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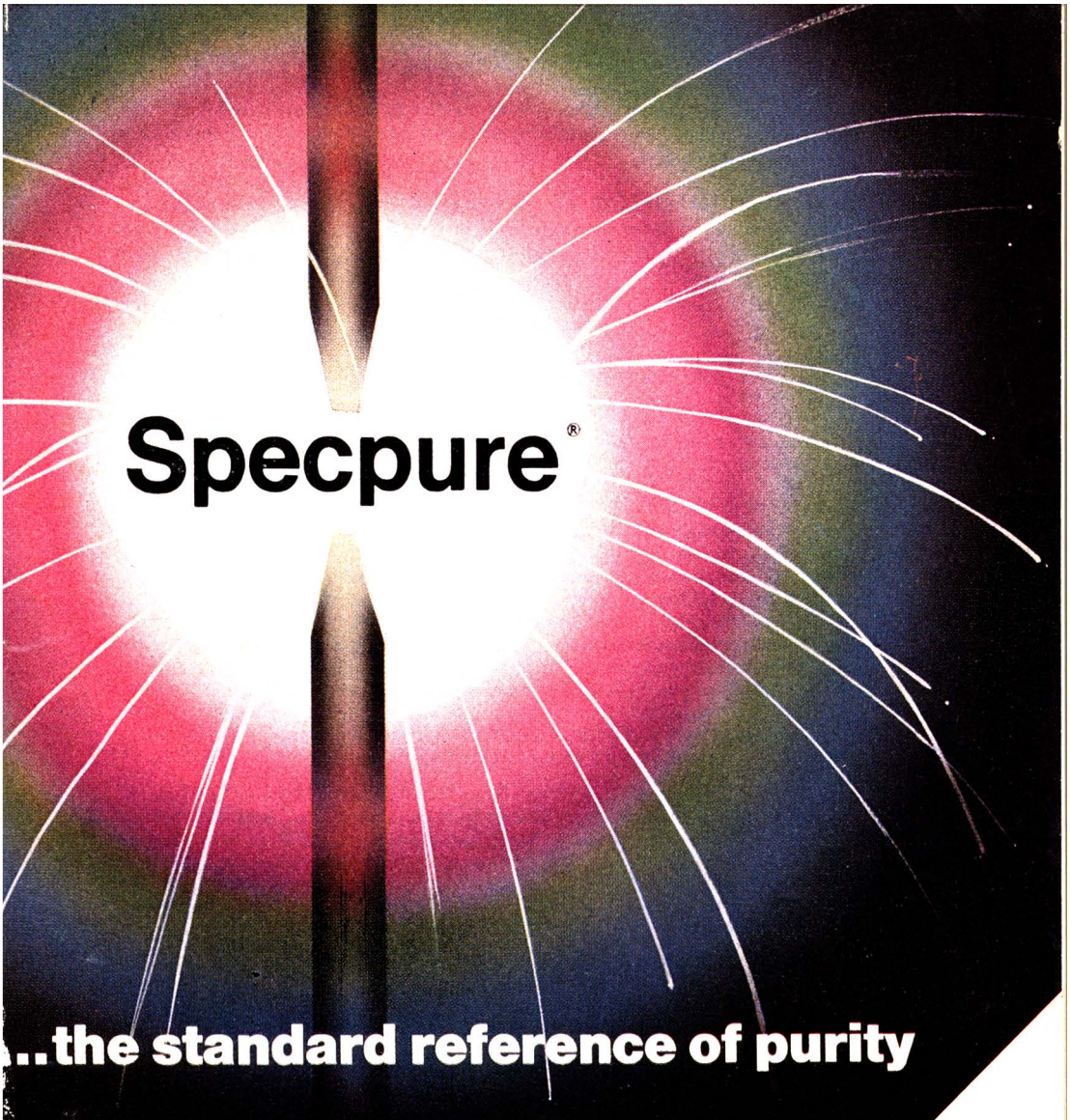

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Summaries of Papers in this Issue

Chemiluminescence in Gas Analysis and Flame-emission Spectrometry

A Review

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J. H. GLOVER

Consultant, 75 Craven Gardens, Wimbledon, London, SW19 8LU.

Analyst, 1975, **100**, 449–464.

The Determination of Barium in Unused Lubricating Oils by Means of Atomic-absorption Spectrophotometry

The use of a mixed-solvent system for the determination of zinc and calcium in unused oils by means of atomic-absorption spectrophotometry has been extended to the determination of barium. The excessive interference that occurs in the determination of barium because of high concentrations of calcium in certain oils has been overcome by modifying the mixed solvent in order to increase sensitivity. The work could then be carried out at much lower concentrations of barium, at which the interference has been shown to be eliminated. The procedure has been applied to a wide range of samples, and results are in good agreement with those obtained by means of X-ray fluorescence.

S. T. HOLDING and J. J. ROWSON

Shell Research Limited, Thornton Research Centre, P.O. Box 1, Chester, CH1 3SH.

Analyst, 1975, **100**, 465–470.

The Determination of Silver in Animal Tissues by a Wet-oxidation Process Followed by Atomic-absorption Spectrophotometry

Silver in animal tissues can be determined at fairly low levels by conventional atomic-absorption spectrophotometry, following wet oxidation. The wet-oxidation stage is difficult when large samples are to be analysed and the final solution must be adjusted in order to minimise losses of silver by adsorption or precipitation. The method has been applied to samples of urine, faeces, individual organs, skin and whole-animal homogenates.

R. C. ROONEY

Rooney and Ward Ltd., Blackwater Station Estate, Camberley, Surrey.

Analyst, 1975, **100**, 471–475.

The Determination of Unsulphonated Primary Aromatic Amines in Water-soluble Food Dyes and Other Food Additives

EEC directives prescribe limits for the amount of unsulphonated primary aromatic amines present in food colouring matters in general and prohibit the presence of particular carcinogenic amines in food dyes and other food additives. Integrated methods based on solvent extraction and thin-layer chromatography have been devised for identification of these amines, followed by the spectrophotometric determination of their condensation products with 4-dimethylaminocinnamaldehyde. The method is also extended to the determination of primary aromatic amines in biphenyl and to the determination of aminoazobenzenes in the dye Fast Yellow. An additional thin-layer chromatographic method is given for the differentiation between the isomers of naphthylamine as their tosyl derivatives.

E. J. DIXON and D. M. GROFFMAN

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

Analyst, 1975, **100**, 476–481.

Determination of 3,5-Dinitro-*o*-toluamide in Feedstuffs and Pre-mixes

A spectrophotometric method is described for the determination of 3,5-dinitro-*o*-toluamide (dinitolmide) in feedstuffs and pre-mixes. Dinitolmide is extracted from the sample with acetone - water (1 + 1). After purification procedures, which include liquid - liquid extraction and chromatography on an alumina column, a coloured complex is formed with sodium hydroxide, which is measured spectrophotometrically.

M. SEVERIJNEN and F. G. BUIZER

Rijkslandbouwproefstation, Kruisherengang 21, Maastricht, The Netherlands.

Analyst, 1975, **100**, 482–484.

An Ion-selective Electrode Method for the Determination of Nitrate in Grass and Clover

A method for determining the nitrate content of grass and clover involving the use of a nitrate-selective electrode is described. The method is rapid, accurate and precise and can be used for samples containing as little as 10 p.p.m. of nitrate-nitrogen.

A. W. M. SWEETSUR and Miss A. G. WILSON

The Hannah Research Institute, Ayr, Scotland, KA6 5HL.

Analyst, 1975, **100**, 485–488.

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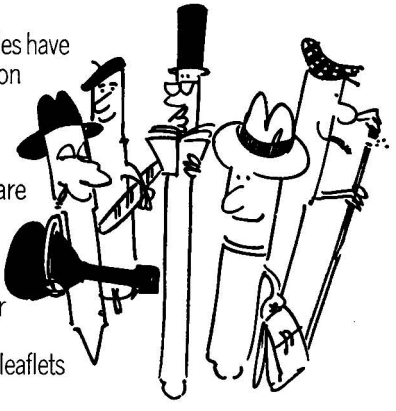

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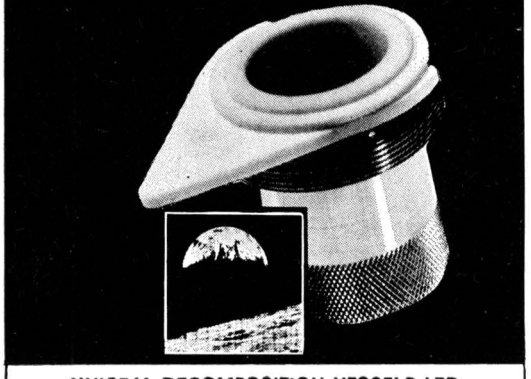

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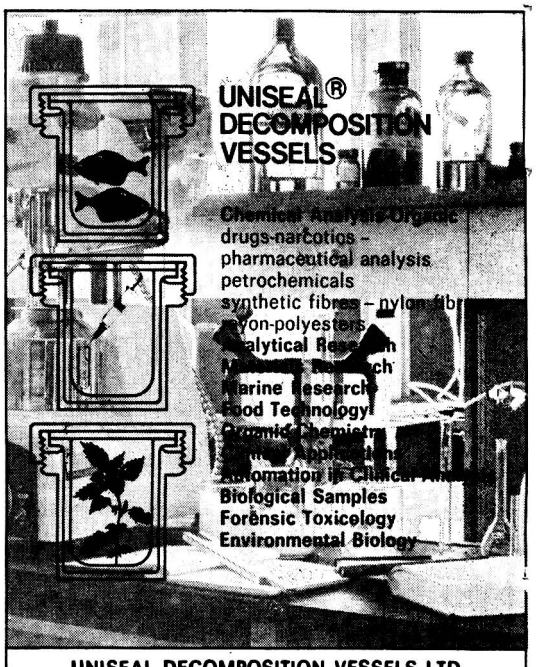

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JULY, 1975 Vol. 100, No. 1192

The Analyst

Chemiluminescence in Gas Analysis and Flame-emission Spectrometry

A Review*

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Summary of Contents

Introduction

Although the phenomenon of chemiluminescence has been known since the middle of the 19th century, it has only recently been introduced as a technique for gas analysis. In this application it has solved a number of problems and has provided a simple and very elegant new method of analysis.

Chemiluminescence can be defined as emission of light as a result of a chemical reaction. There are striking examples to be observed in nature, such as the "afterglows" observed in the upper atmosphere, which are the result of reactions between atomic gases. Another well known manifestation is the bioluminescence of the firefly and other insects.

Studies of chemiluminescence have, until recently, been directed towards an understanding of the mechanism by which energy is transferred within the reacting molecules and elucidation of the energy states concerned. Gas-phase reactions have received a great deal of attention, particularly in relation to upper atmosphere studies, and there is a large volume of literature devoted to this topic. Young and Sharples[1] give a good indication of the extent of previous work and add their own contribution on specific excitation processes that could be involved in the various chemiluminescence reactions of atomic oxygen and nitrogen.

Analytical applications of chemiluminescence began to appear in the late 1950s and consist of solution- and gas-phase techniques. Also, certain flame-emission phenomena are caused by chemiluminescence reactions and many of these have found useful application in analysis. This review will be concerned with gas-phase and flame-emission techniques.

The major factor promoting the use of chemiluminescence methods has been the greatly increased emphasis on air pollution and environmental analysis during the last few years.

* Reprints of this paper will be available shortly. For details see summaries in advertisement pages.

For example, the study of vehicle exhaust emissions exposed the lack of a method of analysis for oxides of nitrogen that would be suitable for use with continuous processes. The wet-chemical methods that were available when this environmental work was begun are more suitable for laboratory use, and there is a certain lack of confidence in the exact interpretation of results obtained by using them. Regulations limiting the composition of vehicle emissions were laid down by the Federal Authorities in America and motor car manufacturers were faced with the necessity of installing analytical equipment for the purpose of conducting exhaust-gas analysis on a production-control basis.

The chemiluminescence technique was applied successfully to this problem in 1969[2,3] and soon gained wide acceptance because of its considerable advantages of convenience, speed, reproducibility and selectivity; so much so, that it is now the standard technique for the measurement of oxides of nitrogen in vehicle emissions. The main advantages of the technique have been indicated by Stevens and Hodgeson[4]; because it is an emission process it is more suited to trace analysis than is absorption spectroscopy, in that a small positive value is being measured against a low background in contrast to the much more difficult measurement of a small difference between two very large absorptions. Further, the requirements for chemiluminescence to occur are such as to confer a high degree of specificity on the technique; and finally, the negative interference of quenching is predictable and can usually be accommodated.

Instruments based on chemiluminescence are fairly easy to produce and extremely simple to operate, so that there is a large incentive to seek other applications for the technique. This review will attempt to present the progress that has been achieved so far.

Theory

The phenomenon of chemiluminescence is the result of the process of chemi-excitation, which involves increasing the total energy of a molecule by means of a chemical reaction. The concept of the excited state is essential to the theory of spectroscopy and is discussed thoroughly in standard textbooks, so that only a very limited treatment of aspects that are directly relevant to chemiluminescence is necessary here.

When an excited molecule emits radiation it undergoes a permitted transition to a lower energy state. The relationship between the change in energy (E) and the frequency of the emitted radiation is given by the following equation:

$$E_1 - E_2 = \Delta E = h\nu$$

where h is Planck's constant and ν is the frequency.

Chemi-excitation is just another way of producing an excited molecule, as distinct from methods such as thermal excitation, irradiation or electronic excitation. The process, followed by emission of the corresponding luminescence, can be written[5]:

$$A + B \xrightarrow{M} C^* + D \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$C^* \longrightarrow C + h\nu \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Indirect chemiluminescence can occur by a reaction such as:

$$A + B + C \xrightarrow{M} AB + C \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Photolysis of certain substances may lead to the formation of excited species:

$$AB + h\nu \longrightarrow A^* + B \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$A + CD + h\nu \longrightarrow AC^* + D \quad .. \quad .. \quad .. \quad .. \quad (5)$$

The term photochemiluminescence has been used to define such reactions as (4) and (5).[6]

The process of excitation has some bearing on the type of radiation obtained and a simple view of the mechanism can be taken by considering the changes in potential energy that occur when an ideal diatomic molecule undergoes regular linear vibrations. Plotting potential energy against the distance between the nuclei gives rise to graph A, shown in Fig. 1, maximum

potential energy occurring when the two nuclei are nearest to each other and therefore subject to maximum repulsive forces. At the equilibrium position, E, the repulsive forces are balanced by the attractive forces and the latter increase, giving rise to more potential energy as the molecule is further stretched. Finally, dissociation occurs at point d.

The horizontal lines on the graphs represent vibrational energy levels. The absorption of energy gives rise to an excited state that can be represented by graph *B* in Fig. 1. Here it is assumed that the time required for quanta to be absorbed is so small as to be negligible compared with the time required for the nuclei to move a significant distance. In this instance, therefore, the energy curve of the excited molecule can be drawn directly above that of the stable molecule and transitions to the ground state can be represented by vertical lines. AB is the most probable $0 \rightarrow 0$ transition, CD the most probable $3 \rightarrow 3$ and so on. A situation of this type would give rise to intense emissions of short wavelength (ΔE is large).

Fig. 2 illustrates the instance when excitation is effected by a process that results in changes in the internuclear distances; for example, an excited molecule that has been produced as a result of chemical change may be in a different state of vibration from that of a normal ground-state molecule. Transitions in this instance may occur as shown, AB being the most probable $0 \rightarrow 4$ and may give rise to changes when ΔE is small and the corresponding emission is weak and of long wavelength. In general, excitation by high-energy sources, such as discharge tubes, will produce a situation corresponding to that shown in Fig. 1, whereas collision excitation and chemi-excitation would be expected to produce the energy changes shown in Fig. 2. For example, Cormier *et al.*[5] distinguish vibrationally excited molecules arising from exothermic gas-phase reactions from electronically excited molecules. The former emit in the infrared region in contrast with ultraviolet and visible-light emissions that result from electronic excitation.

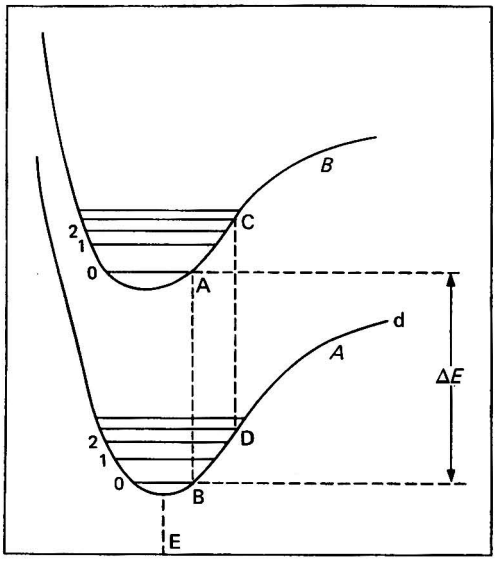


Fig. 1. Graphs of potential energy *versus* distance between nuclei (*A*) and absorption of energy (*B*) for an ideal diatomic molecule.

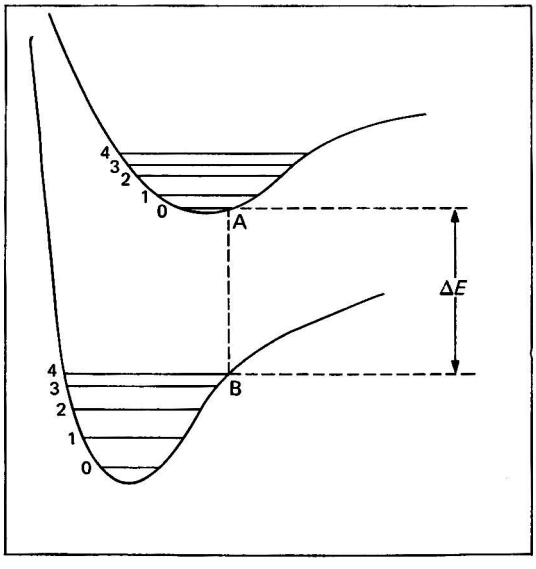


Fig. 2. Graphs of potential energy *versus* distance between nuclei and absorption of energy when excitation is effected by a process resulting in changes in internuclear distances.

Collision excitation can be expressed by constructing potential energy graphs, the simplest form being the association of two atoms, which can be illustrated by graphs of the type shown in Figs. 1 and 2. Reaction between molecules to give excited species is a much more complicated process as it frequently involves a three-body collision. Methods of studying such reactions involve the construction of three-coordinate models in which the collision complex is moving over a potential energy surface with respect to time. The various surfaces correspond to permitted energy states, and can intersect when vibrational energy is converted into electronic excitation.[7]

Efficiency of Chemiluminescence

The amount of radiation emitted as a result of chemi-excitation is obviously a very important factor in considering the analytical usefulness of a particular reaction. The intensity and frequency of the emitted radiation is influenced by the factors referred to in the previous section, but the amount of radiation obtained for a given amount of reactant is dependent on the fate of the excited molecules. An excited molecule can also lose its energy by collision so that the greater the chances of collision in a system, the smaller will be the number of excited molecules available for radiation. There are therefore two opposing factors, the radiative life and the collisional life, which need to be considered in more detail.

The probability (A) that a molecule in energy state 2 will undergo transition to energy state 1 with emission of radiation of frequency ν is given by:

$$A_{2,1} = \frac{8\pi\nu^2}{c^2 N_1} \times \frac{P_1}{P_2}\int_0^{\alpha} \alpha \mathrm{d}\nu$$

where N_1 is the number of molecules per unit volume in state 1; ν is the wavenumber; P_1 and P_2 are the probabilities of the two states; α is the absorption coefficient; and c is the velocity of light.

If there is only one possible transition from state 2, then the radiative life, T, is given by

$$T = \frac{1}{A_{2,1}}$$

but if other transitions are possible, then the reciprocal of the mean life will be equal to the sum of the transition possibilities:

$$\frac{1}{T} = A_{2a} + A_{2b} + A_{2c} \ldots \text{ etc.}$$

Calculations of mean life can be made by using values for N obtained by use of the Boltzmann factor:

$$N_E = e^{-E/kT}$$

where k is the Boltzmann constant and N_E is the number of molecules in any state with energy E. By using calculations of this type, radiative lifetimes of excited species can be estimated, and a typical value is found to be 10^{-8} s.

Collision life can be deduced from kinetic considerations and the following equation gives the number of collisions in unit time (C) that a molecule might be expected to undergo—

$$C = 4\pi n\sigma^2\sqrt{\frac{\overline{RT}}{m}}$$

where n is the number of molecules per millilitre; σ, the diameter of the molecule; and m the mass of the molecule ($m = M_r \times 1{\cdot}66 \times 10^{-24}$ g; M_r is the relative molecular mass).

In the instance of nitrogen at n.t.p., this relationship indicates that a molecule would have 8×10^9 collisions per second, or a collision life of $1{\cdot}3 \times 10^{-10}$ s. The situation is therefore that an excited molecule can undergo at least 100 collisions during the time that it can lose its energy by radiation, and for less favoured transitions the number of collisions may be several orders of magnitude higher. Assuming that collisions will deactivate the molecule, the amount of radiation will be much reduced.

Thus, two important conclusions emerge: one is that chemiluminescent measurements are best made under reduced pressure, so that the possibility of collisions is reduced; the second is that "quenching," or collisional deactivation by a third body, will depend on the nature of the other molecules present in the system. Different diluents will have different effects on the amount of radiation emitted.

An important factor that is omitted from the above simple treatment is the ability of an excited species to survive a number of collisions without deactivation. For example, the NO_2* species is deactivated by one collision, whereas activated mercury atoms appear to survive many collisions. It is therefore more useful to consider the quenching power of a diluent gas, *i.e.*,

$$I_p/I_0 = \frac{1}{(t_r/t_c + 1)} = \frac{1}{(\alpha p + 1)}$$

where I_p and I_0 are the radiation intensities at pressures of the quenching gas of p and 0, respectively; t_r and t_c are radiative and collisional lifetimes; and α is the quenching coefficient.

The over-all efficiency of a chemiluminescent reaction is the sum of the factors discussed, and according to Seitz and Neary[8] rarely exceeds 0·01, as expressed by the equation

$$\text{Efficiency} = \frac{\text{Number of photons emitted}}{\text{Number of molecules reacting}}$$

Bioluminescence, in contrast, is characterised by values for efficiency that approach unity.

The reader is referred to Cormier *et al.*,[5] Laidler[7] and Gaydon[9] for a fuller treatment of the theory, and to Clough and Thrush[10] for a more detailed discussion of quenching.

Gas-phase Reactions

Chemiluminescence in the gaseous phase has very important analytical applications and is widely used in the measurement of air pollution.[4] Well developed commercial analysers are now available, which are based on the application of chemiluminescent reactions. The method used is to mix an excess of reagent gas with the sample gas and then to measure the light produced; the two gas streams are fed into the reaction vessel at constant rates, thus facilitating continuous measurement. The light intensity (I) is proportional to the concentrations of the reactants:

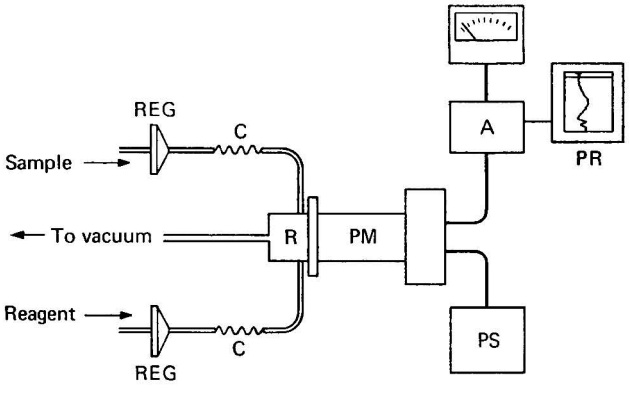
$$I = k\,[\mathrm{R}]\,[\mathrm{X}]$$

where R is the reagent gas and X is the sample gas. If [R] is made large compared with [X] it can be assumed to be constant, then

$$I \propto [\mathrm{X}]$$

The intensity of the emitted light is therefore directly proportional to the concentration of the constituent that is being determined provided that there is a large excess of reagent gas.

The equipment used for the determination is simple and is shown in Fig. 3. It consists of an arrangement for ensuring constant flow-rates of the sample and reagent gases; this is usually a pressure regulator followed by a constriction. The two gases meet in the reactor, which commonly has an end window through which the light passes on, via the appropriate optical filter, into the photomultiplier tube. The reactor exhausts into a vacuum pump, which maintains the desired operating pressure. The electronic circuitry is not complicated



C = Constriction
R = Reactor
PM = Photomultiplier
PS = Power supply
A = Amplifier
PR = Potentiometric recorder
REG = Pressure regulator

Fig. 3. Layout of chemiluminescence gas-phase analyser.

and consists of a high-voltage power supply to power the photomultiplier and an amplifier to facilitate the reading of light intensity.

Ozone

A gas-phase chemiluminescence analyser for ozone was first described in 1965,[11] and was based on the reaction between ozone and ethylene, which gives rise to a continuum of radiation with a peak intensity at 440 nm. A portable analyser based on the same reaction was described in 1970.[12] This instrument consists of a simple mixing chamber, constructed from a 100-ml beaker, that is attached to a photomultiplier assembly (EMI 95365) together with the associated circuitry. Two concentric tubes enter the mixing chamber, the sample air is drawn through the inner tube at the rate of 1 l min^{-1} and ethylene through the outer tube at the rate of 13 ml min^{-1}. The two gases meet at the photomultiplier tube window, where the emitted light is measured.

The instrument was found to give an output that was linear with ozone concentration, based on calibration tests performed against determinations by the iodide method. Under the conditions used the instrument gave a dark-current reading of 2×10^{-10} A and was capable of detecting 1 part per 10^8 of ozone. The chemiluminescence method has been accepted in the USA as a reference method for the determination of ozone concentrations following a number of field trials[13]; modern instruments will detect as little as 0·05 part per 10^8 of ozone with less than 10 s response time and they display linearity over a concentration range of 4 or 5 orders of magnitude. Care should be taken with the disposal of the ethylene effluent from chemiluminescent ozone monitors because of its flammability. Lonneman[14] describes an efficient catalytic disposal unit.

Oxides of Nitrogen

Before the introduction of the chemiluminescence technique, the determination of oxides of nitrogen was a difficult problem in gas analysis. The available methods have been reviewed by Allen[15]; he includes the various chemiluminescent reactions of analytical use. Of these, the reaction between nitric oxide and ozone has received most attention and an analytical application was described by Fontijn and co-workers.[2,3] This work has formed the basis for the design of the commercially available analysers which now find wide use in industry. The kinetics of the nitric oxide and ozone reaction had been studied[10,16,17] before Fontijn's work was published; the reactions are

$$NO + O_3 \longrightarrow NO_2^* + O_2 \quad \ldots\ldots\ldots\ldots \quad (6)$$

$$NO_2^* \longrightarrow NO_2 + h\nu \quad \ldots\ldots\ldots\ldots \quad (7)$$

The light intensity is given by:

$$I = 12 \left\{ \exp \frac{(-4180 \pm 300)}{RT} \right\} \left\{ \frac{[NO][O_3]}{[M]} \right\} s^{-1}$$

for the 600–875-nm region when M is air.

M is the third entity, which may quench the activated NO_2^* by means of the reaction

$$NO_2^* + M \longrightarrow NO_2 + M \quad \ldots\ldots\ldots\ldots \quad (8)$$

The rate constant for the over-all reaction is

$$\frac{-d[O_3]}{dt} = -\frac{d[NO]}{dt} = k[NO][O_3]$$

$$= 1 \times 10^{-7} \text{ l mol}^{-1} \text{ s}^{-1}$$

which is a low enough value to render the consumption of nitric oxide sufficiently small to be negligible and to obtain a uniform emission from the reactor. Clough and Thrush[10] measured the rate constants of the reactions and found that only about 8 per cent. of the nitrogen dioxide is formed in the excited state ($^2B1$). The effect of quenching varies with pressure and reaction (8) is the principal reaction at pressures greater than 0·1 mm. The sum effect is that light emission, reaction (7), occurs only with a very small fraction of the

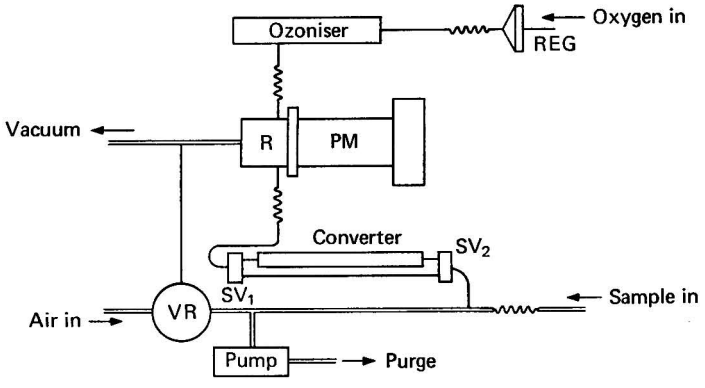
excited molecules. The fact that chemiluminescence is nevertheless a very sensitive method for the determination of nitric oxide illustrates the inherent sensitivity of the technique. Clyne *et al.*[16] analysed the light emitted during the reaction and found it to be a continuum, starting at 600 nm and extending into the infrared region with a maximum at 1200 nm. Although the emission extends over this wide range, only the region between 600 and 875 nm is analytically useful, the upper limit being set by the response of red-sensitive photomultipliers. In practice, a red cut-off filter is used to exclude any possible interfering emissions in the visible region.

The expression for light intensity reduces to

$$I = e^{-k/T}\left(\frac{[NO][O_3]}{[M]}\right)$$

If $[O_3]$ is large and in excess, then the intensity is proportional to [NO] provided that [M] is also constant. M contributes to the total pressure in the system and it is evident that light emission will decrease as the pressure rises. This decrease is caused by quenching, or deactivation by inelastic collisions. The nature of the diluent molecule, M, affects its quenching efficiency and this can be a source of error in chemiluminescent measurements when large concentrations of other "neutral" molecules are present, particularly when using instruments that operate at a pressure near to atmospheric pressure.

The layout of a typical low-pressure chemiluminescence analyser of nitrogen oxides is shown in Fig. 4. Ozone is supplied at a constant flow-rate from a silent discharge cell and is fed via a constriction into the reactor, sample gas is introduced into the reactor at a controlled rate and the reactor is maintained at a constant low pressure by means of the vacuum pump. The sample-flow arrangement provides a rapid purge and a quick response time.



R = Reactor
PM = Photomultiplier
REG = Pressure regulator
SV_1 and SV_2 = Solenoid valves
VR = Vacuum regulator

Fig. 4. Low-pressure NO_x analyser.

A systematic study of the factors affecting the sensitivity of chemiluminescence analysers for the determination of nitric oxide is the subject of a recent paper.[18] The relationship between reactor pressure, pumping speed and residence time in the reactor is discussed, and the point is made that it is necessary to keep the residence time large compared with the radiative life. The importance of efficient mixing is stressed, also the need to choose a photomultiplier with extended response in the infrared region and with low noise and dark current. The authors conclude that the quality and characteristics of the photomultiplier may well be the most important factors in extending the sensitivity of a nitric oxide detector.

The origin of dark current in photomultipliers is discussed by Sharpe,[19] who shows that in tubes with extended red sensitivity cooling to −10 °C is usually all that is needed to

obtain a worthwhile reduction in dark current. Thermoelectric cooling is a convenient method, although a circulatory system is equally satisfactory.

Despite the fact that theoretical considerations favour the use of low pressure in chemiluminescence determinations, there has been a trend in commercial instruments towards operation at near atmospheric pressure. The main reason for this is an attempt to eliminate the vacuum pump, and also to facilitate the production of a smaller, more easily portable unit. Fig. 5 shows the layout used in an ambient pressure monitor. Simple analysers such as these can measure nitric oxide concentrations between 0·1 p.p.m. and 0·1 per cent. with good linearity and a rapid response, but in order to achieve the full sensitivity of which the method is capable it is necessary to reduce the noise level by cooling the photomultiplier tube. Under these conditions a detection limit of 1 part in 10^9 can be achieved.

