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Analyst, 1973, **98**, 465–474.

Mass and Charge Transfer Kinetics and Coulometric Current Efficiencies

Part VI. The Pre-treatment of Solid Electrodes, and a Review of the Effects of Oxidation of Platinum

The nature and condition of working electrode surfaces are set in the context of reaction speeds and current efficiencies. The formation of films and their effects are examined for platinum and other metals and alloys. Adsorption and specific adsorption on electrode surfaces are briefly reviewed. An attempt is then made critically to appraise the current state of the art in respect of the activation and deactivation of electrodes. Methods of cleaning electrodes are canvassed. The more significant theories of activation and deactivation are reviewed with specific reference to platinum electrodes. These theories include the impurity theory, the platinisation theory and the various oxygen-containing surface theories. For the last, the formation of oxides, the measurement of film thickness, the film thickness and the nature of the oxide reducible at 0·6 V are discussed, followed by a selective review of the oxygen-bridge theory, the half-reduced oxide theory and the platinum - oxygen alloy theory. Tentative conclusions are reached. Finally, gold electrodes and their behaviour are briefly examined.

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Analyst, 1973, **98**, 475–484.

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Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

Part IV. The Determination of Catecholamines

(−)-Adrenaline, adrenaline hydrogen tartrate, L-noradrenaline, dopamine hydrochloride, L-dopa, DL-dopa, L-α-methyldopa, D-α-methyldopa and (+)-Corbasil have been determined in amounts down to 0·0001 mequiv by catalytic thermometric titration of their basic and acidic functions. Basic functions were determined by titration with 0·1, 0·01 and 0·001 M perchloric acid by using the ionic polymerisation of α-methylstyrene to indicate the end-point, while acidic functions were determined in a similar manner with tetra-n-butylammonium hydroxide as the titrant and acrylonitrile as the end-point indicator.

The L-dopa contents of tablets and capsules have been determined by using these techniques and the assay results have been compared with those obtained by alternative methods, namely, the recently described B.P. procedure involving non-aqueous titration, and ultraviolet spectrophotometry.

Magnesium stearate, which is used as a lubricant and flow promoter in tablet manufacture, is titrated as a base in the solvents used, but in titrations of the acidic function of catecholamines its effect is negligible.

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Analyst, **1973, 98**, 485–492.

The Determination of Di-n-alkyl Phthalates in Cosmetic Preparations by Gas - Liquid Chromatography

An improved gas-chromatographic method for the direct determination of C_1–C_4 di-n-alkyl phthalates in toiletry samples that contain ethanol is described and a range of perfume essential oils and perfume synthetic chemicals is examined for possible interference.

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Analyst, **1973, 98**, 493–501.

Residues of Prophylactics in Animal Products

Part III. The Determination of Carbarsone in Poultry Meat

A method for the determination of carbarsone in poultry meat is described. The carbarsone is extracted from the sample with methanol and, after clean-up on an ion-exchange column, hydrolysed to arsanilic acid with sodium hydroxide. The arsanilic acid is diazotised, coupled with 2-aminoethyl-1-naphthylamine and determined spectrophotometrically.

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Analyst, **1973, 98**, 502–505.

The Determination of Microgram Amounts of Sulphate by Emission Spectroscopy of Barium with a Nitrous Oxide - Acetylene Flame

The sulphate content of aqueous solutions has been determined indirectly, in the ranges 0·5 to 5·0 and 1·0 to 10·0 p.p.m. from barium emission measurements. By using a slight excess of barium, the sulphate is precipitated in a 50 per cent. solution of propan-2-ol and its concentration is calculated from the decrease in the barium content of the solution. The amount of barium in solution is determined from its emission at 553·55 nm in a nitrous oxide - acetylene flame. The only major flame interference detected is that from the band emission of calcium; 410 p.p.m. of calcium gave an emission intensity equal to that of 1 p.p.m. of barium. Sulphate has been determined in both pure solutions and in synthetic sample solutions containing other electrolytes. Major interferences were noted for potassium and ammonium oxalate, sodium orthovanadate, nickel chloride and to a lesser extent for sodium fluoride and perchloric acid. The sulphur content of biological material, digested by oxygen-flask combustion, has been determined satisfactorily by using this method.

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Analyst, 1973, **98**, 506–511.

Pyridylazonaphthols (PANs) and Pyridylazophenols (PAPs) as Analytical Reagents

Part III. Formation of Copper(II) Complexes and Their Determination in Alloys

The reactions between copper(II) and 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN) and 4-(2-pyridylazo)phenol (*p*-PAP) have been studied by spectrophotometry and graphical analysis, and their equilibrium constants have been determined. A rapid and simple method for the spectrophotometric determination of copper in aluminium alloys has been devised. A solution of the alloy with pH 2 is extracted with an equal volume of a 5×10^{-3} M solution of *o*-α-PAN in carbon tetrachloride and the absorbance of the extract is measured at 590 nm. The method has been checked by the analysis of standard alloys. The effect of interferences is discussed. The extraction behaviour of several cations with *o*-α-PAN is discussed and the sequential separation of copper(II), zinc(II), manganese(II) and lead(II) is outlined.

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Analyst, 1973, **98**, 512–519.

Pyridylazonaphthols (PANs) and Pyridylazophenols (PAPs) as Analytical Reagents

Part IV. Formation of Complexes with Titanium(IV)

The formation of complexes of titanium(IV) with PANs and PAPs has been investigated in order to ascertain the types of complexes that are formed and to elucidate the optical and stability constants of such complexes. The competing effect of hydrolysis on these reactions has also been considered. The reaction with 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN) has been examined in order to determine the analytical utility of this complex formation.

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Analyst, 1973, **98**, 520–524.

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Mass and Charge Transfer Kinetics and Coulometric Current Efficiencies

Part V.* Comparison of Pattern Theory, Tafel, Allen and Hickling and Lewartowicz Methods, and Apparatus and Procedures for Ramping Voltammetry

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A comparison is made of the provenance and application of pattern theory, Tafel, Allen and Hickling and Lewartowicz methods for the determination of charge-transfer kinetic parameters. A general-purpose electrochemical cell is described together with a ramping potentiostat - galvanostat for slow single-sweep voltammetry. The mass-transfer characteristics, which are governed by the geometrical placing of the various appurtenances that dip into the solution and by the stirring speed, were examined by determining the apparent diffusion layer thickness at various stirring speeds for a system of known mass-transfer properties, copper(II) - copper(I). It was further established that the limiting current of reduction of hexacyanoferrate(III) is reproducible and linearly related to concentration.

IN the derivation of pattern theory,[1,2] the thermal diffusion coefficient, D_X, of the species X and the diffusion-layer thickness, δx, were retained as discrete entities. The diffusion-layer thickness in a turbulently stirred solution is a pure fiction. Mass transport is nevertheless a definite and, if conditions such as the stirring speed, solution volume and viscosity and the geometrical placing of the various appurtenances in the cell are held constant, reproducible phenomenon. Although no hydrodynamic treatment of turbulently stirred solutions is possible, Levich[3–6] has shown that mass transport is proportional to the concentration, $[X]_B$, of the active species in the bulk of the solution. It is therefore proper to use an over-all conditional mass-transfer rate constant, k_{mass},[7] which can be determined *in situ* from a voltammetric scan to the limiting current, IL_X, and absorbs all indeterminate quantities, such as the roughness factor, r, of the electrode and the transport number, t_X, of the species X, so that, for a projected area, A, of the electrode

$$\left[\frac{D_X r}{(1 - t_X)\delta x}\right] = k_{mass\ X} = \frac{IL_X}{nFA\,[X]_B} \quad .. \quad .. \quad .. \quad (1)$$

This relationship can be directly substituted in the behaviour equations and solutions for the charge-transfer rate constant, k, and the charge-transfer coefficients, α, for cathodic processes, and $(1 - \alpha)$, or β, for anodic processes. A realistic treatment of mass transfer in electrode processes is thus secured in the practical context. There are many methods in use for the determination of charge-transfer rate parameters. A small selection of the simpler and more popular of these methods will be reviewed together with pattern theory in the context of stirred solutions and coulometric current efficiencies.

PATTERN THEORY

Pattern theory is entirely rigorous when the conditions are satisfied,[1,2] *viz.*, that the charge-transfer overpotential, η_a, is substantial so that the backward reaction is without effect, and $|n\eta_a| \geqslant 0{\cdot}059 \log_{10} (100/\pi)$, where π is the percentage experimental error in the measurement of the current in a voltammetric scan. The method for the determination of k and α (or β) is extremely simple and rapid.[2] A single voltammetric scan is made in the appropriate direction, cathodic or anodic, and the substitution of two points on each wave, including the background for the calculation of current efficiencies, into the appropriate

* For details of Parts III and IV of this series, see reference list on p. 474. For Part VI, see p. 475.

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equations[1,2] will give a quick check. In a careful study, as many points can be taken as desired and subjected to statistical appraisal. The calculation is almost as quickly performed manually as by computer when the time taken to punch the data cards is taken into account. The scan speed should be slow enough to avoid maxima and to allow a reasonable approach to quasi-equilibrium, but not so slow as to produce any significant change in $[X]_B$. Scan speeds between 0·2 and 2·0 mV s^{-1} are suitable. When the process is applied during the course or at the start of a coulometric determination, the quantity of electricity passed and performing desired reactions is easily integrated from the corrected (for unwanted reactions) area under the voltammogram. The method has been thoroughly tested experimentally, as will be shown in later papers, and gives results in concordance with other methods when they have been duly corrected for their deficiencies. One application not mentioned earlier[1,2] is the determination of n′, the number of electrons involved up to and including the rate-determining step.[7] This determination simply involves back-computation of the experimental voltammogram with different integral values of n, excluding those which give values of α greater than unity. Non-integral values of n can be diagnostically useful in working out reaction mechanisms that involve chemical steps. Pattern theory takes full cognisance of mass transfer, but applies only to slow reactions. It applies also to background reactions[2] and therefore to current efficiency calculations.[7] It is not restricted to cases for which the limiting currents and conditional potentials are known.

