exercise employed this substance. It was found, however, that the readily available material was not of the required purity. Therefore, two further internal standards were proposed, ethyl 2-naphthyl ether and dodecan-1-ol. A further collaborative test was conducted with these two substances and the same sample of oil of bay as used previously.

Table I summarises the results obtained in the two investigations. The instructions for the participating laboratories were as follows.

Calibration Mixtures

Pentadecan-1-ol. Into a calibrated flask accurately weigh about 0·2 g of pentadecan-1-ol and 0·1 g of eugenol and dilute the mixture to 10 ml with propan-2-ol.

* For particulars of Parts I–III of this series, see reference list, p. 600.

Ethyl 2-naphthyl ether and dodecan-1-ol. Into a calibrated flask accurately weigh about 0·4 g of ethyl 2-naphthyl ether, 0·2 g of dodecan-1-ol and 0·4 g of eugenol and dilute the mixture to 10 ml with propan-2-ol.

Sample Mixtures

Pentadecan-1-ol. Into a calibrated flask accurately weigh about 0·2 g of pentadecan-1-ol and 0·2 g of sample and dilute the mixture to 10 ml with propan-2-ol.

Ethyl 2-naphthyl ether and dodecan-1-ol. Into a calibrated flask accurately weigh about 0·4 g of ethyl 2-naphthyl ether, 0·2 g of dodecan-1-ol and 0·8 g of sample and dilute to 10 ml with propan-2-ol.

Gas-chromatographic Conditions

The conditions were as described in the Appendix, except in details of stationary phase loading and column temperature; for these variations see below.

Procedure

Chromatograph each mixture three times and measure the appropriate peak heights and retention distances for the internal standard and eugenol peaks.

Calculations

From the chromatogram of each calibration mixture calculate the response factor, f, for that internal standard (eugenol = 1) by using the equation

$$f = \frac{\text{Internal standard response} \times \text{mass of eugenol}}{\text{Eugenol response} \times \text{mass of internal standard}}$$

Determine the mean response factor, f, then use this factor to calculate the percentage of eugenol in the sample, from each chromatogram, by using the equation

$$\text{Eugenol, per cent.} = \frac{f \times \text{Eugenol response} \times \text{mass of internal standard} \times 100}{\text{Internal standard response} \times \text{mass of sample}}$$

In the above equations, the term "response" is taken to mean the product of peak height and retention distance. This assessment of peak area was used for reasons given in an earlier publication by the Sub-Committee.[4]

At least three values for the percentage of eugenol were obtained for each internal standard. It will be noticed that, in the second series, two internal standards were used in the same mixture. In this instance a double set of calculations was necessary.

In practice, some deviations were made from the specified gas-chromatographic conditions. Thus, five of the participating laboratories used 15 per cent. Carbowax 20M columns, two of them used 10 per cent. Carbowax 20M, and one used 5 per cent. Carbowax 20M. Also, three laboratories did not use a column temperature of 160 °C; two of these laboratories used 190 °C and the other 200 °C. There were other minor variations in such parameters as carrier gas flow-rate and sample size. In the experience of the Sub-Committee a reasonable variation in conditions does not have a significant effect on quantitative results. It is necessary, however, to achieve the required separations on the chromatogram. With some samples of oil of bay there is a small peak immediately following the eugenol peak (see Fig. 1). This peak must be sufficiently resolved from the eugenol peak so that it has no material effect on the eugenol peak height.

An inspection of the values given in Table I indicates that, whereas some individual laboratories obtained results that differed according to the internal standard used, the over-all average results from each standard are in close agreement. Thus, the results are not dependent on the choice of internal standard. However, substances closely related to ethyl 2-naphthyl ether are reported to be carcinogenic, so it was decided to avoid any possible risk by not recommending the use of this substance. Therefore, dodecan-1-ol was used as the internal standard for all subsequent work on oil of bay. Some reservations were expressed because dodecan-1-ol is eluted well before eugenol, but the results in Table I show that this is not a disadvantage. Pentadecan-1-ol and ethyl 2-naphthyl ether are eluted between the eugenol and chavicol and would have been well suited to the determination of both of these phenols if a source of pure chavicol had been readily available.

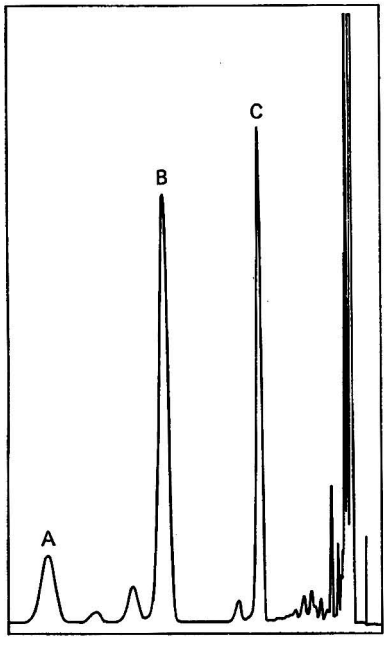

Fig. 1. Typical chromatogram of oil of bay. A, chavicol; B, eugenol; and C, dodecan-1-ol.

Accordingly, the next collaborative exercise consisted in the examination of three different samples of oil of bay, using dodecan-1-ol as the internal standard. The results are shown in Table II. The instructions for the tests were generally as described above, but the mixtures were as specified in the Appendix. The recommended solvent was changed from propan-2-ol

TABLE I

DETERMINATION OF EUGENOL IN OIL OF BAY WITH DIFFERENT INTERNAL STANDARDS

Laboratory	Eugenol found, per cent. Pentadecan-1-ol as internal standard		Ethyl 2-naphthyl ether as internal standard		Dodecan-1-ol as internal standard	
A	51·0					
	53·0	52·1		53·8		53·0
	52·1					
	52·2					
B	52·0		52·4		52·4	
	53·1	52·6	52·3	52·4	52·1	52·2
	52·8		52·4		52·0	
C	52·6		53·5		55·8	
	52·2	52·4	53·4	53·6	55·5	55·6
	52·4		53·9		55·4	
D	52·5		53·6		51·9	
	51·2	51·8	52·9	53·3	52·0	52·2
	51·8		53·3		52·8	
E	53·2		55·7		51·8	
	52·4	52·6	55·4	55·6	52·1	52·1
	52·3		55·7		52·5	
F	52·6		54·6		49·5	
	53·9	53·4	52·7	54·1	51·5	51·0
	53·9		52·8		51·9	
			56·2			
G			52·9		51·3	
			53·1	53·1	52·1	51·8
			53·2		51·9	

to ethyl acetate as some collaborating members reported that oil of bay was not completely soluble in propan-2-ol. In this exercise the majority of the participating laboratories used a column containing 10 per cent. Carbowax 20M, rather than 15 per cent.