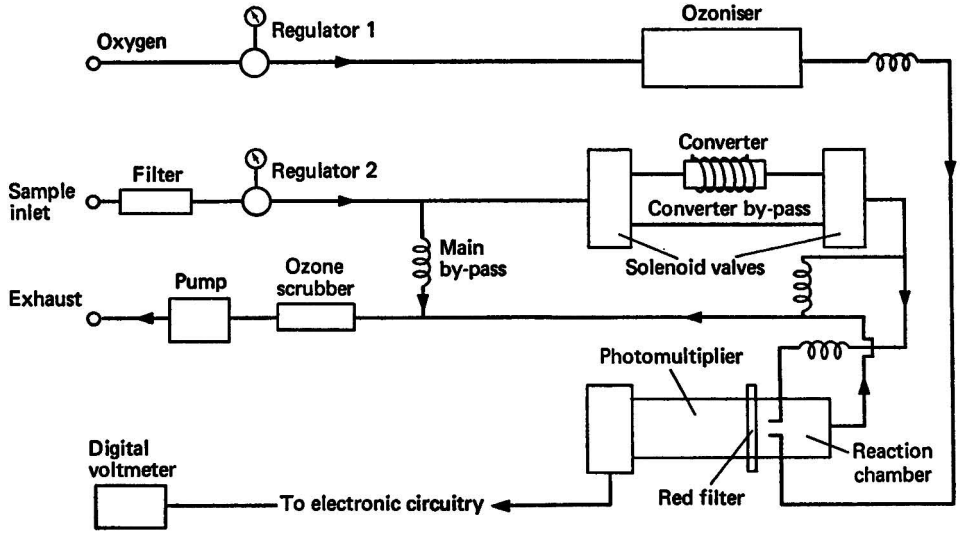


Fig. 5. Ambient-pressure NO_x analyser.

Measurement of Nitrogen Dioxide

While chemiluminescence provides an elegant answer to the problem of nitric oxide determination it is not applicable to nitrogen dioxide, and unfortunately it is the higher oxide that is frequently of more interest. Consequently, there has been a demand for a quick and easy method of converting nitrogen dioxide back into nitric oxide, thus enabling total oxides of nitrogen to be measured. A number of fairly successful methods have been offered, the original one being the thermal converter. This is a stainless-steel tube, heated to 650–700 °C and its operation is based on the thermal decomposition of nitrogen dioxide:

$$2NO_2 \rightleftharpoons 2NO + O_2$$

Breitenbach and Shelef[20] showed this reaction to be complete to the extent of 98 per cent. at 630 °C, provided that the partial pressure of oxygen is kept below 5 mm. At atmospheric pressure in air (oxygen partial pressure 150 mm), only 90 per cent. conversion is obtained. Consequently, the stainless-steel converter is useful only for instruments that operate at pressures below atmospheric pressure.

Another factor influencing the operation of thermal converters is the concentration of nitrogen dioxide. The dissociation of nitrogen dioxide is a second-order reaction and its rate will therefore vary with the nitrogen dioxide concentration; Sigsby *et al.*[21] have pointed out that because of this variation a much longer residence time, or a higher temperature, is needed for low than for higher concentrations of nitrogen dioxide. The stainless-steel converter is subject to serious errors in the presence of ammonia, as this compound is "converted" into nitric oxide; another error arises if carbon monoxide is present in the sample

and the oxygen content is low, when the following reaction is favoured—

$$2CO + 2NO \rightarrow 2CO_2 + N_2$$

These limitations led to the examination of other types of converter and Breitenbach and Shelef[20] examined many materials, concluding that a composite of carbon and molybdenum would convert nitrogen dioxide into nitric oxide with the minimum of interference from other components of the sample (*e.g.*, ammonia and other nitrogen compounds). Modern instruments usually include converters packed with carbon, and in general these are satisfactory. Potential sources of error include adsorptive effects resulting from the use of an unsuitable grade of carbon and catalysis of the reduction of nitric oxide to nitrogen by use of the wrong material to enclose the carbon.

Ethylenic Hydrocarbons

The reaction between ethylene and ozone has already been mentioned, and it can also be applied to the measurement of ethylene. Precisely the same equipment is used as for the measurement of ozone, but with the addition of an ozoniser to provide the reagent. Kummer *et al.*[22] report details of the spectral characteristics of chemiluminescence obtained from the reaction between various ethylenic compounds and ozone. Their results, which are presented in Table I, show that for a specific determination of ethylene a narrow-band filter would be needed in order to prevent interference from other ethylenic compounds. They also show that ethylene is not the best reagent for measuring ozone; a 50-fold increase in sensitivity could be gained by using one of the methyl derivatives.

Finlayson *et al.*[23] extended these studies and distinguished three classes of olefins: 1, terminal olefins, giving a broad emission that peaks at 440 nm; 2, olefins such as *cis*-but-2-ene, giving a broad emission with a peak at about 465 nm plus narrow peaks at 520 and 565 nm; and 3, olefins such as tetramethylethylene, which are dialkyl substituted at a carbon atom of the double bond, giving narrow peaks at 520 nm and broad shoulders at 465 and 565 nm.

TABLE I

CHEMILUMINESCENCE OF SOME ETHYLENIC COMPOUNDS

Compound	Relative emission intensity (ethylene = 1)	Peak wavelength/nm
Ethylene	1	440
Trimethylethylene	50	520
Tetramethylethylene	50	520
2,3-Dimethylbutadiene	8	—
2,5-Dimethylhexa-2,4-diene	30	—

Although no analytical methods based on the ozone - olefin reaction appear to have been developed, it can be seen that the chemiluminescence approach has possible applications in this field.

Sulphur Compounds

A brief study of certain sulphur compounds is presented by Kummer *et al.*[22]; they examined the spectra of emissions obtained by gas-phase reactions with ozone. Results for these reactions are given in Table II and show that sensitive measurements would be possible.

TABLE II

CHEMILUMINESCENCE OF SOME SULPHUR COMPOUNDS

Compound	Relative emission intensity (ethylene = 1)	Peak wavelength/nm
Hydrogen sulphide	25	370
Dimethyl sulphide	200	370
Methanethiol	2000	—

Peroxyacetyl Nitrate

Pitts *et al.*[24] have studied the reactions of peroxyacetyl nitrate and of ozone with triethylamine and suggest a design for an atmospheric monitor for these two oxidants. Peroxyacetyl nitrate is one of the important oxidants formed in photochemical smog, it is a known eye irritant and has been reported in concentrations of about 30 parts per 10^9 in Los Angeles.

The chemiluminescence spectrum obtained from the reaction between peroxyacetyl nitrate and triethylamine is a broad emission with a maximum at about 650 nm, as opposed to the ozone - triethylamine reaction, which gives a peak emission at 520 nm. The authors reported results of trials with various filter combinations and finally selected two cut-off filters for their prototype instrument, one transparent only to wavelengths less than 550 nm and the other transparent only to those greater than 665 nm. By using this combination the ratio of two intensities can be used to give the concentration ratio of the two oxidants, and then calibration with respect to one of them is all that is needed for an absolute measurement of both.

It was found during this work that the chemiluminescent efficiency of the peroxyacetyl nitrate reaction was about ten times that of the corresponding ozone reaction; another feature of the peroxyacetyl nitrate reaction is the long afterglow, which persists for several minutes, even at the lowest concentration.

Carbonyls

Two interesting examples of gas-phase chemiluminescence involve the carbonyls of iron and nickel. These have been shown to take part in chemiluminescent reactions with ozone,[25] the emissions being due to the excited FeO and NiO species and exhibiting maxima in the regions 565, 590 and 620 nm. While no analytical technique based on these emissions has so far been proposed, they are included because metal carbonyls have been found to give rise to interferences in other chemiluminescence methods.

Flame Chemiluminescence

The use of flames for analytical purposes is well established in techniques such as flame-emission spectrometry and atomic-absorption spectroscopy; perhaps less well appreciated are the applications of chemiluminescence occurring in flames. The distinction between chemiluminescence in a flame and radiation due to thermal excitation is not always clear-cut and it is generally accepted that both phenomena can occur simultaneously. However, for the present purpose, consideration will be given only to cool flames in which light emission is well in excess of that which would be expected from thermal excitation, and in which chemiluminescence is the accepted mechanism.

Emissions of this type were described by Gilbert,[26] who used the air - hydrogen flame in the presence of organic compounds, such as alcohols, added to the sample or a hydrocarbon mixed with the hydrogen. Gilbert found that line intensities were enhanced approximately 1000-fold by the addition of the organic species to the flame. That the enhancement was not caused by a higher flame temperature was shown by the addition of oxygen to the flame; this gave only slight intensification at the expense of a higher background emission. Gilbert recorded enhancement with many elements including tin, lead, arsenic, antimony, bismuth, nitrogen and phosphorus.

A good introduction to this subject is given by Gibson *et al.*,[27] who adopted a systematic approach to the elucidation of factors that affect the intensity of flame emissions. After mentioning the contradictory findings that have appeared in earlier literature, these authors pointed out the complexity of the processes that occur when aqueous solutions are atomised into flames in order to produce radiation. They used the Boltzmann distribution equation as a criterion for thermal excitation in a flame:

$$\text{Emission intensity} \propto N^* \propto N^0 e^{-\Delta E/kT}$$

where N^0 corresponds to the concentration of species in the ground state and T is the temperature of the flame.

Ground-state concentrations (N^0) were measured by atomic-absorption spectroscopy and T by the sodium line reversal technique as described in an earlier paper.[28] The enhancement

of emissions by organic solvents was investigated with these methods. The results showed that enhancement with sodium and calcium was due to higher concentrations of ground-state atoms as a result of the more efficient evaporation brought about by the presence of an organic solvent; in the instance of tin, however, the enhancement by propan-2-ol could only be explained by an increase in excited-state concentration. This conclusion confirmed the work of Gilbert,[26] who found that tin gave only a very weak emission in the hydrogen - oxygen flame both with and without the addition of propan-2-ol, although with the cooler hydrogen - air flame a considerable enhancement was obtained with propan-2-ol. The enhancement was difficult to determine because the normal emissions were too weak to measure, but a minimum value of 40 was reported. The mechanism was ascribed to the chemiluminescent reaction:

$$Sn + CH + OH \rightarrow Sn^* + CO + H_2$$

This compares with Gaydon and Wolfhard's proposed mechanism for lead[29]:

$$Pb + H + OH \rightarrow Pb^* + H_2O$$

A comprehensive study of enhancement phenomena has been presented by Buell[30]; he used limited area techniques to investigate the effect of organic solvents on emission intensities. Buell's paper gives excitation potentials and dissociation energies corresponding to over 600 atomic lines, and correlates these with the height in the flame corresponding to maximum emission intensity and the solvent enhancement factor. His results show that as excitation potentials increase, solvent enhancement increases and the height of maximum emission decreases. Solvent enhancing factors are considerable, commonly between one and two orders of ten, and in many instances are recorded as infinite when no emission is obtained without the use of the solvent.

Buell puts forward the argument that if the only function of the solvent is to increase the flame temperature or the evaporation rate, then all enhancements should be of the same order of magnitude. This is obviously not so. In addition, Buell found many spectral lines with excitation potentials in the range 7–9 eV, far in excess of that expected from thermal energy. He concluded that chemiluminescence was the only feasible mechanism that would explain his results.

The papers cited so far have shown the general possibilities of solvent enhancement and a more detailed contribution provides the basis of a sensitive emission method for determining tin.[31] These authors used a direct thermocouple method to determine the temperature of the flame and demonstrated an intense emission at 284 nm with flame temperatures of about 300°C. Chemiluminescence occurred in the presence of alcohols, 40 per cent. propan-2-ol giving the best conditions.

Sulphur and Phosphorus

Sulphur and phosphorus have been determined by chemiluminescent flame emission; these elements, together with the halogens, are beyond the range of atomic-absorption methods because their principal resonance lines are in the far ultraviolet region. This prompted Dagnall *et al.*[32] to investigate the molecular emission of sulphur in cool flames. These workers found that a hydrogen - nitrogen mixture was the most useful fuel gas and they measured the S_2 emission at 384 nm; emission intensity was shown to be dependent on flame temperature, being reduced as the temperature was increased.

It was found that all of the sulphur compounds investigated gave an S_2 emission, including sulphuric acid, sulphates, sulphites, thiosulphates, thiocyanates, sulphur dioxide, hydrogen sulphide and mercaptoacetic acid. The maximum response per molecule was obtained from sulphur dioxide and hydrogen sulphide, while sulphates and sulphuric acid gave only very weak emissions (about 1/600th of that given by sulphur dioxide). The best flame for the analysis of sulphates was found to be a shielded, pre-mixed, cooled hydrogen - air flame. The S_2 emission displayed maximum intensities at 384 and 394 nm.

As the S_2 species is formed via sulphur atom recombination

$$S + S \rightarrow S_2 + h\nu$$

two atoms are needed, and the intensity of emission is proportional to the square of the concentration of the sulphur compound in the flame.

The hydrogen-rich flame has been used extensively as a detector of sulphur-containing species, particularly in gas chromatography, and was first described in a West German patent.[33] An improved version is described by Brody and Chaney,[34] and more recent work is summarised in reference 5.

There are now a number of commercial forms of this sulphur detector, known as the flame-photometric detector, and it has been used in gas-chromatographic studies of atmospheric pollution.[35] The detector consists of a small flame, fed with a hydrogen and inert gas mixture, the primary combustion zone of which is shielded and the cooler secondary zone displays the emission, which is viewed via an interference filter by the photomultiplier. This detector has high sensitivity, but also has a limited range owing to deactivation of the excited species by self-collisional quenching.

The exponential response of the sulphur flame-photometric detector prompted Crider and Slater[36] to suggest the flame luminescence intensification and quenching detector, which operates on the most sensitive part of the response curve. This is achieved by adding sulphur dioxide to the carrier gas, thus obtaining a background luminescence. Eluates from the chromatographic column are detected by their quenching or intensifying effect on the emission.

These workers used a column consisting of a 5-ft length of 0·1-in i.d. PTFE tubing, packed with 9·1 per cent. squalane on 60–80 mesh Gas-Chrom Z, and they measured the detector response to several compounds at various base loadings of sulphur dioxide. Compounds with and without sulphur in their molecules were used and a useful degree of specificity was claimed from the response characteristics.

The behaviour of phosphorus-containing compounds was studied in another paper by Dagnall *et al.*[37] When using the nitrogen - hydrogen diffusion flame the best conditions were found to be in the coolest zone at temperatures between 280 and 300 °C. The emission at 528 nm was used and ascribed to the H–P–O species. Emission intensity was found to vary in a linear manner with concentration when using phosphoric acid over the range 0·2–200 p.p.m. As with sulphur, the response varied with different phosphates and a preliminary cation-exchange separation is advised.

The flame-photometric detector can, of course, be used for detecting phosphorus-containing compounds in gas-chromatographic eluates just as for sulphur compounds, except that a different filter must be used.

Aldous *et al.*[38] described a method for determining sulphur and phosphorus in organic and aqueous media by using a simple filter photometer. They pointed out that because the emission is banded, sensitivity is lost with a highly resolving monochromator, and also demonstrated the advantages to be gained by using a simple filter photometer. Detection limits of 0·08 p.p.m. for sulphur and 0·007 p.p.m. for phosphorus were obtained.

Important features of the method are the need to prevent oxygen from reaching the centre of the flame and quenching the emission, and also the finding that hydrocarbon fragments can quench the emission. Sample treatment methods were recommended in order to avoid these interferences and calibration graphs constructed for sulphur in the range 1–100 p.p.m. and phosphorus between 0·09 and 30 p.p.m.

Halogens

The well known Beilstein test for halogens, based on the green flame obtained by heating on a copper wire, was the basis of a special burner known as the Van der Smissen burner.[39] This device is sensitive, stable, gives a linear response and uses the emission obtained with copper in the presence of a halogen. However, the spectrum consists of emissions due to the species Cu, CuH, CuO and CuOH and is therefore not specific for halogens. Gilbert[40] sought to increase the sensitivity of this technique by using indium instead of copper and expected to measure the resonance line of indium at 451·1 nm. Instead, he observed a very intense emission due to InCl, which shows an intensity maximum at 359·9 nm. The appearance of this line not only conferred even higher sensitivity on the method, but also made it specific for halogens. Gilbert concluded that the InCl was abnormally excited by chemiluminescence because the total power output of the InCl spectrum was found to exceed that of the indium spectrum in the lower part of the flame by one order of magnitude. Gilbert's burner consisted of two jets, the upper completing the combustion of the hydrogen burned at the lower jet. Indium foil was suspended between the jets and the InCl emission was observed in the primary reaction zone of the upper flame.

The indium method was shown to have a very high sensitivity and possess good linearity, and Gilbert suggested that a detection limit of 0·001 μg of chlorine per litre of air should be possible. Gilbert's method has been applied to the measurement of chlorinated pesticides by Herrmann and Gutsche.[41] These workers increased the surface area of the indium by using indium-coated copper - beryllium coils, which were raised above the primary combustion zone and held at a temperature of 200 °C. A ten-fold increase in sensitivity was obtained.

In a second paper[42] a further modification was made, copper - beryllium sheet being used instead of coils of wire. This method was applied to the determination of bromide in urine samples. Sodium gave a strong luminescence background and a prior ion-exchange separation was required. A detection limit of 0·0062 μg of bromine was claimed when using the 375·8-nm InBr emission.

Dagnall *et al.*[43] had noted a similar chemiluminescence when solutions of tin(II) halides were aspirated into cool flames and demonstrated the blue SnCl and red SnH emissions. These emissions were shown to occur in the coolest part of the flame, where the droplets were still evaporating. However, the emissions from tin(II) halides were found to be much less intense than those from the corresponding indium species; the latter were systematically studied in a later paper[44] as a basis for an analytical method for determining halides. These workers record details of the spectra of the three indium halides that exhibit intensity maxima as follows: InCl, 360 nm; InBr, 376 nm; and InI, 410 nm. They used a similar burner to that used in their studies on sulphur and phosphorus[32,37] and established conditions and interference data for chlorides, bromides and iodides.

Detection limits were found to be in the range 1–2 p.p.m., and linear calibration graphs were obtained. Phosphate and sulphate interfered seriously and had to be removed, and interhalogen effects were noted; for example, a large excess of bromide completely destroyed the InI emission. It was also found that chloride enhanced the InI emission. Fluoride cannot be measured by this means as no InF emissions occur; this failure was explained on the basis of the greater stability of the $In^{III}F$ species.

Nitrogen Compounds

A paper describing the detection of nitrogen compounds by means of flame chemiluminescence was presented by Krost *et al.*[45] They used a scanning monochromator to examine emissions from the oxygen - hydrogen flame due to the reaction

$$H + NO \longrightarrow HNO^*$$

$$HNO^* \longrightarrow HNO + h\nu$$

The emission obtained is in the range 660–770 nm. The effect was observed from both nitric oxide and nitrogen dioxide, and the reaction is first order for both reactants in that the emission intensity is proportional to the hydrogen atom and nitric oxide concentrations.

$$I = I_0 [H] [NO]$$

where I is the emission intensity. The reaction mechanism postulated above is supported by the fact that the spectrum obtained with the hydrogen-rich flame is identical with that obtained by Clyne and Thrush[46] by the reaction of nitric oxide with atomic hydrogen produced by a radiofrequency discharge.

The fact that the same spectrum is obtained with both nitric oxide and nitrogen dioxide was explained by the very rapid reaction

$$NO_2 + H \longrightarrow NO + OH$$

which takes place in the hydrogen-rich flame. The paper describes detector design and proposes a shielded, hydrogen-rich flame viewed by two photomultipliers, one with a narrow-band interference filter at 690 nm for measuring the nitrogen luminescence, and the other for measuring the sulphur response at 394 nm. Nitrogen added to the system as a third entity gave the lowest background signal and the most favourable flame conditions.

Studies of the responses showed that ammonia and monomethylamine gave equivalent nitrogen responses but that sulphur dioxide was a source of serious interference in the measurement of nitrogen. The nitrogen compounds that were examined did not interfere in the detection of sulphur.

This detector was shown to have a linear response for nitrogen oxides up to 60 p.p.m. *V/V* with a detection limit of 0·15 p.p.m. *V/V*. The sulphur detection system gave a lowest detection limit of 0·004 p.p.m. *V/V* for sulphur dioxide.

Organic Compounds

In the course of his work on the flame-photometric detector, Crider reported that the addition of chlorinated hydrocarbons intensified the S_2 emission at 405 nm. This effect was investigated further[47] when it was shown that these compounds can emit separately and do not necessarily act as catalysts for the sulphur emission reaction. The spectra obtained when chloroform, ethylene dibromide and methyl iodide were aspirated into cool flames were recorded at various hydrogen to air ratios and optimum conditions were established for maximum emission intensity. The results suggested that low detection limits (0·01 p.p.m. of methyl iodide) were capable of being achieved. Preliminary results were recorded for other simple halogenated aliphatic hydrocarbons.

The technique of aspirating solutions of various organic compounds into cool flames is described in a general study by Dagnall *et al.*[48] They used low-temperature, laminar-flow nitrogen - hydrogen diffusion flames and took measurements in the coolest region of the flame (about 280 °C). Most of the emissions recorded in this paper are chemiluminescent in origin and a characteristic feature is the dependence of emission intensity on experimental variables. Many bands that might have an analytical application were recorded and the authors suggest that cool hydrogen flames could find wider application as gas-chromatographic detectors.

Molecular-emission Cavity Analysis (MECA)

This is a relatively new technique that was pioneered by Belcher *et al.*[49] in which chemiluminescent flame reactions are involved. It presents an alternative to nebulisation into a flame and uses a cavity at the end of a rod into which the sample is deposited. The rod is inserted into the flame so that the cavity is in line with the detector and the spectrum is recorded with respect to time lapse after insertion. Conditions are adjusted so that emission is confined to the cavity and the result is a concentrated and sustained effect giving very high sensitivity. Emissions are sometimes obtained that do not occur with conventional flame techniques, for example from selenium and tellurium,[50] much higher sensitivities are possible, certain sulphur compounds can be detected at the picogram level using the S_2 emissions as with the flame-photometric detector, and phosphorus compounds can be measured by use of the HPO_2 emission. Other examples of the use of MECA are the determination of halogens,[51] arsenic and antimony,[52] and boron and silicon.[49] Injection of oxygen into the cavity can extend the usefulness of the technique when oxides and hydroxides are the emitting species.[53]

Future Developments

A very interesting possibility for the development of new techniques in gas analysis based on chemiluminescence is the use of atomic gases as reagents. One of the best feasibility studies in this field is that by Snyder[54] who examined the possibility of using atomic oxygen to measure air pollutants by means of the general reaction

$$XO + O \longrightarrow XO_2 + h\nu$$

where X is nitrogen, carbon or sulphur. In such reactions the light intensity of the emitted radiation (I) is given by $I = k\,[XO]\,[O]$.

An excess of atomic oxygen was produced by means of a microwave discharge in molecular oxygen, and recordings were taken of the relevant spectra. Both nitric oxide and sulphur dioxide were shown to be capable of being determined with a detection limit of 1 part per 10^9; carbon monoxide gave less favourable detection conditions and a lowest detection limit of about 150 p.p.m. *V/V* was reported. However, there is little doubt that this limit could be improved by at least a factor of ten.

The spectral distributions obtained were studied in some detail and the peak transmission wavelengths were as follows: nitric oxide, 650 nm; carbon monoxide, 400 nm; and sulphur dioxide, 270 nm. The reaction between nitric oxide and atomic oxygen has been studied

extensively and has been used in "titration reactions" of many atomic and free radical species. Cormier *et al.*[5] give a good illustration of the techniques involved and show how the reaction

$$O + NO_2 \longrightarrow NO + O_2$$

can be used to determine the amount of oxygen atoms in a stream of gas. In this instance, when oxygen atoms are in excess, the indicator reaction

$$M + NO + O \longrightarrow NO_2 + M + h\nu$$

gives rise to a whitish green glow. When the flow of nitrogen dioxide is equal to or exceeds that of the oxygen atoms this glow contracts to a very small area round the mixing nozzles. The reaction between nitric oxide and atomic oxygen has been adapted as a laboratory standard for chemiluminescence,[55] and detailed studies of rate constants and quantum yield have been made.[56] Reeves *et al.*[57] also examined the mechanism of this important reaction.

Another method involving oxygen atoms was described by Krieger *et al.*[58]; they showed the possibility of determining unsaturated hydrocarbons by radiative reaction with atomic oxygen. These workers reported a better signal to noise ratio for emissions from the ethylene - atomic oxygen reaction than for the corresponding reaction with ozone. Olefins were found to emit between 700 and 900 nm and acetylene at 600 nm. The kinetics and mechanism of the acetylene reaction have been well studied[59] and in this work the oxygen atoms were produced by the reaction of nitrogen atoms with nitric oxide

$$N + NO \longrightarrow N_2 + O$$

The nitrogen atoms were produced in a microwave discharge. The necessity to use such a discharge in order to produce atomic gases has so far inhibited the application of this type of technique; if an alternative source could be found, then the wider use of atomic gases could give rise to some very attractive methods in gas analysis.

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The Determination of Barium in Unused Lubricating Oils by Means of Atomic-absorption Spectrophotometry

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The use of a mixed-solvent system for the determination of zinc and calcium in unused oils by means of atomic-absorption spectrophotometry has been extended to the determination of barium. The excessive interference that occurs in the determination of barium because of high concentrations of calcium in certain oils has been overcome by modifying the mixed solvent in order to increase sensitivity. The work could then be carried out at much lower concentrations of barium, at which the interference has been shown to be eliminated. The procedure has been applied to a wide range of samples, and results are in good agreement with those obtained by means of X-ray fluorescence.

The development and use of an acidic mixed-solvent system for the determination of zinc and calcium additives in unused lubricating oils by means of atomic-absorption spectrophotometry has been described previously.[1] This solvent system allows inorganic salts to be used as standards and also eliminates the systematic errors that sometimes occur when an air - acetylene flame is used. These errors are largely due to the different chemical constituents of the metal-containing additives in the sample and of the organometallic compounds used as standards. The use of inorganic salts as standards in the determination of zinc and calcium additives had the advantage of permitting simple and inexpensive checks on the validity of the aqueous salt standard whenever it was thought necessary and had the additional advantage of the salts being generally available in pure forms. The same advantages would be obtained if this approach could be extended to the determination of barium additives in lubricating oils.

For the determination of barium it is necessary to use the hotter nitrous oxide - acetylene flame, and many workers[2–5] have reported on the determination, using this flame, of barium in lubricating oils. However, all the methods have depended on the use of oil-soluble organobarium compounds as standards and have employed solvents such as white spirit or wholly aromatic solvents such as xylene. Our experience is that solvents such as white spirit, which give moderate sensitivity for barium, generally give accurate results but that the precision may not be adequate for blending quality control. In addition, with such solvents spectral interference may occur when analysing formulations in which there is a large concentration of calcium in relation to barium. The magnitude of the interference is dependent on flame stoicheiometry. Although Peterson and Kahn[2] analysed oils, including some with a high calcium to barium ratio, they used xylene as solvent, which gives very good sensitivity for barium and so spectral interference would be minimal. They reported single results only for the direct determination of barium using atomic-absorption spectrophotometry but for the small number of samples analysed they obtained good agreement with results obtained by use of X-ray fluorescence.

Our early experience of the use of wholly aromatic solvents in atomic-absorption spectrophotometry for blending quality control was that repeatability was not usually adequate. The burners formerly available were prone to carbon build-up along the slit, which necessitated frequent shutdown for cleaning. Furthermore, the nitrous oxide - acetylene flame is somewhat hazardous and our early assessment was that it should not be used in routine work. However, many instrument manufacturers now incorporate safety features such as switching control units in the gas supply line and bursting discs in the burner assemblies. In addition, on commercial instruments greater emphasis has now been placed on providing fine and steady control of the fuel and support gases, which is essential for good repeatability.

The success of the methods for zinc and calcium, together with the almost universal adoption of the instrumental improvements described above, prompted attempts to extend the use of an inorganic salt standard to the determination of barium additives in lubricating oils by means of atomic-absorption spectrophotometry. This has led to the development of an atomic-absorption procedure for the determination of barium in unused lubricating oils by a direct solvent dilution procedure utilising barium chloride as standard.

Experimental

Development of Solvent System

The mixed solvent used for earlier work had the following composition: cyclohexanone - butan-1-ol - ethanol - concentrated hydrochloric acid - water (10 + 6 + 4 + 1 + 1, *V/V*). This solvent gives a clear-burning flame and seemed likely to be suitable for the determination of barium. However, the sensitivity for barium was so poor that up to 3 g of oil per 100 ml of solvent had to be used for oils of low barium content (*e.g.*, 0·05 per cent. *m/m*). The addition of this amount of oil together with aqueous standard solutions or water balance as well as the ionisation suppressant (1000 p.p.m. of potassium as chloride) produced heterogeneous solutions. An alternative solvent that would overcome this difficulty was therefore sought.

From a limited survey of possible alternatives the most promising appeared to be mixtures of either butan-2-ol or 2-methylpropan-2-ol with white spirit and toluene. Compatibility trials indicated that a mixture, designated solvent N.1 and having the following composition, could accommodate in homogeneous solution 4 g of oil and up to 10 ml of water per 100 ml of standard solution: 2-methylpropan-2-ol - toluene - white spirit (3 + 1 + 1).

Use of solvent N.1 had an additional advantage in that it burned with a steady flame with minimum carbon formation along the burner slit. It was also found that the incorporation of mineral acid in the solvent was not necessary, because the high flame temperature caused complete dissociation of the metal additive. Potassium naphthenate was added as ionisation suppressant instead of potassium chloride because of its better solubility in solvent N.1. It is not necessary to know the suppressant metal concentration accurately, provided that the same amount is added to each solution. To prevent separation of the components the aqueous phase had to be added in the final stage of preparing the calibration and sample solutions; water is added to the sample solutions in order to balance the solvent matrix in relation to the aqueous inorganic salts used in the calibration solutions.

In burner trials of various solvents it had been observed that an increase in the toluene concentration also increased sensitivity. Experiments were also carried out, therefore, with a second solvent, designated solvent N.2 and having the composition: 2-methylpropan-2-ol - toluene (3 + 2).

Results of experiments using solvent N.1 and of experiments using solvent N.2 to obtain greater sensitivity, are reported in Table I.

Analysis of Samples and Interference of Calcium

A number of samples were analysed using solvent N.1. The results obtained are given in Table I, column 5. The standards were prepared from aqueous barium chloride solution, 5 ml, plus the solvent mixture, 95 ml. It can be seen that for samples 1, 2 and 3, which had high concentrations of calcium, there is poor agreement with the results obtained by means of X-ray fluorescence (column 3) and it is inferred that the poor agreement for these samples was associated with the higher calcium concentrations. It is known that at the wavelength (554 nm) of the barium resonance line used for these measurements there is a narrow band due to calcium oxide or calcium hydroxide and therefore it seemed likely that the calcium was responsible for the interference. Although the use of very rich flames reduced the interference, it was not practicable because these flames result in fluctuating signals and hence in poor precision. Furthermore, excessive carbon formations occur along the burner slit.