Tafel plot method

In this well known method,[8] the logarithm of the total current is plotted against the working electrode potential. All too frequently it is applied ignorantly, or with the bland assumption that mass transfer is insignificant; the current is often taken beyond the limiting current of the reaction under examination. Extrapolations of the linear parts, if there are any, of the cathodic and anodic wings of the plot cross at the equilibrium zero-current potential and the logarithm of the exchange current, i_0,[7] from which k can be calculated. The slopes of the cathodic and anodic wings of the plot give α and β. The fallacy lies in the dismissal of mass-transport and mass-transfer overpotential. For extremely fast reactions in solutions that contain a high concentration of the active species, it is possible to have mass-transfer overpotential without detectable charge-transfer overpotential. However, unless a net current passes, $i_c + i_a \neq 0$, there can be no charge-transfer overpotential, and if a net current, however small, flows, there must always be mass transport to carry the current, and therefore mass-transfer overpotential. A charge-transfer overpotential can never arise without an accompanying mass-transfer overpotential, which cannot be ignored. If the potentials in the Tafel plot are corrected for mass transfer, which can be easily done but involves more calculation, then the plot and the parameters derived therefrom will be valid; the plot will have straight parts of the wings unless there are other complications. Without this correction, the wings of the plot can never be linear, and the extrapolations will be of dubious validity. Fundamentally, the Tafel relationship depends on the same assumptions as pattern theory, that $|n\eta_a| \geqslant \log_{10}(100/\pi)$, so that one of the exponential terms in equations (20) and (22) in reference 7, and equation (1) in reference 1,

$$I = n\,FAk\,[Ox]_S^{(1-\alpha)}\,[Red]_S^{\alpha}\left\{\exp\left[\frac{-\alpha nF}{RT}\,\eta_a\right] - \exp\left[\frac{(1-\alpha)nF}{RT}\,\eta_a\right]\right\} \quad .. \quad (2)$$

can be neglected with respect to the other. The Tafel method is therefore restricted to the pattern region. Furthermore, the part outside the exponentials is falsely identified as the exchange current, i_0, which is

$$i_0 = nFAk\,[Ox]_B^{(1-\alpha)}\,[Red]_B^{\alpha} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where the subscript B refers to conditions in the bulk of the solution, and equation (3) is true only when $\eta_a = 0$. To avoid confusion, the relevant section in equation (2) is identified in pattern theory as *IOS* and the subscript S identifies concentrations at the plane of closest approach to the electrode surface:

$$IOS = nFAk\,[Ox]_S^{(1-\alpha)}\,[Red]_S^{\alpha} \quad .. \quad .. \quad .. \quad (4)$$

Only when this correction is made do Tafel plots become valid, and it should be noted that equation (4) contains the unknown parameters that the plot is intended to reveal. This

difficulty is resolved by using equation (5) in reference 7 in order to separate η_a and to correct the potential for mass transport:

$$\eta_a = E_{we} - E_o' - \eta_c \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where the concentration term, ηc, refers to the plane of closest approach[7]:

$$\eta_c = \frac{2{\cdot}303\,RT}{nF} \log_{10} \left\{ \frac{[Ox]_B - \dfrac{I}{n\,FAk_{\text{mass ox}}}}{\dfrac{I}{n\,FAk_{\text{mass red}}} + [Red]_B} \right\} \quad .. \quad .. \quad (6)$$

This procedure is possible only if values for the two limiting currents IL_{ox} and IL_{red} can be obtained and the conditional mass-transfer rate constants calculated. Then η_a becomes a logarithmic function of the current or current density [equations (16) and (41) in reference 1]. The corrected Tafel relationships become equation (7) for cathodic and equation (8) for anodic reactions:

$$\ln I = (\ln IOS) - \frac{\alpha nF}{RT}\,\eta_a \quad .. \quad .. \quad .. \quad .. \quad (7)$$

$$\ln (-I) = (\ln IOS) + \frac{\beta nF}{RT}\,\eta_a \quad .. \quad .. \quad .. \quad .. \quad (8)$$

The Tafel method is therefore restricted to slow reactions, and is not helpful with background reactions. It requires knowledge of limiting currents and conditional potentials.

Allen and Hickling method

Allen and Hickling[9] expanded the second exponential in equation (2), extracted the common term in α and, like Tafel, identified the non-exponential terms as the exchange current, i_o, writing

$$I = i_o \exp\left[\frac{-\alpha nF}{RT}\,\eta_a\right]\left\{1 - \exp\left[\frac{nF}{RT}\eta_a\right]\right\} \quad .. \quad .. \quad (9)$$

then rearranging and taking logarithms:

$$\ln\left\{\frac{I}{\left(1 - \exp\left[\dfrac{nF}{RT}\,\eta_a\right]\right)}\right\} = -\frac{\alpha nF}{RT}\,\eta_a + \ln i_o \quad .. \quad .. \quad (10)$$

They then plotted the left-hand function in equation (10) against η_a, the slope of the line giving α and the intercept giving i_o, from which k could be calculated. This method does not, therefore, neglect one of the exponential terms, and is therefore applicable to faster reactions, in the categories fast and moderate.[1] However, it makes the assumption that the non-exponential terms are constant and equal to i_o (which is true only when $I = \eta_a = 0$), whereas the second term on the right-hand side of equation (10) is properly IOS [equation (4)], which is not constant. In evaluating the functions in equation (10), it is again necessary to know η_a, which can be calculated from equation (5) and the mass-transfer equation (6), but again only if the limiting currents can be measured for the calculation of the mass-transfer rate constants. The further assumption is made that $\alpha + \beta = 1$, although a change in slope as the plot passes from positive (cathodic) to negative (anodic) currents will reveal any change in the charge-transfer coefficient. Even at extremely low currents, the "constant," $\ln i_o$, in equation (10) is not constant unless the reaction is very slow, which vitiates the retention of both exponential terms, and $[Ox]_B$ and $[Red]_B$ are large and equal. Otherwise, the "constant," $\ln (nFAk[Ox]_S^{\beta}[Red]_S^{\alpha})$, requires evaluation and therefore pre-knowledge of the unknowns, k, α and β.

Lewartowicz method

Reviewing earlier work,[10] Lewartowicz took as his starting point the work of Audubert,[11,12] and developed methods[10,13–21] for linearising the Tafel plots of the logarithm of the current *versus* potential with or without making a correction for diffusion, and with or

without making the concentrations of Ox and Red equal. He referenced his potentials to the equilibrium, zero-current potential of the solution of Ox and Red, so that $\eta = E_{we} - E_{eq}$, the total overpotential arising from the passage of current, and then corrected for diffusion by subtracting η_{cs}, for the evaluation of which anodic and cathodic limiting currents must be known:

$$\eta_a = \eta - \eta_{cs} = E_{we} - E_{eq} - \frac{RT}{nF} \ln\left(\frac{1 - I/IL_{ox}}{1 - I/IL_{red}}\right) \quad .. \quad .. \quad (11)$$

The current in equation (2) is then split into partial currents, cathodic I_c and anodic I_a (which is negative), and their values are calculated by using the value of η_a calculated from equation (11):

$$I_c = \frac{I}{1 - \exp\left[\frac{-nF\eta_a}{RT}\right]} \text{ and } I_a = \frac{I}{\exp\left[\frac{nF\eta_a}{RT}\right] - 1} \quad .. \quad .. \quad (12)$$

Anodic currents being negative, the logarithm of the modulus of the partial current is plotted against E_{we}. The slope of the cathodic wing gives α, that of the anodic wing β, and the intercept of the two curves gives the corrected exchange current, i_o, from which k can be calculated and the equilibrium potential of the particular solution, E_{eq}. Lewartowicz took the process a step further by calculating the "ideal" partial currents, which sum to the total "ideal" current I_1; using his notation of a single prime for cathodic and a double prime for anodic reactions,

$$I_1 = I' + I'' \quad .. \quad .. \quad .. \quad .. \quad .. \quad (13)$$

$$I' = \frac{I_c}{1 - \frac{I}{IL_{ox}}} \text{ and } I'' = \frac{I_a}{1 - \frac{I}{IL_{red}}} \quad .. \quad .. \quad .. \quad (14)$$

Plots of the logarithm of the "ideal" partial currents against E_{we} give more effective linearisation than the use of the "real" currents, I_c and I_a. Both current and potential are therefore "corrected" for mass transport, and there is no neglect of either exponential term in equation (2), so the treatment is applicable to moderate and fast reactions. Knowledge of the limiting currents of both anodic and cathodic directions and of the equilibrium, zero-current potential of the system is required, but if the electrode process is fast it is usually possible to measure these quantities experimentally. Lewartowicz's approach deserves more attention: it has tended to be neglected in view of similar approaches by other workers at about the same time.

Curve-fitting methods

Of the foregoing methods, only pattern theory can be used on background reactions,[2] which come into the pattern region at moderate charge-transfer overpotentials because of the larger number of electrons. Before pattern theory had been developed for background reactions, and in default of better methods, curve-fitting methods involving the use of curves computed from the full rigorous theory[7] were perforce used. A family of curves for a series of values of k and a first guess for α, or $(1 - \alpha)$, was plotted by computer using the program VOLTAMMETRY 9 G/P.[7] These curves were then matched with the experimental curves, and the best value of k was chosen. The process was repeated for a series of values of α, the computed curves were offered to the experimental voltammograms and the best value of α was chosen. The process could be repeated as often as necessary in order to refine the charge-transfer parameter values further, but in practice no more than two such essays were required. After development of pattern theory for background reactions, curve fitting was no longer required, because background waves usually came well into the pattern region at solid electrodes.

Summary

It cannot be said that the problem of measuring the rate parameters for all the reactions, including the solvent molecule and ion reactions, in vigorously stirred solutions under the conditions of coulometry has been solved for fast reactions. However, the method of pattern

theory is the simplest of all, involves the least calculation, can be applied at any time before, during and after a coulometric determination thus to detect any change in rate parameters and therefore current efficiencies, and is completely rigorous at adequate charge-transfer overpotentials. It does deal with background reactions,[2] and can be applied without knowledge of limiting currents or equilibrium or conditional potentials. The implementation of the method involves merely a single-sweep voltammetric scan in the appropriate direction using the working electrode and the electrolyte and species under the conditions pertaining to an ensuing coulometric determination, which additionally allows selection of the working potential for potentiostatic determinations and the working current for amperostatic determinations productive of the maximum current efficiency. Equipment has therefore been designed and tested for the examination of coulometrically useful reactions. The design of a ramping potentiostat - galvanostat, together with certain tests, will be described in this paper, and the applications in later papers.

Experimental

The thermostatically controlled cell for voltammetry is shown in Fig. 1. Water maintained thermostatically at 25 $\pm$ 0·05 °C is pumped through the jacket. The lid is machined from Perspex and B19 cones are cemented with Perspex dissolved in chloroform into holes drilled in the lid. The stirrer paddle is a magnetic follower covered with PTFE and fitted

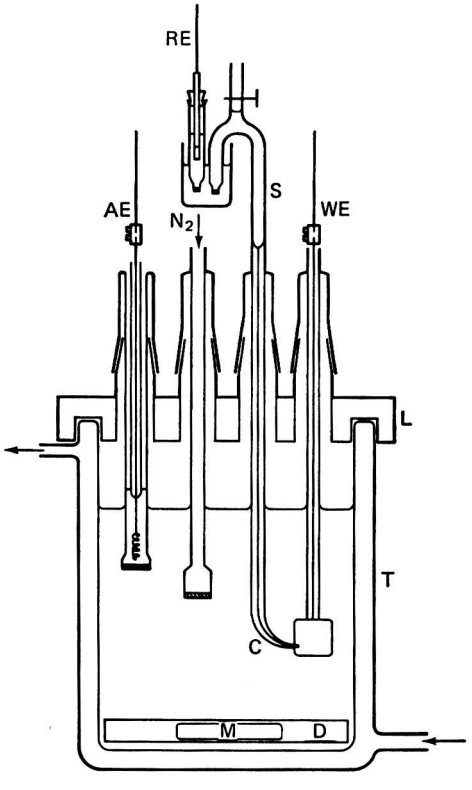

Fig. 1. Thermostatically controlled voltammetric cell: AE, auxiliary electrode in filter stick; RE, reference electrode (in thermostat tank); WE, working electrode; C, Luggin capillary; D, Perspex stirrer disc; L, machined Perspex lid; M, PTFE-coated magnetic follower; N_2, nitrogen inlet to disperser; S, salt bridge between reference electrode and Luggin capillary; and T, jacket of cell thermostat

into a Perspex disc machined so as to be a close fit in the bottom of the cell: this device gave much smoother stirring and therefore lower signal noise than the follower alone. Platinum working electrodes are made of bright platinum sheet, one side and all edges and corners of which are covered with lead-glass, so that one face alone is exposed to the solution. Electrical connection is made by platinum wire spot-welded to the back of the plate, welded in turn to tinned copper connecting wire, and sheathed with lead-glass. The dimensions were measured by means of a travelling microscope. Platinum-wire electrodes consist of 25-mm lengths of 22 s.w.g. wire sealed into soft glass. Gold working electrodes consist of lengths of 22 s.w.g. wire, 1000 fine, on to which a bead of cobalt glass (Plowden and Thomson) is sealed by winding a thread of glass round the heated wire. The hot bead is then sealed to a stem of soft glass and the whole annealed. The exposed wire is trimmed to a length of 25 mm. The auxiliary electrode is a spiral of platinum wire that dips into supporting electrolyte in a porosity 4 filter stick. The reference electrode is either a saturated calomel or a saturated mercury(I) sulphate electrode immersed in a beaker filled with salt-bridge electrolyte and placed in the thermostat, and connected to the Luggin capillary by means of a polythene tube. The Luggin capillary is placed close to the working electrode. In normal operation, the reference electrode is fully protected from polarisation. The cell contents are de-aerated with white-spot nitrogen, scrubbed first with chromium(II) chloride solution and then with distilled water, passing through a porosity 2 disperser. The gas escapes via the rim of the lid.