TABLE II

DETERMINATION OF EUGENOL IN THREE SAMPLES OF OIL OF BAY

	Eugenol found, per cent.					
Laboratory	Sample 1		Sample 2		Sample 3	
A	57·9		41·6		52·0	
	58·2	57·5	41·6	41·5	50·6	51·1
	56·5		41·3		50·6	
B	57·3		42·3		51·7	
	57·3	57·5	42·2	42·3	51·6	51·6
	57·9		42·3		51·6	
C	57·7		42·3		51·9	
	57·5	57·8	42·4	42·4	52·3	52·2
	58·3		42·6		52·4	
D	58·3		40·4		51·6	
	57·6	57·9	40·5	40·5	51·5	51·5
	57·7		40·6		51·5	
E	58·9		41·8		52·2	
	59·6	59·1	41·4	41·7	52·7	52·5
	58·9		41·8		52·5	
F	56·0		43·3		52·3	
	51·5	54·5	43·4	42·9	51·3	50·1
	56·2		42·1		46·8	
G	64·8*		41·6		52·6	
	65·7*	65·2	42·2	41·9	53·7	53·2
	65·1*					
H	58·2		42·0		52·0	
	60·4	59·5	41·4	42·0	49·7	51·1
	59·7		42·7		51·6	
J	58·2		42·0		52·1	
	58·0	57·7	41·4	41·6	50·9	52·1
	57·0		41·3		53·2	

* An apparently anomalous set of results.

Although there is one set of anomalous results in Table II (laboratory G, sample 1), for which no explanation could be found, the results generally show good agreement both within and between laboratories.

The same samples of oil of bay were also examined for total phenols by the alkaline absorption method (British Standard 2073).[2] The results are shown in Table III. The figures are, of course, higher than the eugenol contents in Table II because of the presence of chavicol in the samples.

TABLE III

DETERMINATION OF TOTAL PHENOLS IN THREE SAMPLES OF OIL OF BAY

	Total phenols found, per cent. *V/V*		
Laboratory	Sample 1	Sample 2	Sample 3
A	64, 64	48, 47	55, 54
B	70	48	62
D	69	53	60
E	70	53	61
F	70	49	60
G	70	56	62
H	69, 70	50, 49	60, 61
J	70	54	62

In the next collaborative test, each participating laboratory was asked to prepare three different calibration mixtures, consisting of weighed amounts of eugenol and dodecan-1-ol diluted with ethyl acetate. The proportions of the two substances were such that in one

mixture (mixture 2), the two peaks on the chromatogram were of approximately equal heights, while in the other two, the eugenol and dodecan-1-ol peaks, respectively, were of substantially smaller height. Instructions for preparing the mixtures were as follows.

Mixture 1. Weigh out accurately 0·12 g of eugenol and 0·10 g of dodecan-1-ol, and dilute the mixture to 10 ml with ethyl acetate.
Mixture 2. As for mixture 1, but with 0·20 g of eugenol.
Mixture 3. As for mixture 1, but with 0·28 g of eugenol.

Each mixture was chromatographed three times and the factor, *f*, calculated as described in the Appendix. The results are shown in Table IV. Any variation in the value of *f* between the mixtures would indicate non-linearity of the gas-chromatographic response. It is evident from the results in Table IV that any non-linearity in the instruments used was not significant. Differences between response factors determined in individual laboratories are accounted for by the use of peak height multiplied by retention distance, which does not necessarily reflect an absolute measure of peak areas.

TABLE IV

RESPONSE FACTOR OF INTERNAL STANDARD

Laboratory	Response factor (eugenol = 1) Mixture 1		Mixture 2		Mixture 3	
B	1·070		1·060		1·065	
	1·073	1·07	1·059	1·06	1·065	1·06
	1·067		1·060		1·060	
C	0·924		0·940		0·914	
	0·930	0·92	0·912	0·92	0·921	0·92
	0·914		0·909		0·913	
D	1·202		1·215		1·182	
	1·220	1·21	1·253	1·23	1·199	1·19
	1·220		1·230		1·198	
E	1·427		1·393		1·395	
	1·438	1·43	1·374	1·39	1·399	1·39
	1·415		1·415		1·387	
H	1·050		1·045		1·040	
	1·049	1·05	1·040	1·04	1·042	1·04
	1·048		1·043		1·050	
J	1·399		1·318		1·314	
	1·391	1·35	1·325	1·33	1·312	1·30
	1·247		1·350		1·279	

For the final collaborative exercise, two fortified samples were prepared by mixing a further sample of oil of bay with two different weighed portions of pure eugenol. The fortified samples, together with the unfortified sample of oil of bay, were examined by the method given in the Appendix. Two laboratories also submitted results in which the peak areas had been obtained by means of an integrator.

In order to calculate the recovery of the eugenol added to the fortified samples it is necessary to have an estimate of the eugenol content of the unfortified oil. The recovery values in Table V were calculated by using the value of 50·01 per cent., which is the average value for eugenol content obtained from all participating laboratories (excluding figures obtained with the use of an integrator); Table VI shows recovery values in which the eugenol content of the unfortified oil is taken to be the mean of the three values obtained by the individual laboratory. Table VII corresponds to Table VI except that it contains the results obtained by two laboratories using chromatographs linked to integrators. In these last three tables the eugenol contents of the fortified oils were first calculated from the amounts of oil and eugenol used to prepare them. Then the directly measured values for eugenol content were expressed as a percentage of the calculated value, giving the recovery figures recorded in the tables. In every instance the average value from each laboratory for each sample is shown.

The results shown in Tables II and VI have been analysed statistically in order to establish the relative standard deviations both within and between laboratories,[7] and these variations are shown in Table VIII. The Sub-Committee is grateful to Dr. D. Kealey of Kingston Polytechnic, who carried out the statistical treatment reported in Table VIII.

TABLE V

RECOVERY OF EUGENOL FROM FORTIFIED SAMPLES TAKING 50·01 PER CENT. AS THE MEAN VALUE FOR EUGENOL IN UNFORTIFIED OIL

Theoretical eugenol content of fortified sample 1 = 52·71 per cent.; theoretical eugenol content of fortified sample 2 = 55·02 per cent.