In order to study the effect of calcium in more detail, lean-flame conditions were used to accentuate the interference at the concentrations of interest; concentration *versus* absorbance graphs were plotted (Fig. 1) for both calcium and barium in mixed-solvent solutions containing 1000 p.p.m. of potassium added as naphthenate. The shape of the graph for calcium produced under lean-flame conditions was surprising but shows why lower concentrations of calcium had no visible effect on the result for barium when the normal, richer

TABLE I

COMPARATIVE RESULTS OF ANALYSIS FOR BARIUM

Sample*	Calcium, obtained by X-ray fluorescence, per cent. *m/m*	Barium, per cent. *m/m*, obtained by— X-ray fluorescence	a wet chemical method	atomic-absorption spectrophotometry with— solvent N.1	solvent N.2
1	0·533	0·349	—	0·404	0·355
	0·533	0·347	—	0·415	0·355
2	1·17	0·173	—	0·283	0·179
	1·16	0·171	—	0·290	0·174
3	2·27	0·594	—	0·763	0·635
	2·26	0·586	—	0·783	0·624
4	0·203	0·186	0·189	0·205	0·185
	0·204	0·186	0·191‡	0·203	0·188
5	0·056	0·037	0·039	0·043	0·038
	0·056	0·037	0·040‡	0·043	0·036
6	0·127	1·01	1·02	1·03	0·964
	0·126	0·997	1·02‡	1·05	0·981
7	0·101	0·092	0·100	0·098	0·086
	0·100	0·092	0·102‡	0·100	0·087
8	0·023	0·123	0·126	0·132	0·116
	0·023	0·122	0·126‡	0·130	0·117
9	0·303	0·021	—	—	0·019
					0·019
10	0·257	0·356	—	—	0·353
					0·359
11	0·052	1·09	—	—	1·09
					1·10
12	0·350	0·014	—	—	0·014
					0·014
13	0·392	0·422	—	—	0·440
					0·435
14	0·391	0·436	—	—	0·454
					0·456
15	0·046	0·021	—	—	0·020
					0·020
16	—	—	0·209†	—	0·210
			0·216		0·214
			0·213		
17	—	0·133	—	—	0·127
					0·131
18	—	0·056	—	—	0·055
		0·057			0·055
19	—	0·104	0·111	—	0·104
		0·106	0·108		0·104
20	—	1·07	1·06	—	1·00
		1·08	1·04		1·01

* Samples 1–8 were samples that had been circulated for chemical analysis in an IP correlation programme.
† IP 110 (Method B).
‡ Modified IP 110 (Method B).

flame was used. The results are given in Table I, column 5. Although the graphs in Fig. 1 were produced when a non-standard lean flame was used, they serve to show that if such a flame were used for the barium determination, then at the normal concentration of the analyte in the sample solution (15 mg l^{-1} of barium) any calcium present in a concentration in excess of 15 mg l^{-1} would significantly increase the absorbance reading. However, it is also evident that, even with the lean flame, interference from calcium can be markedly reduced by diluting and working at lower concentrations of calcium and barium. Thus, if the normal flame could be used together with high dilutions of the metal under test then interference from calcium might be eliminated, certainly for concentrations up to the level present in samples 1, 2 and 3.

It was found that although solvent N.2 gave a significant increase in sensitivity, the sensitivity was nonetheless lowered at both the low and high acetylene flow-rates used to obtain the lean and rich flames, respectively. The optimum setting is therefore readily found by

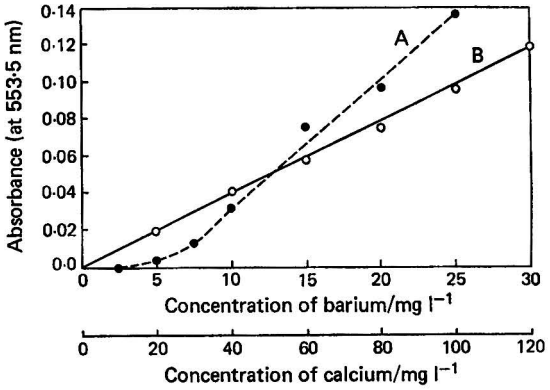

Fig. 1. Absorbance *versus* concentration graphs for A, calcium and B, barium in mixed-solvent solution (Solvent N.1) at the barium resonance line (553·5 nm), using a non-standard lean nitrous oxide - acetylene flame. Acetylene flow-rate, 2·8 l min^{-1}.

adjusting the acetylene flow to find the signal of greatest magnitude using a standard containing a reasonably high barium concentration. The calibration graph now obtainable for barium is shown in Fig. 2, together with a concentration *versus* absorbance graph for calcium plotted for identical conditions. At the 5 mg l^{-1} of barium level interference in samples containing exceptionally high calcium to barium concentration ratios is negligible. The improvement in sensitivity is such that for oils of low barium content only 0·5 g of oil per 100 ml of solution (solvent N.2) is required in contrast to the 3 g of oil per 100 ml of solution required with the original acidic mixed-solvent system.

The improved results for samples 1, 2 and 3 and the results obtained for a number of other oils with solvent N.2 are given in Table I, column 6, and can be compared with those obtained by X-ray fluorescence, column 3, and by wet chemical methods, column 4. Details of the method are given below.

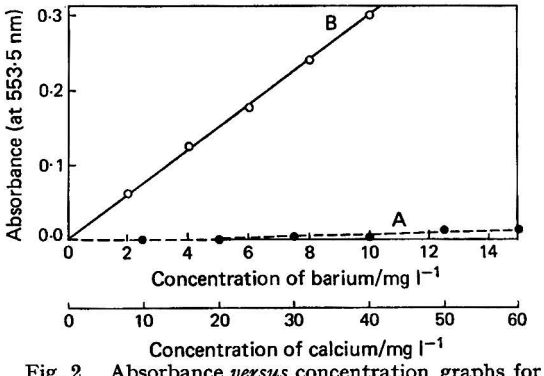

Fig. 2. Absorbance *versus* concentration graphs for A, calcium and B, barium in mixed-solvent solution (Solvent N.2) at the barium resonance line (553·5 nm), using a standard nitrous oxide - acetylene flame. Acetylene flow-rate, 3·6 l min^{-1}.

Determination of Barium in Unused Lubricating Oils by Atomic-absorption Spectrophotometry Using a Mixed-solvent System

The sample, which is contained in a mixed-solvent - aqueous solution, is analysed by means of atomic-absorption spectrophotometry using a nitrous oxide - acetylene flame, and the results are compared with those obtained with barium standards.

Apparatus

An atomic-absorption spectrophotometer fitted with nitrous oxide - acetylene facilities was used; Techtron AA3 and Unicam SP90 instruments are satisfactory. The other equipment required consists of 100-ml calibrated flasks, 5- and 10-ml pipettes, and an Agla 0·5-ml syringe-burette. All glassware should be cleaned with concentrated hydrochloric acid before use.

Reagents

All reagents should be of analytical-reagent grade and de-mineralised water or water of equal purity should be used throughout.

Standard barium solution, 2000 mg l^{-1}. Dissolve 3·557 g of barium chloride ($BaCl_2.2H_2O$) in water and make up to 1 l.

Mixed solvent. Mix 2-methylpropan-2-ol with toluene in the proportions 3 + 2.

Potassium naphthenate solution, 10 000 mg l^{-1} of potassium. Weigh 167 g of potassium naphthenate (obtainable from Durham Raw Materials Ltd., London), which contains 6 per cent. *m/m* of potassium, and make up to 1 l with the mixed solvent.

Preparation of Standards

Add, from an Agla syringe-burette, 0, 0·1, 0·2, 0·3, 0·4 and 0·5 ml of the 2000 mg l^{-1} barium standard solution to successive 100-ml calibrated flasks. Pipette 5 ml of water into each flask, and dilute to approximately 80 ml with the mixed solvent. Then, pipette 10 ml of the 10 000 mg l^{-1} potassium solution into each flask and make up to volume with the mixed solvent.

Preparation of Sample

Weigh into a 100-ml calibrated flask an amount of sample containing between 0·3 and 0·7 mg of barium and add approximately 80 ml of the mixed solvent. Pipette 10 ml of the 10 000 mg l^{-1} potassium solution and 5 ml of water into the flask and dilute to the mark with the mixed solvent.

Procedure

Determine the barium content of the sample by means of atomic-absorption spectrophotometry (using a nitrous oxide - acetylene flame) by comparison with the standards in the usual manner. The sensitivity for barium is dependent on flame stoicheiometry. The optimum setting is found by adjusting the acetylene flow, while spraying a standard, until a maximum absorbance reading is obtained. Typical settings are in the region of 3·6 l min^{-1} of acetylene and 5·5 l min^{-1} of nitrous oxide.

Calculation

Calculate the barium content of the sample by means of the following equation

$$\text{Barium, per cent. } m/m = \frac{a}{w \times 100}$$

where a mg l^{-1} of barium is the reading obtained for the sample solution from the calibration graph and w g is the mass of sample.

Discussion

It is evident from the analytical results that when oils contain a high ratio of calcium to barium the determination of barium by use of atomic-absorption spectrophotometry can be subject to severe interference. The magnitude of the interference is dependent both on the solvent used (*i.e.*, the effect is large for a solvent giving poor sensitivity for barium) and on the stoicheiometry of the flame. By using the solvent N.2, which provides very good sensitivity for the barium determination, the interference is virtually eliminated. Table I shows that there are still some small discrepancies between the results obtained by use of atomic-absorption spectrophotometry and by X-ray fluorescence, although agreement is generally good.

The method developed for barium using the nitrous oxide - acetylene flame complements the acidic mixed-solvent system developed for the determination of zinc and calcium, which

is carried out using the air - acetylene flame. In practice, this means that two different solvent systems and two different flames are used in order to analyse an oil for barium, calcium and zinc. Apart from other considerations, solvent N.2 has poor burning characteristics in the air - acetylene flame and so could not be directly substituted for the acidic mixed-solvent system used in the determination of zinc and calcium. Solvent N.2, however, because of its qualities in the hotter flame, can be used with this flame to determine all three elements, zinc, calcium and barium, with adequate precision.

The majority of the oils in which we are interested contain only zinc or calcium additives, either alone or in combination, and we have shown that these elements can be determined precisely using the relatively safe air - acetylene flame. It is felt at present, therefore, that there is not sufficient justification to carry out a programme of work on the application of the 2-methylpropan-2-ol - toluene solvent in the determination of zinc, calcium and barium in the nitrous oxide - acetylene flame.

The development of a satisfactory procedure for barium means that atomic-absorption spectrophotometric procedures for the three additive elements, zinc, calcium and barium, can be recommended. The results obtained indicate that the methods are suitable for the analysis of samples of unknown composition and also for blending quality control. However, their general applicability can only be adequately tested on an inter-laboratory basis.

Conclusions

Barium alone or in the presence of calcium can be readily determined by atomic-absorption spectrophotometry using a nitrous oxide - acetylene flame. The oil must be diluted in a solvent consisting of 2-methylpropan-2-ol - toluene (3 + 2) plus small amounts of water.

Although calcium and zinc could probably be determined by use of the new procedure for barium a satisfactory atomic-absorption spectrophotometric procedure already exists for their determination and further work is not at present justifiable.

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The Determination of Silver in Animal Tissues by a Wet-oxidation Process Followed by Atomic-absorption Spectrophotometry

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Silver in animal tissues can be determined at fairly low levels by conventional atomic-absorption spectrophotometry, following wet oxidation. The wet-oxidation stage is difficult when large samples are to be analysed and the final solution must be adjusted in order to minimise losses of silver by adsorption or precipitation. The method has been applied to samples of urine, faeces, individual organs, skin and whole-animal homogenates.

There is little information in the literature on the normal levels of silver in animal tissues or on its toxicity. Thus, the work described below was undertaken as part of a toxicological study involving silver taken orally. The experimental animals included mice, rats and monkeys, and the distribution of the silver in the body was studied.

Atomic-absorption spectrophotometry was the technique chosen for this work; the detection limit is good, less than 0·01 μg ml^{-1}, and because interferences are few, sample preparation is fairly simple.

Experimental

The samples to be analysed varied widely in nature and in mass. They included faeces, urine soaked into filter-paper, individual organs from mice, such as liver, kidney or gut, whole-mouse homogenates, whole-rat homogenates and pieces of monkey skin with varying amounts of subcutaneous fat.

Samples were wet oxidised by a method similar to that described by Nangniot,[1] in which the sample is heated gently with a 1 + 1 mixture of nitric and perchloric acids, using about 5 ml of the acid mixture per gram of organic material present.

The smaller samples, containing up to 2–3 g of organic material, responded well to the method, oxidising smoothly and rapidly to give clear solutions and small white final residues. Recoveries of silver added to portions of rat homogenate tended to be low (see Table I) and this was ascribed to the formation of chloride during the final stages of oxidation. The silver was added to the sample as silver nitrate solution before the addition of the acid mixture. The addition of ammonia solution to the final solutions gave quantitative recoveries within the limits of experimental error, *i.e.*, ±3 per cent. over the range 1–100 μg of silver (Table I).

TABLE I

RECOVERY OF ADDED SILVER

Additions were made to a rat homogenate sample that was prepared from an animal that had not been dosed with silver.

Sample size/g	Procedure	Silver added/μg	Silver found/μg
0·2	HNO_3 - $HClO_4$	1·0	0·54, 0·63
0·2	HNO_3 - $HClO_4$	10	8·6, 7·5
0·2	HNO_3 - $HClO_4$	100	95, 92
0·2	HNO_3 - $HClO_4$ - NH_4OH	1·0	0·98, 1·03
0·2	HNO_3 - $HClO_4$ - NH_4OH	Nil	<0·1, <0·1
0·2	HNO_3 - $HClO_4$ - NH_4OH	10	9·9, 10·4
0·2	HNO_3 - $HClO_4$ - NH_4OH	100	102, 98
10	HNO_3 - $HClO_4$ - NH_4OH	1·0	0·96, 0·99
10	HNO_3 - $HClO_4$ - NH_4OH	100	96, 99
10	HNO_3 - H_2SO_4	100	65, 56
10	HNO_3 - H_2SO_4 - NH_4OH	100	72, 68
10	HNO_3 - $HClO_4$ - NH_4OH	Nil	1·0, 0·95
50	HNO_3 - $HClO_4$ - KCN	Nil	4, 7, 5

Oxidation of whole-mouse homogenates and monkey skin samples caused more difficulty because of the larger amount of organic material present, *i.e.*, up to 10 g, and the relatively high fat content. This procedure relies for safety on the formation of a homogeneous solution, which is not obtained with high-fat materials. Fat usually separates and forms a separate upper phase, so that it reacts only to a limited extent with the nitric acid and remains relatively inert until the situation arises in which a fat - hot perchloric acid interface exists; this situation is hazardous. When such an interface occurred the solutions blackened and began a vigorous ebullition as the last traces of nitric acid boiled off. This necessitated continuous supervision of the oxidation and the addition of further nitric acid as this stage began, which was considered potentially hazardous; alternative methods of oxidation were therefore investigated.

Oxidation mixtures that involved the use of sulphuric acid were found to be unacceptable, as copious amounts of calcium sulphate were deposited at the final evaporation stage and arge losses of silver by adsorption were incurred (Table I). Dry ashing was rejected because of the large amounts of objectionable fumes evolved and because the residues obtained were difficult to dissolve. The nitric acid - perchloric acid digestion was therefore retained, despite being recognised as hazardous, as with reasonable care it gave acceptable results.

Whole-rat homogenates caused considerable difficulties in that the animals as supplied were not particularly well homogenised and it appears to be difficult to homogenise effectively an animal of this size. It was agreed with a toxicologist that at least 10 per cent. of the whole body mass would have to be taken in order to ensure a representative sample, and as a male rat weighs up to 600 g, sample masses of 50–100 g were necessary. This range usually corresponded to 150–300 g of homogenate.

Initial attempts to use the nitric acid - perchloric acid oxidation were obviously extremely dangerous, although we have had considerable experience in the wet oxidation of many types of organic material. Five grams of organic matter have usually been regarded as a desirable upper limit and we have never experienced an actual explosion from mixtures of organic matter and perchloric acid. The worst uncontrolled reaction to date has been spontaneous ignition in a beaker, when a reaction mixture had been allowed to blacken and boil to dryness. When using the large, high-fat rat samples it soon became apparent that there was a real danger of an explosion involving tens of grams of material, and a new approach was obviously essential.

Many animal tissues can be dissolved in hot, concentrated nitric acid to give true solutions, and even fat can be distributed by swirling or shaking the vessel to give a relatively homogeneous suspension. If such a solution is added to a large excess of hot perchloric acid in small portions, the organic material will be rapidly oxidised without bringing large amounts of it into contact with perchloric acid. Such a procedure is hazardous in that it requires the handling and transfer of fairly large volumes of hot, concentrated acids, but is much safer from the point of view of risk of explosion.

The procedure given below describes this approach; it should be carried out by an experienced analyst, aware of the dangers, and wearing suitable protective clothing. Gloves and safety glasses are a minimum requirement and all beakers should be handled with tongs. It is very important that the perchloric acid should be boiling, not merely fuming, and that it should be brought back to the boil after each addition of the nitric acid solution and before the next. It is easy to mistake the ebullition of reaction for boiling and if too much organic material is introduced at once, the perchloric acid solution will boil over as a vigorous oxidation takes place.

The solutions obtained from the large rat samples did not respond well to treatment with ammonia solution in order to dissolve the silver chloride. Too much calcium phosphate was present to permit working with final volumes smaller than 500 ml and there was a considerable loss of silver by entrainment (Table I). The use of acidic solutions in order to maintain calcium salts in solution also caused a loss of silver, however, so that it was finally decided to use a cyanide complex, which is soluble and resistant to hydrolysis by dilute acids. There is usually sufficient iron present to complex any excess of cyanide, so that there is little hazard in acidifying the cyanide solution. Recoveries of added silver were found to be good by this method, and the results are given in Table I.

Three procedures are described, for various sample sizes; the smallest sample size compatible with the provision of a representative sample should be used. The results obtained with all three methods are given in Table II.

TABLE II

TYPICAL RESULTS ON TISSUE SAMPLES

Whole-mouse homogenates—

	Total silver/μg
Control	<1
Control	<1

Whole-mouse homogenates and corresponding excreta (all body masses were 20 ± 2 g)—

Silver in whole body, 24 h after dosing/μg	Silver content of faeces in 24 h/μg	Silver content of urine in 24 h/μg
11	10	2·8
5·8	2·4	1·0
12	6·8	3·3
29	5·6	0·9
22	0·4	1·3
36	4·2	2·4

Individual organs—

Time after dosing	Silver in gut/μg	Silver in liver/μg	Silver in kidney/μg
8 h	7·4	1·2	17
48 h	2·0	3·8	24
72 h	3·4	8·2	0·8
7 d	1·4	1·8	0·8

Monkey skin samples—

Monkey	Sample site	Silver/μg	Silver/μg g^{-1}
M1	Chest	3·6	0·5
	Hand	0·6	0·3
	Foot	4·0	1·0
	Muzzle	18	4·7
M2	Chest	1·6	0·2
	Hand	0·6	0·2
	Foot	1·0	0·2
	Muzzle	0·6	0·1
M3	Chest	2·7	0·4
	Hand	4·4	1·6
	Foot	1·2	0·5
	Muzzle	0·8	0·2

Rat homogenates—

Animal	Body mass/g (10–20% taken)	Silver in whole body/μg
1	425	3280
2	515	4710
3	259	21
4	556	125
5	256	5080
6	287	60
7	245	880
8	245	4000

Method

Reagents

Nitric acid, sp. gr. 1·42.
Perchloric acid, sp. gr. 1·54.
Nitric acid (1 + 1). Mix equal volumes of nitric acid (sp. gr. 1·42) and water.
Ammonia solution (1 + 1). Mix equal volumes of ammonia solution (sp. gr. 0·89) and water.
Potassium cyanide solution, 1 per cent. Dissolve 10 g in water and dilute to 1 l.
Tartaric acid solution, 2 per cent. Dissolve 20 g in water and dilute to 1 l.

Procedure 1. Tissue Samples up to 0·5 g (*e.g.*, Individual Organs)

Weigh the sample into a 150-ml squat beaker and add 5 ml each of concentrated nitric and perchloric acids. Cover the beaker and evaporate the contents on a hot-plate at a rate such that the nitric acid is boiled off in 10–15 min and the perchloric acid begins to fume. If the solution darkens at this stage add a few drops of nitric acid in order to clear it. When oxidation has ceased, and the solution is colourless, remove the cover and evaporate the solution to dryness.

Re-dissolve the residue in 2 ml of nitric acid (1 + 1), cool and transfer it to a 25-ml calibrated flask. Add 2 ml of 2 per cent. tartaric acid solution, rinse the beaker with 5 ml of ammonia solution (1 + 1), add the rinsings to the flask and dilute to volume with water.

Determine the amount of silver in this solution by use of atomic-absorption spectrophotometry, using the 328·0-nm resonance line for highest sensitivity; a stoicheiometric air - acetylene flame has been found to give the best results in our laboratory. Standardise the instrument against aqueous silver solutions containing the same concentrations of ammonia and nitric acid.

Procedure 2. Tissue Samples up to 10 g (*e.g.*, Whole Mice)

Weigh the sample into a 600-ml squat beaker and add 30 ml of concentrated nitric acid. Cover the beaker. Boil the mixture gently until the initial reaction and effervescence has subsided, then evaporate it to as small a volume as possible without allowing the solution to boil dry. Add 30 ml of concentrated nitric acid and 30 ml of perchloric acid and evaporate again until incipient fumes of perchloric acid appear. At this stage the solution will probably darken; if darkening occurs, add 1–2 ml of nitric acid to the fuming solution. A vigorous ebullition will occur and as the nitric acid boils off the solution will darken again; the addition of nitric acid should then be repeated. Continue with this procedure until the solution does not darken beyond a clear brown colour; at this stage there must be at least 10 ml of perchloric acid still present, and more should be added if necessary. Make the same additions of extra acids to the blank determination and continue to evaporate to fumes until the solution is colourless or very pale yellow. Remove the cover from the beaker and evaporate the solution to dryness.

Re-dissolve the residue in 5 ml of nitric acid (1 + 1), cool the solution and transfer it to a 50-ml calibrated flask. Add 5 ml of 2 per cent. tartaric acid solution, rinse the beaker with 10 ml of ammonia solution (1 + 1) and add the rinsings to the flask. Make the solution up to volume and determine the silver as in Procedure 1.

Procedure 3. Tissue Samples in Excess of 10 g

Weigh the frozen homogenate and cut portions corresponding to 10–20 per cent. of the homogenate; an amount of sample corresponding to 50–60 g of the animal is the most that can be handled. As many animals cannot easily be homogenised, as large a sample as possible should be taken in order to ensure that it is representative.

Transfer the sample to a 600- or 800-ml squat beaker, add 100 ml of concentrated nitric acid and heat. Reduce the level of heating as necessary if frothing becomes excessive; it may be necessary in extreme cases to use surface heating with an infrared lamp. When the volume has been reduced to about 20 ml, monitor the evaporation continuously and remove the beaker from the heat as soon as the residue begins to darken. Then add 100 ml of concentrated nitric acid and heat the mixture to boiling.

Heat 120 ml of perchloric acid (sp. gr. 1·54) to boiling in a 600- or 800-ml conical flask. Swirl the solution in nitric acid to disperse the fat throughout, and cautiously pour a few millilitres into the boiling perchloric acid. A violent ebullition will take place, and the solution may darken. If the solution darkens appreciably, add a further 50 ml of nitric acid to the squat beaker in order to dilute the organic matter further. Allow the oxidation to reach completion and the solution to clear, and add a further few millilitres of nitric acid solution. Care should be taken that only a few millilitres are added at a time, or the oxidation may become uncontrolled. When all of the solution has been transferred and the final oxidation completed, allow the perchloric acid to cool. Then, add 20 ml of nitric acid to the beaker, transfer the solution in perchloric acid to it and evaporate the whole solution first to fumes of perchloric acid and finally to dryness.

Re-dissolve the residue in 5 ml of nitric acid (1 + 1), and transfer the solution to a 100-ml calibrated flask. Rinse the beaker with water, then with 10 ml of ammonia solution (1 + 1), and add the rinsings to the flask. At this stage calcium phosphate and metal hydroxides will precipitate. Next rinse the beaker with 10 ml of 1 per cent. potassium cyanide solution and again add the rinsings to the flask, and then add 1 + 1 nitric acid dropwise until the precipitate just re-dissolves. Finally, cool the solution, dilute it to 100 ml with water and determine the silver as in Procedure 1.

Discussion

The recoveries of added silver suggest that the method is accurate to about the same degree as the reproducibility, *i.e.*, 10–15 per cent. relative at the blank level, which is usually less than 10 μg of silver with the volumes of reagents required for 50 g of sample. The detection limit is set by the variance of the blank, and is about 0·1 $\mu g\ g^{-1}$ of sample.

The interpretation of the results has more toxicological than analytical significance. It can be seen that the level of silver in unexposed animals is less than 0·1 $\mu g\ g^{-1}$, and that large amounts of silver are absorbed into the tissues following ingestion.

The number of distribution and elimination experiments was small, but it can be seen that in mice a large proportion of the silver is eliminated in the faeces. It also passes fairly rapidly from the gut to the kidneys, where a further substantial fraction is lost via the urine; there is some evidence of retention in the liver for a period of a few days.

The distribution experiments on monkeys were inconclusive, which may be a result of the difficulty of removing skin samples with a constant amount of subcutaneous fat, or it may be caused by the small number of animals involved.

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The Determination of Unsulphonated Primary Aromatic Amines in Water-soluble Food Dyes and Other Food Additives

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EEC directives prescribe limits for the amount of unsulphonated primary aromatic amines present in food colouring matters in general and prohibit the presence of particular carcinogenic amines in food dyes and other food additives. Integrated methods based on solvent extraction and thin-layer chromatography have been devised for identification of these amines, followed by the spectrophotometric determination of their condensation products with 4-dimethylaminocinnamaldehyde. The method is also extended to the determination of primary aromatic amines in biphenyl and to the determination of aminoazobenzenes in the dye Fast Yellow. An additional thin-layer chromatographic method is given for the differentiation between the isomers of naphthylamine as their tosyl derivatives.

Directives in force within the European Economic Community (EEC)[1] require synthetic organic colouring matters for use in foods not to contain more than 0·01 per cent. of free aromatic amines nor more than 0·5 per cent. of intermediate synthetic products other than free aromatic amines. Further, they should not contain 2-naphthylamine, benzidine or 4-aminobiphenyl (xenylamine) or their derivatives. In Britain, the use of these three compounds is prohibited,[2,3] except for special research purposes, and as a result authentic reference samples are difficult to obtain. In general, for purposes of identification the analyst has to rely on the physical data for these substances of unspecified purity quoted in the literature.[1,4,5] Examples of such data are given in Tables I–IV.

Few up-to-date methods for the analysis of food dyes have been published in recent years and those previously available[6–8] are considered to lack sensitivity and specificity. Several gas-chromatographic methods[9,10] are available but are unsuitable because of the difficulty of expressing mixtures of amines as "aniline," as required in the EEC directives, and because gas - liquid chromatography by itself would not give an unambiguous identification. Methods that depend on the production of a diazonium compound, followed by addition of a coupling agent in order to produce a colour, require specific conditions for each amine under test and for each individual coupling agent. Several thin-layer chromatographic methods[5,11,12] are suitable for the separation of the amines under consideration; some of these methods have been adapted to the determination of these amines in dyes, as also have some spectrophotometric[4,13] methods.

The spectrophotometric methods adopted depend on the formation of Schiff's bases and afford quantitative determinations of individual amines that had previously been identified by thin-layer chromatography. Mixtures of amines are more difficult to analyse spectrophotometrically but a semi-quantitative estimate can be made following separation with thin-layer chromatography by comparing the intensities of individual spots with standards. However, the method is not sufficiently sensitive for a fully quantitative determination to be made by scraping off the spots and eluting the amines with solvent in order to give a solution of a known volume.

Section 1 of this paper describes a basic procedure for the identification and determination of unsulphonated primary aromatic amines. However, it is not possible by this procedure to distinguish between 1- and 2-naphthylamines; a suitable method for this purpose is given in section 4. Section 2 describes the modifications necessary to make section 1 applicable to the examination of biphenyl, and section 3 extends the general procedure to the determination of aminoazobenzenes in Fast Yellow.

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1. Determination of Unsulphonated Primary Aromatic Amines in Water-soluble Food Dyes

Apparatus

Separating funnels. 100-ml capacity, with a PTFE stopcock and glass stopper.

Capillary tubes or micropipettes. 5-μl capacity, for spotting samples on to thin-layer chromatographic plates, and 100-μl capacity, for dispensing 3 M hydrochloric acid.

Pipettes. 1- and 5-ml capacity.

Pasteur capillary pipettes.

Filter-paper. Strips of a Whatman low-ash grade filter-paper, approximately 20 × 50 mm.

Cellulose thin-layer chromatographic plates. Prepare the plates as follows: shake 20 g of microcrystalline cellulose powder with 60 ml of methanol for 2 min, then immediately spread the mixture on 200 × 200-mm glass plates to a thickness of 0·25 mm. After air-drying them for 30 min, dry the plates for 10 min at 105 °C. Up to five plates can be prepared in one application by using the above amount of mixture. Equally suitable are 200 × 200-mm ready-made plates of 0·1-mm thickness, which are obtainable from E. Merck.

Test-tubes. 10-ml capacity, pointed and graduated, with ground-glass necks and stoppers.

Recording spectrophotometer. Suitable for scanning in the range 350–600 nm.

Spectrophotometer cells. 10-mm path length.

Reagents

Sodium hydroxide solution, 1 M.

Toluene. Analytical-reagent grade.

Hydrochloric acid, 3 M.

Methanol. Analytical-reagent grade.

Propan-2-ol. Analytical-reagent grade.

Mobile solvent for thin-layer chromatography. Dissolve 1·0 g of sodium acetate in about 10 ml of distilled water and add 0·10 ml of glacial acetic acid. Transfer the mixture, with aqueous washings, into a 100-ml calibrated flask and make the volume up to the mark with water.

4-Dimethylaminocinnamaldehyde (DAC) solution A. Dissolve 0·1 g of DAC in about 50 ml of methanol, carefully add 5 ml of concentrated sulphuric acid, transfer the mixture plus washings with methanol into a 100-ml calibrated flask and make the volume up to the mark with methanol.

DAC solution B. Dissolve a mixture of 0·1 g of DAC and 0·1 g of *p*-toluenesulphonic acid in about 10 ml of propan-2-ol, transfer the mixture plus washings with propan-2-ol into a 100-ml calibrated flask and make the volume up to the mark with propan-2-ol.

4-Dimethylaminobenzaldehyde solution (DAB). Dissolve 1 g of DAB in about 10 ml of methanol, add 4 ml of concentrated hydrochloric acid, transfer the mixture plus washings with methanol into a 100-ml calibrated flask and make the volume up to the mark with methanol.

Procedure

Weigh accurately about 3 g of dye into a separating funnel containing 50 ml of water. Shake the funnel in order to dissolve the sample. Add 10 ml of 1 M sodium hydroxide solution and mix, then add 5 ml of toluene and shake the funnel for 1 min. Allow the layers to separate and discard the lower aqueous layer. Add another 5-ml portion of 1 M sodium hydroxide solution and shake the funnel for 1 min. If on separation the lower layer is coloured (caused by partition of the dye between the organic and inorganic phases) discard the lower layer and repeat the above extractions with alkali. Finally, wash the toluene extract with two 10-ml portions of water and discard the washings. Dry the inside of the stem of the separating funnel, then insert a 20 × 50-mm rolled-up strip of Whatman low-ash filter-paper.

Transfer the toluene extract into a graduated, pointed ground-glass test-tube containing 100 ± 2 μl of 3 M hydrochloric acid, stopper the tube and shake it vigorously for about 30 s. Allow the acid to sink to the bottom of the tube (it may be helpful to tap the side of the tube) and carefully remove as much as possible of the toluene with a Pasteur capillary pipette, the toluene being discarded. Spot two 5-μl portions of the remaining acidic solution about 100 mm apart, and each 20 mm from the bottom of a cellulose thin-layer chromatographic plate, and spot 5 μl of appropriate standards alongside each spot under test. Run the

chromatogram in a tank containing the prescribed mobile solvent until the solvent has travelled about 150 mm above the base-line. Allow the plate to dry, cover one set of standards and sample with a glass plate and spray the remaining set with DAC solution A. Cover the other half of the plate, then spray with DAB solution. Compare the positions and colours of spots in the sample with the standards. The chromatographic behaviour of some amines that may be present is shown in Table I.

TABLE I

CHROMATOGRAPHIC PROPERTIES OF DAB AND DAC DERIVATIVES OF SOME AMINES

	R_F value on cellulose TLC plate	Colour produced with DAB spray	Colour produced with DAC spray
Aniline	0·88	Yellow	Pink
p-Toluidine	0·86	Yellow	Orange - pink
1-Naphthylamine	0·47	Yellow	Purple - pink
2-Naphthylamine	0·46	Yellow	Bluish pink
4-Aminobiphenyl	0·34	Yellow	Bluish pink
Benzidine	0·32	Orange - pink	Blue

By using this technique it is not possible to differentiate between 1- and 2-naphthylamines, for which purpose a separate method is described in section 4.