RAMPING POTENTIOSTAT—

The essentials of a simple potentiostat are shown in Fig. 2, the heart of which is the control amplifier, CA, which operates in the manner of all operational amplifiers so as to maintain the potential of the inverting input or summing junction, S, at the same potential as its non-inverting input, which is shown as connected to earth. The summing junction is therefore a virtual earth. The negative feedback loop includes the auxiliary and reference electrodes and the cell electrolyte, and the amplifier passes a current through auxiliary and working electrodes so as to bring the potential of the working electrode with respect to the reference electrode to equality with a command potential, which is pre-set by a signal generator, R_2E_2. The amplifier CA is therefore in the configuration of a linear combiner. In order

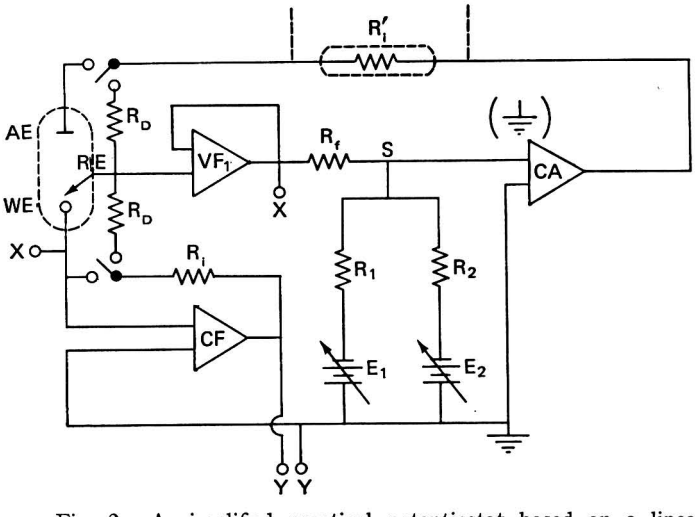

Fig. 2. A simplified practical potentiostat based on a linear combiner: CA, potentiostat control amplifier; S, summing junction; VF_1, voltage follower on the reference electrode; CF, current follower; X,X, output to X-axis of recorder; Y,Y, output to Y-axis of recorder; R_i (R_i'), current-measuring standard resistor; R_f, feedback resistor of CA; R_1 and R_2, input resistors for signal generators E_1 and E_2, respectively; R_D, dummy load resistors; AE, auxiliary electrode; RE, reference electrode; WE, working electrode; and CA, VF_1 and CF, Philbrick P65AU differential operational amplifiers

to prevent the reference electrode from being polarised, a second operational amplifier, VF_1, is connected between the reference electrode and the feedback resistor, R_f, of CA. VF_1 provides a low impedance output to supply current to R_f while offering a very high input impedance and therefore drawing a minimum current through the reference electrode. VF_1 is a voltage follower in the non-inverting mode at unity gain. The potential of the working electrode can be measured against the reference electrode either directly with a high-impedance meter with a recorder output to the X amplifier of an X - Y recorder, or at the points XX, which can be taken direct to the recorder, or via a pH meter. The measurement of the cell current poses certain problems, as do the earthing and placement of the signal generators. These topics have been reviewed by Schwarz and Shain.[22] The current is measured in terms of a voltage drop across a standard resistor, R_1, which could be placed between the working electrode and earth. This arrangement, however, would form an extra component in the command voltage provided by the signal generator, and an unwanted voltage in the output of the control amplifier. Such a resistor could be placed in the line to the auxiliary electrode as represented by R_1' in Fig. 2. Neither end of the resistor is then at earth potential and, although the input to the Y-axis amplifier of the recorder can be floated, this position of the resistor was found to be productive of excessive noise. Instead, therefore, an operational amplifier in the current follower mode, CF, is placed in the working electrode to earth path, thus holding the working electrode at virtual earth, and the calibrated resistor, R_1, forms the feedback loop of the current follower, the output of which with respect to earth is taken to the Y-axis of the recorder. Unless the cell with its electrodes and electrolyte solution are in circuit, the control amplifier will slam into saturation when trying to obey impossible commands, and so dummy load resistors, R_D, are switched in in place of the cell when the latter is not in use. A second signal generator, R_1E_1, is included, which can be used to set the starting voltage of a scan, and forms the third input to the linear combiner, CA.

In the complete seven-amplifier circuit shown in Fig. 3, a second voltage follower, VF_2, is added between the output from the current follower and the X - Y recorder, because the latter is a low-impedance instrument (10^4 to $10^8\ \Omega$). The amplifiers used are capable of producing currents of only $\pm 2{\cdot}2$ mA at ± 11 V, and so booster amplifiers, B, are included in the feedback loops of the current-driving amplifiers, the control amplifier, CA, and the current follower, CF, so as to increase the available current to 100 mA. The pre-set starting potential signal generator is retained in the form of a Mallory cell and a 15-turn Helipot potentiometer. The potential command signal generator shown as R_2E_2 in Fig. 2 is replaced by a ramp generator, RG, which takes the simple form of an integrator, for which the output potential is given by

$$E_{out} = -\frac{1}{R_3C}\int_0^t E_{in}\,dt \qquad (15)$$

By integrating a constant, stable input voltage, the output voltage increases linearly with time. The ramp speed is controlled by R_3 and C; in practice, it was found convenient to use a fixed capacitance of 10 μF and a four-decade resistance box, such as was formerly used in d.c. differential electrolytic potentiometry.[23] The integrator can be arranged so as to hold a given command potential by open-circuiting the input, and then the instrument becomes an ordinary potentiostat. Ramping in either direction is secured by reversal of the polarity of the input voltage to the integrator; re-setting to zero is unnecessary and the ramp can be reversed at any potential. The integrator is re-set to 0 V by discharging the capacitor, C, through the shorting resistor and re-set switch.

The loose circuitry, batteries, resistors, capacitors, switches, etc., are mounted on circuit boards inside an aluminium box, and connections to the cell electrodes and the recorders are co-axial. The box is provided with a number of Cinch sockets, into which the canned operational amplifiers and boosters and the power supplies can be plugged as required. The whole assembly is very flexible and circuit alterations can be made very rapidly.

OTHER EQUIPMENT—

E.I.L. Vibron 39A pH meters were used for the measurement of potential at high impedance, or as impedance transducers. Honeywell-Brown 10-mV strip-chart recorders with chart speeds of 1 to 360 in h^{-1}, and a Hewlett-Packard Moseley 7035A X - Y recorder

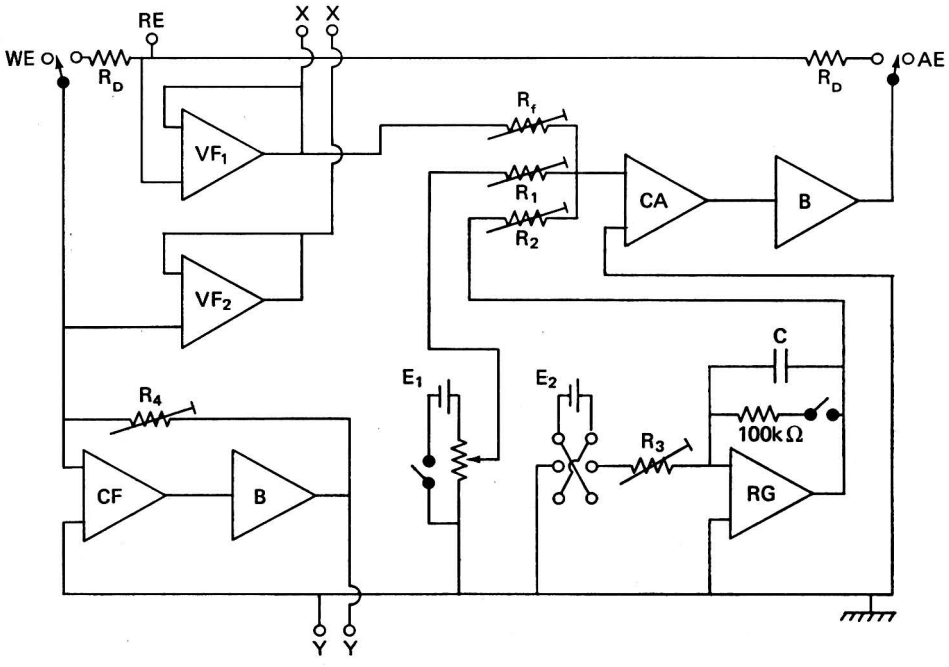

Fig. 3. The seven-amplifier ramping potentiostat: RG, Philbrick P65AU integrating operational amplifier; B, Philbrick P66A current booster amplifiers; VF_2, voltage follower on the working electrode; XX, output to X-axis amplifier of recorder; YY, output to Y-axis amplifier of recorder; C, integrating capacitor, 10 × 1 μF ± 10 per cent. 160-V d.c. polyester capacitors, Mullard Series C296 AA/A; E_1, starting potential signal generator, 5·4-V Mallory cell or 2·0-V accumulator; E_2 supply battery for ramp generator, as E_1; R_f, feedback resistor of CA = R_1 = 1 to 100 kΩ in five steps; R_2, 100 Ω to 1 MΩ resistors in seven steps; R_3, integrating resistor, 100 kΩ to 100 MΩ in decades; R_4, calibrated current-measuring resistor, 10, 100 or 1000 Ω; other components as in Fig. 2

of sensitivity 1 mV to 10 V in^{-1}, input impedance 10^4 to 10^8 Ω, depending on range, and a writing area of 10 by 7 inches were used. The operational amplifiers in the present phase of the work were as shown in Table I.

TABLE I

OPERATIONAL AMPLIFIERS

Type	D.c. open loop gain	Output	Drift
Philbrick P65AU differential	⩾2 × 10^4	±11 V at ±2·2 mA	50 μV d^{-1} 30 μV $°C^{-1}$
Philbrick SP456 chopper-stabilised	⩾10^8	±10 V at ±20 mA	1 μV $week^{-1}$ 0·1 μV $°C^{-1}$
Solartron AA1023 chopper-stabilised	⩾10^8	±100 V at ±12 mA	20 μV d^{-1}
Philbrick P66A current booster	—	±12 V at ±100 mA	—

Power supplies included either a Philbrick PU3, ±12 to 15 V at 1 A per line, or lead - acid accumulators, ±14 V and 80 A h, and transformer-supplied 6·3 and 115 V, 50 Hz for the SP456 amplifiers. A Solartron AS 1411 constant-current source[24] was used for integrator calibration. The high-precision potentiometer[24] was a Cropico P3, and Cropico RS 1 resistance standards were used. The stroboscope was an EMI type 6, and the thermoregulator a B.T.L. Circon.