Laboratory	Eugenol found, per cent. Sample 1		Eugenol found, per cent. Sample 2		Recovery, per cent. Sample 1	Recovery, per cent. Sample 2
A	51·3		54·8			
	52·4	52·03	54·5	54·77	98·7	99·6
	52·4		55·0			
B	52·3		55·1			
	52·8	52·53	55·3	55·33	99·7	100·6
	52·5		55·6			
C	53·1		54·7			
	52·5	52·83	54·9	54·93	100·2	99·8
	52·9		55·2			
D	53·1		54·2			
	53·0	53·23	55·3	54·70	101·0	99·4
	53·6		54·6			
E	53·1		53·9			
	53·9	53·30	55·6	54·63	101·1	99·3
	52·9		54·4			
F	53·8		56·1			
	53·0	53·23	55·1	55·40	101·0	100·7
	52·9		55·0			
H	52·5		55·3			
	52·6	52·67	54·9	55·20	99·9	100·3
	52·9		55·4			
J		51·80		55·10	98·3	100·1

TABLE VI

RECOVERY OF EUGENOL FROM FORTIFIED SAMPLES TAKING THE MEAN OF THREE VALUES AS THE EUGENOL CONTENT OF UNFORTIFIED OIL

Laboratory	Eugenol found in bay oil, per cent.		Calculated eugenol content, per cent. Sample 1	Calculated eugenol content, per cent. Sample 2	Mean eugenol* found, per cent. Sample 1	Mean eugenol* found, per cent. Sample 2	Recovery, per cent. Sample 1	Recovery, per cent. Sample 2
A	48·7							
	49·4	48·97	51·73	54·08	52·03	54·77	100·6	101·3
	48·8							
B	50·0							
	50·4	50·20	52·89	55·19	52·53	55·33	99·3	100·3
	50·2							
C	50·2							
	50·3	50·20	52·89	55·19	52·83	54·93	99·0	99·5
	50·1							
D	50·1							
	49·9	50·03	52·73	55·04	53·23	54·70	100·9	99·4
	50·1							
E	49·9							
	51·6	50·73	53·39	55·67	53·30	54·63	99·8	98·2
	50·7							
F	50·6							
	50·7	50·40	53·08	55·37	53·23	55·40	100·3	100·1
	49·9							
H	49·7							
	49·8	49·67	52·39	54·71	52·67	55·20	100·5	100·9
	49·5							
J		49·90	52·61	54·92	51·80	55·10	98·5	100·3

* Individual figures as given in Table V.

Conclusions

The quantitative determination of eugenol in commercially available samples of oils of bay (*P. racemosa*) can be satisfactorily achieved by use of a gas - liquid chromatographic procedure.

TABLE VII

RECOVERY OF EUGENOL FROM FORTIFIED OILS AS CALCULATED FROM INTEGRATOR RESULTS

Laboratory	Eugenol found in bay oil, per cent.		Calculated eugenol content, per cent. Sample 1	Calculated eugenol content, per cent. Sample 2	Eugenol found, per cent. Sample 1		Eugenol found, per cent. Sample 2		Recovery, per cent. Sample 1	Recovery, per cent. Sample 2
E	50·1				52·7		54·5			
	50·0	50·10	52·80	55·10	52·6	52·60	55·0	54·87	99·6	99·6
	50·2				52·5		55·1			
H	50·5				53·2		55·7			
	50·2	50·27	52·96	55·25	52·8	52·93	55·3	55·47	99·9	100·4
	50·1				52·8		55·4			

Based on experience of using a range of operating conditions the Sub-Committee recommends the procedure given in the Appendix, which should provide accurate results for the determination of the eugenol content with an acceptable level of precision and should reduce variations, both within and between laboratories, to a satisfactory level. The linearity experiments show that satisfactory results are obtainable when applying the procedure to oils that contain a higher or lower eugenol content than those used in the collaborative exercise. The recovery experiments show that satisfactory levels of accuracy can be obtained when determining eugenol at the levels likely to be found in commercial samples.

TABLE VIII

RELATIVE STANDARD DEVIATIONS

	Results	Within laboratories, per cent.	Between laboratories, per cent.	Over-all, per cent.
Table II	Sample 1..	1·8	4·8	5·1
	Sample 2..	0·9	1·0	1·8
	Sample 3..	2·3	1·1	2·5
Table VI	Bay oil	0·8	1·0	1·3
	Eugenol found: sample 1	0·8	0·8	1·1
	Eugenol found: sample 2	0·9	0·2	0·9

APPENDIX

Recommended Method for the Determination of Eugenol in Oil of Bay (*P. racemosa*) by Gas - Liquid Chromatography

Operating Conditions

It is essential that, throughout the determination, the operating conditions are maintained as constant as is practicable. It is also essential to use the detector - amplifier system within its linear range.[8]

Detector	Flame ionisation
Stationary phase	Carbowax 20M
Support	Chromosorb W, acid washed, dimethyldichlorosilanised, 80–100 mesh, or equivalent
Stationary phase loading ..	About 10 per cent. *m/m*
Column*	5–9 ft; outside diameter $\frac{1}{8}$–$\frac{1}{4}$ in; glass or stainless steel
Column temperature	Isothermal, 160 °C
Injection	On-column or flash between 160 and 225 °C
Chart speed	12 in h^{-1} (minimum)
Internal standard	Dodecan-1-ol, purity by gas - liquid chromatography not less than 99 per cent. by the method of peak normalisation

* It is the experience of the Sub-Committee that gas-chromatographic columns are becoming standardised in metric sizes and the nearest metric equivalent would be satisfactory.

Eugenol	Purity by gas - liquid chromatography not less than 99 per cent. by the method of peak normalisation
Sample size	Such that the internal standard and eugenol peaks fall within the linear range
Peak heights	Internal standard and eugenol within 40–75 per cent. of full-scale deflection
Gas flow-rate	To give satisfactory instrument performance
Solvent	Ethyl acetate

Determination of the Factor, *f*, for the Internal Standard (Eugenol = 1)

Carry out all weighings to an accuracy of 0·2 mg. Weigh out about 0·20 g of eugenol and 0·10 g of dodecan-1-ol into a calibrated flask and dilute the mixture to 10 ml with ethyl acetate. Inject 1·0 μl, or such other volume of the solution as will ensure a response within the linear range of the method, into the chromatograph and calculate the factor, *f*, to three decimal places from the equation

$$f = \frac{h \times d}{h_e \times d_e} \times \frac{m_e}{m}$$

where h_e is the height of the eugenol peak, d_e the retention distance of the eugenol peak, h the height of the dodecan-1-ol peak, d the retention distance of the dodecan-1-ol peak, m the mass of dodecan-1-ol and m_e the mass of eugenol. Repeat the operation twice on the same solution and use the mean of the three values of f in the calculation of the eugenol content of the sample.

Determination of the Eugenol Content of the Sample

Weigh out about 0·40 g of sample and 0·10 g of dodecan-1-ol into a calibrated flask and dilute the mixture to 10 ml with ethyl acetate. Inject 1·0 μl, or such other volume as will ensure response within the linear range of the method, into the chromatograph and calculate the eugenol content of the sample to two decimal places from the equation

$$\text{Eugenol, per cent.} = f \times \frac{h_e \times d_e}{h \times d} \times \frac{m}{m_s} \times 100$$

where h_e is the height of the eugenol peak, d_e the retention distance of the eugenol peak, h the height of the dodecan-1-ol peak, d the retention distance of the dodecan-1-ol peak, m the mass of dodecan-1-ol, m_s the mass of sample and f the mean response factor.

Repeat the operation twice with the same solution and report the mean of the three results, correct to one decimal place.