The absolute detection limits for the DAC spray are 10 ng of 4-aminobiphenyl and 2-naphthylamine and 2 ng of benzidine, which are equivalent to 0·07 and 0·02 $\mu g\ g^{-1}$, respectively, when 5 μl are spotted from 3-g samples.

Spectrophotometric Determination of Amines

The identity of the amines may have been indicated by the thin-layer chromatographic method described in the second paragraph of Procedure and the operator can then refer to the appropriate standards, the spectrophotometric properties of which are shown in Table II. Confirmation of identity can be made by spectrophotometric analysis as described below.

To the remainder of the acidic solution (or to a 5-μl aliquot of this solution if the presence of large amounts of aniline or toluidine has been indicated) and to another tube containing 100 μl of 3 M hydrochloric acid (to be used as a blank), add 1 ml of DAC reagent B and make the volumes up to a total of 6 ml with methanol. Mix and allow the solution to stand for 20 min. Scan the absorption spectrum of the colour produced (if any) in the range 350–600 nm by means of a recording spectrophotometer, using 10-mm cells with the reagent blank as reference. Note the absorbance at the wavelength of maximum absorption and determine the amount of amine present by comparison with appropriate standards, as shown in Table II.

TABLE II

SPECTROPHOTOMETRIC PROPERTIES OF THE AMINE STANDARDS

	$\lambda_{max.}$ for DAC derivative/nm	Absorbance of 10 μg in 6 ml of final solution (10-mm cell)
Aniline	518	0·46
p-Toluidine	521	0·75
1-Naphthylamine	512	0·33
2-Naphthylamine	534	0·79
4-Aminobiphenyl	534	0·62
Benzidine	576	0·64

The results should be expressed as aniline unless another amine is known to be present. With a mixture of amines the results should be expressed in terms of the predominant amine.

Discussion of Experimental Details

In the final stage of the extraction procedure it was established that the amines should be extracted as their hydrochlorides so as to prevent losses caused by the relatively high volatilities of free aniline and toluidine. Extraction into a large volume of hydrochloric acid followed by evaporation down to a small volume proved laborious and gave rise to several unknown interferences in both the thin-layer chromatographic and spectrophotometric determinations. As far as possible it is necessary to exclude water from the amine - DAB and amine - DAC reactions, which are of the condensation type. The spectrophotometric method

with DAC can tolerate the presence of up to 2 per cent. of water, above which content the sensitivity[4] is markedly reduced.

Several acids were tried in the spectrophotometric procedure before finally deciding on the use of *p*-toluenesulphonic acid. Mineral acids such as hydrochloric or sulphuric acid caused great changes in sensitivity with different acid concentrations,[4] as well as producing unstable complexes. They were, however, satisfactory for use in the DAB and DAC spray reagents. Trichloroacetic acid produced more stable colours but only at very high acid concentrations. *p*-Toluenesulphonic acid, although producing less sensitive colours, formed very stable solutions at low acid concentrations with all the amine complexes tested, reaching a maximum colour intensity within 20 min.

2. Determination of Unsulphonated Primary Aromatic Amines in Biphenyl

Biphenyl, which is often used as a preservative for oranges and other citrus fruits, is liable to contain trace amounts of amines. An EEC directive[14] requires that biphenyl shall contain not more than 2 mg kg^{-1} of aromatic amines, expressed as aniline. The proposed method is essentially the same as that described in section 1 for dyes, apart from a variation in the extraction procedure.

Procedure

Dissolve 3 g of biphenyl in 5 ml of toluene and transfer the solution into a separating funnel with the aid of a further 5 ml of toluene. Add 2 ml of 3 M hydrochloric acid and 50 ml of water and shake the mixture for 1 min. Allow the layers to separate and run the lower aqueous layer into a second separating funnel containing 10 ml of 1 M sodium hydroxide solution and 5 ml of toluene. Proceed as in the first and second paragraphs of the procedure in section 1.

3. Determination of 2- and 4-Aminoazobenzenes in Fast Yellow

Fast Yellow dye (C.I. Number 13015), also known as Acid Yellow 9 and Food Yellow 2, is manufactured by the disulphonation of 4-aminoazobenzene which, with the 2-isomer, occurs as an impurity in the dye. Apart from the general requirement, the EEC directive[1] specifically requires that Fast Yellow (E105) shall contain not more than 10 mg kg^{-1} of unsulphonated aromatic amines. In the procedure for the determination of amines in dyes (section 1), these substances exhibit some properties that may mistakenly be taken as evidence for the presence of benzidine. The following method permits individual spectrophotometric determinations to be made of the extracted isomers after separation by column chromatography.

Several improvements in the method described in the EEC directive[1] have been made, namely an approximate ten-fold reduction in the amounts of sample and solvent, and the use of toluene instead of chlorobenzene Toluene has two advantages over chlorobenzene for use in this method. Firstly, it does not form emulsions when shaken with aqueous solutions as does the latter. Secondly, it is more convenient to use a solvent that has a lower density than water when several washes with water are required.

Apparatus

Chromatographic column. Glass, with PTFE stopcock and Quickfit joint at the neck, length at least 200 mm and internal diameter approximately 15 mm.

Separating funnel. Cylindrical, 100 ml in capacity, with ground-glass spout to fit the neck of the chromatographic column.

Reagents

Alumina. Brockmann activity II, equivalent to a 3 per cent. water content. For preparation of the alumina column for chromatography, weigh accurately 20 g of alumina into a beaker, add a few millilitres of toluene, mix and pour the slurry into the chromatographic column fitted with a small cotton-wool plug at the bottom. Run off the toluene until the meniscus coincides with the top of the column of alumina.

Procedure

Dissolve 2·0 g of Fast Yellow in 20 ml of water and transfer the solution into a separating funnel with the aid of a further 30 ml of water. Proceed thereafter as in the first paragraph

of the procedure in section 1. Transfer the toluene extract into a test-tube and reduce the volume of the solution to about 0·5 ml by evaporation. Transfer the solution and a small amount of toluene used for washings on to the prepared alumina column and elute it with toluene. Collect separately the first 30 ml of eluate, which contains the 2-aminoazobenzene, and the next 40 ml containing the 4-isomer. Make the volume of each fraction up to 50 ml with toluene and scan each solution on a spectrophotometer over the range 300–500 nm. Note the wavelength of maximum absorbance of each solution and the absorbance at that wavelength and compare the values with the standards shown in Table III.

TABLE III

SPECTROPHOTOMETRIC PROPERTIES OF THE AMINOAZOBENZENES

	$\lambda_{max.}$/nm	Absorptivity (1 mg ml^{-1}, 10-mm path length)
2-Isomer	414	40
4-Isomer	376	110

4. Detection and Identification of 1- and 2-Naphthylamines in Water-soluble Food Dyes and Other Food Additives

With neither the cellulose thin-layer chromatographic method nor the spectrophotometric method is it possible to distinguish satisfactorily between 1- and 2-naphthylamines in the presence of each other or in the presence of large amounts of other amines. However, their tosyl derivatives can be resolved by the following method, which affords further confirmation of the identity of amines determined by the thin-layer chromatographic and spectrophotometric methods. Of other amine derivatives that were investigated for suitability for thin-layer chromatography, only the dansyl derivative gave reasonable separation of 1- and 2-naphthylamines. The spots produced by the dansyl derivatives were more compact and more strongly fluorescent than those of the tosyl derivatives but the presence of hydrolysis and other products gave a chromatogram that was too complex for positive identification.

Apparatus

Alumina thin-layer chromatographic plate. Dimensions 200 × 200 mm and 0·1 mm thick, with a pH of approximately 9 (obtainable from Eastman Organic Chemicals).

Reagents

Mobile solvent. n-Hexane - chloroform (2 + 1 *V*/*V*).
p-*Toluenesulphonyl fluoride.*

Procedure

Prepare the amine mixture in toluene as described in the first paragraph of the procedure in section 1. Transfer this solution into a graduated, pointed test-tube containing approximately 10 mg of *p*-toluenesulphonyl fluoride. Hold the tube over a steam-bath and slowly pass air into the tube until the volume of the solution is reduced to about 0·1 ml. Spot 5 μl of this solution about 20 mm from the bottom of an alumina thin-layer chromatographic plate and spot 5 μl of available appropriate standards alongside. Run the chromatogram in the hexane - chloroform mobile solvent until the solvent front has travelled about 150 mm from the base-line. Allow the plate to dry and then observe the spots under ultraviolet radiation. Note the positions and colours of the spots and compare them with standards (Table IV).

TABLE IV

PHYSICAL PROPERTIES OF AMINE TOSYLATES

	R_F value on alumina	Fluorescence under ultraviolet radiation
Aniline	—	Not observed
p-Toluidine	—	Not observed
1-Naphthylamine	0·62	Sky blue
2-Naphthylamine	0·57	Dark blue
4-Aminobiphenyl	0·59	Dark blue
Benzidine	0·11	Dark blue

Discussion

Although the tosyl derivatives of 1- and 2-naphthylamine are not completely resolved by the alumina thin-layer chromatographic method, either isomer can be observed in the presence of at least a ten-fold excess of the other as they give different fluorescent spectra. Large excesses of aniline or *p*-toluidine, which are liable to interfere in 1- and 2-naphthylamine determinations by the cellulose thin-layer chromatographic method, do not interfere under the reaction conditions described in section 4; 4-aminobiphenyl or benzidine would not normally be expected to occur with the naphthylamines and in any event would have already been identified by the latter method.

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Determination of 3,5-Dinitro-*o*-toluamide in Feedstuffs and Pre-mixes

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A spectrophotometric method is described for the determination of 3,5-dinitro-*o*-toluamide (dinitolmide) in feedstuffs and pre-mixes. Dinitolmide is extracted from the sample with acetone - water (1 + 1). After purification procedures, which include liquid - liquid extraction and chromatography on an alumina column, a coloured complex is formed with sodium hydroxide, which is measured spectrophotometrically.

3,5-Dinitro-*o*-toluamide (dinitolmide) is a coccidiostat that is incorporated in feedstuffs, usually at a concentration of 125 mg kg^{-1}. The current spectrophotometric methods for determining dinitolmide in feedstuffs and pre-mixes[1–3] have the disadvantage that they require the use of several highly toxic agents, such as acetonitrile, dimethylformamide and methylamine.

In a search for less toxic agents, we found that under certain conditions Janovsky's colour reaction[4,5] could be applied to the determination of dinitolmide. We further found that for a suitable range of concentrations this colour reaction obeyed Beer's law. Based on Smith's method,[6] we extracted dinitolmide from the sample with a water - acetone mixture. Dinitolmide can be extracted from this medium with organic solvents, and of these solvents dichloromethane proved to be the most suitable for our purpose because of its relatively low toxicity.[7] However, Janovsky's reaction could not be directly applied to these extracts; further purification was necessary. Previous experience with chromatography had shown that dinitolmide when dissolved in methanol will pass through a column of alumina without being absorbed and without loss, while substances that may interfere are adsorbed.

Method

Apparatus

Chromatographic columns. These were made of glass, of internal diameter 10 mm and length 200 mm, narrowed at one end, which was plugged with cotton-wool. For the preparation of the columns, transfer into a column a slurry of 4–5 g of alumina with dichloromethane. Prepare a separate column for each sample less than 5 min before use.

Rotary evaporator.

Spectrophotometer.

Reagents

All reagents should be of analytical-reagent grade.

Acetone.

Acetone - water (1 + 1).

Dichloromethane.

Methanol.

Alumina. E.g., Merck, Darmstadt, 1097, activity grade II–III.

Sodium hydroxide solution. Dissolve 15 g of sodium hydroxide in water and dilute to 100 ml with water.

Hydrochloric acid, 5 mol l^{-1}.

Dinitolmide reference standard.

Procedure

Weigh accurately 2–25 g of sample containing 0·25–50 mg of dinitolmide into a 300-ml glass-stoppered conical flask. For feeds containing 125 mg kg^{-1} of dinitolmide, a sample size of 5 g is usually taken. Add 100·0 ml of acetone - water (1 + 1) and agitate the mixture for 1 h. Filter it through filter-paper, rejecting the first 15 ml of filtrate. Prepare from the filtrate a solution in acetone - water (1 + 1) containing 2·5–20 mg l^{-1} of dinitolmide [dilution factor (F) = final volume divided by initial volume].

Transfer 20·0 ml of this solution into a 100-ml separating funnel. Add 5 ml of hydrochloric acid (5 mol l^{-1}), mix and add 25 ml of dichloromethane. Agitate the mixture gently by inverting the funnel ten times. After the phases have separated, draw off the dichloromethane layer through a cotton-wool plug into a 250-ml evaporating flask. Extract the aqueous layer three times more with 25-ml portions of dichloromethane, shaking the funnel well for 1 min each time. Collect all dichloromethane extracts in the same evaporating flask. Reduce the volume of the combined extracts to about 5 ml at 35 °C in a rotary evaporator. Pour this solution on to a freshly prepared chromatographic column, washing the evaporating flask and column four times with 4-ml portions of dichloromethane.

Elute the dinitolmide with 30 ml of methanol. Collect the eluate in a 100-ml evaporating flask and evaporate it to dryness at 50 °C in a rotary evaporator. Dissolve the residue in 5 ml of acetone by heating the mixture for a few seconds in a water-bath at 50 °C. Cool and transfer the solution into a 25-ml calibrated flask. In order to ensure complete dissolution of the residue, repeat this procedure with three more 5-ml portions of acetone, adding them to the flask. Make the volume up to the mark with acetone and mix.

Transfer 5·0 ml of this solution into a 50-ml conical flask and add, by pipette, 0·40 ml of sodium hydroxide solution. Swirl the flask carefully for 10 s. Read the absorbance, within 3 min of adding the sodium hydroxide solution, on a spectrophotometer at 576 nm in a 10-mm cell against water as reference solution. Prepare blanks by adding 0·40 ml of water to 5·0 ml of sample solution and 0·40 ml of sodium hydroxide solution to 5·0 ml of acetone. Subtract both values from the sample value.

After taking into account these blank values, the amount of dinitolmide present in the samples can be calculated by reference to a standard graph.

Preparation of Standard Graph

Weigh accurately 40·0 mg of dinitolmide reference standard into a 100-ml calibrated flask. Dissolve the standard in and dilute to volume with acetone. Mix well, transfer 10·0 ml of the solution into a 200-ml calibrated flask, dilute to volume with acetone and mix the solution well. Transfer 5·0, 10·0, 20·0, 30·0 and 40·0-ml portions into separate 50-ml calibrated flasks, dilute to volume with acetone and mix. Transfer, from each flask, 5·0 ml into separate 50-ml conical flasks and proceed with the colour development and spectrophotometry as described above.

Calculation

Calculate the content, w (g per 100 g), of dinitolmide in the sample of feed or pre-mix from the following equation:

$$w = 2{\cdot}5 \times \rho \times F/m$$

where ρ mg per 5 ml is the concentration of dinitolmide in the sample solution; F, the dilution factor; and m g, the amount of sample of feed or pre-mix taken for analysis.

Shortened Method for Analysis of Pre-mixes Containing more than 1 g of Dinitolmide per 100 g

Pre-mixes containing more than 1 g of dinitolmide per 100 g can be examined by the following method. Weigh accurately a sample containing 40–50 mg of dinitolmide into a 300-ml conical flask. Add 100·0 ml of acetone and agitate the mixture mechanically for 15 min. Filter it through filter-paper, rejecting the first 15 ml. From this filtrate, prepare a solution with acetone so as to contain between 2 and 15 mg l^{-1} of dinitolmide. Transfer 5·0 ml of this solution into a 50-ml conical flask and proceed with the colour development and spectrophotometry as described above.

Calculate the amount, w (g per 100 g), of dinitolmide in the sample of pre-mix from the following equation:

$$w = 2 \times \rho \times F/m$$

(symbols as used above).

Results and Discussion

Contents or dinitolmide down to 10 mg kg^{-1} can be determined by the above method. Other chemotherapeutic additives to feedstuffs (amprolium, ethopabate, nitrofurazone,

furazolidone, nitrovin, nicarbazin, acetyl enheptin, furnicozon, dimetridazole, meticlorpindol, buquinolate, decoquinate, methylbenzoquate, sulfaquinoxaline, sulfamezathine, sulfacetamide, monensin, tetracycline, oxytetracycline, penicillin, streptomycin, zinc bacitracin, tylosin, oleandomycin, virginiamycin and spiramycin) do not interfere when present in the usual amounts. For instance, a content of nitrovin of 100 mg kg^{-1} (usually 10 mg kg^{-1}) gave a "dinitolmide recovery" of 10 mg kg^{-1}. Dinitrobenzamide interfered, but could be distinguished from dinitolmide by using concentrated ammonia solution instead of sodium hydroxide solution. Dinitolmide did not react with ammonia to give a blue colour.

The ratio of acetone to sodium hydroxide solution of 5·0:0·40 gives a coloured complex, which is stable for about 3 min and the absorbance must therefore be read within this time. The above method has been in normal routine use in our laboratory for almost 2 years and has given full satisfaction.

Results

We incorporated dinitolmide into several feed ingredients: feathermeal, fishmeal, grassmeal, maize gluten feed and meatmeal. All these ingredients showed low blank values (less than 3 mg kg^{-1}). When medicated with dinitolmide (125 mg kg^{-1}) the method gave complete recoveries, except with meatmeal, when 95 mg kg^{-1} was recovered.

Results for a commercial poultry feed that was medicated with 125 mg kg^{-1} of dinitolmide are given in Table I. The blank value of the feed was less than 1 mg kg^{-1}. The average value for the dinitolmide recovered was 124 mg kg^{-1} with a standard deviation of 4 mg kg^{-1}, and the limit of error for a 95 per cent. probability level was 2 mg kg^{-1}.

TABLE I

RECOVERY OF 3,5-DINITRO-*o*-TOLUAMIDE IN 24 DETERMINATIONS ON A COMMERCIAL POULTRY FEED MEDICATED WITH 125 mg kg^{-1}

Dinitolmide recovered/mg kg^{-1}			
128	126	127	127
124	119	122	126
128	128	127	115
128	127	121	113
117	121	123	127
125	125	119	122

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An Ion-selective Electrode Method for the Determination of Nitrate in Grass and Clover

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A method for determining the nitrate content of grass and clover involving the use of a nitrate-selective electrode is described. The method is rapid, accurate and precise and can be used for samples containing as little as 10 p.p.m. of nitrate-nitrogen.

Methods currently exist for the determination of nitrate-nitrogen in plant materials by using a nitrate-selective electrode,[1–4] but they have a lower limit of sensitivity of approximately 100 p.p.m. of nitrate-nitrogen. Hence they cannot be applied directly to grass and clover, which may contain much lower amounts of nitrate. This paper describes a procedure for the determination of nitrate in rye-grass, Blanca white clover and mixtures of them down to a lower sensitivity level of 10 p.p.m. of nitrate-nitrogen.

Experimental

Reagents

All the reagents were of AnalaR quality except when stated otherwise. De-ionised water was used throughout.

Buffer extraction solution. The concentration of this solution was 2·5 times that used by Milham *et al.*,[3] *i.e.*, 0·025 M with respect to aluminium sulphate, 0·025 M to silver sulphate, 0·050 M to boric acid and 0·050 M to sulphamic acid (microanalytical grade). The pH was adjusted to 3·0 with 0·1 M sulphuric acid.

Apparatus

Electrodes

A nitrate-selective membrane electrode (Corning Instruments Ltd.) was used in conjunction with a Corning silver - silver chloride glass double-junction reference electrode, the outer compartment of which was filled with 1 M sodium sulphate solution.

Electrometer

A Corning - EEL, Model 101, instrument was used. Nitrate-nitrogen concentrations of more than 5 p.p.m. in the test solution were read directly on the activity scale. Concentrations of between 1 and 5 p.p.m. were read from a calibration graph.

Procedure

Two procedures were employed, depending upon whether the sample contained more (A) or less (B) than approximately 500 p.p.m. of nitrate-nitrogen.

(A). Weigh approximately 0·1 g of sample, add 5 ml of buffer extraction solution and 5 ml of water. Mix in order to moisten all of the sample and shake the mixture gently for 5 min. Add 5 ml of buffer solution to 5-ml portions of potassium nitrate standard solutions containing an appropriate range of nitrate-nitrogen. Calibrate the electrometer with the standard solutions at 20 °C according to the operating instructions. Measure the nitrate-nitrogen concentration of the sample.

(B). Weigh approximately 1·0 g of sample, add 5 ml of buffer extraction solution and 5 ml of water. Mix, shake the mixture for 5 min and leave it for at least 2 h to complete the extraction. Calibrate the electrometer as for (A), and measure the nitrate-nitrogen concentration of the sample.

NOTE—

The test solution can be stirred mechanically in order to enhance the attainment of equilibrium, but this was found not to shorten the response time.

Results and Discussion

Fig. 1 shows the relationships obtained between millivolt readings and nitrate-nitrogen concentrations for standard potassium nitrate aqueous and buffered solutions containing between 0·14 and 7000 p.p.m. The difference between the curves was doubtless due to the difference in the ionic strengths of the solutions, but above concentrations of about 5 p.p.m. each approximates closely to that predicted by the Nernst equation.

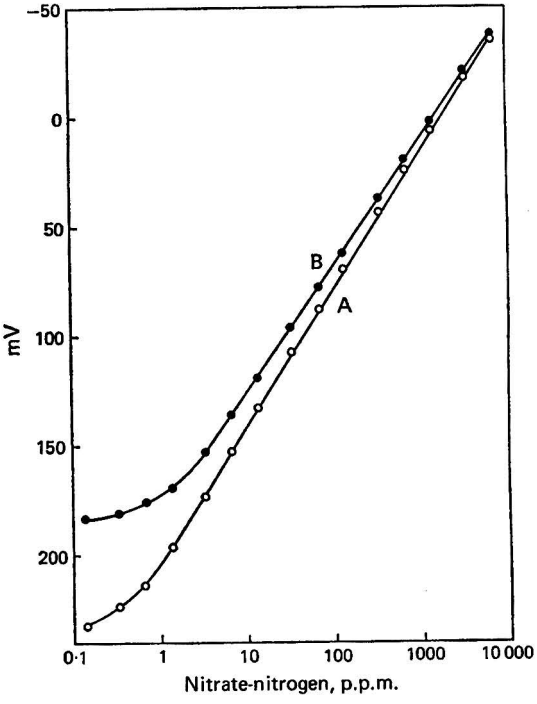

Fig. 1. Nitrate-selective electrode calibration graphs for aqueous (A) and buffered (B) standard solutions.

Attempts to use the buffer described by Milham *et al.*[3] in conjunction with 1·0-g samples of grass resulted in erroneously high nitrate values being obtained, probably as a result of incomplete removal of interfering ions. Table I demonstrates that the use of the more concentrated buffer solution described above resulted in similar nitrate recoveries for different amounts of the same grass. Although good nitrate recoveries could be obtained from 1·5 g of grass per 10 ml of test solution (5 ml of buffer solution plus 5 ml of water), the electrode response was very slow (about 5 min) and therefore this level of grass was not used for routine measurements.

TABLE I

EFFICIENCY OF BUFFER SOLUTION FOR NITRATE EXTRACTION

Grass	Ratio of sample (g) to buffer solution (ml)	Nitrate-nitrogen, p.p.m. Test solution	Nitrate-nitrogen, p.p.m. Dried grass
1	0·1 : 10	8	800
	0·3 : 10	25	833
	1·0 : 10	82	820
	1·5 : 10	122	814
2	0·1 : 10	35	3500
	0·3 : 10	104	3463
	1·0 : 10	384	3480
	1·5 : 10	518	3455

It was found that complete nitrate recoveries could be obtained for 0·1-g samples using procedure A outlined above. However, Table II demonstrates that a 2-h incubation period after shaking is required for complete recovery from the 1·0-g samples, and that an increase in the shaking period had little effect on the recoveries.

TABLE II

EFFECT OF SHAKING AND INCUBATION TIMES ON NITRATE RECOVERIES

Sample	Amount analysed/g	Shaking time/min	Incubation time/h: 0	0·5	1	2	4	6	24
			Nitrate-nitrogen, p.p.m.						
Grass	1·0	5	370	365	421	482	468	459	471
		20	360	372	435	473	470	468	476
Grass	0·1	5	718	705	705	714	720	716	722
		20	716	721	718	716	720	722	715
Clover	1·0	5	10	11	14	16	15	16	16
		20	10	10	13	16	16	15	16
Clover	0·1	5	2950	2940	2920	2950	2910	2930	2910
		20	2930	2930	2950	2920	2920	2950	2930

Duplicate determinations were made of nitrate-nitrogen concentration on 41 dried grass, grass plus clover or clover samples by the electrode method and the standard AOAC procedure.[5] The relationship obtained is shown in Fig. 2. The regression equation was

$$[\text{nitrate (electrode)}] = -2{\cdot}92 + 0{\cdot}97\ [\text{nitrate (AOAC)}]$$

and the correlation coefficient of 0·9981 was highly significant. The slightly lower values obtained with the electrode method may have been caused by salt interferences.[3] Duplicate sub-samples were taken from 27 grasses or clovers and paired nitrate-nitrogen determinations made on each by the electrode method. Eight of the 54 paired determinations differed by more than 2 per cent. and three by more than 5 per cent. The means of three of the 27 duplicate determinations differed by more than 2 per cent. and one by more than 5 per cent. The accuracy, precision and repeatability of the method are therefore good.

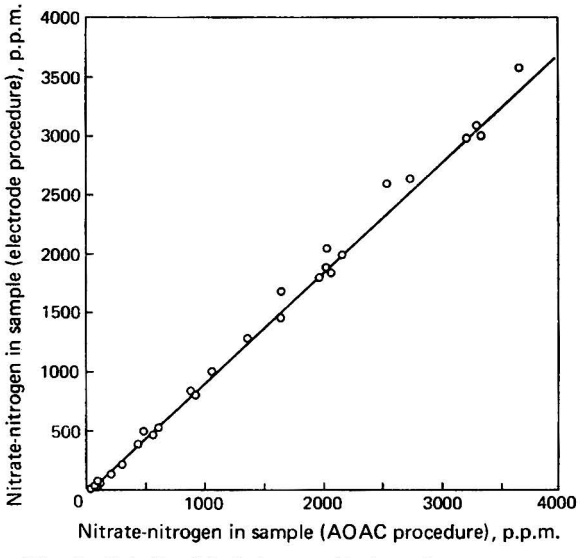

Fig. 2. Relationship between nitrate - nitrogen concentration of grass and clover determined by the electrode and the AOAC procedures.

Table III demonstrates that the recoveries of added nitrate to low, intermediate and high nitrate-containing samples are complete.

It can be concluded that the procedure described provides a simple, accurate and rapid method for the analysis of grasses and clovers containing as little as 10 p.p.m. of nitrate-nitrogen.

TABLE III

RECOVERY OF ADDED NITRATE BY THE ION-SELECTIVE ELECTRODE METHOD

Sample	Nitrate-nitrogen added, p.p.m.	Total nitrate-nitrogen in test solution, p.p.m.	Nitrate-nitrogen determined in test solution	
			p.p.m.	Recovery, per cent.
Grass	0	2	2	—
	35	37	37	100·0
	70	72	73	101·4
	140	142	141	99·3
	350	352	350	99·4
	700	702	706	100·6
Grass + clover ..	0	29	29	—
	35	64	64	100·0
	70	99	100	101·4
	140	169	167	98·6
	350	379	382	100·9
	700	729	734	100·7
Clover	0	310	310	—
	35	345	344	97·1
	70	380	379	98·6
	140	450	452	101·4
	350	660	657	99·1
	700	1010	1005	99·3

The authors thank Dr. D. Reid for his statistical analysis and Miss N. McGregor and Mrs. J. Cuthbertson for their technical assistance.

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Polarographic Studies on Some Organic Compounds of Arsenic

Part I. Substituent Effects and the Arsonic Acids

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A study has been made of the polarographic behaviour of 12 arsonic acids. They give rise to a single well defined cathodic wave in acidic solutions below pH 3. The wave height is diffusion controlled and proportional to the concentration in the range 10^{-5} to 10^{-3} M. The arsonic acids are reduced irreversibly to arsenobenzenes. The current-potential relationships have been examined and a linear relationship was found between the half-wave potential and the polar Hammett substituent constants. The use of polarography has been proposed for the quantitative functional analysis of the arsonic group, for the specific determination of nitrophenylarsonic acids and arsanilic acid in mutual mixtures, and for the specific determination of phenylarsonic acid and phenyl arsenoxide, also in mutual mixtures.

The organic compounds of arsenic have many important applications in agriculture and industry and also in the laboratory as analytical reagents or ligands in co-ordination chemistry. Despite an extensive literature on other aspects of their chemistry, very little work has been carried out on their electrochemical behaviour. Also, most of the techniques for the analysis of these compounds do not differentiate between the different oxidation states.

For these reasons it was decided to undertake a comprehensive study of the polarographic behaviour of these compounds. The present paper deals with the arsonic acids, while the following two papers in this series are concerned with phenyl arsenoxide and triphenylarsine oxide.

The arsonic acids have three major fields of application: as additives in animal feedingstuffs for growth promotion,[1] as reagents for the determination of heavy metals[2] and as a starting material in the synthesis of other organic compounds of arsenic.[3] A few medical applications remain, for example in the treatment of African trypanosomiases.[4] Most of the methods for the determination of the arsonic acids involve the breaking of the arsenic–carbon bond by oxidation or reduction followed by the determination of either the inorganic arsenic or the organic residue, thus not differentiating the arsonic acids from the considerably more toxic arsenoxide, in which the arsenic is present in a lower oxidation state. In this paper a polarographic method is proposed for the specific determination of phenylarsonic acid in the presence of phenyl arsenoxide.

The arsonic acids were first reported to be polarographically active by Breyer[5] in 1938 and by Maruyama and Furuya[6] in 1957, while the present authors made a study of the products of the macro-scale reduction of phenylarsonic acid at a large mercury pool.[7] However, no really detailed study of the classical polarography of these compounds has been made. The present paper attempts to undertake this task.

Experimental

Apparatus

Current - potential curves were recorded using the Polariter PO4 polarograph in conjunction with the drop-life timer DLT1 and the dropping-mercury electrode assembly E65, all manufactured by Radiometer of Copenhagen, who also produced the capillary. The electrolytic cell was a Kalousek cell, modified to suit the electrode assembly, with a saturated calomel electrode.

Instantaneous current *versus* time curves were recorded oscillographically using equipment

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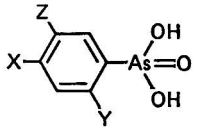
designed and built in the Department. The stabilised potential source had a tolerance of ±1 mV and the current could be measured to a precision of 0·5–1 per cent., while the response time was of the order of milliseconds.

Microcoulometry was carried out with the dropping-mercury electrode in a vessel designed by Manoušek. A device was used to keep the level of the fallen mercury constant.

All pH values were measured with a standard Pye pH meter and glass and calomel electrodes.

Reagents

Compounds of the following general description were examined



in which the substituents were as follows:

X	Y	Z	Name
H	H	H	Phenylarsonic acid
OH	H	H	4-Hydroxyphenylarsonic acid
OH	OH	H	2,4-Dihydroxyphenylarsonic acid
NH_2	H	H	4-Aminophenylarsonic acid (4-arsanilic acid)
H	NH_2	H	2-Aminophenylarsonic acid (2-arsanilic acid)
OH	H	NH_2	3-Amino-4-hydroxyphenylarsonic acid
CH_3	H	H	4-Methylphenylarsonic acid (4-tolylarsonic acid)
Cl	H	H	4-Chlorophenylarsonic acid
NO_2	H	H	4-Nitrophenylarsonic acid (nitarsone)
H	NO_2	H	2-Nitrophenylarsonic acid
H	H	NO_2	3-Nitrophenylarsonic acid
OH	H	NO_2	3-Nitro-4-hydroxyphenylarsonic acid (roxarsone)
OH	OH	NO_2	5-Nitro-2,4-dihydroxyphenylarsonic acid

and also n-propanearsonic acid ($CH_3CH_2CH_2AsO_3H_2$).