VOLTAMMETRIC PROCEDURE—

The voltammetric cell was initially thoroughly leached so as to condition it, cleaned with chromic acid, very thoroughly washed and again conditioned with pure electrolyte

solution. The supporting electrolyte was placed in the cell and de-aerated for at least 20 minutes. During this period, the working electrode was given the appropriate pre-treatment so as to "clean" or "activate" it. The Luggin capillary end of the salt bridge was filled with cell electrolyte by application of suction to the Y-piece shown in Fig. 1. The ramping potentiostat was run in to thermal equilibrium with the dummy load resistors switched in, and the integrator re-set switch closed. The balance of the differential amplifiers was checked and adjusted if necessary by means of the built-in pre-set. At the end of the de-aeration, the working and auxiliary electrodes were positioned with care so as to ensure constant geometry. The electrodes were then connected to the potentiostat, the starting potential was pre-set and the cell switched into circuit. After holding the starting potential for 15 s, the shorting switch on the integrator was opened and the Mallory cell switched into its input. The required portion of the current - voltage curve was recorded on the X - Y recorder. The scan was then repeated after addition of the de-aerated sample. If required, the working electrode was pre-treated before each scan.

RESULTS OF PERFORMANCE TESTS—

The characteristics of the cell and the method of stirring were investigated by scanning a 10^{-3} M solution of copper(II) chloride in 1·0 M potassium chloride adjusted to pH 2·0 with hydrochloric acid, because this sample gives well defined voltammetric waves at platinum electrodes, and the thermal diffusion coefficient of copper(II) ion in this medium has been determined.[25]

At first, the PTFE-covered follower alone was used for stirring, but gave an uneven effect and fluctuations on the current axis of the voltammogram. When fitted into the Perspex disc, much smoother stirring was achieved. This disc was used thereafter unless the electrolyte was such as to attack Perspex. In 7 and 10 M sulphuric acid, the follower alone proved satisfactory because the high viscosity of such solutions smoothed out the stirring to acceptable noise levels. The limiting current at constant stirring speed varied with the positioning of the working electrode, and it was therefore necessary to ensure constancy of geometrical placing of the various appurtenances that dipped into the solution. The orientation of the working electrode with respect to the flow of the solution was adjusted to that giving the maximum limiting current, which proved to be the condition with the exposed face of the electrode at right-angles to the direction of flow of the solution. The speed of rotation of the stirrer disc was measured with a stroboscope.

The effect of stirring speed on the limiting current for the reduction of copper(II) was investigated. Assuming that r and t_X in equation (1) are constant, a value of δx was calculated with the aid of the reported value of D_X.[25] The relationship between δx and stirring speed is shown in Fig. 4. Whatever interpretation may be placed on δx, its value approaches a minimum of about 10^{-3} cm at high stirring speeds, and is nearly constant at speeds in excess of about 6 Hz. This is fortunate in that it permits an ordinary stirrer motor to be

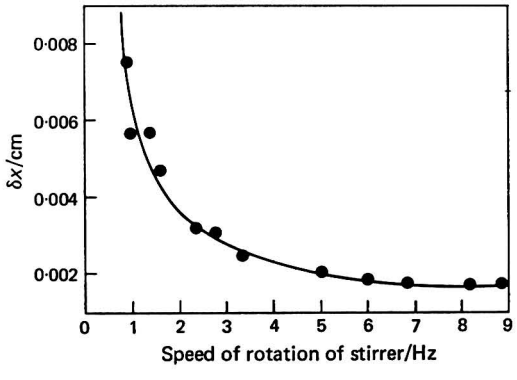

Fig. 4. Effect of stirring speed on the apparent thickness of the diffusion layer. Reduction of 10^{-3} M copper(II) in 1·0 M potassium chloride solution at pH 2·0

run on an unstabilised mains supply at a sufficiently high speed without small fluctuations in speed having any material effect on the mass-transfer process.

The dependence of the limiting current on the concentration of the electroactive species was examined for potassium hexacyanoferrate(III) in 1·0 M hydrochloric acid. It was found that the relationship

$$IL \propto [Fe(CN)_6^{3-}]^{1.00 \pm 0.005}$$

was obeyed over the concentration range of 10^{-5} to 6×10^{-3} M hexacyanoferrate(III), which indicates that a stable and reproducible mass-transfer condition is readily attainable.

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NOTE—References 1, 2 and 7 are to Parts III, IV and I of this series, respectively.

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Mass and Charge Transfer Kinetics and Coulometric Current Efficiencies

Part VI.* The Pre-treatment of Solid Electrodes, and a Review of the Effects of Oxidation of Platinum

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The nature and condition of working electrode surfaces are set in the context of reaction speeds and current efficiencies. The formation of films and their effects are examined for platinum and other metals and alloys. Adsorption and specific adsorption on electrode surfaces are briefly reviewed. An attempt is then made critically to appraise the current state of the art in respect of the activation and deactivation of electrodes. Methods of cleaning electrodes are canvassed. The more significant theories of activation and deactivation are reviewed with specific reference to platinum electrodes. These theories include the impurity theory, the platinisation theory and the various oxygen-containing surface theories. For the last, the formation of oxides, the measurement of film thickness, the film thickness and the nature of the oxide reducible at 0·6 V are discussed, followed by a selective review of the oxygen-bridge theory, the half-reduced oxide theory and the platinum - oxygen alloy theory. Tentative conclusions are reached. Finally, gold electrodes and their behaviour are briefly examined.

With very few, if any, exceptions, the primary overriding consideration in the rate of the charge-transfer process at a working electrode, and therefore in the current efficiency of the desired process, is the condition of the electrode surface. The coulometric current efficiency is a function of the relative rates of wanted and unwanted electrode processes, and all rates are affected, often to greatly differing degrees, by the condition of the electrode surface. No matter how carefully the rate parameters and solution conditions are determined, or how accurately the total current, I, and working electrode potential, E_{we}, are measured at one instant in time, the mere act of using the electrode can change the charge-transfer kinetic parameters by several orders of magnitude. Particularly is this true of solid electrodes, but the assumption that liquid electrodes are self-cleaning is over-optimistic. Nor are the many forms of carbon electrode free from these effects.

For any particular reaction at any particular kind of electrode, there is a maximum inherent speed that is governed by the activation energies of the transition states. Commonly, this maximum speed is attained at a perfectly clean electrode, and it is not possible to generalise on how perfect cleanliness is achieved or what it means. From the point of view of current efficiency, it is well to assume this maximum speed for all unwanted reactions, including the background solvent reactions, and to monitor at frequent intervals the real speed of the desired reaction. Unless the reaction is very fast, the anodic and cathodic directions occur at potentials that are sufficiently different to engender a change in the nature of the electrode surface, and the apparent over-all conditional rate constant, k, will seldom be the same for both anodic and cathodic reactions. Theory states that the charge-transfer coefficient for the cathodic direction is α, and that for the anodic direction is necessarily $(1 - \alpha)$, but, while this may hold for fast reactions, the difference in potential between the two directions of reaction again makes it unlikely that the cathodic and anodic charge-transfer coefficients will add up to unity, and so the designation of β for the anodic charge-transfer coefficient retains some practical usefulness.

The most generally used solid electrode material is platinum. A highly subjective, and selective, review of the pre-treatment and oxidation of platinum in the restricted context of coulometry is appropriate at this stage as a preliminary to the report of practical results. It cannot be emphasised too often that it is essential in any report fully to specify the electrode pre-treatment and solution purification. The objective of electrode pre-treatment is to give

* For Part V of this series, see p. 465.

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a reproducible electrode surface, which can be regained at any time by exact repetition of the pre-treatment. The pre-treatment of electrolyte solutions has not only the objective of discovering and removing any electroactive impurities in them, but also of removing trace amounts of surfactant materials that may be detectable only by their malign influence on electrode behaviour. De-ionised water and surfactant detergents should be avoided at all costs.

Films on platinum

The nature, condition and participation in reactions of the electrode surface is a complicated and contentious subject, extremely difficult experimentally to investigate and little productive of unequivocal interpretation, as the extensive literature attests. Noble metals are by no means inert,[1] and both chemical and anodic oxidation produce similar attacks.[2] Baker and McNevin[3] found that oxidised surfaces slowed the oxidation of arsenic(III), and thereafter adopted a cathodic pre-treatment of platinum as a routine procedure.[4] They ascribed the interference of trace amounts of antimony to the formation of a film of the insoluble antimony oxide Sb_2O_4 on the electrode surface.[4] Bard[5] identified hydrated tin oxide films on cathodes. Lingane and co-workers[6] detected oxidation of platinum during the oxidation of iodine to iodate, and later provided chemical evidence of anodic attack[7] and film formation in chloride media,[8] although the precise interpretation of the evidence was challenged.[9] Davis[10] observed oxidation of platinum in the chronopotentiometry of iron(III) and cerium(IV), and found that repeated reduction of the electrode was necessary in the oxidation of hydroxylamine.[11] Anson found that the reduction of iodate was much faster at an oxidised electrode,[12] first postulating an oxygen-bridge mechanism and then platinisation.[13] Lingane found that an oxide film stopped the oxidation of oxalate.[14] This effect was ascribed to prevention of the adsorption of oxalic acid, which is a necessary preliminary to its oxidation.[15] Organic electrode processes are strongly affected by the condition of the electrode,[16] and the rate constant of the hexacyanoferrate(III) - hexacyanoferrate(II) process at an oxidised electrode is only one tenth of that at a clean electrode.[17] Kozawa[18] ascribed humps in voltammograms for alkaline solutions to the formation of oxides or hydroxides. Laitinen and Enke[19] showed that the process of oxidation is slow, and postulated a hydroxyl radical mechanism. Feldberg, Enke and Bricker[20] favoured chemisorption followed by a two-step process. Meyell and Langer[21] showed that the oxide layer thickened with increasing anodisation potential until it became constant at potentials above 1·8 V. Lingane[22] showed that the reduction of oxygen to water at platinum without the intermediate formation of hydrogen peroxide was fast at an electrode that had one tenth of a monolayer of oxide on its surface, but was slow at a reduced electrode. He suggested that a chemical reaction between oxygen and platinum occurred, followed by reduction of the oxide; this suggestion was attacked by Anson and King,[13] but was supported by Sawyer and Interrante,[23] who ascribed the decreasing speed of reaction with time to ageing of the oxide film, and found the speed at a pre-reduced electrode to be so slow that the electrode retained the characteristics of a reduced surface even in oxygen-saturated solutions.