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8. Primavesi, G. R., Scott, C. G., Nelson, F., and Wirth, M. M., *J. Inst. Petrol.*, 1967, **53**, 367 (Appendix II, p. 377).

NOTE—References 4, 5 and 6 are to Parts I, II and III of this series, respectively.

Book Reviews

LOW ENERGY ELECTRONS AND SURFACE CHEMISTRY. By G. ERTL and J. KÜPPERS. *Monographs in Modern Chemistry, Volume* 4. Pp. 251. Weinheim/Bergstrasse: Verlag Chemie. 1974. Price DM98.

This monograph has 11 chapters, eight of which can fairly be described as technique description chapters (2, "Auger Electron Spectroscopy"; 3, "Electron Energy Loss Spectroscopy"; 4, "Photoelectron Spectroscopy"; 5, "Appearance Potential Spectroscopy"; 6, "Field Emission Spectroscopy"; 7, "Ion Neutralization Spectroscopy"; 8, "Work Function and Contact Potential"; 9, "Low Energy Electron Diffraction"). The remaining chapters are: Chapter 1, "Basic Concepts"; Chapter 10, "Vibrations at Surfaces"; and Chapter 11, "Processes in Adsorbed Layers."

The subject area is certainly ripe for a full length book, there being no other comparable work to date. The stated aim of the monograph is to provide a treatment of the interaction of low-energy electrons with solids for the non-expert that is introductory in character and more comprehensive than many of the original papers and reviews. In this I consider the authors are only partially successful because too many subjects are packed into the 250 pages. The result is that for some of the subjects there is insufficient explanation for them to be fully understood by the non-expert without recourse to other material (for instance, in the chapter on" Photoelectron Spectroscopy" the term "relaxation energy" is mentioned several times without any explanation of its meaning). Nevertheless, because an attempt has been made to be introductory, the coverage of some subjects is not deep or wide enough to add much to the numerous reviews or the individual papers available to those people already working in the fields concerned; the coverage is also uneven. Thus, whereas the first eight chapters take up 126 pages, Chapter 9 on low-energy electron diffraction (LEED) commands 60 pages to itself and a fair proportion of Chapter 10 is also concerned with LEED. While the treatment of the chemical applications of Auger and photoelectron spectroscopy is rather cursory, there is lengthy discussion on domain structures, disordered structures, and faceting and stepped surfaces in LEED.

Despite the above doubts about the balance and advisability of compressing so many subjects into a book of this length the general standard of the material actually covered is very high, obviously having been thoroughly and carefully planned. The large numbers of clear diagrams and figures are of great assistance and the chapter on LEED is as good as any over-all review that I have come across.

The authors assert that the frontier between physics and chemistry in surface science is almost non-existent, but for all that the book will probably be better received by those considering themselves to be surface physicists rather than chemists. At a price of about £18, which British scientist of either persuasion is going to buy a personal copy? C. R. BRUNDLE

SELENIUM. Edited by RALPH A. ZINGARO and W. CHARLES COOPER. Pp. xx + 835. New York, Cincinnati, Toronto, London and Melbourne: Van Nostrand Reinhold Co. 1974. Price £25·20.

The authors state in the preface of this book that their objective is to provide a treatise that would meet the needs of a broad spectrum of readers, including teachers and students, research workers and industrialists. They have done this admirably, and I make no apology for listing below the titles of each chapter, as I feel that this is the best way to "quantify the spectrum."

The chapters are: "The History, Occurrence, and Properties of Selenium"; "Recovery and Refining of Selenium"; "The Structure of Selenium"; "The Interaction with Light of Phonons in Selenium"; "Optical and Electrical Properties of Selenium"; "The Structural Aspects of Selenium Chemistry"; "Coordination Compounds in which Selenium Functions as the Donor Atom"; "The Organic Chemistry of Selenium"; "Biochemistry of Selenium"; "Analytical Chemistry of Selenium"; "The Toxicology of Selenium and its Compounds"; "Selenium in Agriculture"; "Selenium in Glass"; "Selenium and Selenium Compounds in Rubber and Plastics"; "Metallurgical Aspects and Uses of Selenium"; "Selenium in Electrophotography."

The fact that individual chapters are written by authorities in their own field is reflected in the high over-all standard of the book, which gives a complete picture ranging from the discovery of the element to its chemical and physical properties, and its industrial applications.

The analytical content of the book is rather small and amounts to only some 6 per cent. of the total number of pages. The chapter on the analytical chemistry of selenium is not intended to

be a detailed method book but rather a comprehensive survey of the methodology available. Nevertheless, it does contain many useful references to specific analytical procedures, and also some useful information concerning the separation and isolation of selenium, its detection and identification, and the determination of selenium in specific materials. I found the discussion on the decomposition, dissolution and other methods of preliminary treatment of inorganic and organic materials to be of particular interest.

The analytical chemist will certainly benefit from using this book as a general encyclopaedia. For example, the chapters on co-ordination chemistry and the organic chemistry of selenium are well worthwhile, and a knowledge of toxicity, biochemistry, and the divers industrial and agricultural uses quoted, is desirable in anyone involved in the determination of selenium in a variety of samples.

I found the book to be informative and well presented. I consider that it is of value to the analyst but believe that the high price of this comprehensive treatise may well act as a deterrent against its widespread usage.

J. WARREN

THE INFRARED SPECTRA OF MINERALS. Edited by V. C. FARMER, *Mineralogical Society Monograph No.* 4. Pp. x + 539. London: Mineralogical Society. 1974. Price £16; $38.

Infrared spectroscopy is commonly regarded as being chiefly applicable to organic materials, and relatively little attention is paid to its potential in the inorganic field. The Mineralogical Society have clearly recognised the dearth of adequate text and reference books covering the use of infrared spectroscopy for examining minerals and have published this volume to meet the need.

The book, following the pattern of so many, is not the work of one man but is a compilation from thirteen authors, each contributing one or more chapters. This type of book often fails to achieve its full purpose as, frequently, the individual contributions do not fit together to form an entity, but in this book it is clear from the contents and their arrangement that great care has gone into the selection of authors and their topics. The result is that the book forms a wide-ranging, one could almost say comprehensive, treatise on the application of infrared to minerals.

The first few chapters are probably best described as general and theoretical; they deal with applications, instruments and techniques, followed by chapters on such topics as symmetry, dynamics, vibrational and Raman spectra. Most of the remainder of the book deals with infrared spectroscopy of classes of minerals, *e.g.*, oxides, borates, carbonates, orthosilicates, chain, ring and layer silicates, silica and three-dimensional silicates, phosphates and sulphates. The last three chapters are concerned with industrial applications to cements, ceramics and glasses.

The book brings together a mass of information in a readily accessible manner, and such a compilation has been lacking for some time. The Mineralogical Society should be complimented on its publication and the book deserves to be brought to the attention of all those interested in the structure of minerals.