Aqueous stock solutions of 5×10^{-3} M concentration were prepared, solubility permitting, in distilled water.

Some of the reagents were obtained from Kodak Ltd., BDH Chemicals Ltd. and Koch-Light Laboratories Ltd., while others were prepared by Dr. K. Vadasdi of the Hungarian Academy of Sciences, Budapest. All reagents were subjected to elemental analysis and thin-layer chromatography in order to check their purity. All substances examined gave carbon and hydrogen values that approximated to the theoretical values to within an error of less than 2–3 per cent.

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, phosphoric 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. In each instance analytical-reagent grade reagents were used.

Experimental Techniques

After de-aeration of the solution with oxygen-free nitrogen, the current - potential curve was recorded at the slowest available scan rate, 100 mV min^{-1}, with a drop lifetime of 2·4 s and a blanking time of 85 per cent. Capacitative damping was not required.

The height of the mercury column was measured with a millimeter scale, and the values were corrected for back-pressure, as described by Heyrovský and Kůta.[8]

The wave height and the half-wave potential were measured in each instance by the same standard graphical construction.[9] In linear regression analysis by the method of least squares carried out on the experimental data, care was taken to choose the most error free of the two sets of data as the independent variable. All tolerance intervals given are for a 95 per cent. probability.

In the comparison of the graphs of wave height *versus* the height and the square root of the height of the mercury column, the coefficients of correlation provide proof as to which

is the more linear only if the deviation of the points from the regression line due to non-linearity is significantly greater than the random scatter of the points. As the variation in wave height is small in these measurements, an alternative method involving the intercept of the regression line on the axis for the height or the square root of the height of the column was also employed.

It can be proved that the intercepts depend only on the set of heights of the mercury column used and the exponent, which should be 0 for kinetic control, 0·5 for diffusion control and 1 for adsorption control.[10] Comparison was made of the predicted intervals with the experimentally obtained intercepts and their tolerance intervals.

Microcoulometry was carried out on 2 ml of de-aerated solution placed in the Manoušek cell. The tip of the capillary was positioned just below the surface of the solution so as to ensure the maximum stirring effect by the falling mercury drops. A potential corresponding to the upper plateau of the wave was maintained across the cell, by the Polariter PO4, for 8 h. The current - potential curve was recorded at 30-min intervals, allowance being made for the time required to record the curve. Additional stirring was obtained by a rapid burst of nitrogen through the solution every 5 min. The total current passed was obtained by integration of the wave height over the period of electrolysis, and the decrease in concentration from the decrease in the wave height, from which the number of electrons consumed per molecule was obtained.

Results and Discussion

The arsonic acids were examined polarographically at various pH values from 1 to 13. The simplest behaviour was shown by those compounds which do not possess a nitro group. These derivatives gave rise to a single, well defined cathodic wave i_1 at 800–1000 mV in acidic solutions below pH 3 (Fig. 1). At higher pH values the wave becomes merged with

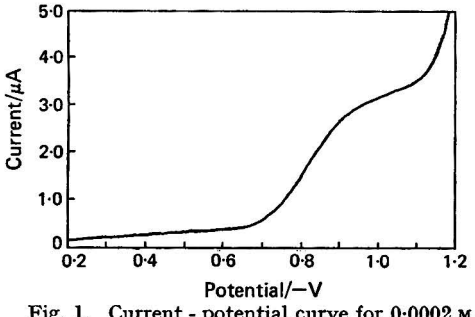


Fig. 1. Current - potential curve for 0·0002 M solution of phenylarsonic acid in 0·1 M HCl.

the decomposition current of the supporting electrolyte and so no further cathodic behaviour is shown throughout the entire pH range.

The nitro-substituted phenylarsonic acids give rise to two waves of approximately equal height (Fig. 2). The more negative wave i_1 is basically similar to the wave produced by the

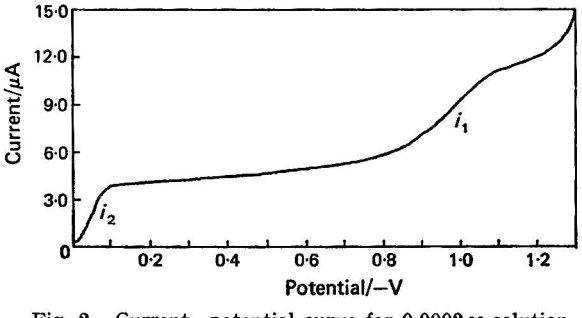


Fig. 2. Current - potential curve for 0·0002 M solution of 4-nitrophenylarsonic acid in 0·1 M HCl.

TABLE I

LINEAR REGRESSION ANALYSIS OF WAVE HEIGHT *versus* CONCENTRATION DATA FOR SEVERAL PHENYLARSONIC ACIDS

Derivative	Concentration/ mmol l^{-1}	Slope of graph: Slope (μA/ mmol l^{-1})	Slope of graph: Tolerance (μA/ mmol l^{-1})	Intercept on the wave height axis: Intercept/ μA	Intercept on the wave height axis: Tolerance/ μA	Intercept on the concentration axis: Intercept/ mmol l^{-1}	Intercept on the concentration axis: Tolerance/ mmol l^{-1}	Coefficient of correlation	Standard error of estimate/μA	Mean value of the wave height/μA
H	0·1–1·0	16·29	0·34	0·07	0·21	−0·004	0·023	0·999 67	0·14	9·03
4-OH		16·19	0·29	0·09	0·18	−0·006	0·020	0·999 76	0·11	8·99
2,4-DiOH		16·42	0·66	0·00	0·41	0·000	0·044	0·998 80	0·26	9·03
4-NH_2		16·61	0·20	0·10	0·13	−0·006	0·013	0·999 89	0·08	9·23
2-NH_2		16·89	0·24	−0·05	0·15	0·003	0·016	0·999 84	0·10	9·24
3-NH_2-4-OH		14·44	1·38	0·00	0·44	0·000	0·041	0·998 64	0·12	4·19
4-CH_3		16·21	0·23	0·01	0·14	−0·001	0·016	0·999 85	0·09	8·93
4-Cl		15·84	0·45	0·09	0·14	−0·005	0·012	0·999 88	0·04	4·68
4-NO_2		17·59	0·81	−0·34	0·50	0·019	0·051	0·998 41	0·32	9·29
2-NO_2		7·82	0·82	0·95	0·51	−0·122	0·122	0·991 89	0·32	5·25
3-NO_2		16·80	0·87	−0·15	0·54	0·009	0·057	0·998 00	0·34	9·09
3-NO_2-4-OH		16·14	0·70	0·02	0·43	−0·001	0·047	0·998 60	0·27	8·90
5-NO_2-2,4-diOH		15·29	0·84	0·23	0·53	−0·015	0·061	0·997 72	0·33	8·64
n-Propane-AsO_3H_2		7·03	0·28	0·13	0·17	−0·018	0·044	0·998 84	0·11	3·99
H	0·01–0·1	16·00	0·30	0·017	0·019	−0·0011	0·0021	0·9997	0·012	0·897
4-OH		16·05	0·19	−0·002	0·012	0·0001	0·0013	0·9999	0·008	0·881
4-NH_2		16·56	0·27	0·007	0·017	−0·0004	0·0018	0·9998	0·011	0·918
2-NH_2		16·60	0·39	0·011	0·024	−0·0007	0·0026	0·9996	0·015	0·924
3-NH_2-4-OH		15·61	0·21	0·001	0·013	−0·0001	0·0015	0·9999	0·008	0·860
4-Cl		15·84	0·43	0·002	0·026	−0·0001	0·0030	0·9995	0·017	0·873

other arsonic acids and can be observed only below pH 3. The more positive wave i_2 can be observed over the entire pH range and is believed to be due to the reduction of the nitro group to the hydroxylamine derivative. In addition, the 2-nitro- and 3-nitrophenylarsonic acids give rise to a third poorly defined reduction phenomenon, i_3, between the waves i_1 and i_2, observable only at the lowest pH values, which is believed to involve the hydroxylamine derivative. The 5-nitro-2,4-dihydroxy derivative displays a maximum of the first kind on the wave i_2 while the 3-nitro-4-hydroxy derivative gives rise to a maximum of the second kind on the upper plateau of the wave i_2. The present work is concerned only with the wave i_1.

None of the arsonic acids give rise to any anodic phenomena throughout the pH range 1–13.

Characterisation of the Limiting Current

The relationship between the wave height of the wave i_1 and the concentration was examined for ten concentrations of each derivative in the range 1×10^{-4} to 1×10^{-3} M (solubility permitting) and for a further ten concentrations of six of the compounds in the range 1×10^{-5} to 1×10^{-4} M. Table I gives the results of linear regression analysis on the data with concentration as the independent variable and shows that a strong proportional relationship exists between the wave height and the concentration, behaviour typical of diffusion or kinetically controlled processes.

The standard error of the estimate for those phenylarsonic acids without a nitro group is of the order of 1–2 per cent. of the average current, in both concentration ranges, while for the nitro compounds the value is 3–5 per cent. of the average current owing to the slightly less well defined lower plateaux of the latter derivatives. The slopes of these graphs are equal within experimental error, indicating that the substituents examined have no significant effect on the wave height. These results tend to suggest diffusion rather than kinetic control. The deviant behaviour of the 2-nitro derivative is probably due to the overlap of the processes i_1 and i_3, while that of the n-propanearsonic acid is most likely to be due to its different molecular geometry and the poorly defined upper plateau that is almost overlapped by the decomposition current of the supporting electrolyte.

The relationship between the wave height and the height of the mercury column was determined by linear regression analysis of wave height *versus* the height and the square root of the height of the mercury column as independent variables, for 2×10^{-4} M solutions of each derivative and eight heights of the mercury column.

The high positive values of the coefficients of correlation (Table II) are not consistent with kinetic or catalytic hydrogen waves, while the higher values of the coefficients against the square root of the height of the column suggest diffusion rather than adsorption control.

TABLE II

LINEAR REGRESSION ANALYSIS OF WAVE HEIGHT i_1 *versus* THE HEIGHT h AND SQUARE ROOT OF THE HEIGHT $h^{\frac{1}{2}}$ OF THE MERCURY COLUMN DATA FOR SEVERAL PHENYLARSONIC ACIDS

	Intercept with the h axis			Intercept with the $h^{\frac{1}{2}}$ axis			Coefficient of correlation	
Derivative	Intercept/cm	Tolerance/cm	Predicted value for diffusion current/cm	Intercept/cm$^{\frac{1}{2}}$	Tolerance/cm$^{\frac{1}{2}}$	Predicted value for adsorption current/cm$^{\frac{1}{2}}$	i_1 *vs.* h	i_1 *vs.* $h^{\frac{1}{2}}$
H	−54·72	10·24	−52·10	−0·183	0·549	3·574	0·9979	0·9987
4-OH	−52·57	12·75	−52·10	−0·023	0·469	3·574	0·9966	0·9998
2,4-DiOH	−83·16	27·63	−52·10	−2·114	1·502	3·574	0·9901	0·9938
4-NH_2	−62·58	11·08	−59·25	−0·207	0·259	3·790	0·9966	0·9995
2-NH_2	−58·07	10·80	−55·63	−0·157	0·297	3·683	0·9979	0·9995
3-NH_2-4-OH	−57·04	6·98	−59·25	0·136	0·153	3·790	0·9986	0·9998
4-CH_3	−58·38	10·02	−59·63	0·042	0·155	3·800	0·9976	0·9999
4-Cl	−52·62	8·24	−55·63	0·181	0·677	3·683	0·9982	0·9972
4-NO_2	−55·73	11·72	−59·25	−0·231	0·453	3·790	0·9958	0·9985
2-NO_2	−51·25	21·91	−59·25	0·487	1·489	3·790	0·9869	0·9832
3-NO_2	−51·67	18·83	−59·25	0·458	1·314	3·790	0·9888	0·9869
3-NO_2-4-OH	−57·21	15·83	−59·25	0·147	0·672	3·790	0·9927	0·9968
5-NO_2-2,4-diOH	−58·61	17·69	−59·25	0·062	0·777	3·790	0·9910	0·9958
n-Propane-$As_3O_3H_2$	−38·75	14·67	−59·25	1·274	1·187	3·790	0·9917	0·9871

The coefficients of correlation do not by themselves offer sufficient proof and so the intercepts of the regression line on the axis for the height or square root of the height of the mercury column were compared with the predicted intercepts for diffusion and adsorption controlled processes and for the set of heights of the column used (see under Experimental Techniques). The predicted intercepts for diffusion control fell within the confidence intervals of the experimentally obtained intercepts, while those for adsorption control in each instance did not.

The instantaneous current during the drop lifetime was displayed oscillographically as a function of time at a series of potentials along the length of the rising part of the wave for 2×10^{-4} M solutions of each derivative. The logarithm of the instantaneous current, measured from the photographic record, was plotted against the logarithm of the time. These graphs (Fig. 3) are somewhat curved, which result is partly a general characteristic of irreversible processes and also partly due to residual depletion effects carried on from the previous drop. As both of these effects are 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.

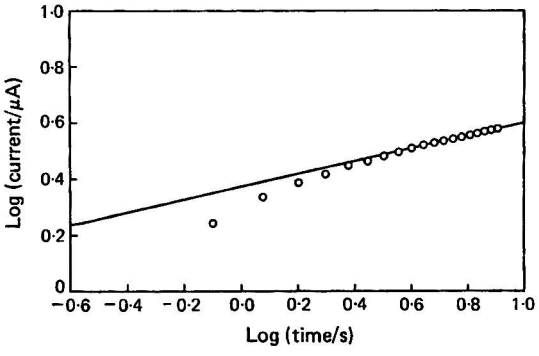


Fig. 3. Logarithmic analysis of current - time curves for phenylarsonic acid at −0·975 V.

As the potential rises the exponent x, given in Table III, decreases as it approaches the theoretical value of 0·19 for a diffusion controlled process at the beginning of the upper plateau.[11] (The theoretical values for adsorption and kinetic control are −0·33 and 0·67, respectively.) Above this potential the exponent increases, possibly because the plateau overlaps somewhat with the base of the large hydrogen wave of the supporting electrolyte that follows. The increase of the exponent towards the base of the wave suggests that the wave is irreversible, although the theoretical value of 0·66, at the base of the wave, for most irreversible processes, is not in fact attained.

The Current - Potential Relationship

The reversibility of the process was further investigated by logarithmic analysis of the shape of the wave. Five functions of the current were plotted against the electrode potential over the rising part of the wave, corresponding to six possible current - potential relationships and six different types of process, for a series of different concentrations of phenylarsonic acid. The functions of current used were log $[(i_d - i)/i]$ corresponding to most irreversible and simple reversible reductions,[12] log $(i_d - i)$ for reversible reduction to an insoluble product,[13] log $[(i_d - i)^2/i]$ corresponding to reversible reduction followed by reversible dimerisation,[12] log $[(i_d - i)/i^2]$ for the reversible reduction of a dimer[12] and, finally, log $[i_d(i_d - i)^2/i^3]$ corresponding to a reversible reduction followed by an irreversible dimerisation.[12] In these functions i_d is the limiting diffusion current and i the current at each potential.

Table IV gives the predicted value of $2{\cdot}303\,RT/\alpha nF$ (V), the reciprocal of the slope, calculated for each of the possible functions of current by linear regression analysis with potential as the independent variable. In no instance is the value a simple fraction of 0·059 V, indicating that the process is irreversible.

The coefficients of correlation[14] are consistently higher for the graphs of log $[(i_d - i)/i]$ *versus* potential, suggesting that this function of the current has the most linear relationship

TABLE III

SLOPE (x) OF THE LOGARITHM OF THE INSTANTANEOUS CURRENT *versus* THE LOGARITHM OF THE TIME GRAPH AT VARIOUS POTENTIALS FOR SEVERAL PHENYLARSONIC ACIDS

Potential/V	H	4-OH	2,4-DiOH	4-NH_2	2-NH_2	3-NH_2-4-OH	4-CH_3
−1·200	—	—	—	—	—	—	—
−1·150	—	—	—	—	—	—	—
−1·100	0·26	0·25	0·23	0·25	0·28	0·27	0·27
−1·050	0·25	0·23	0·20	0·22	0·26	0·24	0·25
−1·000	0·24	0·20	0·21	0·19	0·23	0·21	0·22
−0·975	0·22	0·23	—	—	—	—	—
−0·950	0·26	0·25	0·29	0·27	0·23	0·26	0·27
−0·925	0·29	0·26	—	—	—	—	—
−0·900	0·27	0·27	0·31	0·30	0·26	0·28	0·28
−0·875	0·29	0·30	—	—	—	—	—
−0·850	0·29	0·31	0·34	0·31	0·29	0·30	0·30
−0·800	0·33	0·35	0·38	0·36	0·32	0·35	0·35
−0·750	0·37	0·41	0·45	0·43	0·35	0·40	0·39
−0·700	—	—	—	—	—	—	—

Potential/V	4-Cl	4-NO_2	2-NO_2	3-NO_2	3-NO_2-4-OH	5-NO_2-2,4-diOH	n-Propane-AsO_3H_2
−1·200	—	—	—	—	0·24	0·24	0·24
−1·150	—	0·26	—	—	0·21	0·20	0·25
−1·100	—	0·24	—	0·24	0·23	0·24	0·25
−1·050	0·25	0·23	0·23	0·25	0·26	0·26	0·28
−1·000	0·24	0·25	0·20	0·22	0·25	0·27	0·30
−0·975	—	—	—	—	—	—	—
−0·950	0·21	0·27	0·21	0·22	0·28	0·29	0·33
−0·925	—	—	—	—	—	—	—
−0·900	0·24	0·29	0·20	0·24	0·29	0·29	0·39
−0·875	—	—	—	—	—	—	—
−0·850	0·26	0·30	0·23	0·27	0·32	0·33	—
−0·800	0·28	0·33	0·27	0·29	—	—	—
−0·750	0·29	—	0·28	0·34	—	—	—
−0·700	0·31	—	0·29	—	—	—	—

with the potential. However, by themselves the coefficients of correlation do not offer sufficient proof. For each of these functions of current and the corresponding current-potential relationship, there exist equivalent relationships between the half-wave potential and the logarithm of the concentration.[12,13] 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

TABLE IV

PREDICTED VALUE OF $2{\cdot}303\,RT/\alpha nF$ (V) FROM GRAPHS OF SEVERAL FUNCTIONS OF CURRENT *versus* POTENTIAL AT VARIOUS CONCENTRATIONS OF PHENYLARSONIC ACID

Concentration / mmol l^{-1}	Function of current: Log $\frac{i_d - i}{i}$	Log $(i_d - i)$	Log $\frac{i_d - i}{i^2}$	Log $\frac{(i_d - i)^2}{i}$	Log $\frac{i_d (i_d - i)^2}{i^3}$
0·035	0·116	0·274	0·0738	0·0816	0·0451
0·055	0·117	0·259	0·0751	0·0803	0·0456
0·075	0·119	0·289	0·0751	0·0844	0·0461
0·095	0·125	0·268	0·0736	0·0807	0·0449
0·115	0·117	0·288	0·0734	0·0832	0·0451
0·125	0·116	0·286	0·0749	0·0841	0·0460
0·200	0·121	0·292	0·0761	0·0854	0·0467
0·500	0·121	0·299	0·0759	0·0862	0·0467
0·700	0·119	0·288	0·0757	0·0847	0·0464
1·000	0·120	0·286	0·0760	0·0845	0·0465

should shift to more negative potentials at a rate of 2·303 $RT/\alpha nF$ (V) for an increase in concentration of one decade, while for the remaining processes considered the shift is to more positive potentials at an equal rate. A coefficient of correlation of 0·1025 that was well below the critical value was found between the half-wave potential and the logarithm of the concentration of ten solutions of phenylarsonic acid, confirming that the function log $[(i_d - i)/i]$ is linearly dependent on potential.

The reciprocal of the slope of this graph gives the actual value of 2·303 $RT/\alpha nF$. It can be seen (Table IV) that this is a constant that is independent of concentration, with a value of 0·119 ± 0·005 V, corresponding to a value of 0·49 for the product, αn, of the transfer coefficient and the number of electrons involved in the potential-determining step. This value is typical of those for most irreversible processes.

The effect of pH on the value of 2·303 $RT/\alpha nF$ was investigated for a 2×10^{-4} M solution of phenylarsonic acid at seven pH values. Once again the coefficients of correlation indicated that the graphs of log $[(i_d - i)/i]$ *versus* potential gave the best linear relationship. The value of 2·303 $RT/\alpha nF$ from these graphs (Table V) was found to increase only slightly with increased pH. Above pH 2·2 the wave becomes more ill-defined as it merges with the decomposition current of the supporting electrolyte and logarithmic analysis becomes no longer practical.

TABLE V

VALUE OF 2·303 $RT/\alpha nF$ (V) FROM GRAPHS OF LOG $[(i_d - i)/i]$ *versus* POTENTIAL AT SEVERAL pH VALUES

pH	$\frac{2{\cdot}303\,RT}{\alpha nF}$
(0·1 M HCl)	0·121
1·20	0·120
1·40	0·121
1·60	0·125
1·80	0·129
2·00	0·127
2·20	0·132

The logarithmic analysis was repeated for each substituted phenylarsonic acid at four concentrations and two pH values. Again the pattern of the coefficients of correlation indicated that the graphs of log $[(i_d - i)/i]$ *versus* potential had the best linear relationship. The value of 2·303 $RT/\alpha nF$ (Table VI) from these graphs is again independent of concentration and increases only slightly with the increase in pH. The standard deviation associated with each value is 0·005–0·01 V. With the exception of the 4-chloro derivative, all the phenylarsonic acids give rise to equal values, within experimental error, of 2·303 $RT/\alpha nF$, that is, they are independent of substitution.

TABLE VI

VALUE OF 2·303 $RT/\alpha nF$ (V) FROM GRAPHS OF LOG $[(i_d - i)/i]$ *versus* THE POTENTIAL AT FOUR CONCENTRATIONS AND TWO pH VALUES FOR SEVERAL PHENYLARSONIC ACIDS

Derivative	Concentration/mmol l^{-1} in 0·1 M HCl				Concentration of 0·2 mmol l^{-1} at pH 1·8
	0·2	0·5	0·7	1·0	
H	0·121	0·121	0·119	0·120	0·132
4-OH	0·115	0·117	0·115	0·116	0·127
2,4-DiOH	0·116	0·115	0·116	0·116	0·126
4-NH_2	0·119	0·120	0·120	0·119	0·128
2-NH_2	0·117	0·116	0·118	0·117	0·125
3-NH_2-4-OH	0·122	0·121	0·122	0·123	0·131
4-CH_3	0·113	0·114	0·114	0·112	0·124
4-Cl	0·151	0·149	0·150	0·150	0·166
4-NO_2	0·126	0·125	0·122	0·123	0·130
3-NO_2	0·126	0·124	0·127	0·124	0·136
3-NO_2-4-OH	0·121	0·123	0·124	0·124	0·129
5-NO_2-2,4-diOH ..	0·123	0·125	0·124	0·123	0·129

Effect of pH

A completely different method of determining the value of $2{\cdot}303\,RT/\alpha nF$ is offered by measuring the change of half-wave potential with pH. The wave height and the half-wave potential were measured for 2×10^{-4} M concentrations of each derivative in hydrochloric acid - potassium chloride mixtures that differed by 0·2 pH unit in the pH range 1·0–2·2. For phenylarsonic acid and 4-hydroxyphenylarsonic acid the measurements were carried out at pH up to 2·8; however, above pH 2·2, as the wave begins to merge with the decomposition current of the supporting electrolyte, the wave becomes too ill-defined for precise measurement and so work was continued for the other derivatives at pH below 2·4. The wave height was found to be independent of pH.

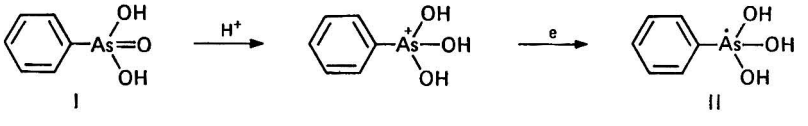
For each derivative a linear relationship was found to exist between the half-wave potential and the pH, without any discontinuities. As the first pK values[15] of the arsonic acids lie in the pH range 3–5, this would indicate that the electroactive form in the solution is the free acid. The slope of the graph gives the product of the value of $2{\cdot}303\,RT/\alpha nF$ from the current - potential relationship and the number of protons involved in the reduction prior to the potential-determining step. For each derivative this slope was an integral multiple only of the reciprocal of the slope of the graphs of log $[(i_d - i)/i]$ *versus* potential, again confirming the linear dependence of this function on potential, and is illustrated for the unsubstituted acid by a comparison of Table IV and the slope from Table VII. For phenylarsonic acids without a nitro group, agreement is excellent within experimental error (Tables VI and VII). In each instance the integral is one corresponding to one protonation in the

TABLE VII

SLOPE (V per pH unit) OF THE HALF-WAVE POTENTIAL *versus* pH GRAPH FOR SEVERAL PHENYLARSONIC ACIDS

Derivative	$\frac{dE_i}{d(pH)}$	Tolerance	Derivative	$\frac{dE_i}{d(pH)}$	Tolerance
H in HCl - KCl	−0·112	0·003	4-CH_3	−0·110	0·006
H in H_3PO_4 - KH_2PO_4	−0·101	0·026	4-Cl	−0·155	0·007
4-OH	−0·115	0·007	4-NO_2	−0·106	0·010
2,4-DiOH	−0·113	0·005	2-NO_2	−0·086	0·009
4-NH_2	−0·123	0·010	3-NO_2	−0·110	0·004
2-NH_2	−0·123	0·006	3-NO_2-4-OH	−0·096	0·005
3-NH_2-4-OH	−0·122	0·007	5-NO_2-2,4-diOH	−0·104	0·007

reduction prior to the potential-determining step. If, as is generally true with most irreversible reductions, the addition of the first electron is the potential-determining step, then this result suggests that a rapid protonation of the free acid occurs prior to the first electrochemical step:



I II

For the nitrophenylarsonic acids agreement is not so good, with the slopes of the half-wave potential *versus* pH graphs about 10 per cent. below average and the value of $2{\cdot}303\,RT/\alpha nF$ about 10 per cent. above average. These results are most likely due to the slightly less well defined plateaux of these waves.

The measurements were repeated for the unsubstituted phenylarsonic acid in a phosphoric acid - potassium dihydrogen orthophosphate buffer. Within experimental error identical results were obtained, indicating that the data discussed above are dependent on pH and not on the composition of the buffer.

These results indicate that the process fulfils the relationship that is typical for most irreversible processes—

$$E = E_{i,pH=0} + \frac{2{\cdot}303\,RT}{\alpha nF} \log \frac{(i_d - i)}{i} - \frac{2{\cdot}303\,RT}{\alpha nF} \times pH$$

Substituent Effects and the Half-wave Potential

It has been shown, with the exception of the 4-chloro derivative, that (*a*) the wave heights of the derivatives are equal, that is, the number of electrons transferred are equal, (*b*) the slope of the graphs of half-wave potential *versus* pH are equal (within 10 per cent.) and (*c*) the values of $2{\cdot}303\ RT/\alpha nF$ from the analysis of the wave shape are equal (within 10 per cent.).

These facts fulfil the conditions necessary for a significant study of the effect of substitution on the polarographic behaviour of the phenylarsonic acids by a graphical comparison of half-wave potentials with the Hammett substituent constants.[16] The half-wave potential was measured under precisely identical conditions for a 2×10^{-4} M concentration of each derivative in 2×10^{-2} M hydrochloric acid that was 5×10^{-2} M in potassium chloride and the results are given in Table VIII, together with the corresponding polar Hammett substituent constants, which were obtained from a review by Jaffe.[17] For the nitro derivatives the substituent constant corresponding to the hydroxylamine reduction product is given.

TABLE VIII

HALF-WAVE POTENTIALS, HAMMETT SUBSTITUENT CONSTANTS AND pK_1 VALUES FOR SEVERAL PHENYLARSONIC ACIDS

Derivative	Half-wave potential/V	Hammett constant	pK_1 value	Derivative	Half-wave potential/V	Hammett constant	pK_1 value
H	−0·945	0·00	3·58	4-Cl	−0·905	+0·23	—
4-OH	−0·9625	−0·37	4·05	4-NO_2	−1·000	−0·34	—
2,4-DiOH	−0·990	—	4·40	2-NO_2	−0·860	—	—
4-NH_2	−0·980	−0·66	4·35	3-NO_2	−0·950	−0·04	—
2-NH_2	−0·9375	—	4·45	3-NO_2-4-OH	−1·015	−0·41	—
3-NH_2-4-OH	−0·975	−0·53	—	5-NO_2-2,4-diOH	−1·030	—	—
4-CH_3	−0·955	−0·17	3·78	n-Propane-AsO_3H_2	−1·11	—	—

The 4-amino-, the 3-amino-4-hydroxy-, the 4-hydroxy-, the 4-methyl- and the unsubstituted phenylarsonic acids fall on a straight line in the graph of half-wave potential *versus* the polar Hammett substituent constants (Fig. 4), with a coefficient of correlation of 0·9946, the high value of which further confirms that these derivatives follow essentially the same reduction mechanism. In this regression analysis purely polar substituent constants were used, which do not take into account mesomeric effects, thus suggesting that mesomeric effects are not involved in the reduction.

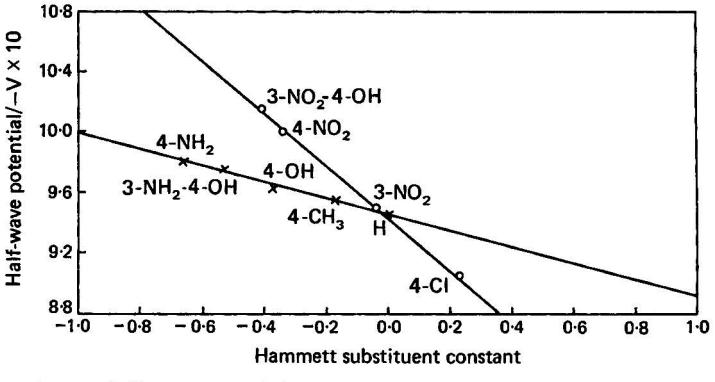


Fig. 4. Half-wave potential *versus* polar Hammett substituent constants for some phenylarsonic acids. ○, $\rho_\pi = 0{\cdot}173$; and ×, $\rho_\pi = 0{\cdot}056$.

The half-wave potential for these derivatives obeys the relationship

$$E_{\frac{1}{2},x} = 0{\cdot}0530\sigma_x - 0{\cdot}945\ \text{V}$$

If the polarographic reaction constant, 0·053 ± 0·010 V, is compared with other polarographic reaction constants given in the literature,[18] it can be seen that the constant has a much lower value than most, that is, the effect of substitution on the half-wave potential is small. It has already been proposed that the potential-determining step is the reduction of a protonated cation, that is, the formation of a neutral transition state, which could be expected to yield a low reaction constant. Further, the low value combined with a lack of mesomeric effects indicates that the reduction does not directly involve the benzene ring but is confined to the arsonic group itself. The central importance of the arsenic atom is confirmed by the fact that the phosphorus analogue, phenylphosphonic acid, was shown not to be electroactive.

With the nitro compounds the situation is complicated as the nitro group itself undergoes reduction at the potential of the reduction of the arsonic group. Linear regression analysis was carried out on the half-wave potential of the wave of the latter reduction *versus* the substituent constants corresponding to the nitro group and its possible reduction products, the hydroxylamino and amino groups, both polar and mesomeric constants being employed. The polar substituent constants for the hydroxylamine reduction product gave the only coefficient of correlation (0·9991) above the critical value. This suggests that the more positive wave, i_2, is a four-electron reduction of the nitro group to the hydroxylamine derivative, which was confirmed by microcoulometry. However, the results for the nitro derivatives using these substituent constants fall on a separate line from those for the other derivatives (Fig. 4), which line passes close to the point corresponding to the unsubstituted phenylarsonic acid, according to the relationship

$$E_{i,x} = 0{\cdot}173\sigma_x - 0{\cdot}943 \text{ V}$$

The cause of this separate line is not clear, but a possibility is that it might involve a different orientation or a change of orientation of the molecule at the electrode as a result of the preceding reduction of the nitro group.