Other metals have been examined less extensively. Palladium and gold are oxidised in the same way chemically and anodically.[2] Gold[23–26] is particularly attacked in media that contain complexing ions such as cyanide and chloride, but is otherwise[25] more resistant than platinum to oxidation. Attack of single-crystal silver depends on the crystal face exposed to the solution.[27] Alloys of gold and platinum[28,29] show interesting properties, as do those of gold with palladium, silver and platinum.[30] Iridium migrates preferentially to the surface of platinum - iridium alloys.[31]

Adsorption on electrodes

Adsorption in general, or specific adsorption, of reactant, product or other species may be a necessity, a nuisance or a disaster, and again has an extensive literature; some has already been quoted.[9–12,15] Anson has made many contributions.[32–41] He ascribed activity to very light platinisation of the electrode on reduction of the oxide,[32] found that perchloric acid could not replace sulphuric acid in chemical treatment with iron(II),[12] and ascribed the accelerated reduction of iodate[12] and vanadate at a pre-oxidised electrode to electrolytic platinisation.[13] The same reason was adduced by Bard[42] for the accelerated oxidation of hydrazine at a.c. biassed activated electrodes. Feldberg, Enke and Bricker[20] contended that

the activity resided in a half-reduced oxide. Adsorption effects were detected in studies of the iron(III) - iron(II) system,[33] but chronopotentiometric evidence of the adsorption of iron ions[37] was traced to capillary cracks in the glass-to-metal seal of the electrode,[36] but adsorption of iodide[7,34] and prevention of adsorption of oxalate[14,15] by oxide films is confirmed. Anson and Schultz[15] postulate that adsorption of certain species, such as oxalate, iodide, hydrogen peroxide, arsenic(III), thiocyanate, nitrite, ethylenediaminetetraacetatocobaltate(II) and methanol, is a necessary preliminary to their oxidation, and adsorption and oxidation are inhibited by oxide films. Adsorbed bromide catalyses the anodic oxidation of ethylenediaminetetraacetatocobaltate(II) and the product contains no bromide.[38] Adsorption of ethylenediaminetetraacetatocobaltate(III) ion on a platinum cathode inhibits the reduction of the unadsorbed complex ion.[39] Preferentially adsorbed ions, such as iodide, will displace adsorbed complex ions and so eliminate the interference. The cobalt(II) complex reduction product is also adsorbed. Mercury cathodes extensively adsorb the cobalt(II) complex,[40] but not the tris(ethylenediamine)cobalt(III) cation,[41] contrary to previous findings.[43,44] Interface and film inhibition at mercury electrodes have been reviewed by Oeder, Seiler and Fischer.[45] Unusual adsorption effects at mercury in the reduction of flavin nucleotide have been observed.[46] Murray and co-workers[47–52] used several techniques in adsorption studies at mercury electrodes. Reduction of mercury(I) and bismuth(III) is inhibited by adsorbed lead iodide, bromide and thiocyanate[47,49]; charge transfer is slowed by a chemical step at an adsorption-blocked surface in the reduction of copper(II) in tartrate media by brucine. Supporting electrolyte effects in non-aqueous media have been examined,[51] and adsorption has been deliberately used in electrochemical masking with an adsorbed metal complex.[52] Adsorbed lead selectively penetrable by different ions was used in the analysis of silver - mercury(II) mixtures. Interpretative studies by Wopschall and Shain[53–55] set out to test a theory[53] by examination of the reduction of methylene blue,[54] wherein the product is strongly adsorbed, and of the azobenzene - hydrazobenzene system,[55] wherein there is a succeeding chemical reaction of the adsorbed reactant. Returning to platinum, thin-layer cells have been used in adsorption studies.[56–58] Adsorption of EDTA complexes of cobalt and iron,[57] and of iodine - iodide systems[58] has been examined. Napp and Bruckenstein[59] revealed the risks of unsuspected adsorption in a ring-disc electrode study of 0·5 M hydrochloric acid at potentials from 0·25 to 1·25 V. They challenged earlier work,[8] and ascribed the observed behaviour to a trace amount of copper(II) in the medium, which is reduced to and adsorbed as copper(I) on the electrode surface. Gileadi[60] has reviewed the adsorption of uncharged molecules on solid electrodes.

Activation and deactivation of electrodes

A caveat against de-ionised water and detergents has already been entered. Flaming, or dipping in alcohol and flaming, will only poison the electrode. Treatment with chromic acid is strongly to be deprecated: not only does chromium(VI) oxidise the surface, but chromium species are tenaciously adsorbed on platinum and block entirely such reactions as the reduction of vanadium(V).[61,62] Reduction does not remove the chromium, and washing so prolonged as to remove it will result in a deactivated electrode. Chemical stripping in fresh, warm aqua regia, followed by washing with quartz-distilled water,[63] will remove gross contamination; anodisation in hydrochloric acid serves much the same purpose. This stripping stage can be followed by chemical reduction[64] or cathodisation. Commonly, however, several cycles of anodisation and cathodisation in purified sulphuric acid, finishing with a protracted cathodisation, are used, and hydrogen adsorbed on or dissolved in the electrode is removed potentiostatically at 0·25 to 0·4 V. It is not possible to generalise further. In use, the electrode will be deactivated and need fresh pre-treatment. Setting aside specific adsorption, discussed above, of the very many theories of electrode behaviour, it is worth examining three, which have commanded considerable attention in the past, or appear to have present value. These theories are the impurity theory, the platinisation theory, and a group that may be termed oxygen-containing surface theories, and all are specifically directed to platinum.

The impurity theory—

The malign influence of surface-active impurities was demonstrated in 1937 by Frumkin's group,[65–67] and 30 years later[68] was used in their determination. Ion-exchange resins gave

water of low conductance, but 10^{-6} M in surfactants against 10^{-7} M for double-distillation in quartz. Charcoal columns removed most of the surfactant impurities but introduced ionic impurities, which may or may not be of importance. We have found charcoals to be ineffective for sulphuric acid purification, but both alumina[69] and charcoal[70,71] have been used successfully. We have found adsorption on a large electrode to be successful, but erratic, for sulphuric acid[61]; Bockris and co-workers[72,73] have used this method. Barker[74] has used a combination of column and electrolytic methods. Trial and error is the only general approach to purification of solvents and electrolytes, and little is known of the nature of surfactants; both organic and inorganic materials can be responsible.[75,76] One difficulty may be removed at the cost of introducing another, and particular care is needed in treating sample solutions.[70]

James[76] used solution cleaning methods in a study of the effect of impurities on the iron(III) - iron(II) and hydrogen ion - hydrogen gas processes at platinum. He recorded the current at constant potential of a small, spinning platinum-wire electrode. In untreated iron(III) - iron(II) in perchloric acid, an electrolytically activated electrode showed a current that decreased with time, until it became equal to the current at an untreated electrode and then remained constant. After charcoal "cleaning" of the solution, both treated and untreated electrodes behaved alike, this time in the manner of the initial behaviour of a treated electrode in an untreated solution. He concluded that the electrolytic treatment removed impurities from the surface of the electrode, which, on exposure to a cleaned solution, did not become dirty again. Similar observations were recorded for the hydrogen system, but the charcoal treatment, whether or not followed by electrosorption, did not entirely remove surfactants from the sulphuric acid. Both before and after "cleaning" of the electrolyte, the rate of decay of the current was accelerated by increasing the rotation speed of the electrode. This effect was to be expected when deactivation depended on migration of impurities to the electrode. James concluded that deactivation arose by re-adsorption of solution impurities, and not solution ions, and that it was unlikely that slow dissolution of hydrogen gas in the metal surface contributed to deactivation. Damjanovic, Genshaw and Bockris,[73] studying oxygen reduction in acidic media, supported the view that impurities exerted a profound effect.

It is notable that scrupulously clean solutions in equally clean vessels containing perfectly clean electrodes acquire impurities even when the system is completely sealed.[61,77,78] Warner, Schuldiner and Pierama[78] found that platinum electrodes kept on open circuit in the solution remained clean for several weeks, but when in continuous use, impurities built up, and they concluded that the impurities originated from the electrodes. This conclusion need not be true. An impurity theory is impossible to disprove because the level needed to interfere with electrode processes is so low that the impurity cannot be detected other than by its interference. Acceptance of a form of impurity theory does not reveal the full picture, and other effects require consideration.

THE PLATINISATION THEORY—

Smooth platinum gives no zero-current hydrogen-ion response, as it is unable to catalyse the rate-determining combination of hydrogen atoms. When coated with finely divided palladium-black or platinum-black, the effective surface area is multiplied several thousand-fold and contains the necessary energetic sites for the catalysis. The platinisation theory argues for an increase in surface area and catalytic power, and states that anodisation - cathodisation cycling produces light platinisation on reduction of oxide. Certainly initially bright surfaces are quickly dulled and etched by this treatment. Anson and King[13] used 60-Hz a.c., probably of a very rough waveform, and produced a grey to black deposit, and Shibata[79,81] observed a similar phenomenon, claiming that the oxide was PtO_2. Hoare,[82] however, investigated a.c. polarisation of platinum, rhodium, palladium, iridium and gold, and found that only those metals which are capable of dissolving hydrogen, *i.e.*, platinum and palladium, formed roughened surfaces, and ascribed the break-up of the surface to alternate dissolution and removal of hydrogen. One might ask whether roughness increases the charge-transfer rate. Anson and King[13] did not purify their reagents, and the activation may simply be removal of impurities.[70] Shibata[81] repeatedly distilled his hydrochloric acid in a quartz still, but this is still not unequivocal in view of the work of Berezina and Nikolaeva-Fedorovich.[68] Shibata cleaned platinum electrodes, aged them for 2 weeks in hydrogen, oxidised them chemically or anodically, and determined the amount of oxide chronopotentiometrically. Oxides formed in either way showed a single halt at 0·6 V and the amount increased with time

of oxidation. Prolonged oxidation produced what seemed to be a second oxide, which was reducible at 0·3 to 0·2 V. All electrodes showed the same initial activity in hydrogen production, and the decay in activity was a function of the oxidation time. At the extreme, anodisation for 28 hours at 100 mA cm^{-2} gave an electrode that showed a negligible loss of activity after 30 hours of continuous use. The dependence of decay rate on oxidation time seems to exclude adsorption of impurities for deactivation, and Shibata opined that the platinised layer produced was unstable, but had a high initial activity that decreased as the platinised layer recrystallised to a stable form.

Shibata's work suggests that impurities alone cannot account for deactivation, but this neither proves the platinisation theory nor disproves the impurity theory. Reports of surface recrystallisation[83–85] have been challenged by Gilman,[86,87] who found the deactivation to be dependent on stirring speed, which argues for impurity deactivation. Warner, Schuldiner and Pierama[78] used clean electrodes and electrolyte and showed that well annealed platinum was more active than unannealed or drawn platinum in the reaction

$$Pt-O + H_2 \rightharpoonup Pt + H_2O$$

but annealing was without effect on simple electrochemical reactions. The work of Khazova, Vasil'ev and Bagotskii[88] would seem effectually to disprove the platinisation theory. They studied the catalytic and electrochemical activities of smooth and platinised platinum over the roughness factor range from 2 to 6500, and found the kinetic principles for chemisorption and electro-oxidation to be the same for all electrodes. The maximum rate of either type of reaction in relation to the true surface area *decreased* by an order of magnitude from smooth to highly platinised electrodes; the sharpest change occurred in the roughness factor range from 10 to 1000, and further increase in roughness did not affect the rates. Observation of transients absolved mass transfer from the decrease in rate, so platinisation reduces the charge-transfer rate constant.