H. BENNETT

HANDBUCH DER PHOTOMETRISCHEN ANALYSE ORGANISCHE VERBINDUNGEN. By B. KAKÁČ and Z. J. VEJDĚLEK. Band 1 and 2. Band 1, pp. viii + 718; Band 2, pp. vi + 598. Weinheim/Bergstrasse: Verlag Chemie. 1974. Price DM320.

Although many colour reactions for organic compounds are known there are fewer text-books available on the spectrophotometric analysis of organic compounds than of inorganic compounds. This may well be due to the fact that organic reactions are generally less selective and the mechanisms are often less well understood. Nevertheless, there is undoubtedly a need for a comprehensive collection of spectrophotometric methods for the determination of organic compounds. Renewed interest in this field was stimulated when Feigl turned his main attention from inorganic spot-tests to organic spot-tests and later published "Organic Spot-Tests." Although these tests were qualitative in nature, many of them formed the basis for the development of quantitative methods. Feigl's work also showed that many of these tests could be made selective and this undoubtedly encouraged further studies in organic spectrophotometric analysis.

The chapters in the present two volumes are arranged according to the compound class as used in Beilstein, apart from the last three chapters, which describe the following: saccharides and their compounds; amino-acids, peptides and proteins; and steroids and related compounds. The 18 chapters are divided further according to compound sub-classes and the selected methods are then finally classified according to the reagent or reaction used.

The contents are very clearly set out. The principle is stated and this is followed by observations on the selectivity of the reaction. The working details follow the conventional pattern. Tables are provided containing references to the determination of individual members of the series.

More than 6000 compounds are classified in 671 tables; this provides some idea of the magnitude of the work. The coverage is both exhaustive and up to date. Especially useful is the first of the three indexes, which describes more than 500 reagents and lists the compound classes that they can determine. The other two indexes are the conventional author and subject indexes and these are extensive because of the vast coverage. There are very few errors and it would perhaps be carping to single these out. However, on page 416 "Perjodate" should be "Perjodide," and there seems to be a mistake in the Foreword, on page vi, where it states that there is a "lack of not-completely specific reagents and colour reactions."

This is an excellent treatise on a subject that has tended to become badly neglected. It can be recommended to all laboratories concerned with organic analysis. R. BELCHER

THE CHEMISTRY OF ELECTRODE PROCESSES. By ILANA FRIED. Pp. x + 225. London and New York: Academic Press. 1973. Price £4·90; $13·75.

Such a title from such an authority immediately raises great expectations of a text that could be of valuable use in teaching. Eager browsing, however, produced some disquiet over the treatment and irritation from the frequency of errors, and so the book was read from beginning to end. The result was disappointing. The keenest eye will miss errors in proof reading, and some inconsistencies in symbols can be overlooked, but there are rather too many of them. This fault destroys the confidence with which one would like to place such a text in the hands of students, and it does not do justice to either authoress or publisher. Spelling mistakes are irritating: stoicheiometry becomes stechiometry three times on p. 62 and again on p. 64; Levitch, p. 38; Tailor series, p. 53; quater wave, Fig. 81; berilium, p. 182; alluminate, p. 188; absorb for adsorb, p. 179; diestuff, p. 154.

Sudden changes in nomenclature are disconcerting, and the bewildering array of typefaces and symbols for the same thing does not improve the bad image of electrochemistry in this respect. The list of symbols is out of date and contains contradictions. The use of vector typefaces for non-vector quantities, *e.g.*, $\boldsymbol{M}$ for concentration of *HCl* (*sic*) on pp. 105, 117 and 134, italicisation of identifiers, units, and even *Na*F (twice on p. 79, also pp. 82 and 84), the inversion of ch to hc, p. 89, the contradictory η_{minax}, p. 152, the contradiction that fluoride "is not" (p. 79) and "is" (pp. 83 and 95) specifically adsorbed on mercury, the change of subscript letter "l" into numeral "1" on p. 130, the antiquated -ous/-ic nomenclature and other such infelicities increase the reader's irritation. The use of the term interphase does not commend itself as a replacement for the familiar interface. Dr. Fried is hardly to be blamed for mishandling prepositions and producing less than perfect English, but surely the sub-editors should have given some assistance with this.

None of this will do more than annoy those who know the subject, but the student or newcomer, to whom the book is addressed, may be puzzled or even misled, which is a pity because there is need for a book such as this. The avowed intention is to provide an introduction to real electrochemistry that is simple rather than rigorous, and which will lead on to more advanced texts, a bibliography of which is supplied; there are no references. This intention is carried through at some sacrifice: the theoretical sections often evade rather necessary explanations; the practical section does not carry sufficient information to permit a technique to be evaluated, let alone implemented; and the technology section makes interesting reading and includes many knowledgeable hints but is rather superficial. This text is not, therefore, usable on its own, but requires recourse to other sources for practical implementation of the material. This may very well be welcomed, because many of the advanced texts make arduous going even for the experienced.

A brief introduction sets the context; it is unfortunate that a derivative of methane is named instead of ethane on p. 2. The galvanic cell, basic definitions and concepts are then treated without taking any previous knowledge for granted, but with a heavy thermodynamic bias, followed by electrode kinetics with a properly more kinetic bias. Chapter 4 deals with the electrode - solution interphase qualitatively and quantitatively, and Dr. Fried does not evade adsorption and its effects, which are usefully discussed. This first half of the book is reasonably well produced, although the usual tangle of symbology requires close study, and there are some ambiguities.

Chapter 5 covers techniques of measurement, measurements at equilibrium, steady-state and transient measurements and spectroscopy; this last topic is dismissed with absurdly short coverage and the reference to Bair's "Introduction to Chemical Instrumentation" of 1962 could well have

been transferred to Strobel's text of 1973. Cautionary mention of ground loops on p. 126 would have been appropriate. The final chapter on technological aspects deals first with electrodic methods of analysis so superficially as to be of little use, making unspecific reference to "general texts." The difference between buffer solutions and pH standards is not appreciated, and the treatment of pH measurement and polarised electrode titrimetry is too naïve. Non-aqueous potentiometry is dismissed because of lack of knowledge, and the advantage of amperometric titrimetry is declared to be that "the concentration range of the titrated solutions is 10^{-2}–10^{-4} Molar, a concentration range where other titration methods usually fail." Likewise, "Coulometry at a *constant potential* is the most accurate method of analysis known," (my italics) on p. 172, and the subsequent statements will cause eyebrows to elevate, while statements on p. 174 reveal a fundamental ignorance of coulometry. The remainder of the chapter deals with electroplating, corrosion, batteries and fuel cells, and electrochemical production, again rather superficially but with some interesting and illuminating asides.