The 4-chloro derivative also seems to fall on this line, which could, however, merely be a chance effect. The deviation of this derivative from the main line is not unexpected as the deviation of the 4-chloro derivatives in many other reduction series has been observed.[19] This deviation is believed to be due to the fact that the highly polarisable nature of the chlorine atom causes it to take up the electron and therefore act as a "bridge" between the electrode and the electroactive group, thus involving a different orientation at the electrode and altering the electrode kinetics.[19] A deviant value was also observed for $2{\cdot}303RT/\alpha nF$.

As no substituent constants were available for the *ortho* derivatives, the half-wave potentials of six derivatives were compared graphically with the pK_1 value of the acid (Table VIII) obtained by potentiometric titration with barium hydroxide solution under an atmosphere of nitrogen. The four 4-substituted derivatives fell on a straight line (Fig. 5) as in the Hammett plot, as could be expected from the dependence of the pK_1 value on the polar Hammett substituent constants.[15]

$$E_{i,x} = -0{\cdot}438pK_1 - 0{\cdot}788 \text{ V}$$

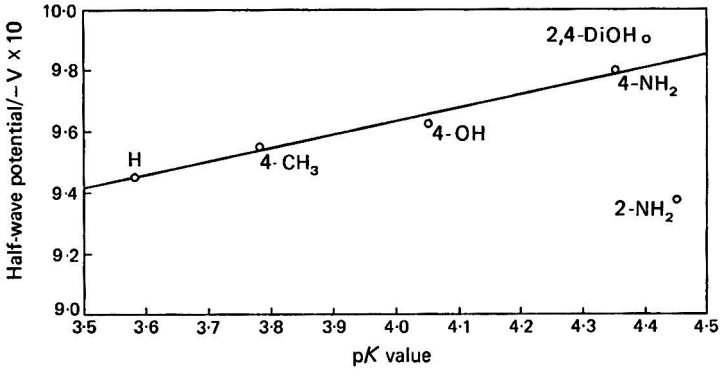


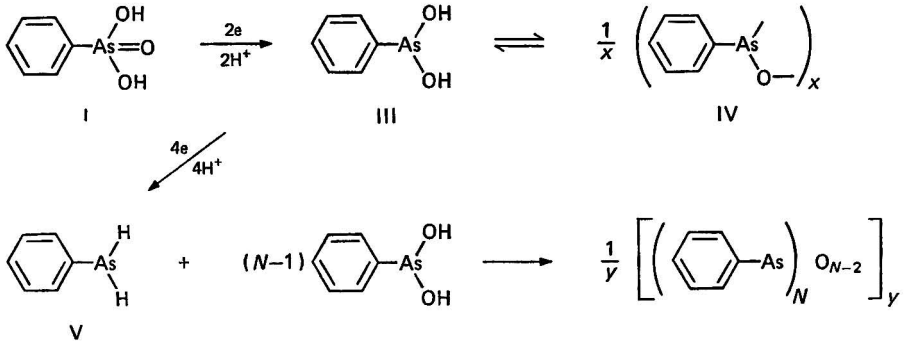
Fig. 5. Half-wave potential *versus* pK_1 value for some phenylarsonic acids.

Both the *ortho* derivatives deviate, the 2,4-dihydroxyphenylarsonic acid being reduced at a slightly more negative potential and the 2-amino derivative at a significantly more positive potential than the line would predict, indicating that, as is usual, steric and hydrogen bonding effects between the two groups must be considered.

The Reduction Mechanism

The number of electrons consumed per molecule in the reduction was directly determined for the 4-hydroxy- and the unsubstituted phenylarsonic acids at two different initial concentrations 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 4·05 $\pm$ 0·15 for both compounds. As the other derivatives have equal wave heights and have shown very similar behaviour, it is reasonable to assume that four electrons are also consumed per molecule of these derivatives. Similar work on the more positive wave i_2 of 5-nitro-2,4-dihydroxyphenylarsonic acid also gave a value of four electrons per molecule; as the waves i_1 and i_2 are equal in height this finding also confirms the value of four electrons for the wave i_1.

A detailed study of the products of a macro-scale reduction of phenylarsonic acid at a large mercury pool formed the basis of a previous publication.[7] From this study we proposed the following reaction path for the macro-scale reduction:

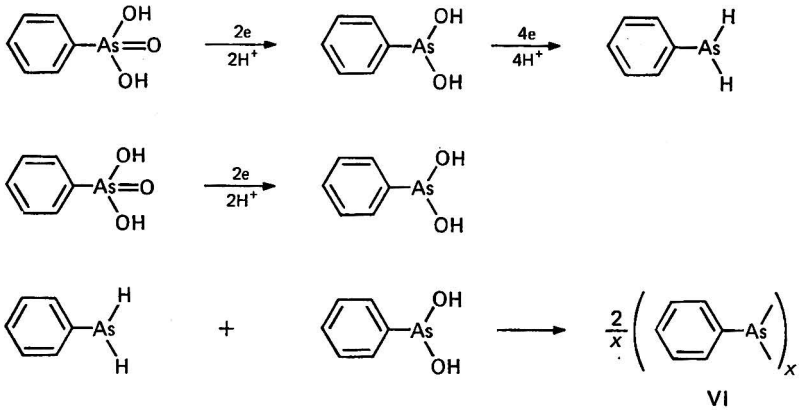


in which N depends on the exact reaction conditions.

In polarography with totally different reaction conditions, such as the use of much lower concentrations, one would not expect the reaction to follow exactly the above path but could expect the chemistry of the reaction to follow the same general pattern.

It has been proposed in an earlier section that the potential-determining addition of the first electron, preceded by a rapid protonation, yields the phenyltrihydroxyarsine radical, **II**. The addition of a further electron - proton pair and the subsequent loss of a molecule of water would yield the monomeric form of phenyl arsenoxide (phenylarsonous acid), **III**, as above. At the concentrations used in polarography, however, the precipitation of the polymeric form, **IV**, of phenyl arsenoxide is highly unlikely to occur.

The most significant difference from the macro-scale reduction would be expected to be in the stoicheiometry of the reaction between phenylarsine, **V**, and phenyl arsenoxide, **III**. In the absence of precipitation, phenylarsine, being the final product of the electrochemical stage of the reaction, could be expected to be in excess over the intermediate phenyl arsenoxide. If this is true the phenylarsine would react with the minimum number of moles of phenyl arsenoxide, that is, an equal number of moles of each would react to form the oxygen-free polymer arsenobenzene, **VI**. In a separate polarographic study[20] it has been shown that phenyl arsenoxide is reduced at these potentials to arsenobenzene, thus suggesting the reaction path below for the polarographic reduction of phenylarsonic acid. This reaction path requires eight electrons for the reduction of two molecules of phenylarsonic acid, which is in good agreement with the average of four electrons per molecule obtained by microcoulometry.



VI

The close similarity of behaviour of the other derivatives would indicate that they also are reduced by following this reaction path.

Analytical Applications

The derivatives of phenylarsonic acid give rise to a single, well defined diffusion-controlled wave in 0·1 M hydrochloric acid. The wave height is reproducible, proportional to concentration in the range 10^{-5}–10^{-3} M and is independent of pH. These are suitable conditions for analytical application.

In analyses of known solutions of phenylarsonic acid 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 and methanol were found not to interfere.

The phenylarsonic acids without a nitro substituent have similar standard errors of estimate (1–2 per cent.) of the calibration line (Table I), indicating an accuracy similar to that for the unsubstituted acid in the determination of these compounds. The close values of the half-wave potential, within 50 mV, result in a single wave from mixtures of different substituted phenylarsonic acids, thus preventing differentiation of the derivatives. However, as the slope of their calibration graphs are equal within experimental error, this wave height can be used to determine the total concentration of the arsonic group in such mixtures.

The nitrophenylarsonic acids have higher standard errors of estimate (3–5 per cent. of the average current) of the calibration line for the "arsonic" wave i_1. For the determination of these compounds it is better to use the more positive wave i_2 for which a value of 1–2 per cent. was obtained.

The most important applications of the arsonic acids are as additives in animal feedstuffs.[1] Polarography offers a suitable method of analysis for the concentrations involved.[21] The most important derivatives for these purposes are the 4-amino-(arsanilic acid) and 3-nitro-4-hydroxy-(roxarsone) phenylarsonic acids. The use of the wave i_2 to determine the nitro derivative and the wave i_1 to determine the total arsonic acid content permits the specific analysis of both compounds in mutual mixtures with an error of 3–5 per cent.

In a separate study,[20] phenyl arsenoxide (the lower oxidation state) was found to give rise to two well formed diffusion-controlled waves in 0·1 M hydrochloric acid at more positive potentials (—0·1 and —0·4 V) than the wave due to phenylarsonic acid. As all three waves are separated by 300–400 mV and do not mutually interfere, polarography offers a simple, rapid and reliable method for the specific analysis for the two compounds in mutual mixtures in the range 10^{-5}–10^{-4} M. The use of the standard addition technique with five additions was found to give an error for both of about 2 per cent. Most of the available methods of analysis do not differentiate the two oxidation states, which differ greatly in their toxicity.

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The Polarography of Fast Red E

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The dye Fast Red E, 2-hydroxy-1,1′-azonaphthalene-4′,6-disulphonic acid, disodium salt, is reduced at the dropping-mercury electrode in a single four-electron wave over the pH range 2·0–11·3, with $E_{\frac{1}{2}}$ values ranging from −0·08 to −0·61 V *versus* S.C.E. The rising portion of the polarographic wave is markedly influenced by the adsorption of depolariser but well defined limiting currents that are proportional to concentration in the range examined, *viz.*, 10^{-3}–10^{-4} M, are obtained.

The object of this investigation was the study and interpretation of the polarographic reduction of Fast Red E, the disodium salt of 2-hydroxy-1,1′-azonaphthalene-4′,6-disulphonic acid (C.I. 16045).

Azo compounds have been the subject of a number of studies utilising the dropping-mercury electrode. As a result, reduction processes in aqueous media that lead to formation of the corresponding hydrazo compound (1) and formation of amines (2) have been recognised.[1]

$$\phi\text{–N=N–}\phi' + 2e^- + 2H^+ \rightarrow \phi\text{–NH—NH–}\phi' \quad .. \quad .. \quad (1)$$

$$\phi\text{–N=N–}\phi' + 4e^- + 4H^+ \rightarrow \phi NH_2 + \phi' NH_2 \quad .. \quad .. \quad (2)$$

In addition, an intermediate step has been described in which electron-transfer step (1) is followed by disproportionation of the hydrazo compound.[2,3]

$$2\phi\text{–NH—NH–}\phi' \rightarrow \phi NH_2 + \phi' NH_2 + \phi\text{–N=N–}\phi' \quad .. \quad .. \quad (3)$$

When this step is rapid the over-all stoicheiometry is the same as for step (2), but if this condition is not fulfilled the number of electrons consumed per azo molecule will appear to lie in the range 2–4.

The stability of the hydrazo compound is determined by the nature of its substituent groups. Electron withdrawing groups are conducive to stability, and electron releasing groups have the opposite tendency.[4,5] The mechanism of the disproportionation involves acid and/or base catalysis and consequently in some instances the full range of behaviour can be observed over a pH range.[2,5]

Experimental

A commercial sample of Fast Red E was recrystallised twice from 50 per cent. ethanol. The final purity, as found by titration with titanium(III),[6] was 93·5 per cent. on an anhydrous basis. The supporting electrolyte solutions used were each made up to an ionic strength of 0·8 by addition of potassium chloride solution when necessary; the solutions were well buffered by ensuring that the concentration of the minor buffer component was at least 80 times greater than the highest electroactive concentration used.[7] The measured pH values quoted were within range of those expected from pK_a values of the acid component of the buffer solutions. The solutions were as follows:

pH 2·0: 0·08 M phosphoric acid, 0·08 M sodium dihydrogen orthophosphate, 0·72 M potassium chloride.

pH 2·95: 0·08 M sodium hydroxide, 0·16 M citric acid, 0·72 M potassium chloride.

pH 4·3: 0·24 M sodium hydroxide, 0·16 M citric acid, 0·48 M potassium chloride.

pH 5·6: 0·40 M sodium hydroxide, 0·16 M citric acid, 0·08 M potassium chloride.

pH 6·6: 0·08 M sodium dihydrogen orthophosphate, 0·08 M disodium hydrogen orthophosphate, 0·48 M potassium chloride.

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pH 8·5: 0·08 M ammonia, 0·80 M ammonium chloride.
pH 9·7: 0·08 M ammonia, 0·08 M ammonium chloride, 0·72 M potassium chloride.
pH 10·6: 0·80 M ammonia, 0·08 M ammonium chloride, 0·72 M potassium chloride.
pH 11·3: 0·08 M disodium hydrogen orthophosphate, 0·08 M trisodium orthophosphate, 0·08 M potassium chloride.

A manual polarograph of conventional design was employed and a saturated calomel electrode of area 2·5 cm², separated from the working dropping-mercury electrode by a short agar bridge, served as counter and reference electrode. Solutions contained 0·01 per cent. of gelatin and were oxygen free. The apparatus was tested by using a solution of cadmium(II); the literature value for E (*i.e.*, −0·60 V *versus* S.C.E. in 0·1 M potassium chloride solution)[8] was duplicated and therefore no correction for internal resistance was made. Measured limiting currents were corrected by subtraction of the residual current at the same potential and mercury column heads (h) were corrected for the back-pressure. The drop-time method was used to obtain the electrocapillary curves and all experiments were conducted at 25 ± 0·1°C.

Results and Discussion

Limiting Currents

Well defined plateau regions were observed on polarograms of each of the buffer solutions (Fig. 1). A linear dependence of the mean limiting current (i_L) on depolariser concentration (c) in the range 10^{-3}–10^{-4} M was established in each instance. These lines passed through the origin and their respective gradients (i_L/c) are given in Table I. In addition, the dependence of the limiting current on the mercury head in the range 30–55 cm was investigated, with Fast Red E at a concentration of 5×10^{-4} M. In each buffer system i_L was found to be directly proportional to $h^{\frac{1}{2}}$. These results show that the limiting currents are diffusion controlled, and hence the disproportionation reaction (3) either plays no part in the reduction process or otherwise is a very rapid step.

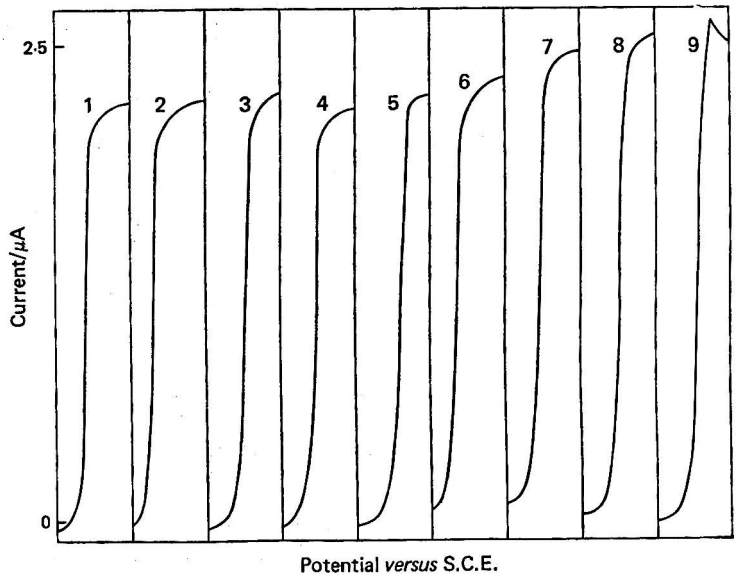

Fig. 1. Actual polarograms, uncorrected for residual currents, of 5×10^{-4} M Fast Red E. pH values of buffer solutions: 1, 2·0; 2, 3·0; 3, 4·3; 4, 5·6; 5, 6·6; 6, 8·5; 7, 9·7; 8, 10·7; and 9, 11·3. Curve 1 starts at 0·00 V, 2 at −0·10 V, 3 at −0·15 V, 4 at −0·25 V, 5 at −0·30 V, 6 at −0·40 V, 7 and 8 at −0·45 V and 9 at −0·50 V *versus* S.C.E. (200 mV abs.).

The mercury flow-rate (m) and drop time (t) were recorded at appropriate plateau potentials and the term $i_L/cm^{\frac{2}{3}}t^{\frac{1}{6}}$ was found to be essentially uniform over the pH range studied (see Table I), indicating a common stoicheiometry for the reduction process. According to the Ilkovič equation, this term can be identified with the product $607nD^{\frac{1}{2}}$, where n is the number

of electrons involved in the reduction process and D is the diffusion coefficient. For $n = 2$, the values calculated for the diffusion coefficients were in the range 8·2–11·2 × 10^{-6} cm^2 s^{-1}, while for $n = 4$, the range was 2·1–2·8 × 10^{-6} cm^2 s^{-1}. The latter values only are within the range of compilations for azo dyes,[9,10] suggesting that the polarographic reduction of Fast Red E follows the over-all scheme (2).

TABLE I

LIMITING CURRENTS AND HALF-WAVE POTENTIALS

pH	$\frac{i_L}{c}$ / μA m^3 mol^{-1}	m/mg s^{-1}	t/s	$\frac{i_L}{cm^{\frac{2}{3}}t^{\frac{1}{6}}}$	$E_{\frac{1}{2}}$*/V *versus* S.C.E.
2·0	4·38	0·9088	5·90	3·47	−0·08
3·0	4·48	0·9079	5·87	3·56	−0·16
4·3	4·62	0·9079	5·80	3·68	−0·26
5·6	4·40	0·9087	5·70	3·51	−0·35
6·6	4·50	0·9061	5·65	3·60	−0·42
8·5	4·64	0·9100	5·58	3·71	−0·48
9·7	4·84	0·9155	5·50	3·87	−0·54
10·7	5·10	0·9144	5·50	4·08	−0·56
11·3	5·12	0·9190	5·48	4·08	−0·61

* Measured at a depolariser concentration of 5 × 10^{-4} M.

Waveforms

Half-wave potentials become more negative with increasing pH, as is to be expected for processes that consume protons. In addition, the $E_{\frac{1}{2}}$ values tend to become slightly more negative with increasing depolariser concentration, *e.g.*, by 40 mV for a ten-fold concentration change at pH 4·3. Similar trends have been reported in the reduction of both amaranth[11] and azobenzene.[12] In well buffered media the effect has generally been ascribed to specific adsorption of the reactant and/or electrode product.[12]

Typical normalised polarograms are shown in Fig. 2, and these possess the common feature of a uniform initial development followed by a more rapid rise in current. This characteristic is illustrated by the logarithmic graphs shown in Fig. 3. The deviation from linearity was most marked at pH values 6·7 and 11·3, in the latter instance a small but distinct maximum being evident (see Fig. 1).

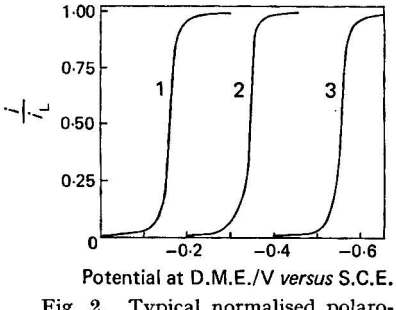

Fig. 2. Typical normalised polarograms. Fast Red E concentration, 5 × 10^{-4} M. pH values: 1, 3·0; 2, 5·6; and 3, 10·7.

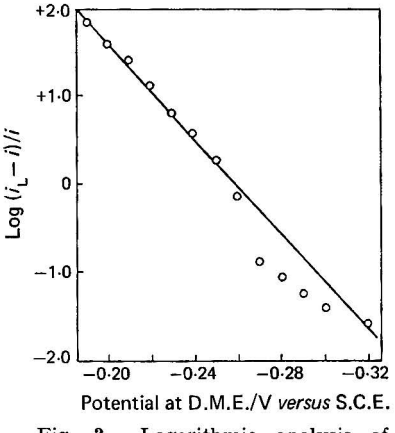

Fig. 3. Logarithmic analysis of polarogram. Fast Red E concentration, 5 × 10^{-4} M. Supporting electrolyte, pH 4·3.

The electrocapillary curve at pH 4·3, shown in Fig. 4, has a discontinuity in the potential region where the polarographic current begins to increase rapidly (about −0·25 V *versus* S.C.E.). This effect was confirmed at pH values 6·7 and 10·7. The lowering of interfacial tension in the potential region in which dye molecules are present in the vicinity of the electrode indicates that the depolariser is adsorbed throughout the reduction process. The continuous develop-

ment of the early part of the electrocapillary curve favours the interpretation that the equilibrium surface concentration of the electroactive species is not disturbed by electrolysis; however, the onset of the discontinuity signifies a net removal of the adsorbed electroactive species, with the interfacial tension tending to return to that displayed by the background solution.

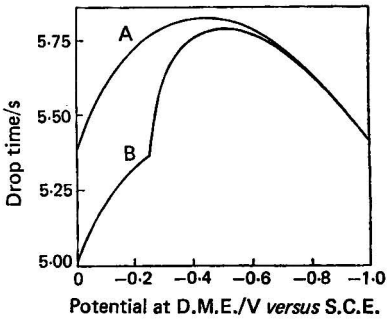

Fig. 4. Electrocapillary curves. Supporting electrolyte, pH 4·3: A, without depolariser and B, with 5×10^{-4} M Fast Red E.

Guidelli[13] has presented a theoretical treatment for the reduction of adsorbed depolariser. This treatment generates the classic Brdička post-wave when the electron-transfer step is assumed to be very rapid. In other instances, the charge-transfer overpotential on the "normal" wave displaces it to more negative potentials so as to merge with the "adsorption" wave. The morphology of the theoretical, irreversible waves[13] resembles those of Fast Red E in that an initial slow development is followed by a rapid rise in current.

Polarography of Related Dyes

Although Fast Red E no longer appears on the list of permitted food colours, other 1,1′-azonaphthalene dyes (*viz.*, amaranth and Ponceau 4R) are currently accepted.[14] Amaranth was the subject of an earlier investigation[11] in which a four-electron reduction process with a value for the diffusion coefficient (D) of $6{\cdot}9 \times 10^{-6}$ cm^2 s^{-1} was established. For completeness, a brief study of Ponceau 4R was made by the methods referred to above. At pH 8·5 a four-electron reduction process was again indicated, based on a value for D of $4{\cdot}4 \times 10^{-6}$ cm^2 s^{-1}, with an $E_{\frac{1}{2}}$ value of −0·61 V *versus* S.C.E. Such data may prove useful in the design of analytical methods should maximum levels for the addition of food colours be legislated for in the future.

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Complexometric Analysis of Magnetic Materials by Means of Automatic Titrations

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An analytical method based on automatic potentiometric titrations was developed for the analysis of magnetic material (*e.g.*, $Ba_2Zn_2Fe_{12}O_{22}$). The elements were separated by extracting iron(III) as its chloride in diisopropyl ether and by precipitating barium as its sulphate in the presence of disodium dihydrogen ethylenediaminetetraacetate (EDTA). After separation, the iron and zinc were complexed with EDTA, buffered at pH 5 with ammonium acetate solution and back-titrated with standard iron(III) chloride solution by using platinum-wire and saturated calomel electrodes. Barium sulphate was dissolved in EDTA and ammonia solution, buffered at pH 10·1 and back-titrated with standard zinc nitrate solution at mercury | mercury - EDTA and saturated calomel electrodes. A complete analysis of the magnetic material was performed on as little as 10 mg of sample. A Tacussel Titrimat automatic titrator was used for all of the determinations. Results accurate to within 0·35 per cent. were obtained. A similar procedure can also be used to analyse different magnetic samples.

When growing single crystals of hexagonal ferrites ($Ba_2M_2Fe_{12}O_{22}$), for example, by the flux method, various compositional phases are often obtained, and it is interesting to determine the chemical composition of these phases. If the mass of the samples is limited to a few tens of milligrams, ethylenediaminetetraacetate (EDTA) is a very versatile reagent for investigating the metallic composition of such ferrite samples.

In the literature some studies of the analysis of ferrite by chelatometric methods have been reported. Répàs,[1] Barcanescu *et al.*[2] and Vasyutin *et al.*[3] chromatographically separated the elements before commencing the quantitative analysis. Přibil and Vesely[4] employed separate aliquot methods, following extraction of the iron. Further, these authors used visual end-point methods and based the determinations of the single elements on control of the solution pH or on the use of masking agents.

We analysed zinc ferrite samples by using potentiometric titrations, which were preferred to visual methods because they are more reliable. The automatic technique, which gives excellent precision, was used to achieve the necessary accuracy of results. To compensate for the poor selectivity of the reagent the elements were isolated by using a simple procedure, in which iron was extracted as its chloride into diisopropyl ether and barium was precipitated as the sulphate.

The same technique can be applied to the analysis of nickel and copper ferrites. Cobalt, manganese and magnesium ferrites can also be analysed by using similar procedures, which consist in performing a suitable potentiometric titration of the cations after separation of iron and barium.

Method

Apparatus

A Titrimat automatic titrator (Tacussel) was used for the potentiometric titrations. It consists of a measure plug-in unit (TAT 4) associated with a potentiometric recorder (EPL 2) and a reagent-supply system (Electroburap syringe-burette). The plug-in unit also controls a galvanometer fitted with two adjustable control indexes. The counter resolution was 10 μl.

The indicator electrode used in the iron - EDTA titrations was a Tacussel platinum-wire electrode, while for the determination of barium it was a Radiometer P_{902} mercury-cup electrode. For all titrations a Tacussel C_4 saturated calomel electrode was used as the

reference electrode. The pH measurements were carried out on a Sargent - Welch NX pH meter.

Separating funnels with PTFE stopcocks were employed for iron extractions.

Reagents

Hydrochloric acid, sp. gr. 1·18, ammonia solution, sp. gr. 0·880, and nitric and sulphuric acids were BDH Aristar-grade products.

Diisopropyl ether. Reagent grade, supplied by C. Erba, was used without further treatment.

Standard iron(III) chloride solutions, 0·01 and 0·05 M. These solutions were prepared by dissolving the appropriate amount of 99·998 per cent. pure iron wire in hydrochloric acid. Hydrogen peroxide was added to ensure complete oxidation of the iron and the excess was evaporated off by heating gently. Finally, the solution was partially neutralised (to pH 1·5–2) with ammonia solution.

Standard zinc nitrate solution, 0·01 M. This was obtained by dissolving 99·999 per cent. pure zinc wire in 2·5 N nitric acid and neutralising with ammonia solution.

Standard disodium dihydrogen ethylenediaminetetraacetate solutions, 0·01 and 0·05 M. These solutions were prepared from the salt purified by use of the method reported by Welcher.[5]

Synthetic samples of ferrite. These were obtained by mixing iron(III), zinc and barium oxides in a 3 : 1 : 1 molar ratio. The metallic content of each oxide was accurately standardised by classical gravimetric procedures.

Mercury - EDTA solution, 0·0025 M. This solution was prepared by mixing equivalent amounts of mercury(II) nitrate and EDTA solutions.

Ammonia buffer solution, pH 10·1. This buffer was obtained by dissolving 16 g of ammonium nitrate in 28 ml of ammonia solution (sp. gr. 0·880) and diluting to 1 l with double-distilled water.

Analytical Procedure

Weigh the sample and dissolve it in the hydrochloric acid by using the most suitable process for the particular ferrite to be examined. A complete dissolution of zinc ferrite samples is achieved in about 20–30 min by warming the compound gently with a few millilitres of concentrated hydrochloric acid in a covered beaker. In order to avoid losses of volatile chlorides, the temperature should not exceed 50–60 °C. To the solution add a few drops of 100-volume hydrogen peroxide and evaporate off the excess reagent, then cool the solution and measure the volume exactly. Dilute the solution carefully with water until it is 8 M in hydrochloric acid, transfer it into a separating funnel and extract with an equal volume of diisopropyl ether. Separate the phases and repeat the extraction three or four times with about 80–100 ml of the ether. Combine the organic extracts and back-extract the iron by washing three times with about 20 ml of water, shaking the separator for about 1 min on each occasion. Combine the washings in a beaker, then add a sufficient amount of standard EDTA solution and solid ammonium acetate until a pH of 5 has been reached. Titrate potentiometrically the excess of EDTA with standard iron(III) chloride solution.

Contemporaneously with the above procedure slowly evaporate the solution containing zinc and barium chlorides and when as much as possible of the acid has been eliminated dilute the solution with water and evaporate it a further two or three times. Control the pH of the solution by adding ammonia solution or hydrochloric acid, if necessary, until a pH of 1–1·5 is obtained. Add a sufficient amount of standard EDTA solution and heat to incipient boiling. Precipitate the barium with hot 1 N sulphuric acid, let it stand for 1 h, then filter and wash the precipitate with hot water until no chlorides are present in the wash solution. Collect the filtrate and wash solutions in a beaker, add solid ammonium acetate to raise the pH to 5 and titrate the excess of EDTA with standard iron(III) chloride solution. Calculate the zinc concentration.

Transfer the filter containing barium sulphate into the original beaker, add a sufficient amount of standard EDTA solution and about 2 ml of concentrated ammonia solution, then heat the mixture to boiling. At this stage barium sulphate is usually completely dissolved; if it is not, heat for 5 or 10 min and remove the filter-paper after carefully washing it. Next add 20 ml of ammonia buffer and 2 or 3 drops of mercury - EDTA solution and remove any dissolved oxygen from the solution by passing nitrogen through it for about 15 min. Dip the electrodes and the needle of the syringe-burette into the solution and de-gas for a

few more minutes. Finally, titrate the excess of EDTA with standard zinc nitrate solution and calculate the barium concentration.

Automatic Titrations

All of the potential - titrant volume traces were automatically recorded at controlled speed such that the reagent addition and the synchronous chart speed were correspondingly slowed in the high slope region of the trace. The equivalence point was determined exactly by the slowest speed at the point of inflection (Fig. 1).

In the course of performing routine analyses, a known standard is first titrated in order to find the equivalence potential, which needs to be pre-set on the meter dial by use of the adjustable index. The instrument then automatically permits reagent to flow and records until the pre-set potential is attained.

Results

The entire procedure described above was verified by analysing powdered synthetic mixtures of iron(III), zinc and barium oxides, having a molar composition of 3:1:1. The results are summarised in Table I. Typical titration graphs, automatically recorded, are shown in

TABLE I

ANALYSIS OF IRON, ZINC AND BARIUM OXIDE SYNTHETIC MIXTURES

Iron/mmol			Zinc/mmol			Barium/mmol		
Taken	Found*	Standard deviation	Taken	Found*	Standard deviation	Taken	Found*	Standard deviation
0·0842	0·0844	$1·0 \times 10^{-4}$	0·0140	$0·0140_5$	$1·3 \times 10^{-4}$	0·0140	0·0140	$1·0 \times 10^{-4}$
0·1683	0·1682	$0·8 \times 10^{-4}$	0·0280	0·0279	$1·3 \times 10^{-4}$	0·0280	0·0281	$0·8 \times 10^{-4}$
0·2525	0·2522	$1·4 \times 10^{-4}$	0·0420	0·0419	$1·3 \times 10^{-4}$	0·0420	$0·0420_5$	$1·7 \times 10^{-4}$
0·4208	0·4211	$2·5 \times 10^{-4}$	0·0701	0·0703	$0·6 \times 10^{-4}$	0·0700	0·0698	$0·8 \times 10^{-4}$
0·8397	0·8399	$2·5 \times 10^{-4}$	0·1401	0·1405	$1·3 \times 10^{-4}$	0·1401	0·1402	$1·3 \times 10^{-4}$

* Average of four determinations.

Fig. 1. Table II contains the results of a number of determinations of iron and zinc or cobalt in the same solution. After the removal of chloride, iron was first titrated with EDTA at pH 3, in the presence of ammonium acetate, at platinum and saturated calomel electrodes. Afterwards the solution pH was increased (to 5 with sodium acetate for zinc and to 8·5 with ammonia and ammonium nitrate for cobalt) and the second metal was then titrated at mercury | mercury - EDTA and saturated calomel electrodes.