OXYGEN-CONTAINING SURFACE THEORIES—

This awkward term attempts to include several theories ranging from the formation of stoicheiometric oxide films to dissolution of oxygen in platinum. The subject is contentious in the extreme, presents enormous experimental difficulties, absorbs a huge research effort, not least in fuel cells,[89,90] and has produced a formidable literature, of which this review can do no more than skim the surface in the special context of coulometry, as previously adumbrated.[64] Dispute continues over the nature of oxidised platinum, originating from the difficulty in distinguishing chemisorption from stoicheiometric phase oxides. Confusion is the more confounded by lack of reproducibility of the results (or inadequate reporting) of the cathodic-stripping determination of the amount of oxygen present. Contentions that films scarcely exceed a monolayer[91–93] are difficult to prove. Electron and X-ray diffraction are useless, but ellipsometry assisted by coulometric stripping appear at present to offer a reasonable guide.[94,95] At potentials cathodic to 0·98 V, there was no ellipsometric evidence of oxide; the sensitivity was about 0·01 nm. At 0·98 $\pm$ 0·01 V, a film of 0·02 nm thickness suddenly appeared and grew linearly with increasing potential to 1·6 V, when the thickness was 0·55 nm; higher potentials were not examined. Coulometry[94] indicated that a 0·05-nm thick film was present below 0·98 V, and that the film thickened at about the rate indicated by ellipsometry up to 1·6 V, but the film was always 0·05 nm thicker than was found by ellipsometry. A complete monolayer would be about 0·3 nm thick. Ellipsometry depends on a difference in optical constants of the film and the surrounding medium, and the difference between chemisorbed oxygen and water is too small to show up. Below 0·98 V, the coulometrically detectable oxygen was interpreted as being chemisorbed, and above 0·98 V a phase oxide was formed. The coexistence of chemisorbed oxygen and phase oxide had been predicted earlier.[96]

Film thickness—Chronopotentiometric reduction after conventional anodisation indicates a single oxide reducible at 0·6 V,[7,14,19,78] and coulometry supports the view that no more than a monolayer is formed,[7,8,14,20,78,91,93,97,98] amounting to 0·3 to 0·74 mC cm^{-2} depending on the roughness factor, but there is some evidence of thicker films.[1,81,96] Endeavours by Cooksey and Bishop to establish precise growth-rate laws have been frustrated by poor reproducibility, but have emphasised that the competitive formation of molecular oxygen during oxidation has too often been ignored. Stronger anodisation yields thicker films,[21,81,94,99–101] and in some instances a second reduction halt at 0·2 to 0·3 V has been noted.[82,101,102] Meyell and Langer[21]

have reported many platinum to oxygen ratios, including a "tight" PtO. Their de-oxygenation procedure was inadequate, and Hoare[103] rightly suggests that unremoved oxygen contributed to the stripping current. Visscher and Devanathan[94] reported a linear relationship between the stripping charge and the potential of oxide formation (Bishop and Cooksey find this to be a gross oversimplification); even with a roughness factor of 2·5,[21] oxide formed at 1·7 V required 2 mC cm^{-2} for complete reduction, implying a layer six to seven atoms thick. Kolthoff found that layers 0·3 to 1·4 mC cm^{-2} thick were formed on chemical oxidation, depending on the nature of the oxidant and the time of attack. Prolonged anodisation gave second halts at 0·3 V[81] or 0·2 V,[101] and the amount of low-voltage oxide increased with anodisation time. Shibata[81] reached 36 mC cm^{-2}, which corresponds, at a roughness factor of 2·5, to forty oxygen atoms per surface platinum atom.

The nature of the oxide reducible at 0·6 *V*—This has been extensively researched, as Young's[104] and Hoare's[105] books and Hoare's extended review[103] show. The re-interpretation[9] of Anson and Lingane's chemical stripping results[7] has been mentioned; the same objections to other work can be raised.[100] Hoare[103] canvasses a wide variety of likely and unlikely oxides. The most widely held view is that moderate anodisation produces PtO or $Pt(OH)_2$ in hydrated form, and probably some chemisorbed oxygen as well, but drastic conditions are required in order to produce platinum(IV) oxide, which is the reverse of the situation with sulphide, where PtS_2 is formed but not PtS.[106] Decreases, often drastic, in the rate of the charge-transfer process at oxide-filmed electrodes have been reported.[13,14,32,42,70,107] Müller[108] demonstrated that the rate of the charge-transfer process is proportional to the surface area of the platinum that remains unoxidised. Reports[12,107–109] of reactions accelerated by oxide filming have mostly been discounted[32,42]; what has been observed can be interpreted as reaction at a bare platinum surface after the oxide has been chemically stripped.

THE OXYGEN-BRIDGE THEORY—

The analogy with the oxygen-bridge mechanism in homogeneous oxidation - reduction reactions is tempting but false. An attempt[110] was made to explain an observed "reversibility" of freshly anodised platinum electrodes on a basis of an "oxygen bridge" structure of the oxide layer, claimed to accelerate the iron(III) - iron(II) and cerium(IV) - cerium(III) reactions. Anson first lent support to this interpretation,[12] but later changed to the platinisation theory.[13] Davis[107] found that heavy oxide films suppressed the iron(III) - iron(II) reaction as earlier predicted,[3] and the vanadium(V) - vanadium(IV) reaction, but claimed that a light film, less than a monolayer, facilitated oxygen-bridge formation and accelerated these reactions. He suggested that oxide formation occurred at grain boundaries, but this suggestion was conclusively challenged.[111] James[76] demonstrated that a simple oxygenated surface could not account for platinum activation. At a potential of 0·8 V, at which the oxide should be stable, he found a considerable decay in activity with time; contrarily, prolonged anodisation produced long-lived activity in hydrogen evolution at 0·0 V, at which the oxide should have been completely destroyed.

THE "HALF-REDUCED OXIDE" THEORY

Studying the oxidation of platinum in perchloric acid, Feldberg, Enke and Bricker[20] concluded that film formation was a two-step process, the first being slow formation of $Pt(OH)_x$ and the second fast oxidation to $Pt(O)_x$. On cathodic stripping, reduction of $Pt(O)_x$ to $Pt(OH)_x$ was fast, but the slow reduction of $Pt(OH)_x$ to bare platinum was complete only after prolonged potentiostatic reduction, while the process stopped at $Pt(OH)_x$ on amperostatic reduction, the current then being used in the generation of hydrogen. If the quantity of electricity required to form the oxide is Q_a and for reduction is Q_c, then on the first cycle Q_a/Q_c should be 2, decreasing to unity. This, by and large, is so. The arguments against this theory, that the half-reduced oxide is the active condition, are the same as for the oxygen-bridge theory.[76] The observations can be interpreted in several ways. Vetter and Berndt[112] adduced different reactions, saying that the oxide was formed from water by a four-electron step, and reduced to hydrogen peroxide by a two-electron step. Breiter,[113] using low-frequency cyclic voltammetry, found that Q_a/Q_c started at greater than 2 but did not fall below 1·18, which agrees with our findings,[64] and considered that the observations were contrary to previous theories.[20,112] He suggested that Q_a included oxidation of organic impurities,[114,115] while Q_c was little affected because reduction of the oxide occurred at

potentials negative to 0·9 V, when such reactions became very slow. He also claimed that whereas oxygen atoms dissolve readily in platinum, their desorption is slow. Preferring the first explanation, he regarded Q_c as a measure of the "electrochemical cleanliness" of the system. Gilman[86,87] reached similar conclusions. We suggest[61,64] that the formation of molecular oxygen occurs during anodisation, and that this oxygen in part diffuses away from the electrode and is therefore not reduced during cathodisation.

THE PLATINUM - OXYGEN ALLOY THEORY—

Breiter's suggestion about oxidisable impurities[114,115] may well be right, but his suggestion of dissolution of oxygen in platinum is supported by other work[116] in which a pulse and decay technique was used. Perhaps the most significant work at the time of writing is that of Hoare,[92,103,105,117–127] who ascribes the activity of pre-anodised electrodes to oxygen dissolved in the surface layers of the bulk metal.[126] Oxygen has been reported to dissolve in massive platinum to a depth of three to four layers before saturation is reached.[102,116,128–130] Schuldiner and Warner[116] found a direct connection between oxygen dissolved in platinum and the catalytic activity of the metal. Hoare based his hypothesis[126] on earlier work,[92,119,122] and made solutions of reagent grade chemicals in triply distilled water and pre-electrolysed the solutions for at least 24 hours before use,[117] and was thus reasonably assured of impurity-free media. Further, he found that oxidation in concentrated nitric acid produced an electrode that displayed a steady potential of 1·225 V for over 24 hours when immersed in 1·0 M sulphuric acid saturated with pure oxygen, in accord with prediction.[121] Such an electrode also catalysed the reduction of oxygen and hydrogen peroxide in acidic media.[122,126] A minimum of 50 hours' soaking was needed in order to produce an electrode of stable activity, which is much longer than is necessary in order to remove impurities or to coat the metal with oxide. This result is compatible with the postulate that oxygen dissolved in the metal to give a "platinum - oxygen alloy" electrode.[92] A cathodic chronopotentiogram of one of Hoare's activated electrodes has been quoted[76] as showing a single arrest at 0·7 V similar to that of conventionally anodised electrodes. Hoare reports that when his activated electrodes were immersed in hydrogen-saturated acidic solution, they took 40 minutes to reach the same potential as a reduced electrode, compared with 4 minutes for conventionally anodised electrodes.[119,127] Breiter[131] reported that nitrogenous species were tenaciously adsorbed on platinum soaked in nitric acid, but Hoare could not detect such species.[127]

Hoare's prolonged treatment with nitric acid seems to produce electrodes with unique properties, which he attributes to the platinum - oxygen alloy, but it can equally be postulated that prolonged anodisation produces not only a surface-phase oxide but also some of this alloy or solid solution, which suffices to activate the electrodes towards oxidation - reduction systems. The oxide layer would be stripped chemically or cathodically, but the oxygen in solid solution would be removed only very slowly.[126] Hoare does not offer a mechanism; this is probably not yet possible. Much of the earlier work can be re-interpreted in the light of Hoare's suggestion. Particularly, Shibata's results[81] concerning prolonged anodisation could be explained by the extensive formation of the platinum - oxygen alloy, with consequent long-lived activity, rather than by platinisation.

CONCLUSIONS

There can be no finality yet: hot debate continues. No sooner has one read one paper that appears to be convincing and conclusive, than another of contrary view that is equally convincing and conclusive appears. Tentatively, however, desorption and adsorption of impurities can be said to contribute substantially to activation and deactivation processes, respectively, and at the moment Hoare's platinum - oxygen alloy theory seems to be the most attractive. Chemical oxidation[1,2] seems to produce the same oxide, which can be stripped at 0·6 V, as anodisation, but electrochemical activation is to be preferred. There are many reports,[103,104,120,132] some of considerable antiquity,[133–139] of yellow or red films formed on platinum by means of a periodic current of 50 to 60 Hz, with a d.c. bias. Such films could well consist of oxide. In our laboratory, extremely hard, scratch-proof gold films have been produced by precisely symmetrical waveforms, but the rough waveforms from such an iron-cored inductance as a transformer gave black films that could be wiped off with a moist paper tissue.

GOLD ELECTRODES

The behaviour of gold is simpler because neither hydrogen nor oxygen is soluble, and has raised less controversy. An oxidised surface renders gold a poor electrode for oxidation - reduction reactions,[25,70] but as soon as the oxide has been reduced, reactions proceed at normal speed. Hoare has examined gold as a possible oxygen electrode,[103,123–125] but it seems[118,140] that gold gives a mixed potential in oxygen-saturated solutions. Potentiostatic measurements[123] in acidic oxygen solutions indicate a partial layer of oxygen to be adsorbed between 0·9 and 1·3 V, which is a good electrical conductor. Above 1·36 V, Au_2O_3, probably hydrated, is formed and is not a good conductor,[112] so that passivation occurs between 1·36 and 1·60 V. Reports[2,141] of the formation of Au_2O and AuO are not held in much regard. A trans-passive region between 1·60 and 2·0 V has been attributed to the migration of gold atoms through the loosely packed Au_2O_3 film.[142] Such migration produces an oxide film that is much thicker than a monolayer.[25,143,144] Reduction of an electrode anodised at high potentials gives considerable roughening, attesting to a thick porous oxide film.[140] Baumann and Shain[25] recommend oxidation - reduction pre-treatment, and ascribe the improved activity to the removal of surface impurities. This improvement is probably the main consequence of electrolytic pre-treatment, although roughening may give minor benefit. No attempts have been made to explain the activity of gold electrodes on a basis of an oxygen-containing surface. Charging curves for gold[20,23,145] indicate that $Q_a = Q_c$, which accords with the insolubility of oxygen in gold. The proposal of oxygen diffusion along grain boundaries[23] has been challenged[111] on the grounds of lack of evidence. Gold is more resistant[25] than platinum to chemical oxidation in non-complexing media, but gold dissolves readily in media that contain cyanide or halide ions. This attack starts at 0·6 V in chloride media, and so severely restricts the use of gold as an anode.