The choice of texts at student level is limited, and this book does deal with electrode processes as opposed to electrolyte solutions, a topic adequately treated in many texts. Perhaps unduly great expectations have led to inevitable disappointment, and the matter is one of proportion, so that had this come from a less eminent source the assessment would have been higher. The first half of the book presents a reasonable account of theory if one is prepared to adapt to the nomenclature and system of symbols used. Certainly, teachers in search of a text of this nature should give this one serious consideration.

E. BISHOP

THERMAL METHODS OF ANALYSIS. Second Edition. By WESLEY W. WENDLANDT. *Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications, Volume* 19. Pp. xvii + 505. New York, London, Sydney and Toronto: Wiley-Interscience. 1974. Price £14·60.

The appearance of the first edition of this book in 1964 marked a milestone in the history of thermal analysis, in that it provided the first treatise ever to cover the wide range of techniques that come under this umbrella term and brought to the notice of chemists many thermoanalytical techniques that had been successfully used in other fields. It is reasonable to suppose, indeed, that the first edition was one of the catalysts for the thermal analysis "explosion" that has resulted in the second edition having, with the exception of part of one chapter, to be completely rewritten.

Almost two thirds of the book is concerned with thermogravimetry, derivative thermogravimetry, differential thermal analysis (DTA) and differential scanning calorimetry (DSC) and their applications, particularly to analytical chemistry. The theory behind the methods is reviewed, components of apparatus and experimental technique are discussed, and some pieces of equipment with interesting features are described. No detailed description of commercial instruments is given in view of other recent publications and the fact that developments in instrumentation can outdate any such account very rapidly. The other subjects covered are evolved gas detection (EGD) and evolved gas analysis, spectroscopic, photometric and optical techniques (some of which are particularly associated with the author and his co-workers), the use of heating curves and DSC in purity determinations, dilatometry, thermomechanical methods, electrical conductance, emanation thermal analysis, thermoluminescence, thermomagnetic methods, torsional braid analysis and oxyluminescence. Examples of applications are given throughout and all have some analytical relevance. The penultimate chapter considers the place of digital and analogue computers in thermal analysis and the final chapter reproduces recommendations of the Standardization and Nomenclature Committees of the International Confederation for Thermal Analysis (ICTA) relating, respectively, to the reporting of thermoanalytical results and nomenclature in thermal analysis. This list constitutes an impressive coverage of the subject, the only major technique dropped in the second edition being thermometric titrimetry, which has, in any event, always been regarded as a border-line case.

Much of the text has been compiled by summarising or extracting information from original papers, with the result that several symbols can be used for the same entity in the space of a few pages (*e.g.*, pp. 48–53), statements are occasionally out of context, as in the description of the Whitehead and Breger apparatus on p. 233, where "in the dimensions shown" is superfluous and meaningless, and results seemingly at variance are quoted in close proximity. Some apparent discrepancies in DTA results could have been explained had the significance of sample – thermocouple configuration and the effect of the use of thermocouples with different characteristics been

discussed. Indeed, a rather more critical approach throughout would have been helpful to the novice. In some chapters (particularly four and five) most reference numbers in figure legends are incorrect and in one legend (p. 221) all six references are erroneous; this, fortunately, should raise no problems for the careful reader, as the correct reference numbers are given in the text. A few misattributions have arisen from the fact that the journal references cited are mainly American; thus, EGD was developed in eastern Europe long before 1960 (pp. 319–320).

These criticisms should not, however, be allowed to detract from the value of this important book, the compilation of which is a tremendous achievement for a single author. Professor Wendlandt is indeed to be commended for the boundless energy and breadth of vision that have enabled him to produce such a wide-ranging treatise. It is particularly gratifying to note that the nomenclature recommendations of ICTA are employed throughout and one can sympathise with the author in the difficulty he experienced in Chapter 10 when dealing with "heating curves" *versus* "thermal analysis" in the restricted sense of that word. The facts that only sporadic reference is made to the 1972 literature and that the preface is dated July 1973 give some indication of the time currently required to compile and publish a volume of this nature. A brief, but possibly adequate, index is provided and the very few misprints noted reflect a commendably high quality of proof-reading.

This book can be wholeheartedly recommended to all who are interested in thermal analysis and even seasoned thermal analysts will find therein much to stimulate their palates. The price is by no means excessive for the amount of information collected; indeed, the book, these days, is excellent value for money. R. C. MACKENZIE

HANDBOOK OF MOISTURE DETERMINATION AND CONTROL. Volume 1. By A. PANDE. Pp. xii + 266. New York: Marcel Dekker Inc. 1974. Price $28·50.

Volume 1 is the first of four volumes on the topic of moisture determination; in justification of the apparently lavish treatment, the author states that "many scientific disciplines (for instance, chemistry, physics, statistics and electronics) are covered and are synthesised in the techniques of moisture determination and control."

This volume contains four chapters, the first of which is concerned with properties of water, the mechanism of moisture absorption and statistical techniques and sampling. Chapter 2 is concerned with gravimetric methods, Chapter 3 with azeotropic distillation and chromatographic methods, while Chapter 4 is concerned with the Karl Fischer method.

The material covered in the first chapter is sketchily treated in some respects and over-elaborated in others and there are typographical errors that make nonsense of the text, for example, "The conductivity of water is not appreciably altered by ionized impurities at the p.p.b. level, nor is it affected by the presence of ionized impurities."

The chemical properties of water merit 14 lines, in which the hydroxyl ion is not mentioned, while the electrical and dielectric properties occupy some six pages of script, with many repetitions of the same information. Half of the chapter is concerned with the elaboration of simple statistical principles and a description of sampling procedures.

The treatment of gravimetric methods is comprehensive, there are descriptions of the different direct-reading balances and thermobalances in the discussion on automatic balances and the author digresses into the use of balances for measurement of surface tension. Thermal analysis techniques are described in detail with special reference to the analysis of clay minerals and the examination of coking coals.

Chapter 3 is concerned with azeotropic distillation and gas-chromatographic methods. The Dean and Stark method and its various modifications are described in detail, much of which is repetitious. A study of the relative merits of the solvents benzene, toluene and xylene in the recovery of water from glycerol is reported in detail but there are errors in the tabulated data.

Combinations of distillation methods with alternative end-measurement techniques, such as infrared spectrometry and Karl Fischer titration, are described and discussed. One set of comparative determinations by using four variations of the methods described is presented; unfortunately no details of the six samples analysed are given. The chapter continues with descriptions of gas-chromatographic instrumentation; again, there are repetitions and diversionary discussions on topics other than that under review. The chapter is concluded with a brief description of thin-layer and dry-column chromatography but no "in context" applications are given; also, it is difficult to accept that literature references that are 10 years old describe "new methods."