TABLE II

TITRATION OF IRON AND ZINC (OR COBALT) AT DIFFERENT pH VALUES

Taken/mmol		Found/mmol		Error, per cent.	
Iron	Other metal	Iron	Other metal	Iron	Other metal
0·4070	0·0810*	0·3340	0·1560*	−18	+92
0·4260	0·1100*	0·3820	0·1580*	−10	+44
0·8610	0·1020†	0·8430	0·1080†	−2	+6
0·4230	0·1020†	0·3880	0·1380†	−8	+35
0·4690	0·1020†	0·4160	0·1610†	−11	+58

* Zinc. † Cobalt.

In Table III the results of iron, zinc and barium determinations in zinc ferrite samples grown in our laboratory by the flux (sodium oxide) method are given. Two series of four analyses are reported, referring to different compositional phases, and a comparison with the results obtained by classical methods is given. Iron was determined gravimetrically, as iron(III) oxide, and titrimetrically by use of the dichromate method. Zinc was determined gravimetrically, as the pyrophosphate, and chelatometrically, by using EDTA and xylenol orange indicator. Barium was precipitated and weighed as barium sulphate.

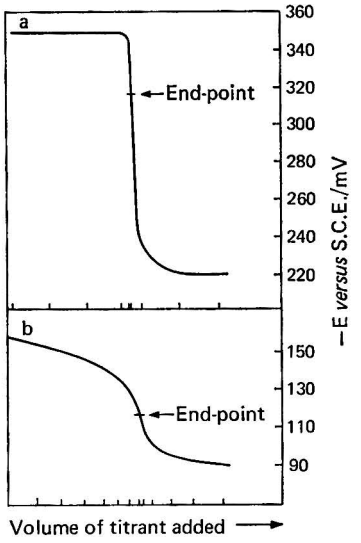

Fig. 1. Typical automatically recorded titration curves for: (a), iron(III) - EDTA + EDTA (5×10^{-5} mol) titrated with 0·01 M iron(III) chloride solution at pH 5 at platinum and saturated calomel electrodes; and (b), barium - EDTA + EDTA (5×10^{-5} mol) titrated with 0·01 M zinc nitrate solution at pH 10·1 at mercury|mercury - EDTA and saturated calomel electrodes. The vertical lines on the abscissa are time-marking automatically recorded in the chart margin by a built-in time base that delivers a pulse every 30 s.

Discussion

We have verified that the methods based on the control of pH are not well suited to the potentiometric determination of iron(III) mixed with cations that are detectable at higher pH values (such as zinc, nickel, cobalt, etc.). In fact, by titrating at a pH less than 5, the reaction of iron(III) with EDTA is unsatisfactory; the results obtained are low and not reproducible (Table II). The common masking agents (such as triethanolamine, fluorides and cyanides) are not suitable in this instance. It was proved that after use of complexing agents the cations cannot be titrated potentiometrically with EDTA, as unsatisfactory graphs are obtained. The separate aliquot method, by means of which an element is determined by the difference between two titrations, usually entails a summation of the errors and therefore gives inaccurate determinations.

The extraction of iron by the method of Dodson, Forney and Swift[6] was preferred to precipitation. A very satisfactory separation of iron from other cations was obtained in a short time provided that the hydrochloric acid concentration in the aqueous phase was kept strictly in the range 7·8–8·5 mol l^{-1}. Spot tests, carried out with a hexacyanoferrate(II) solution, showed that four extractions with a total of about 80 ml of diisopropyl ether were enough to remove about 5 mg of iron(III) from 25 ml of a solution in 8 M hydrochloric acid. By shaking the organic phase with double-distilled water two or three times, iron(III) chloride is completely back-extracted in aqueous solution; this step was also verified by the hexacyanoferrate(II) spot test. Any bivalent iron that was formed during the ferrite growth processes would be oxidised to iron(III) during dissolution of the sample. Therefore, the total iron was separated and determined by the described procedure.

Přibil, Koudela and Matyska[7] reported that at pH 5, in the presence of ammonium acetate, iron(III) can be either directly titrated or back-titrated with EDTA at platinum and saturated

calomel electrodes. We preferred back-titration because more regular potentiometric graphs were obtained with this technique. Further, by raising the pH of the solution in the presence of an excess of EDTA, the precipitation of slightly soluble iron compounds was avoided.

The method of Přibil, Koudela and Matyska[7] for the determination of zinc was preferred to the potentiometric method of Reilley and co-workers,[8,9] because of the interferences from a chloride medium.

The separation of barium was conveniently carried out by its precipitation as the sulphate; EDTA, added before this stage in the analysis, prevents co-precipitation.[10]

TABLE III

RESULTS FOR ZINC FERRITES

	Iron found, per cent.			Zinc found, per cent.			Barium found, per cent.	
	Automatic titration	Gravimetry	Manual titration	Automatic titration	Gravimetry	Chelatometry	Automatic titration	Gravimetry
Phase A								
Probe 1	47·58	47·72	47·42	10·78	10·65	10·70	19·46	19·39
Probe 2	47·52	47·54	47·48	10·72	10·72	10·84	19·52	19·50
Probe 3	47·65	47·75	47·35	10·70	10·78	10·85	19·50	19·58
Probe 4	47·55	47·80	47·45	10·75	10·78	10·92	19·50	19·40
Mean value	47·57	47·70	47·42	10·74	10·73	10·83	19·49	19·47
Mean deviation, per cent.	±0·09	±0·17	±0·08	±0·25	±0·44	±0·58	±0·10	±0·50
Phase B								
Probe 1	46·21	46·48	46·40	7·81	7·92	7·92	17·78	17·85
Probe 2	46·29	46·30	46·24	7·85	7·70	7·95	17·70	18·20
Probe 3	46·25	46·38	46·28	7·79	7·75	7·80	17·82	17·92
Probe 4	46·22	46·42	46·25	7·80	7·72	7·87	17·80	17·75
Mean value	46·24	46·39	46·29	7·81	7·77	7·88	17·77	17·93
Mean deviation, per cent.	±0·06	±0·12	±0·11	±0·22	±0·93	±0·63	±0·22	±0·75

Conclusions

The proposed procedure offers an accurate and time-saving method for carrying out the routine analysis of ferrite. Samples weighing as little as a few milligrams can be analysed in a total time of about 4 h, including sample dissolution and cation separation and determination. Reasonably accurate results, within 0·35 per cent., can be obtained with a small number of simple operations.

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Precipitation of Silver Molybdate from Homogeneous Solution by Use of Diamminesilver(I) Reagent

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An investigation has been carried out on the application of diamminesilver(I) reagent to the precipitation of molybdate. Addition of ammonia solution to a silver salt results in the formation of the complex diamminesilver(I), which when heated gradually decomposes to give silver ions in solution and ammonia, which is volatilised slowly, with a corresponding decrease in pH. The liberated silver ions react with molybdate to form a dense precipitate of silver molybdate.

Diamminesilver(I) was first used as a reagent by Firsching for the separation of equimolar mixtures of chloride, bromide and iodide[1] and for the quantitative determination of phosphate.[2] Varughese and Vaidya[3] and Varughese and Somasekhara Rao[4] found this reagent useful for the precipitation of chromate and tungstate, respectively, from homogeneous solution. This paper describes the suitability of this reagent for the precipitation of molybdate from homogeneous solution.

Materials

Reagents

A stock solution of molybdenum was prepared by accurately weighing 6·4440 g of pure sodium molybdate ($Na_2MoO_4.2H_2O$) (P.O.C.H. Polska, Poland), dissolving it in double-distilled water and diluting to 1 l. The molybdenum content was determined by the 8-hydroxyquinolinate method.[5] From the results, the purity of the sodium molybdate was found to be 99·9 per cent.

The diamminesilver(I) reagent was prepared by adding 20 ml of ammonia solution (sp. gr. 0·910) to 40 ml of 1 M silver nitrate (AnalaR) solution and diluting to 100 ml.

The other reagents used were of analytical-reagent grade.

Apparatus

A Geiger-Müller counter (Medical Spectrometer, Bhabha Atomic Research Centre, Bombay, India) was used for measurement of radioactivity and a Cahn RG electrobalance was used to record thermograms. For pH measurements a Beckman pH meter was used and a Carl Zeiss Jena Amphival was used in order to prepare photomicrographs. Particle measurements were made with a MIN-8 micrometer.

Results and Discussion

Experiments were carried out on the effect of pH and the concentrations of precipitant and ammonium nitrate on the completeness of precipitation of molybdate.

Variation of pH

A solution of sodium molybdate containing molybdenum-99 as tracer was prepared as follows. Molybdenum-99 tracer was prepared by irradiating sodium molybdate in a reactor at the Isotopic Division, Bhabha Atomic Research Centre. A 6·4-g amount of labelled sodium molybdate was accurately weighed and dissolved in water and the solution made up to 1 l. This solution was standardised by use of 8-hydroxyquinolinate and the activity of a 25-ml portion, which contained 63·8 mg of molybdenum, was measured and found to be 61 923 counts min^{-1}.

A solution containing 25 ml of the labelled sodium molybdate solution (*i.e.*, 106·4 mg of molybdate), 5 ml of 1 M ammonium nitrate solution and 10 ml of reagent solution in a total volume of 200 ml was heated on a water-bath. Samples were removed at intervals, cooled, filtered and the pH values of the filtrate measured. The molybdate content of the filtrate was determined by measuring the activity. The results are given in Table I.

The maximum precipitation was found to occur between pH 7·0 and 7·4. The amount of molybdate in the filtrate was about 50 μg when 106·4 mg of molybdate were taken for precipitation in this pH range.

TABLE I

DETERMINATION OF MOLYBDATE IN THE FILTRATE (100 ml) BY USING MOLYBDENUM-99 AS TRACER

pH value of filtrate	Molybdate in filtrate/mg
8·2	65·31
8·0	41·50
7·8	25·01
7·7	13·54
7·5	4·80
7·4	0·05
7·3	0·03
7·2	0·02
7·1	0·02
7·0	0·02

Variation of Concentrations of Silver Nitrate and Ammonium Nitrate

Portions (200 ml) of solution containing 106·4 mg of molybdate, 5 ml of 1 M ammonium nitrate solution and various amounts of silver nitrate with a sufficient volume of ammonia solution were heated on a water-bath until the pH of the solution reached 7·0–7·4, which was indicated by phenol red. The results are given in Tables II and III.

It can be seen that the minimum amount of silver nitrate required for complete precipitation of 106·4 mg of molybdate is 2 mmol. Excess of silver nitrate up to 40 mmol does not affect the determination in the presence of 5 mmol of ammonium nitrate.

TABLE II

EFFECT OF SILVER NITRATE ON THE PRECIPITATION OF MOLYBDATE

Amount of ammonium nitrate = 5·0 mmol.

Silver nitrate taken/mmol	Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
0·50	106·40	40·42	38·0
1·00	106·40	74·49	70·0
1·50	106·40	104·30	98·0
1·65	106·40	104·70	98·4
1·75	106·40	106·00	99·6
2·00	106·40	106·40	100·0
3·00	106·40	106·40	100·0

TABLE III

EFFECT OF EXCESS OF SILVER NITRATE ON THE PRECIPITATION OF MOLYBDATE

Amount of ammonium nitrate = 5·0 mmol.

Silver nitrate taken/mmol	Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
5·0	106·40	106·38	99·98
10·0	106·40	106·46	100·05
20·0	106·40	106·35	99·95
30·0	106·40	106·44	100·04
40·0	106·40	106·60	100·19

The experiments were repeated with the minimum requisite amount of silver nitrate (2 mmol) and with various amounts of ammonium nitrate and the results are shown in Table IV. It was observed that ammonium nitrate, in the concentration range studied, has no effect on complete precipitation. When using various concentrations of ammonium nitrate the final pH of the solution after precipitation is found to vary progressively. However, no appreciable error in the determination resulted from this variation. Therefore, it can be inferred that in the presence of the minimum requisite amount of silver nitrate, variation of pH in the range 6·2–8·2 has little effect on the determination.

TABLE IV

EFFECT OF AMMONIUM NITRATE ON THE PRECIPITATION OF MOLYBDATE WHEN USING THE MINIMUM AMOUNT OF SILVER NITRATE

Amount of silver nitrate = 2 mmol.

Ammonium nitrate taken/mmol	pH	Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
0·0	8·2	106·40	106·00	99·63
5·0	7·2	106·40	106·41	100·01
10·0	7·0	106·40	106·52	100·11
25·0	6·5	106·40	106·25	99·86
50·0	6·2	106·40	106·20	99·81

Further experiments were carried out on the effect of using different amounts of ammonium nitrate in the presence of excess of reagent (5 mmol of silver nitrate) and the results are reported in Table V. The results indicate that accurate values can be obtained by employing 5–10 mmol of ammonium nitrate. Outside this range the measured recoveries were too high. Above pH 7·4 (*i.e.*, when less ammonium nitrate was used) the high recovery is presumably due to the formation of silver(I) oxide and several blank experiments, without molybdate ion, were conducted with 5 mmol of silver nitrate and with amounts of ammonium nitrate below 5 mmol, other conditions being identical. It was found that significant amounts of silver(I) oxide were precipitated, depending on the concentration of ammonium nitrate. Significant amounts of silver oxide were not precipitated when 5 mmol or more of ammonium nitrate were used. No definite explanation could be given for the high results below pH 7·0, *i.e.*, when more than 10 mmol of ammonium nitrate were used. Some experiments were conducted in order to establish the cause of these high results. Silver molybdate was precipitated from solutions containing various amounts of ammonium nitrate in the presence of excess of silver nitrate and the precipitates thus obtained were observed under the microscope. This examination revealed the presence of two distinct types of crystal representing different structures. It is thought that when the pH of the solution is less than 7·0, polymerisation of molybdate may take place. However, the presence of silver polymolybdate could not be confirmed.

TABLE V

EFFECT OF AMMONIUM NITRATE ON THE PRECIPITATION OF MOLYBDATE WHEN USING EXCESS OF SILVER NITRATE

Amount of silver nitrate = 5·0 mmol.

Ammonium nitrate taken/mmol	pH	Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
0·0	8·2	106·40	120·50	113·25
5·0	7·2	106·40	106·38	99·98
10·0	7·0	106·40	106·25	99·86
25·0	6·5	106·40	107·65	101·17
50·0	6·2	106·40	108·52	101·99

Thus it appears that ammonium nitrate plays a significant role in the precipitation. Although accurate results are obtained by taking 2 mmol of silver nitrate for every 100 mg of molybdate even in the absence of ammonium nitrate, this condition cannot be achieved for samples of unknown molybdate content. A higher concentration of reagent may be used in order to ensure complete precipitation and as a result higher values for the recovery are likely to be obtained (Table V). However, this difficulty can be overcome if the precipitation is carried out in the presence of ammonium nitrate (Table V). It is concluded that 5–10 mmol of ammonium nitrate should be the amount used in the determination of molybdate in the presence of silver nitrate as precipitant even when larger amounts of silver nitrate are used for precipitation.

Effect of Ammonium Nitrate on Particle Size

Precipitates were prepared by the method of volatilisation of ammonia in the absence of ammonium nitrate and also in the presence of ammonium nitrate (5 mmol). Precipitates of silver molybdate were also prepared by a direct method.[6] From the photomicrographs it

was observed that the precipitate obtained by direct precipitation is amorphous whereas the precipitate obtained by precipitation from homogeneous solution in the absence of ammonium nitrate is slightly improved and has crystalline form. The precipitate obtained in the presence of 5 mmol of ammonium nitrate is more developed, with rhomboidal crystals. The dimensions of the larger crystals are 0·576 × 0·352 mm and those of the smallest are 0·096 × 0·080 mm (measured micrometrically). The proportion of small crystals was about 80 per cent.

Determination of Molybdate

Recommended Procedure

Add 5 ml of 1 M ammonium nitrate solution to a sample solution containing 20–200 mg of molybdate in a beaker and adjust the volume to approximately 200 ml. Then add 10 ml of the reagent solution and 1 ml of 0·1 per cent. phenol red indicator solution. Heat the beaker and contents on a steam-bath for about 3½ h, maintaining the volume by occasional addition of distilled water. The completeness of the precipitation is indicated by the change of the red colour of the supernatant to light yellow. Precipitation commences after heating for about 1 h. The pH of the supernatant at room temperature was found to be between 7·0 and 7·4. Filter the precipitate of silver molybdate (Ag_2MoO_4) on a weighed G_4 sintered-glass crucible, dry it at 175 °C for about 30 min and then re-weigh it. Typical results obtained are shown in Table VI.

A thermogravimetric study revealed that the precipitate has a constant mass up to 700 °C.

TABLE VI

DETERMINATION OF VARIOUS AMOUNTS OF MOLYBDATE

Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
21·280	21·292	100·06
21·280	21·295	100·07
21·280	21·298	100·08
42·560	42·584	100·06
42·560	42·550	99·76
42·560	42·572	100·03
85·120	85·100	99·98
85·120	85·143	100·03
85·120	85·088	99·96
106·400	106·450	100·05
106·400	106·370	99·97
106·400	106·390	99·98
106·400	106·410	100·01
106·400	106·420	100·02
212·800	212·860	100·03
212·800	212·720	99·96
212·800	212·700	99·95

TABLE VII

PRECIPITATION OF SILVER MOLYBDATE IN THE PRESENCE OF FOREIGN ANIONS

Source of anions	Concentration/ mmol l^{-1}	Molybdate taken/mg	Molybdate found/mg	Recovery, per cent.
CH_3COONa ..	100·0	106·40	106·28	99·89
CH_3COONa ..	200·0	106·40	100·60	94·55
$NaNO_3$	150·0	106·40	106·54	100·13
KNO_3	50·0	106·40	106·32	99·92
K_2SO_4	100·0	106·40	106·28	99·89
K_2SO_4	150·0	106·40	103·90	97·67
Na_2SiO_3	1·0	106·40	106·68	100·27
Na_2WO_4	0·025	106·40	106·28	99·89
Na_2WO_4	0·050	106·40	107·20	100·75
Na_2HPO_4	0·40	106·40	106·90	100·47
Na_2HPO_4	0·60	106·40	110·70	104·04
CH_3COONH_4 ..	100·00	106·40	106·15	99·76
CH_3COONH_4 ..	200·00	106·40	95·41	89·67
$(NH_4)_2SO_4$	100·00	106·40	106·25	99·86
$(NH_4)_2SO_4$	200·00	106·40	102·60	96·43

Determination of Molybdate in the Presence of Foreign Ions

The recommended method was applied to a solution containing 106·4 mg of molybdate and some chosen anions. The results are shown in Table VII.

Good recoveries of molybdate were obtained in the presence of large amounts of acetate, sulphate and nitrate. Small amounts of silicate, tungstate and phosphate can be tolerated. The absence of interference by sulphate in this method is an advantage.

Separation from molybdate of some heavy metals, as their hydroxides, could be successfully accomplished prior to precipitation of silver molybdate from the molybdate ions in solution.

Application of the Method

The amount of molybdenum(VI) oxide in three samples of molybdic acid was determined by using the proposed method and the results were compared with those obtained with the procedure involving 8-hydroxyquinolinate. The values are given in Table VIII. The results indicate that the method can be reliably employed for the determination of molybdenum(VI) oxide. The sample solution was prepared by dissolving the sample in dilute ammonia solution.

TABLE VIII

DETERMINATION OF MOLYBDENUM(VI) OXIDE

Sample	Mass of sample taken/mg	Mass of molybdenum(VI) oxide found by— proposed method/mg	8-hydroxyquinolinate method/mg
Molybdic acid	100·0	90·01	89·95
Molybdic acid	100·0	84·15	84·60
Molybdenum(VI) oxide	60·0	59·99	60·02

The molybdenum content of an impure sample of molybdenum metal was also determined by using this procedure (Table IX). The sample solution was prepared by fusing about 1 g of the metal with 8 g of fusion mixture and 2 g of potassium nitrate, disintegrating the melt with water, and then diluting to 250 ml. The values obtained for the molybdenum content were compared with those obtained by the method involving 8-hydroxyquinolinate.

TABLE IX

DETERMINATION OF MOLYBDENUM IN IMPURE MOLYBDENUM METAL

Molybdenum metal taken/mg	Molybdenum found by— proposed method/mg	8-hydroxyquinolinate method/mg	Molybdenum content, per cent.
100·0	85·5	85·5	85·5
100·0	85·5	85·5	85·5
150·0	128·2	128·3	85·5
150·0	128·2	128·2	85·5

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A Selective Method for the Determination of Molybdenum Using Toluene-3,4-dithiol

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A method is described for the colorimetric determination of molybdenum. The procedure involves the extraction of molybdenum(VI) from solution in hydrochloric acid with tri-n-butyl phosphate. The tri-n-butyl phosphate extract is then complexed with toluene-3,4-dithiol in glacial acetic acid - tri-n-butyl phosphate - orthophosphoric acid medium.

This procedure enhances the selectivity, sensitivity and reproducibility of the molybdenum - dithiol reaction.

A number of methods are available for the determination of molybdenum that are based on the formation of its complexes with thiocyanate and toluene-3,4-dithiol (dithiol). Of these, the tin(II) chloride - thiocyanate method is considered to be the most satisfactory, being more selective than any method based on the use of dithiol.

The molybdenum(VI) ion gives a thiocyanate complex. It is believed that in acidic solution and in the presence of tin(II) chloride molybdenum(VI) is reduced to molybdenum(IV), which then disproportionates to molybdenum(V) and molybdenum(III).[1] A number of solvents have been used for the extraction of the complex formed with thiocyanate. Various ions, *e.g.*, cobalt, uranium, tungsten(VI) and ruthenium interfere in the method.

The dithiol complex is known to yield a dark green precipitate in a mineral acid medium and this precipitate can be extracted with polar and non-polar solvents. The reaction is considered to be less selective than formation of the thiocyanate complex because of the large number of interfering ions, *e.g.*, lead, bismuth, copper, cadmium, antimony, cobalt, nickel, tin and tungsten(VI), which react in acidic solution, and iron(III), manganese and ruthenium, which react in alkaline solution. Various methods have been devised in order to eliminate these interferences.[2–7]

In the method described in this paper, molybdenum(VI) in solution in a water sample is extracted from the solution (acidified with 6 N hydrochloric acid) with tri-n-butyl phosphate (TBP). The extraction is fairly selective. The molybdenum(VI) ion is extracted quantitatively, together with substantial amounts of tungsten(VI) and iron(III) and much smaller amounts of other elements. The extract is then complexed with dithiol in a glacial acetic acid - TBP - orthophosphoric acid medium. This medium has the advantage that it suppresses the reaction of dithiol with tungsten(VI), iron(III) and the trace amounts of the other elements that have been co-extracted with the molybdenum. The selectivity of the method is thereby enhanced.

The molar absorptivity of the molybdenum(VI) - dithiol complex in the proposed medium is higher than the value obtained with the usual extraction solvents that are employed when the complexation reaction takes place in a mineral acid medium. The sensitivity of the proposed method is therefore higher. The use of this medium has the further advantage that turbidity problems arising from the restricted solubility of dithiol in mineral acids and the concomitant necessity for extraction of the complex with an immiscible solvent is eliminated. This advantage improves the reproducibility of the method.

Experimental

Reagents

Toluene-3,4-dithiol reagent solution. Solutions of dithiol are notoriously unstable and have attracted considerable attention.[8,9] It has been found, however, that solutions of dithiol prepared in the manner indicated below are stable for several weeks when stored in a refrigerator. The solution should be discarded when it shows evidence of deterioration, due possibly

to the formation of the disulphide. The test solution is prepared by dissolving 1 g of dithiol in 100 ml of 10 per cent. sodium hydroxide solution that contains 1·0 g of mercaptoacetic acid. The solution should be stored in a refrigerator.

Glacial acetic acid, analytical-reagent grade.

Tri-n-butyl phosphate, analytical-reagent grade.

Orthophosphoric acid, analytical-reagent grade.

Stock standard solution of molybdenum. An amount (1·840 g) of analytical-reagent grade ammonium molybdate was dissolved in water contained in a 1-l standard flask and the solution was diluted to the mark. This solution contained 1000 μg ml^{-1} of molybdenum.

Test solutions of standard molybdenum. The stock solution was serially diluted to obtain the working standard solutions containing the requisite amounts of molybdenum.

Apparatus

The apparatus described under Method was used. The absorbance measurements were made by using a Beckman, Model DB, spectrophotometer with 1-cm cuvettes. The reference cuvette was filled with double-distilled water.

Method

A 5-ml aliquot of the TBP extract (obtained according to the procedure described under Extraction of molybdenum(VI) ion from solution in hydrochloric acid with tri-n-butyl phosphate) was pipetted into a 50-ml standard flask. Glacial acetic acid (25 ml) and 2 ml of orthophosphoric acid were added and the solution was mixed, five drops of dithiol reagent were added and the flask was swirled to mix the contents. The volume was made up to the mark with glacial acetic acid and the solution allowed to stand for 3 h. Finally, the absorbance was measured in a Beckman spectrophotometer using 1-cm cuvettes. The reference cuvette was filled with double-distilled water.

Reproducibility and Calibration

In order to study the reproducibility of the method, the procedure described above was slightly modified.

The molybdenum stock solution was diluted with glacial acetic acid to give four solutions, containing 5, 10, 15 and 20 μg ml^{-1} of molybdenum. Five-millilitre aliquots of each of these solutions contained 25, 50, 75 and 100 μg of molybdenum, respectively.

A 5-ml aliquot of the relevant solution of molybdenum in acetic acid was pipetted into a 50-ml standard flask followed by 5 ml of TBP (saturated with 6 N hydrochloric acid), 20 ml of glacial acetic acid and 2 ml of orthophosphoric acid, then 5 drops of dithiol reagent were added and the contents of the flask swirled to mix. The procedure described under Method was then followed. Reagent blanks were prepared in the same manner as described above except that 5 ml of glacial acetic acid were used instead of the molybdenum solution.

Analyses were carried out in quadruplicate at each of the four concentrations (together with reagent blanks, also in quadruplicate). The results of analytical interest were obtained by subtracting the mean absorbance of the reagent blanks from the absorbance of the solution in each instance. The reagent blanks gave a mean absorbance of 0·008 with an over-all standard deviation of $0{\cdot}0011_2$.

The procedure was repeated on five different occasions and the results were studied statistically. The between-batch standard deviations were not significantly larger (at the 95 per cent. confidence level) than the within-batch standard deviations. The results were therefore pooled to obtain over-all standard deviations. The means of the 20 absorbance values (corrected for the absorbance of the reagent blank) obtained at each of the four concentrations were used to plot the calibration graph (see Table I).

The calibration graph is linear, passing through the origin, and can be represented by the equation:

$$Y = 0{\cdot}384 \times 10^{-2} X$$

where Y is the absorbance determined as described under Method and X μg is the concentration of molybdenum in the 50-ml standard flask.

TABLE I

OVER-ALL STANDARD DEVIATIONS CORRESPONDING TO VARIOUS LEVELS OF MOLYBDENUM

Amount of molybdenum in 50-ml standard flask/μg	Mean absorbance at 705 nm in 1-cm cuvette (corrected for blanks)	Over-all standard deviation in absorbance units	Degrees of freedom
25	0·094	$0·0015_6$	19
50	0·192	$0·0021_2$	19
75	0·288	$0·0025_4$	19
100	0·384	$0·0030_0$	19

Development of Optimum Conditions for the Determination of Molybdenum

The technique described under Method represents the optimum conditions for the reaction. These conditions were arrived at after studying the following factors.

Choice of acid mixture

The complexation reaction requires a low pH, hence glacial acetic acid, formic acid and mixtures of acetic and formic acids were tried. Finally, a mixture of glacial acetic and orthophosphoric acids was selected. Formic acid alone was unsuitable because the solubility of the complex in this acid was not satisfactory, giving rise to turbidity. Further, the reaction with tungsten(VI) was not suppressed in this medium. Mixtures of acetic and formic acids were also unsatisfactory.

Glacial acetic acid gave clear green solutions and clear and colourless blanks. It also suppressed the reactions of tungsten(VI), iron(III) and trace amounts of other metals that are co-extracted with the molybdenum. The molar absorptivity, however, was less than the optimum value that could be attained, but this optimum value was reached when orthophosphoric or hydrochloric acid was added to the medium. Control was easier with orthophosphoric acid and the addition of 4 per cent. acid also had the advantage that the time of reaction was decreased. However, concentrations of orthophosphoric acid greater than 4 per cent. slowed down the reaction. The presence of TBP (inevitable in the principal analytical complexation) tended to lower the molar absorptivity of the complex. This tendency was overcome by the use of orthophosphoric acid. The presence of small amounts of hydrochloric acid from the TBP extract did not cause any turbidity problems.

Absorption graph

In the medium used (glacial acetic acid - TBP - orthophosphoric acid) the molybdenum - dithiol complex showed absorbance maxima at 705 and 440 nm and a minimum at 555 nm. The peak at 705 nm was preferred to that at 440 nm because of greater linearity of the calibration graph and the lower absorption of the reagent blanks. The complex had a molar absorptivity of 18 425 l mol^{-1} cm^{-1} at 705 nm.

Molar absorptivity

Comparison of the molar absorptivities of the molybdenum - dithiol complex in various media showed that the value in the selected medium (acetic acid - TBP - orthophosphoric acid) was higher than the values obtained with the extraction solvents used when the reaction was effected in solution in sulphuric acid, as described by Bickford *et al.*[7]

The results are reported in Table II.

Complexation reaction

The complexation reaction is slow but is completed in 3 h. The colour is stable for over 24 h.

Extraction of Molybdenum(VI) Ion from Solution in Hydrochloric Acid with Tri-n-butyl Phosphate

It has been reported that molybdenum(VI) is quantitatively extracted into TBP from solution in hydrochloric acid.[10,11] Further, Edge *et al.*[12] used extraction into TBP from solution in hydrochloric acid for concentrating molybdenum for a subsequent spectrochemica determination. Dhara and Khophar[13] used a lithium chloride - hydrochloric acid medium

and the molybdenum was extracted with a TBP - chloroform mixture. The determination of molybdenum was completed by using the thiocyanate reaction.

When this work was undertaken it was felt that the selectivity of the molybdenum - dithiol reaction could be improved by first effecting a selective extraction of molybdenum(VI) into TBP and subsequently carrying out the complexation in a non-aqueous medium, which would suppress some of the reactions of dithiol.

TABLE II

MOLAR ABSORPTIVITIES OF THE MOLYBDENUM - DITHIOL COMPLEX IN VARIOUS MEDIA

Medium	Molar absorptivity at maximum/ l mol^{-1} cm^{-1}
Methylene chloride	14 396
Chloroform	13 433
Carbon tetrachloride	15 066
Butyl acetate	16 833
Glacial acetic acid	14 966
Glacial acetic acid - TBP (9 + 1)	13 623
Glacial acetic acid - TBP - orthophosphoric acid	18 425

Method of extraction

The extraction was carried out in a series of separating funnels, each containing 100 μg of molybdenum in 25 ml of hydrochloric acid ranging in acid concentration from 3 to 8 N. Each solution was extracted with a 5-ml portion of TBP (saturated with hydrochloric acid of the appropriate concentration) by shaking the separator slowly and continuously for 5 min. The TBP layer was allowed to separate completely and was then transferred to a 50-ml standard flask and treated as described under Method.

The absorbance of the solution (corrected for the absorbance of the reagent blank) was used to obtain a value for the percentage of molybdenum extracted by comparison with the value obtained for 100 μg of molybdenum as described under Reproducibility and Calibration.

$$\text{Molybdenum extracted, per cent.} = \frac{\text{Absorbance reading of solution under test}}{\text{Absorbance reading for 100 } \mu\text{g of molybdenum}} \times 100$$

$$= \frac{\text{Absorbance reading of solution under test}}{0{\cdot}384} \times 100$$

Each solution in hydrochloric acid was then subjected to a second extraction under the same conditions and the molybdenum in each TBP fraction was determined as described.

The values for the percentage of molybdenum extracted in the first and second extractions from the various solutions in hydrochloric acid are reported in Table III.