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Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

Part IV.* The Determination of Catecholamines

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(−)-Adrenaline, adrenaline hydrogen tartrate, L-noradrenaline, dopamine hydrochloride, L-dopa, DL-dopa, L-α-methyldopa, D-α-methyldopa and (+)-Corbasil have been determined in amounts down to 0·0001 mequiv by catalytic thermometric titration of their basic and acidic functions. Basic functions were determined by titration with 0·1, 0·01 and 0·001 M perchloric acid by using the ionic polymerisation of α-methylstyrene to indicate the end-point, while acidic functions were determined in a similar manner with tetra-n-butylammonium hydroxide as the titrant and acrylonitrile as the end-point indicator.

The L-dopa contents of tablets and capsules have been determined by using these techniques and the assay results have been compared with those obtained by alternative methods, namely, the recently described B.P. procedure involving non-aqueous titration, and ultraviolet spectrophotometry.

Magnesium stearate, which is used as a lubricant and flow promoter in tablet manufacture, is titrated as a base in the solvents used, but in titrations of the acidic function of catecholamines its effect is negligible.

The determination of catecholamines in biological specimens normally requires the use of trace analysis techniques such as fluorimetry and chromatography, as the concentrations usually encountered rarely exceed the microgram per gram level and are often in the sub-nanogram per gram range.[1] In contrast, catecholamines that form the active constituents in pharmaceutical preparations are present therein in relatively large amounts, and can be determined conveniently at the milligram per gram level or at even higher concentrations.

For the routine assay of L-dopa [L-3-(3,4-dihydroxyphenyl)alanine], noradrenaline, adrenaline hydrogen tartrate and noradrenaline hydrogen tartrate, non-aqueous titration with perchloric acid is recommended.[2,3] For the determination of catecholamines in formulations the more selective spectrophotometric methods are usually used,[4,5] presumably in order to avoid interference from excipient material. However, in recent monographs,[6] non-aqueous titration is the method prescribed for the assay of the L-dopa content of tablets and capsules.

Thermometric procedures for the determination of organic acids and bases in amounts down to about 10 μg are described in Parts I[7] and II.[8] In these determinations a monomer capable of ionic polymerisation is added to a non-aqueous solution of the acid or base prior to titration. The end-point is indicated by a rise in temperature, which results from the ionic polymerisation of the monomer and which is initiated by the titrant following neutralisation of the sample.

The present paper describes the application of these thermometric procedures to the determination of some catecholamines of medical importance, namely (−)-adrenaline, adrenaline hydrogen tartrate, L-noradrenaline, dopamine hydrochloride, L- and DL-dopa, L- and D-α-methyldopa, and (+)-Corbasil. It is particularly concerned with the development of suitable solvent systems for these rather intractable compounds. The formulae of the compounds are shown in Fig. 1. Determinations have been carried out on the pure compounds and also on L-dopa tablets and capsules, and both the basic and acidic functions have been determined. As in the previous investigations, perchloric acid was used as the titrant and α-methylstyrene as the end-point indicator for the determination of basic functions, while tetra-n-butylammonium hydroxide was used as the titrant and acrylonitrile as the end-point indicator for the determination of acidic functions. The results obtained with L-dopa and

* For details of Parts I, II and III of this series, see reference list on p. 492.

with formulations that contain it are compared with those obtained by using the B.P. non-aqueous titration method[6] and ultraviolet spectrophotometry.

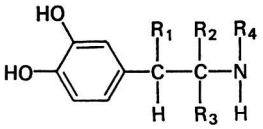

Fig. 1. Structural formulae of the catecholamines

	R_1	R_2	R_3	R_4
(−)-Adrenaline	OH	H	H	CH_3
(+)-Corbasil	OH	CH_3	H	H
DL-Dopa	H	COOH	H	H
Dopamine	H	H	H	H
α-Methyldopa	H	COOH	CH_3	H
L-Noradrenaline	OH	H	H	H

Experimental

Reagents—

Glacial acetic acid and propan-2-ol were of analytical-reagent grade and acrylonitrile, 1,2-dichloroethane, dimethylformamide and α-methylstyrene were laboratory-reagent grade materials. All were dried over molecular sieve 4A before use. Oxalic acid (analytical-reagent grade) was dried at 110 °C for 3 hours before use.

Other solvents and reagents were laboratory-reagent grade materials and were used as received.

Perchloric acid, 0·1 M solution in acetic acid—Prepare this solution and standardise it by the method described in Part I.[7] Prepare 0·01 and 0·001 M solutions by diluting the 0·1 M titrant with 1,2-dichloroethane.

Tetra-n-butylammonium hydroxide, 0·1 M in toluene - methanol—Laboratory-reagent grade material was used as received. Prepare 0·01, 0·002 and 0·001 M solutions by adding appropriate volumes of toluene - propan-2-ol mixture (3 + 1) to the 0·1 M reagent. Standardise the solutions against benzoic acid (analytical-reagent grade) in dimethylformamide by the thermometric method.

Catecholamines—

(−)-Adrenaline, L-noradrenaline, L-dopa, DL-dopa, dopamine hydrochloride and adrenaline hydrogen tartrate were laboratory-reagent grade materials. All other catecholamines were gifts: L-dopa tablets (normal and sustained release) and capsules from Brocades (Great Britain) Ltd., L-α-methyldopa and D-α-methyldopa from Merck, Sharp and Dohme, and (+)-Corbasil from Dr. S. Jones (Department of Pharmacy, Chelsea College).

Apparatus—

Manual method—Use the apparatus described in Part III[9] and the 15-ml titration flask described in Part II.[8]

Automatic method—Use the motor-driven syringe described in Part III. An 8-ml titration flask is adequate for the present work.

Procedure

A. Titration of the basic function—

Manual method—Weigh about 0·1 mequiv of the sample into the titration vessel, add 0·5 ml of formic acid and mix well, then add 2·0 ml of acetic acid and 3 ml of α-methylstyrene. Add the titrant (0·1 M perchloric acid) at the rate of about 0·4 ml min^{-1} to within 0·3 ml of the end-point, noting the temperature at 15-s intervals, and complete the titration with addition of titrant at a rate not exceeding 0·2 ml min^{-1}.

Automatic method—Add the titrant at a constant rate (not exceeding 0·2 ml min^{-1}) to a mixture of the sample, solvent and monomer in the 8-ml titration flask that is appropriate to the titrant concentration (see Table I). Record the temperature and titrant volume on a millivolt chart recorder (20-mV scale) at a chart speed of 600 mm h^{-1}.

Suitable amounts of sample, solvent and monomer are given in Table I.

TABLE I

AMOUNTS OF SAMPLE, SOLVENT AND MONOMER RECOMMENDED FOR USE WITH THE DIFFERENT CONCENTRATIONS OF TITRANT

	Titrant—Perchloric acid		
	0·1 M	0·01 M	0·001 M
Sample/mequiv	0·1 to 0·02	0·01 to 0·002	0·001 to 0·0001
Solvents—			
Formic acid/ml	0·5	0·2	0·05*
Acetic acid/ml	2·0	0·2	0·2
1,2-Dichloroethane/ml ..	—	0·6	0·75
Propylene carbonate/ml ..	—	1·1	—
α-Methylstyrene/ml ..	0·5	0·9	2·0

* With dopamine hydrochloride use the solvent mixture prepared by mixing 0·1 ml of formic acid, 0·5 ml of acetic acid and 0·4 ml of 1,2-dichloroethane.

The end-point of the titration is measured, as in Part I,[7] at the "upturn" temperature. This is the point where the titration curve leaves the tangent drawn to the "horizontal" portion of the curve in the vicinity of the temperature rise.

B. TITRATION OF ACIDIC FUNCTIONS—

All determinations were carried out by using the automatic method. Use the same general procedure as that used for titrations of the basic function but with the recorder set at 100 mV full scale. Prepare titration solutions by adding 2 ml of acrylonitrile to a solution of the sample in either 1 ml of dimethylformamide (suitable for dopamine hydrochloride, adrenaline hydrogen tartrate and α-methyldopa) or 1 ml of dimethylformamide containing an approximately equivalent and accurately known amount of *p*-toluenesulphonic acid [suitable for adrenaline, L-noradrenaline, L- and DL-dopa and (+)-Corbasil].

Appropriate amounts of sample for 0·1 M titrant (tetra-n-butylammonium hydroxide) are: 0·1 to 0·02 mmol of (−)-adrenaline, (+)-Corbasil and L-noradrenaline; 0·05 to 0·01 mmol of L- and DL-dopa, dopamine hydrochloride and α-methyldopa; and 0·04 to 0·007 mmol of adrenaline hydrogen tartrate. With 0·01, 0·002 and 0·001 M titrant, use correspondingly smaller amounts of sample.

The end-point of the titration is measured, as in Part II,[8] by the method of Vaughan and Swithenbank,[10] in which it is located as the point where the tangent to the main heat rise leaves the curve at its lower temperature end.

B.P. ASSAY METHOD—

This method, which involves titration of a solution of the sample (about 500 mg of active constituent) in a formic acid - acetic acid mixture with 0·1 M perchloric acid, with Oracet blue as the indicator, is described in detail in the British Pharmacopoeia.[6]

ULTRAVIOLET SPECTROPHOTOMETRY—

Extract an amount of sample containing about 50 mg of L-dopa with 40 ml of 0·1 M hydrochloric acid. Filter and, by using 0·1 M hydrochloric acid as the diluent, make the volume up to 100 ml, then dilute 10 ml of this solution to 100 ml. Measure the absorbance of a 1-cm layer of the solution at a wavelength of 280 nm and determine the L-dopa content by comparing the result obtained with values obtained with a "pure" sample of L-dopa.

TITRATION OF MAGNESIUM STEARATE—

Basic function—Add magnesium stearate (0·03, 0·06 or 0·1 mequiv) to a mixture of 0·5 ml of formic acid and 2 ml of acetic acid. Stir the mixture for 5 minutes, add 0·5 ml of α-methylstyrene and titrate the solution according to procedure A (automatic method).

Basic function after precipitation of magnesium as the oxalate—Mix magnesium stearate (0·3, 0·6 or 1·0 mequiv) with 10 ml of formic acid, filter, wash the insoluble material with formic acid and make the volume of filtrate up to 20 ml with formic acid. Add 0·5 g of

dry oxalic acid, stir and allow the solution to stand for 4 hours. Filter and, by using 0·5 ml of the filtrate, determine the basic function as above.

Acidic function—Add magnesium stearate (0·03, 0·06 or 0·1 mequiv) to 1 ml of a 0·1 M solution of benzoic acid in dimethylformamide and titrate the total acidity by using procedure B.

Results and discussion

The sample must be partially soluble, at least, if consistent results are to be obtained by the thermometric method. Except for D- and L-α-methyldopa, which are soluble in dimethylformamide, the catecholamines examined in the present investigation were virtually insoluble in neutral and weakly basic organic solvents.