The last chapter is given over to a detailed description of the Karl Fischer technique and its applications. This is by far the best chapter of the book. Overall, the impression is that the book is reasonably well produced by the off-set litho process, but there are a number of examples of mistyped words and wrong use of words, for example, "indigenous" for "ingenious." As a part of a four-volume work this book is expensive for the information contained; the amount of repetition and over-description of technique, together with the wasteful use of page area, is such that the reader could reasonably expect a more condensed and wider coverage of the subject matter; perhaps the whole work should be contained in two volumes rather than four. R. SAWYER

THE DETERMINATION OF VINYL CHLORIDE. A PLANT MANUAL. Edited by W. THAIN. Pp. viii + 92. London: Chemical Industries Association Limited. 1974. Price £20.

This book has been compiled by a specialist committee of the Vinyl Chloride Committee of the Chemical Industries Association Ltd. It represents the considered views of an analytical team on the analytical methods that are required and recommended for the control of vinyl chloride concentrations in and around manufacturing and process plants; these plants are concerned with vinyl chloride monomer and poly(vinyl chloride) polymer as well as copolymers of vinyl chloride and other monomers.

This important work, in effect, represents the analyst's contribution to the wider aspects of the Vinyl Chloride Committee's work, *viz.*, the clarification of the nature and extent of the hazard to health from vinyl chloride monomer and the establishment of conditions and actions necessary to safeguard the health of personnel making or using vinyl chloride or processing materials made from it.

Essentially, the analytical methods developed by this team of analysts concern the following.

1. The determination of vinyl chloride monomer in air by one of three methods, *viz.*, (a) gas chromatography, or, as the reviewer still prefers to call the procedure, vapour-phase separation, (b) the use of an infrared gas analyser and (c) trapping a sample of air, followed by gas chromatography of the trapped sample.
2. The determination of vinyl chloride monomer in poly(vinyl chloride)-free aqueous effluents by a gas-chromatographic procedure. In an effluent that may contain poly(vinyl chloride) in suspension the test determines only the vinyl chloride that is soluble in the aqueous medium.
3. The determination of vinyl chloride monomer in poly(vinyl chloride) by both rapid and high-sensitivity methods.
4. The determination of vinyl chloride in copolymers of vinyl chloride with other monomers.

The methods that are proposed have been worked out in great detail, *e.g.*, when vinyl chloride is being determined in the atmosphere using an infrared gas analyser, full details are given of the instrument itself, and of its mode of operation in the test. Information is provided on the scope of the test, the hazards of operation, sample preparation and interfering substances. Finally, due attention is paid to the calibration of the instrument and the expression of the results of the application of the test to particular atmospheres.

Other tests that involve gas-chromatographic procedures are dealt with in great detail and on similar lines to the above.

The reviewer is of the opinion that this book would be even more valuable if each standard method was made complete in itself and unsatisfactory cross-referencing was avoided. A complete reappraisal of the information provided in the "Scope of the method" preamble to each standard method to include most of the material on pages 5–32 would be well worthwhile. Further, the inclusion in Chapter 4 of information on analytical methods, instruments and procedures that have not achieved the full status of standard methods may, indeed, confuse the plant operator.

Finally, there is the question of the price of the book. Even in these inflationary times £20 is a lot of money but, of course, it may be argued that in this first edition of the book a great deal of information is provided about analytical methods that have not been published previously in the analytical literature. J. HASLAM

METHODICUM CHIMICUM. Edited by FRIEDHELM KORTE. Volume 1. ANALYTICAL METHODS. Part A. Purification, Wet Processes, Determination of Structure. Pp. x + 1–628. Part B. Micromethods, Biological Methods, Quality Control, Automatization. Pp. x + 629–1218. New York, San Francisco and London: Academic Press. 1974. Price $98; £47·05.

This is a heavyweight book in every sense, and, faced with more than 1200 pages covering a very wide range of analytical methods and techniques, the reviewer has a daunting task. I hope,

therefore, that I will be forgiven for listing sub-titles and chapter headings, but I feel that this is the only sensible way that I can convey to the reader some idea of the over-all scope and of the coverage of his special interests.

The two books form the first volume of an 11-volume series covering different aspects of chemistry and which, to quote the Editor-in-Chief ". . . provides a guide to rapid and reliable detection of the method suitable for the solution of the problem concerned." Volume 1 is entitled "Analytical Methods," Part A being sub-titled "Purification, Wet Processes, Determination of Structure," and Part B "Micromethods, Biological Methods, Quality Control, Automatization" (yes, it really does say automatization, and I will return to the subject of the English later).

Each volume contains a substantial number of articles by different authors and these are grouped into the following chapters. 1. A 20-page section by H. Kaiser on "Foundations for the Critical Discussion of Analytical Methods." 2. "Methods of Separation," 12 reviews in 156 pages covering everything from distillation to the various forms of chromatography. 3. Nine reviews, totalling 70 pages, on different aspects of "Determination of Classes of Compounds and Functional Groups by Chemical Methods." 4. "Importance of Chemical Transformation for Analytical Purposes," a short piece by Giesselmann on methods for increasing the volatility and solubility of organic compounds. 5. "Spectroscopic and Photometric Methods," 18 reviews totalling 220 pages. 6. "Fragmentation methods," a 34-page survey of mass spectrometry, field ionisation and "special" mass spectrometry, "Elektronbrenzen" and pyrolysis of polymers. 7. "Diffraction Methods," 70 pages, five reviews including one on electron microscopy. 8. "Equilibrium and Kinetic Methods"; polarography, ion-resonance spectroscopy and determination of tautomeric equilibria, all in 45 pages. 9. "Special Physical Methods," incorporating eight specialised articles covering briefly such topics as relative molecular mass, calorimetry and dipole moments. 10. "Trace Analysis of Elements," seven reviews of well known techniques in 126 pages. 11. "Methods for the Determination of Essential Organic Components," 182 pages on pesticides, food additives, water, mineral oils, fats, oils and waxes, and industrial gases. 12. "Carbohydrates, Proteins, Nucleic Acids," six reviews totalling 54 pages. 13. "Biochemical and Biological Methods," 52 pages covering enzymatic, microbiological, toxicological and pharmacological methods, and biochemical transformations. 14. "Development Trends in Analytical Methods," four short essays.

The editor is to be congratulated on successfully performing the Herculean task of producing a reasonably coherent whole from over one hundred contributors. The majority of the authors are German, with a smattering of other nationalities, and one wit in my laboratory suggested "Encyclopaedia Germanica" as a possible alternative title. Let me hasten to add that the edition under review is produced entirely in English, but its Teutonic origin is displayed by the use of some rather odd words and sentences here and there. An example is "automatization," and another is a section heading "Chemical Calometry," which has just sufficient plausibility to make one wonder whether this is a new technique that one has not yet heard about. Although they occur rather frequently, taken in context they tend to amuse rather than confuse. Nevertheless to the pedant they will be an irritant that ought to have been removed by more careful English editing.