It can be seen that molybdenum was extracted quantitatively from solution in hydrochloric acid of concentration greater than 6 N. For the determination of molybdenum, as described in this paper under Method, extraction was effected from solution in 6 N hydrochloric acid.

TABLE III

EXTRACTION OF MOLYBDENUM(VI) FROM SOLUTION IN HYDROCHLORIC ACID

Concentration of hydrochloric acid/N	Molybdenum extracted, per cent. 1st extraction	2nd extraction
3	96	4
4	98	2
5	99·5	0·5
6	100	—
8	100	—

Interferences

The behaviour of a number of ions that could interfere in the determination of molybdenum was studied at a level of interferent of 1000 μg. Portions (25 ml in volume) of two solutions

(6 N in hydrochloric acid), the first containing 1000 μg of the interfering ion alone, and the second containing 1000 μg of the interfering ion together with 100 μg of molybdenum(VI), were each extracted once with 5 ml of TBP (saturated with 6 N hydrochloric acid) as was described earlier. The TBP layer was allowed to separate completely, transferred to a 50-ml standard flask and treated as described under Method.

The following results were obtained. For the solutions containing 1000 μg of interfering ion alone, the extracts in TBP gave clear and colourless solutions with absorbance values hardly different from the average absorbance value of the reagent blanks. For the solutions containing 1000 μg of the interfering ion, together with 100 μg of molybdenum(VI), the recoveries of molybdenum in the presence of the interfering ions were as shown in Table IV.

TABLE IV

RECOVERY OF MOLYBDENUM(VI) IN THE PRESENCE OF INTERFERING IONS

Interfering ion	Amount of molybdenum recovered/μg
Pb	100·5
Cd	100
Cu	99
Co	98·5
Ni	98·5
Zn	100
As	96
Bi	97·5
Sn	98
Fe(II)	99·5
Fe(III)	98·5
W(VI)	100

I thank the Government Analyst, Sri Lanka, for permission to carry out this work.

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Communication

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

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

Elimination of Interferences in the Determination of Arsenic and Antimony by Hydride Generation Using Molecular Emission Cavity Analysis (MECA)

Although numerous papers on the hydride generation technique for the determination of arsenic, antimony, selenium, tellurium and some other elements have been published recently, few have discussed or investigated the effects of interfering elements.[1] Smith[1] has shown, however, that such interferences are numerous and important. For example, he found that gold, germanium, nickel, platinum, palladium, rhodium and ruthenium interfered seriously in the determination of arsenic and antimony by atomic-absorption spectrophotometry using the hydride system, and that silver, bismuth, cobalt, copper, selenium and tin also gave appreciable suppression of the signal. No means has been suggested for eliminating such interference effects.

The hydride generation system can also be applied to the determination of arsenic and antimony by MECA,[2] and both elements can be determined simultaneously by using this technique.[3] Arsine and stibine are generated by injection of 1 ml of sample solution, 0·1 N in hydrochloric acid, on to 20–30 mg of sodium borohydride, in a nitrogen-purged system, and are carried to the MECA cavity by the nitrogen flow. A previous study[2] had shown that silver and copper ions suppressed the arsenic and antimony emission intensity, and a further study[3] has shown that Co^{2+}, Ni^{2+}, Zn^{2+}, Fe^{3+}, Bi^{3+} and Cd^{2+} also cause suppression (Table I). Tin gives an emission in the cavity and thus is a positive interferent. Al, Mn(II), Hg(II), Ba, Cr(III), Se(IV), Te(IV), Ge(IV), Si, Pb, SO_4^{2-}, $C_2O_4^{2-}$, acetate and PO_4^{3-} (50 p.p.m.) are without effect under the conditions used.

TABLE I

EFFECT OF INTERFERING IONS AND OF EDTA ON ARSENIC AND ANTIMONY EMISSION INTENSITY

	Change in emission intensity,* per cent.			
Ion added	As (10 p.p.m.)	As + EDTA†	Sb (25 p.p.m.)	Sb + EDTA†
Co^{2+}	−62	2	−27	−4
Ni^{2+}	−69	−4	−65	−3
Zn^{2+}	−41	3	0	1
Fe^{3+}	−33	−5	−16	0
Bi^{3+}	−23	2	−14	−1
Cd^{2+}	−14	3	−9	−3
Cu^{2+}	−22	−7	−14	−4
Ag^{+}	0‡	−4	−23‡	2

* Compared with emission in the absence of interfering ion.
† Test solution made 0·01 M in EDTA.
‡ AgCl precipitates; supernatant injected. In 0·1 N HNO_3, suppression is 25 per cent. and 39 per cent. for arsenic and antimony emissions, respectively.

The results are the same if peak area (as in Table I) or peak height measurements are used. The coefficients of variation for the determination of 10 p.p.m. of arsenic and 25 p.p.m. of antimony, using peak area measurements, were 4·3 and 2·7 per cent., respectively.

If the test solution is made 0·01 M in EDTA, the suppressive effects are almost entirely eliminated (Table I). When interfering elements are present, borohydride reduction gives a precipitate (usually black), probably of the finely divided metal. In the presence of EDTA, no precipitation

occurred from solutions containing Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} or Fe^{3+}, although Bi^{3+}, Cu^{2+} and Ag^{+} still formed some black precipitate. Hence the EDTA appears to be preventing or, for the last three ions, slowing down, the reduction of the interfering ions, and thus preventing or diminishing possible adsorptive or reactive capture of the arsine or stibine, with consequent suppression of the emission intensity. EDTA has no effect on the interference of tin.

As the generation procedure is common to MECA and atomic-absorption spectrophotometry, the releasing effect of EDTA should be equally applicable to the latter technique.

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Book Reviews

PRINCIPLES AND TECHNIQUES OF SCANNING ELECTRON MICROSCOPY. BIOLOGICAL APPLICATIONS. Edited by M. A. HAYAT. Volume 1. Pp. xiv + 273. Volume 2. Pp. xviii + 171. New York, Cincinnati, Toronto, London and Melbourne: Van Nostrand Reinhold Company. 1974. Price: Volume 1, £11·95; Volume 2, £10·60.

PRINCIPLES AND TECHNIQUES OF ELECTRON MICROSCOPY. BIOLOGICAL APPLICATIONS. Edited by M. A. HAYAT. Volume 4. Pp. xvi + 216. New York, Cincinnati, Toronto, London and Melbourne: Van Nostrand Reinhold Company. 1974. Price £10·60.

The preface to the first volume "Principles and Techniques of Scanning Electron Microscopy—Biological Applications" (SEM I for short) states that it is the first of a planned three-volume series. The contents pages of SEM 2 indicate that six volumes are already anticipated in this series. The preface to SEM 2 (a great deal of it similar to SEM 1 and to the preface of "Principles and Techniques of Electron Microscopy—Biological Applications," TEM 4 for short) states that the editor is encouraged to know that the first volume has been received favourably, thereby ringing the first tone of doubt about the rest of the contents, for the volumes were received for review in the same post. The same editor has dealt with six volumes in the TEM and SEM series, as well as producing a volume entitled "Basic Electron Microscopy Techniques" (1972) and the first volume of another series entitled "Electron Microscopy of Enzymes. Principles and Methods" (1973) since TEM 1 in 1970. Jack of all trades? He is certainly no master editor, as there is a great deal of duplication of material between the series, within the individual volumes and, to a certain extent, within the separate chapters. (Take, for example, the topic of critical point drying, which is the subject of a chapter in TEM 4 and SEM 1 and also considered by half the other authors in SEM 1 and SEM 2.)

The editor claims that his contributory authors are scholars, but has failed to edit out their numerous errors. He claims that areas of disagreement are pointed out, yet it is a fact that a reader referring to individual chapters in these books may receive totally opposing advice, and no indication that the opposite opinion exists, let alone within the same covers.

The ten years 1964–1974 since the introduction of the first commercial SEM in Cambridge have seen some 15 manufacturers and several thousands of SEMs enter the field. The greatest rate of expansion of interest has occurred during the 1970s; it is happening now. Rapid publication of relevant books is therefore servicing a much felt need. Nevertheless, much more stringent pre-writing planning and post-writing correction by editors competent in both the physical and biological aspects of SEM would have been desirable, and still is desirable for future volumes.

SEM 1 begins with a short review of SEM operating principles that is too short to serve as an introduction to the chapters which follow. Chapter 2 deals with critical point drying, a fundamental and important method for the preservation of morphological detail following the removal of the aqueous phase from biological specimens that is carried out in the great majority of cases in their preparation for the SEM. Chapter 3, on cryotechniques, deals with the possibility of, and the problems associated with, the examination of specimens in which the water is retained during both preparation and examination in the SEM by freezing it in place, thus minimising damage due to the crystal growth causing displacement of the specimen solids by the use of cryoprotectives and rapid deep-cooling agents. This technique, the procedures of shrinkage-free drying by vacuum sublimation of ice and exposure of hidden internal features by fracturing the ice are all treated, much too briefly, in 10 pages, contrasting with the more thorough, but rambling treatment of critical point drying (60 pages). Chapter 4 is called "Frozen Resin Cracking Method and its Application in Cytology" and deals essentially with the use of a particularly effective cryoprotective, which, like all of its kind, is incompatible with the living condition and must be used only with chemically killed and stabilised (fixed) tissue samples. The author fails to point out that identical results can be obtained by first substituting the tissue water with any solvent that freezes to a relatively amorphous or glass-like solid. Many such solvents are used in the preparation of tissues for critical point drying, and the extra steps of substitution with epoxy resin prior to freezing and fracture, and the later thawing and removal of the epoxy resin, are unnecessary and do not add to the opportunities for the acquisition of information. Chapter 5 deals with the preparation of stereoscopic pairs of SEM (and TEM) images for display by projection; it is eminently practical, although the author would have us reverse the green = starboard, red = port international convention that is also used for anaglyph images!

Chapter 6, on the low magnification study of uncoated specimens, is grossly inadequate. The two most important points here are first, that all biological non-conductors can be photographed using back-scattered electrons only, even at comparatively high magnifications, and secondly, that the problems described by biologist SEM users as "charging" are caused by the periodic discharging of parts, or the whole, of the specimen. It is the period at which discharging occurs that limits the ability to record images by photographing a CRT. If the recording frame time is less than the discharging period, which is almost always the case at conventional TV scanning speeds, then no charging artifacts are seen. The secret then, is to photograph a TV display. If it is necessary to reduce noise, then the exposure can last for many frames. This will not spoil the resolution at low magnifications.

Chapters 7 to 11 deal with non-animal specimens, discussing the special problems encountered with spores (Chapter 7), the free surfaces of higher plants (Chapter 8), plant cell walls and intracellular structures (Chapter 9), intracellular structures of plant cells (again, Chapter 10) and wood (Chapter 11). This last remarkable material can be boiled and cut with blades and still reveal important details for the SEM user. This is not the case with the majority of biological tissues, which are to be treated with great respect and by a number of the methods, which the diligent reader will be able to work out for himself from the ideas presented here.

The second volume of the SEM series begins with two chapters on cathodoluminescence of SEM specimens. The first rightly admits defeat, in that the author cannot provide an example of a useful practical application and states that "there is no self-evident reason to use cathodoluminescence techniques (in the SEM) in preference to optical fluorescence microscopy." The second, although extremely brief, shows one good illustration of the distribution of a supposedly electron-excited luminescent herbicide on a plant leaf; had the experimental details behind this feat been detailed, so that alternative image formative processes could be rigidly excluded, the reader would be properly impressed. The title of the third chapter, on silver as a stain, might be misleading, but the likelihood of this method having a practical usefulness will be forgotten by the time that the end is reached. Chapter 4 deals with "Sections Incubated in the Histochemical Media." In fact, there is the basis for a solid advance in the future here through the use of scanning transmission electron microscopy (STEM) and X-ray microanalysis, but the present chapter is so primitive that it cannot be considered as encouraging that belief. The author is apparently unacquainted with both the theoretical and practical difficulties of the subject; he claims that great advances will follow the use of field emission source SEMs because of the higher probe resolution and intensity. Probe resolution in X-ray analytical terms is limited by both the ability of the specimen to withstand particular electron irradiation intensities and the spread of electrons in the (target) specimen.

Chapter 5 deals with SEM and TEM examination of the same specimen; that this can be done is obvious, although the present authors do not demonstrate any outstanding feats by having done so. A far better chapter on the same topic, including practical methods for the selection of single living cells by phase-contrast light microscopy, is found as chapter 3 of TEM 4.

The soft tissues of marine teleosts are considered as a separate problem in Chapter 6, as are ciliated epithelia in Chapter 7, embryonic and foetal tissues in Chapter 8, and lung tissues in Chapter 9. There are special problems in the fixation of these classes of tissue, but beyond that, the approaches to the treatment of all animal soft tissues can be unified and no service is done by having so many chapters. The author of Chapter 6 does not understand that fixative solutions should be isotonic in respect of their electrolyte concentrations excluding the fixative radicals. Chapter 7 adds nothing other than some attractive pictures and its author might be recommended to abandon the extravagant gold plating procedure she describes and to invest in an argon atmosphere (10^{-1} torr) sputter-coating unit to put effective, yet economical, conductive surface layers on her "hairy" specimens. The variety of preparative treatments for bone and other (living) hard tissues and for fossils are described in Chapters 10 and 11 and would raise the eyebrows, if not the hopes, of those working with soft tissues.

The fourth volume of the conventional electron microscopy series (TEM 4) contains eight chapters, seven of which are concerned with serious methods for particular kinds of morphological analyses. The other, which is the first, on optical shadowing with the TEM, demonstrates a way of taking occasional impressive pictures by the Schlieren method. In terms of specimen analyses, however, the value of the method remains doubtful and one can look forward to further development of practice and ideas before it is commended to the attention of TEM users.

Chapter 2, on relative mass determination in dark-field electron microscopy, demonstrates the potential of the TEM given the availability of suitable methods for calibration to detect and

measure masses as small as "not-too-small macromolecules." The author believes that it should be possible to measure relative molecular masses of 3000 Dalton ($4{\cdot}5 \times 10^{-21}$ g) if suitable supporting substrates are developed in the future. Chapter 3 returns to straight morphological microscopy, but is admirable for drawing attention to the possibilities of finding the same cell in light microscope, SEM and TEM preparations.

Chapter 4 deals with a technique called "denaturation mapping of DNA." Denaturation of DNA is performed by elevating temperature or pH, when A–T-rich segments "strand" more readily than G–C-rich areas. The process is "frozen," the strands are mounted and shadowed, and, assuming that the specimen and support are flat, the distance of doubled regions along the otherwise single strand of DNA is measured as if along a road on a map.

Chapter 5 describes a method for the preparation of polysomes from cardiac muscle, which is probably appropriate for several other tissues. The point, however, lies in the procedure for counting monosomes, diribosomes - decaribosomes, etc. The author does not mention the possibility of automatically counting the polysome fractions by appropriate image-analysis techniques, although his one electron micrograph suggests that this should be feasible.

Chapter 6 is also concerned with particle counting in a TEM context, although in this instance to derive quantitative descriptions of the concentrations of suspensions of virus particles. Various approaches to the problem of uniformly sedimenting the virus particles are shown. Again, some of the images suggest a good future for automatic counting methods.

Chapter 7 describes the preparation of incinerated preparations of thin sections to provide a qualitative description of ash distribution, with reference to cell architecture at the scale of cell-organelles. Cold ashing in an oxygen plasma seems to be the most favoured method in that more of the original specimen and its morphology are retained. One would agree with the author that it is a method sufficiently well based to be added to the standard techniques of TEM, but that its future would be rosier if developed in conjunction with electron-probe X-ray microanalysis (XRMA). The latter is the subject of the last chapter (8). The authors attempt to review methods for the preparation of biological tissues for XRMA, yet fail to proffer any firm advice as to the best approach. This would, according to the latest work by the probe group in Cambridge (Echlin, Gupta and Hall), lie in the direction of using thin frozen sections, with the tissue rapidly frozen and maintained at cryogenic temperatures. The technical procedures involved here are formidable, yet the results are unique in terms of the resolution and reliability of analyses referred to cell and tissue structure.

TEM 4 is altogether a better volume than SEM 1 and SEM 2. We hope that the later volumes of the latter series will mature into something more valuable, as SEM users themselves mature from a "look-see" to a "let's measure" attitude. A. BOYDE

NEUTRON ACTIVATION ANALYSIS TABLES (ANALYSE PAR ACTIVATION). By J. C. LECLERC and A. CORNU, with the technical collaboration of A. GINIER-GILLET. Pp. vi + 64. London, New York and Rheine: Heyden and Son Ltd. 1974. Price £5; $14; DM41.

This booklet is a collection of tables of data on the properties of radionuclides which are formed by neutron activation. The range of half-lives extends from 11 s to 30 years, so that it cannot be used for the very short-lived nuclides that are used in pulsed neutron work or cyclic activation. The tabulation of gamma-energies ranges from 37 keV to 4 MeV, and includes many more entries than the earlier work by Adams and Dams (*J. Radioanalyt. Chem.*, 1969, **3**, 99), but these energies are rounded off to the nearest keV. This is surprising because measurements of low energies are usually accurate to $\pm 0{\cdot}2$ keV or $\pm 0{\cdot}01$ keV for many calibration lines. The format adopted allows three columns where most books have two, but the volume is an awkward shape for library shelves (17·5 cm high × 26 cm wide). The quality of the printing is good and I noticed only one misprint (Ir 192 for Ir 194 on p. 62). The price of 7·8 p per page (22 cents per page) is excessive compared, say, with Adams and Dams compilation at 4 cents per page. H. J. M. BOWEN

THE POISONED PATIENT: THE ROLE OF THE LABORATORY. Edited by RUTH PORTER and MAEVE O'CONNOR. *Ciba Foundation Symposium* 26 (*new series*). Pp. viii + 325. Amsterdam, Oxford and New York: Associated Scientific Publishers. 1974. Price $21·25; Dfl55.

Ralph Goulden, speaking at the SAC Centenary Celebrations, said "the analytical chemist must . . . interact to the full with his non-analytical colleagues, firstly to ensure that the analytical problems with which he is being presented are worth solving, secondly, to pinpoint other areas

upon which his analytical expertise can profitably be brought to bear and, finally, to contribute as far as he can in areas beyond the purely analytical by virtue of his numerate and quantitative experience" (*Analyst*, 1974, **99**, 937). Reading this report of a symposium in which analytical toxicologists were quite clearly "interacting" with physicians, pathologists and pharmacologists proved to be a stimulating experience. A wide range of analytical techniques is paraded for critical evaluation and related to the prime objective of saving the life of the poisoned patient. The role of the laboratory in programmes for the cure of drug addiction, the exploration of drug interactions and the early observation of unexpected side effects are all discussed and these are perhaps good examples of the second function referred to above.

An attractive feature of the book is the verbatim report of the discussions and it is particularly pleasing to see that analytical chemists at the symposium were applying their "numerate and quantitative experience" to methods of treatment, such as haemodialysis and forced diuresis. Some of the participants may regret that their contributions to the "brainstorming" sessions are permanently recorded but they should be reassured that all the contributors add to the value of the book as an intellectual exercise. Clearly this is a book that all concerned with analytical toxicology must read. Because of its study of the relationship between the user and provider of the service it is worthy of attention by all professional analytical chemists. P. G. W. COBB

ATOMIC ABSORPTION AND FLUORESCENCE SPECTROSCOPY. By G. F. KIRKBRIGHT and M. SARGENT. Pp. x + 798. London, New York and San Francisco: Academic Press. 1974. Price £17.

It would be no easy task to cite any innovation in analytical inorganic chemistry that has made such an impact and so completely changed the *modus operandi* of trace determinations as atomic-absorption (including fluorescence) spectroscopy. Equally difficult would be the assignment of naming two experimenters who have made a greater combined contribution than the authors of this publication, to this branch of chemistry, over the past five to ten years.

Their book is, essentially, a text covering the principles and applications of the discipline, with a strong practical bias, including a detailed coverage of the hardware of atomic-absorption (and fluorescence) spectrometers, *e.g.*, light sources, atom cells, dispersive systems, etc. This extensive coverage, however, has not been to the detriment of the theoretical aspects of the subject, which are adequately covered. In this latter respect the authors have aimed successfully at being neither misleading nor over-simple in their approach; the balance, overall, is about right.

The book is well illustrated throughout its fourteen chapters, and is, presumably, the first to present in any worthwhile detail an integrated treatment of atomic-absorption and atomic-fluorescence spectrometry. This integrated presentation has many advantages, for example, it provides a ready means of assessing the pros and cons of these related techniques when the analyst has to decide which of them is most likely to be the best for a particular determination.

Selections of the book's named chapter headings, chosen at random, are: "Theory of Atomic Absorption Measurements" (there is also a similar chapter on fluorescence measurements), "Non-Flame Absorption and Fluorescence Cells," "Introduction of Liquid Samples into Flame Atom Cells," "Practical Techniques of Atomic Absorption and Fluorescence Spectroscopy" and "Analytical AAS and AFS Characteristics of the Elements and Applications Data." To exemplify the subsections given in each of the chapters, the pages comprising "Atomic Absorption and Fluorescence Instrumentation," cover "Radiation detectors," "Signal processing," "Enhancement of signal-to-noise ratios," "Optical systems," "Burner systems" and "References."

To attempt to assign this book to any level of expertise might be misleading. The careful selection of information it contains, much of which is based on the authors' own experimentation and first-hand experience, is such that the book is likely to have a wide appeal to both the inexperienced and the more knowledgeable in these fields.

In most branches of, and at every level of expertise in, this rapidly expanding field of the analytical sciences, this book, which is presented in a clear and unequivocal style and with numerous supporting references, is destined, and deserves, to be well received. W. T. ELWELL

CHEMICAL ANALYSIS OF ORGANOMETALLIC COMPOUNDS. Volume 2. ELEMENTS OF GROUPS IVA-B. By T. R. CROMPTON. *An International Series of Monographs, No.* 4. Pp. x + 163. London, New York and San Francisco: Academic Press. 1974. Price £5; $13·25.

It was the author's original intention to publish a single, comprehensive volume, which dealt with the analytical chemistry of organometallic compounds. The general increase in interest in these compounds, however, is reflected in the fact that, in order to do full justice to the subject

it has been necessary to split it into three volumes. This volume (Volume 2) follows Volume 1, published in January 1974, and a review of Volume 3 follows below.

Although this volume purports to be a review of the analytical chemistry of the organometallic compounds of Group IVA and IVB elements it is almost entirely devoted to organosilicon compounds. The Group IVA elements, titanium, zirconium, hafnium and thorium, are covered in three pages and the other Group IVB elements, germanium, tin and lead, are to be covered in Volume 3.

Accepting that this volume is a critical review of the analytical chemistry of organosilicon compounds it would appear to be very useful to persons engaged in this field. The arrangement of the material is logical, particularly in view of the diversity of the techniques covered and the varying complexity of the different types of organometallic compounds. Each chapter commences with a general discussion of the determination of elements and functional groups and is followed by the application of different techniques to the analysis of the different classes of compounds. Gas chromatography, column, paper and thin-layer chromatography and spectroscopy of organosilicon compounds are well covered in separate sections. The volume includes many detailed analytical procedures that enable workers to apply the method without reference to the original source. The volume includes many diagrams and tables of useful information, which are excellently laid out.

The international literature has been surveyed until the end of 1972 and the references are well chosen in support of the text. The book is written in a very lucid fashion and at £5 should be of inestimable value to workers in both academic and industrial situations who use organosilicon compounds in either pure or applied research. P. B. SMITH

CHEMICAL ANALYSIS OF ORGANOMETALLIC COMPOUNDS. Volume 3. ELEMENTS OF GROUP IVB. By T. R. CROMPTON. *An International Series of Monographs, No.* 4. Pp. x + 211. London, New York and San Francisco: Academic Press. 1974. Price £6·50; $17·25.

Under the generic title, *The Analysis of Organic Materials*, a review of the first of three volumes to be published as No. 4 in this series of monographs has already appeared in *The Analyst* (1974, **99**, 614) and a review of Volume 2 appears above.

Volume 3 of this continuing, relatively new analytical series deals with elements in the conventional Group IVB, the three major chapters being devoted entirely to germanium, tin and lead.

The presentation of this volume is similar to that of Volume 1, and the high technical standard that enabled the reviewer to report favourably on that volume is commendably maintained.

To quote from the first review: "Here is a publication that is destined to be well received by the busy analyst (and others) who requires reliable information quickly without undue recourse to the original literature." W. T. ELWELL

ENZYME HANDBOOK. First Supplement. By THOMAS E. BARMAN. Pp. ix + 517. Berlin, Heidelberg and New York: Springer-Verlag. 1974. Price $21·20; DM51·80.

This supplement provides data on 430 enzymes that were described after the publication of the original "Enzyme Handbook" in 1969, which described some 800 enzymes. The arrangement of information is as before, giving details of sources, molecular properties, kinetic parameters and other relevant information, including appropriate references. In addition, the supplement classifies about 60 enzymes discovered after the publication of "Enzyme Nomenclature" (Elsevier, Amsterdam, 1972). The supplement is a welcome updating of the "Enzyme Handbook," and will be of value to all analytical chemists who utilise enzymes in their work. A. TOWNSHEND

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Polarographic Studies on Some Organic Compounds of Arsenic Part I. Substituent Effects and the Arsonic Acids

A study has been made of the polarographic behaviour of 12 arsonic acids. They give rise to a single well defined cathodic wave in acidic solutions below pH 3. The wave height is diffusion controlled and proportional to the concentration in the range 10^{-5} to 10^{-3} M. The arsonic acids are reduced irreversibly to arsenobenzenes. The current - potential relationships have been examined and a linear relationship was found between the half-wave potential and the polar Hammett substituent constants. The use of polarography has been proposed for the quantitative functional analysis of the arsonic group, for the specific determination of nitrophenylarsonic acids and arsanilic acid in mutual mixtures, and for the specific determination of phenylarsonic acid and phenyl arsenoxide, also in mutual mixtures.

A. WATSON and G. SVEHLA

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

Analyst, 1975, **100**, 489–502.

The Polarography of Fast Red E

The dye Fast Red E, 2-hydroxy-1,1′-azonaphthalene-4′,6-disulphonic acid, disodium salt, is reduced at the dropping-mercury electrode in a single four-electron wave over the pH range 2·0–11·3, with $E_{\frac{1}{2}}$ values ranging from −0·08 to −0·61 V *versus* S.C.E. The rising portion of the polarographic wave is markedly influenced by the adsorption of depolariser but well defined limiting currents that are proportional to concentration in the range examined, *viz.*, 10^{-3}–10^{-4} M, are obtained.

F. E. POWELL

Department of Science and Food Technology, Grimsby College of Technology, Nuns Corner, Grimsby, South Humberside.

and C. J. SNOWDEN

Ross Foods Limited, Grimsby, South Humberside.

Analyst, 1975, **100**, 503–506.

Complexometric Analysis of Magnetic Materials by Means of Automatic Titrations

An analytical method based on automatic potentiometric titrations was developed for the analysis of magnetic material (*e.g.*, $Ba_2Zn_2Fe_{12}O_{22}$). The elements were separated by extracting iron(III) as its chloride in diisopropyl ether and by precipitating barium as its sulphate in the presence of disodium dihydrogen ethylenediaminetetraacetate (EDTA). After separation, the iron and zinc were complexed with EDTA, buffered at pH 5 with ammonium acetate solution and back-titrated with standard iron(III) chloride solution by using platinum-wire and saturated calomel electrodes. Barium sulphate was dissolved in EDTA and ammonia solution, buffered at pH 10·1 and back-titrated with standard zinc nitrate solution at mercury | mercury - EDTA and saturated calomel electrodes. A complete analysis of the magnetic material was performed on as little as 10 mg of sample. A Tacussel Titrimat automatic titrator was used for all of the determinations. Results accurate to within 0·35 per cent. were obtained. A similar procedure can also be used to analyse different magnetic samples.

V. FANO and F. LICCI

Laboratorio Maspec, CNR, Via Spezia, 73, 43100 Parma, Italy.

Analyst, 1975, **100**, 507–511.

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Precipitation of Silver Molybdate from Homogeneous Solution by Use of Diamminesilver(I) Reagent

An investigation has been carried out on the application of diamminesilver(I) reagent to the precipitation of molybdate. Addition of ammonia solution to a silver salt results in the formation of the complex diamminesilver(I), which when heated gradually decomposes to give silver ions in solution and ammonia, which is volatilised slowly, with a corresponding decrease in pH. The liberated silver ions react with molybdate to form a dense precipitate of silver molybdate.

KAZA SOMASEKHARA RAO and V. G. VAIDYA

Department of Chemistry, Government College of Engineering and Technology, Raipur (M.P.), India.

Analyst, 1975, **100**, 512–516.

A Selective Method for the Determination of Molybdenum Using Toluene-3,4-dithiol

A method is described for the colorimetric determination of molybdenum. The procedure involves the extraction of molybdenum(VI) from solution in hydrochloric acid with tri-n-butyl phosphate. The tri-n-butyl phosphate extract is then complexed with toluene-3,4-dithiol in glacial acetic acid - tri-n-butyl phosphate - orthophosphoric acid medium.

This procedure enhances the selectivity, sensitivity and reproducibility of the molybdenum - dithiol reaction.

(The late) M. E. M. S. de SILVA

Government Analyst's Laboratory, Colombo 7, Sri Lanka.

Analyst, 1975, **100**, 517–521.

Elimination of Interferences in the Determination of Arsenic and Antimony by Hydride Generation Using Molecular Emission Cavity Analysis (MECA)

Communication

R. BELCHER, S. L. BOGDANSKI, E. HENDEN and A. TOWNSHEND

Chemistry Department, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT.

Analyst, 1975, **100**, 522–523.

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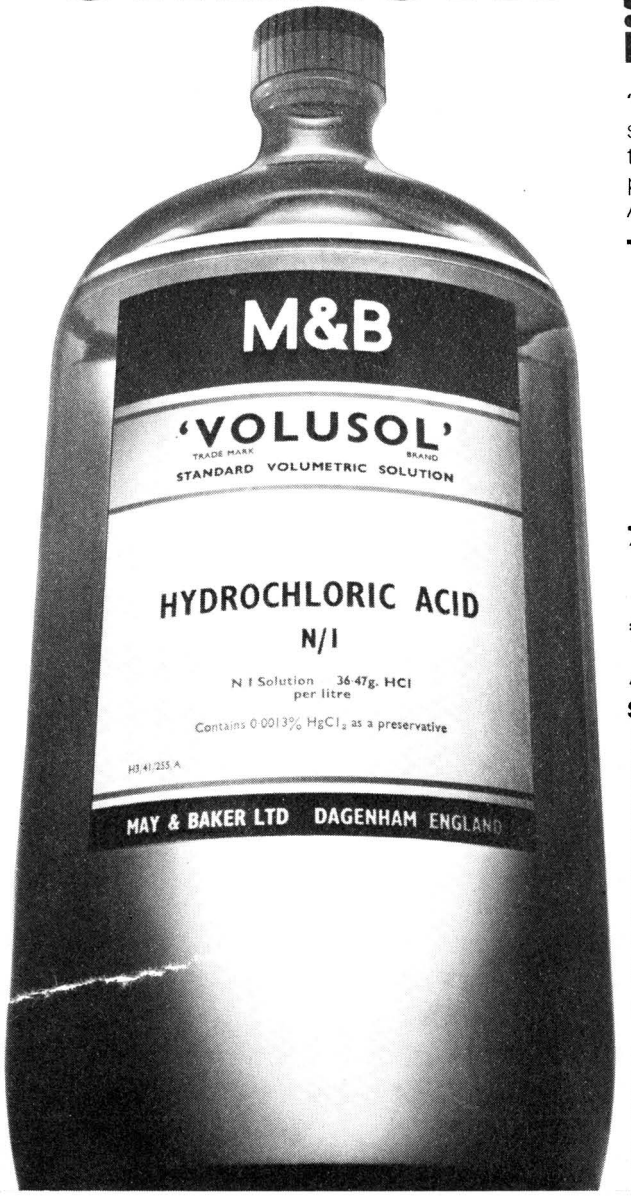

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