The general style is that of a collection of review articles of the type that *The Analyst* does so well, and with few exceptions they are well written, although I am not sure that many of them are as critical (in the "compare and contrast" sense) as they might be. The Editor says in his preface that the literature has been surveyed up to January 1st, 1972; my impression of the parts that I know something about is that they are a fairly good distillation of the work of the 1960s, and as such usually give an excellent introduction to a given topic. Their value in this respect is enhanced by the practice of giving not only extensive references with the texts but also a bibliography of key reference works.

So far I have concentrated on giving the reader some idea of what the content of the book is, but it might be almost as valuable to some people to outline what it is not. Firstly, it is not the sort of book that one would turn to for the very latest advances in a particular technique, the delays and difficulties in putting the whole thing together preclude that. Because of this, incidentally, I feel that the last chapter on development trends should have been omitted; interesting and well written as the articles are they will not age well in a book that will obviously have a long shelf-life. Secondly, it is not the sort of book that the inorganic chemist will want or need. The whole book is concerned with organic analysis, using the term in its broadest sense, and there is nothing in it on, for example, analysis of metals, minerals, etc. Thirdly, the price is such that I find it hard to believe that the individual chemist will buy it; it is certainly a book for the library.

To sum up, the book should be regarded as a reference book giving clear and concise introductions to most of the methods and techniques used in modern organic analysis. It should be most valuable for those institutions, both industrial and academic, where almost any branch of organic chemistry is practised, and I would certainly recommend the libraries of such places to buy it.

G. E. PENKETH

INSTRUMENTATION FOR MONITORING AIR QUALITY. *A Symposium Sponsored by the Environmental Protection Agency, the National Center for Atmospheric Research, and Committee D-22 on Methods of Sampling and Analysis of Atmospheres, Boulder, Colo.*, 14–16 *Aug.* 1973. *ASTM Special Technical Publication* 555. R. C. BARRAS, Symposium Chairman. Pp. x + 192. Philadelphia: American Society for Testing and Materials. 1974. Price $15·25.

This book consists of 16 papers on various aspects of the sampling and analysis of atmospheres. There are three papers on instruments used for the measurement of sulphur dioxide, two on oxides of nitrogen, four on hydrocarbons and two on instrumentation for the measurement of ozone. A further paper considers automated wet-chemical methods for air pollution analysis and the last four papers consider aspects of the analysis of air-borne particulate matter.

The papers have been written by those actively engaged in the application or development of instrumentation and sampling equipment for air quality control in the United States. One or two papers are written by members of companies marketing versions of the equipment that is described; naturally, the details in these instances are drawn from the make of instrument with which the authors are most familiar.

An attractive feature of the reviews is the emphasis placed on the practical problems of the operation and maintenance of instrumentation over long periods in what are often dirty environments. One example of such perceptive comment runs as follows: "it cannot be stressed enough that with automated networks the need for a sufficient number of qualified personnel, together with ancillary support personnel and services, are critical for success. A high degree of automation and computer data processing does not mean that an existing staff can be replaced by machines. Usually the reverse is true, that is, an increase in staff (quantity and quality)" (p. 40). In another paper the authors state: "it has been stated so frequently in the past that an analysis can be no better than the calibration of the method, we are reluctant to belabor the issue. Nonetheless, today, more than ever, the importance of this concept is difficult to overemphasize." These, and other, insights give this book an authoritative ring. The reviews are in some cases quite short and lack details of the various instruments that are discussed. Fairly complete references are given, which compensates to some extent for the generalised treatment. Inevitably, much of the information is already out of date as newer versions of the old principles, and indeed some new principles, have emerged, *e.g.*, fluorescence spectroscopy for measurement of sulphur dioxide, now commercially available.

Some of the papers are only marginally concerned with instrumentation, *e.g.*, that on filter media. They are, however, a contribution to the sub-topic of sampling and analysis. The book is well presented to a high editorial standard although there are some errors. Table 1 (p. 127) suggests that impurities in air-filter materials are in concentrations up to 669 g cm^{-2}. Presumably this should be $\mu g\ cm^{-2}$ but the reference (2), when checked, does not contain the information cited for this table.

The book will be of interest to those desirous of acquainting themselves with the basic tools for monitoring air quality. It will also be of interest to those who are involved with the analysis of air quality, using instrumentation acquired in the period covered by this book. It should eventually be superseded by a new edition, which would describe the solutions to the problems that have been identified.

H. N. M. STEWART
(Warren Spring Laboratory)

APPOINTMENTS VACANT

Use of Hydroxypropylmethylcellulose in the Determination of Chloride, Bromide or Iodide

A number of surface-active substances have been added to solutions of chloride, and the latter has been titrated with silver nitrate solution in the presence of an adsorption indicator. Although the use of agar was found to offer some advantages, that of hydroxypropylmethylcellulose gave rise to end-points that were much superior. The same additive also greatly facilitated the determinations of bromide and iodide. Attempts to determine these halides in admixture were, however, unsuccessful.

M. KAPEL, J. C. FRY and D. R. SHELTON

Procter Department of Food and Leather Science, University of Leeds, Leeds, LS2 9JT.

Analyst, 1975, **100**, 570–572.

Polarographic Studies on Some Organic Compounds of Arsenic Part II. Phenyl Arsenoxide

A study has been made of the polarographic behaviour of phenyl arsenoxide. Below 1×10^{-4} M concentration this compound gives rise to two well defined cathodic waves in acidic solutions below pH 2. The wave heights are diffusion controlled and proportional to concentration in the range 1×10^{-5} to 1×10^{-4} M. The current - potential relationships and the effect of pH, the electrode parameters and other factors have been investigated. With these and microcoulometric data the reaction path for both waves has been elucidated. The splitting of both waves at concentrations above 1×10^{-4} M by inhibition effects has been examined. The use of polarography has been proposed for the quantitative specific determination of phenyl arsenoxide and phenylarsonic acid in mutual mixtures.

A. WATSON and G. SVEHLA

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

Analyst, 1975, **100**, 573–583.

Polarographic Studies on Some Organic Compounds of Arsenic Part III. Triphenylarsine Oxide

A study has been made of the polarographic behaviour of triphenylarsine oxide. It gives rise to a single cathodic wave in 0·1 M hydrochloric acid, which is considerably complicated by inhibition effects. These effects are removed by the addition of a surface-active agent, thus yielding a well formed diffusion controlled wave the height of which is proportional to concentration (up to 1×10^{-3} M) and independent of pH. The current - potential relationships are discussed. On the basis of microcoulometric data and thin-layer chromatography the product of the electrode reaction has been shown to be triphenylarsine. Triphenylarsine oxide can be determined quantitatively in the presence of triphenylarsine by polarography.

A. WATSON and G. SVEHLA

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

Analyst, 1975, **100**, 584–592.

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

Part IV. Determination of Eugenol in Oil of Bay (*Pimenta racemosa, Miller*)

Report prepared by the Essential Oils Sub-Committee.

ANALYTICAL METHODS COMMITTEE

The Chemical Society, Burlington House, London, W1V 0BN.

Analyst, 1975, **100**, 593–600.

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