For the determination of the basic function adrenaline and L-noradrenaline can be dissolved in acetic acid, but with L- and DL-dopa and (+)-Corbasil it is necessary first to dissolve the sample in formic acid before adding acetic acid. To avoid separation of the phases when α-methylstyrene is added to the sample solution there must be present a sufficient excess of acetic acid over the α-methylstyrene. A homogeneous solution can also be achieved by addition of propylene carbonate to mixtures of formic and acetic acids and α-methylstyrene. Formic acid and, to a lesser extent, acetic acid reduce end-point sharpness, particularly in titrations with 0·01 and 0·001 M titrants. With the weaker titrants the proportion of formic and acetic acids is kept to a minimum and 1,2-dichloroethane is used as a diluent. Details of suitable solvent systems are given in Table I.

For titration of the acidic functions, dimethylformamide is a satisfactory solvent for adrenaline hydrogen tartrate and dopamine hydrochloride, as well as for the α-methyldopas. The other catecholamines can be dissolved conveniently in dimethylformamide containing about 1 equiv of *p*-toluenesulphonic acid. It is, of course, necessary to subtract the volume of titrant consumed by the latter from the final titration volume.

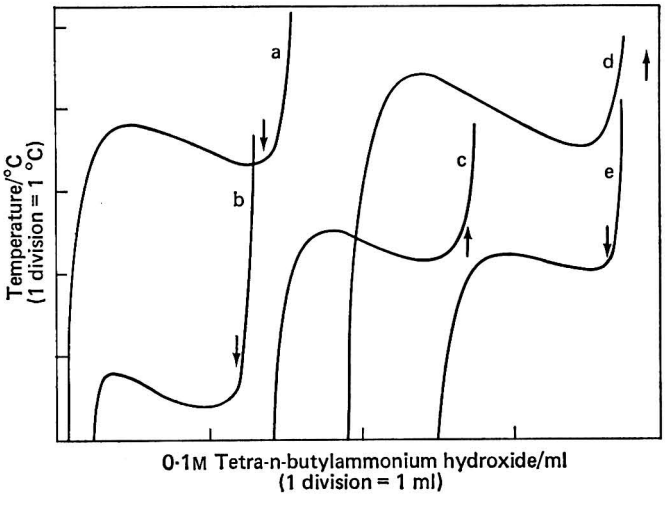

Fig. 2. Thermometric titration of the acidic functions of catecholamines in suspension in weakly basic media

	a	b	c	d	e
Compound/mg	N, 21·5	C, 16·6	A, 21·4	D, 19·0	A, 18·8
Solvent/ml	F, 2	F, 2	P, 1	H, 1	M, 2
Acrylonitrile/ml	1	1	2	2	1

Compounds—N, L-noradrenaline; C, (+)-Corbasil; A, (−)-adrenaline; and D, DL-dopa

Solvents—F, dimethylformamide; P, *NN*-diethyl-3-aminopropionitrile; H, hexamethylphosphoramide; and M, *N*-methylmorpholine

Arrows indicate theoretical end-points

It is possible to titrate these latter catecholamines in dimethylformamide alone, and also in *N*-methylmorpholine, *NN*-dimethyl-3-aminopropionitrile and hexamethylphosphoramide, although they are almost insoluble in these solvents, but the precision of the determination is not good. In the course of the titration there is initially a sharp rise in temperature until the combination of the heat evolved and added titrant brings about dissolution of the sample. The temperature then slowly decreases as neutralisation of the sample continues; when neutralisation is complete the usual sharp temperature rise occurs. The resultant S-shaped titration curve (Fig. 2) is characteristic of the particular catecholamine. The lack of precision of the determination may be caused by the temperature fluctuations.

In determinations of both the basic and acidic groups, amounts of sample were chosen so as to give titration volumes in the range 0·1 to 2·0 ml. In this range, calibration graphs were linear for the 0·1, 0·01 and 0·001 M titrants and 0·002 M tetra-n-butylammonium hydroxide. In determinations of the acidic functions only one of the hydroxyl groups of the catechol moiety could be titrated (see Table III, Part II). Thus the dopa and α-methyldopa isomers and dopamine hydrochloride were determined as dibasic acids and adrenaline hydrogen tartrate was determined as a tribasic acid.

Typical titration curves obtained by using the manual method for the determination of the basic function are shown in Fig. 3. Curves of similar shape were obtained with the automatic apparatus, which was used for most of the determinations including those carried out in order to establish the precision of the method, both for basic and acidic functions. The results of the precision measurements are summarised in Tables II and III. The coefficients of variation are similar in order to those obtained with the simpler bases and acids examined in previous papers,[7,8] and lie in the range 0·2 to 1·76 per cent.

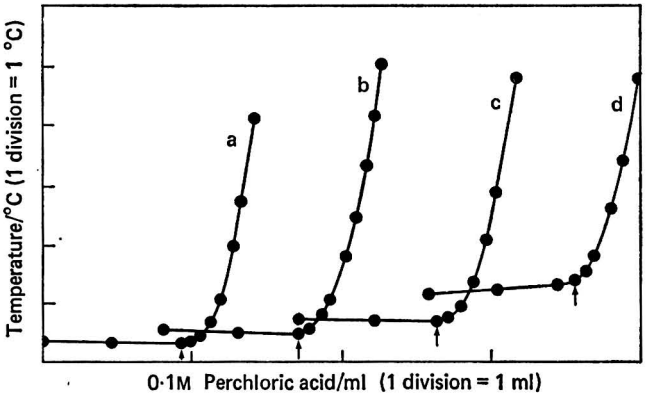

Fig. 3. Thermometric titration curves obtained in determinations of the basic functions by the manual method

Compound/mg	
a L-Dopa, 19·8	b (−)-Adrenaline, 18·0
c L-Noradrenaline, 16·9	d Dopamine hydrochloride, 19·1

Arrows indicate theoretical end-points

With the 0·001 M titrants catecholamines could be determined in amounts down to about 20 μg by titration of the basic function and to less than 20 μg by titration of the polyfunctional acids.

A comparison of the results given in Table II with those in Table III indicate that titration of either the basic or the acidic groups should be a satisfactory procedure for the assay of the catecholamines although, with compounds containing two or more acidic functions, titration of the acidic function has the advantage of higher sensitivity. The L-dopa content of formulations, namely, ordinary tablets, sustained-release tablets and capsules, has been

determined by titration of the basic function and of the titratable acidic functions. The same procedures as those used for titration of the pure catecholamines were used.

The results of these determinations have been compared with those obtained by two methods that are currently used for the assay of L-dopa and its formulations, namely, the recently described B.P. method[6] and ultraviolet spectrophotometry of a solution of the L-dopa in aqueous hydrochloric acid. It can be seen from the summary of the comparative study, shown in Table IV, that the results obtained by the B.P. method, ultraviolet spectrophotometry and thermometric titration, with one exception, do not differ by more than 1·6 per cent., and can be considered to be comparable. The exception is the value obtained in the thermometric titration of the acidic functions of capsules which, at 95·2 per cent., is significantly higher than those obtained by the other methods. This higher value may possibly be attributed to acidic excipient material, *e.g.*, stearic acid or citric acid.

TABLE II

RESULTS FOR PRECISION FROM THE THERMOMETRIC TITRATION OF CATECHOLAMINES WITH 0·1 TO 0·001 M SOLUTIONS OF PERCHLORIC ACID

Catecholamine	Amount/ mg	Titrant molarity*	n†	Mean titre/ml	Standard deviation	Coefficient of variation, per cent.
(−)-Adrenaline	18·36	0·1	4	1·04	0·006	0·56
DL-Dopa	19·56		5	1·01	0·009	0·88
L-Dopa (tablets)	29·52		5	1·04	0·005	0·50
L-Dopa (sustained-release tablets)	31·22		4	1·27	0·011	0·91
L-Dopa (capsules)	20·08		3	0·94	0·016	1·23
(−)-Adrenaline	1·84	0·01	3	1·43	0·006	0·40
(−)-Adrenaline	0·18	0·001	3	0·86	0·015	1·76
L-Noradrenaline	0·17		3	0·95	0·014	1·49
Dopamine hydrochloride ..	0·19		3	1·24	0·007	0·58

* Nominal value.
† Number of determinations.

When the B.P. method is used for the assay of L-dopa formulations, care must be taken in deciding when the end-point has been reached as the indicator, titrant and L-dopa can be adsorbed on to the surface of insoluble excipient material in the sample. If such adsorption is likely, it is advisable to stir the titration solution for about 3 minutes between additions of titrant near the end-point so as to ensure that all of the titrant is consumed. This operation will, of course, increase significantly the time required for each determination.

TABLE III

RESULTS FOR PRECISION FROM THE THERMOMETRIC TITRATION OF CATECHOLAMINES WITH 0·1 TO 0·001 M SOLUTIONS OF TETRA-n-BUTYLAMMONIUM HYDROXIDE

Catecholamine	Amount/ mg*	*p*-Toluene-sulphonic acid/mg	Titrant molarity†	n‡	Mean titre/ml	Standard deviation	Coefficient of variation, per cent.
(−)-Adrenaline ..	15·63	18·98	0·1	3	1·93	0·017	0·89
Dopamine hydrochloride	9·48	—		4	1·03	0·006	0·63
L-Dopa (tablets) ..	15·07	18·98		3	2·50	0·005	0·20
(−)-Adrenaline ..	0·31	1·90	0·01	3	0·41	0·005	1·23
DL-Dopa	0·97	1·90		3	2·18	0·025	1·16
Dopamine hydrochloride	0·94	—		3	1·03	0·006	0·59
Dopamine hydrochloride	0·095	—	0·002	3	0·84	0·012	1·46
DL-Dopa	0·029	0·057	0·001	3	1·70	0·027	1·61

* In 1 ml of dimethylformamide.
† Nominal value.
‡ Number of determinations.

Some formulations contain small amounts of magnesium stearate, *e.g.*, from 0·5 to 2 per cent., as a lubricating agent for ease of tabletting and as a flow promoter. It was found that magnesium stearate dissolved in formic acid is titrated as a base when the thermometric method is used. An addition of 2 per cent. of magnesium stearate increases the titre by

0·66 per cent., which is not insignificant in the assay of catecholamines in formulations. Magnesium can be precipitated as a solvated oxalate from formic acid solutions by the addition of an excess of oxalic acid. However, trace amounts are difficult to remove by precipitation because the oxalate is slightly soluble in formic acid and, after filtration, the solution still contains about 0·01 mequiv ml^{-1}. This amount is equivalent to 10 per cent. of the titre when one is determining catecholamines at the 0·1 mequiv ml^{-1} level.

TABLE IV

COMPARISON OF METHODS FOR THE DETERMINATION OF THE L-DOPA CONTENT OF FORMULATIONS

Sample	B.P. method of assay	Ultraviolet spectrophotometry	Thermometric titration: Basic function	Thermometric titration: Acidic functions
Levodopa tablets (normal)	67·9	68·7	67·2	67·7
Levodopa tablets (sustained release) ..	74·2	74·3	74·9	73·9
Levodopa capsules	92·3	93·2	91·3	95·2
L-Dopa	98·9	(100)*	99·5	100·9

* Calibration standard.

All determinations were carried out on the same bulk sample of each formulation type, prepared by grinding together twenty dosage forms. Values given are per cent. *m/m*, and are the average of three determinations.

In contrast, the effect of magnesium stearate on the titration of acidic functions is small and can be attributed partly, but not wholly, to small amounts of free stearic acid. The addition of 2 per cent. of magnesium stearate to L-dopa would increase the titre by about 0·1 per cent., and for all practical purposes this effect can be ignored. The results of a study of the titration of the basic and acidic functions of relatively large amounts of magnesium stearate are summarised in Fig. 4.

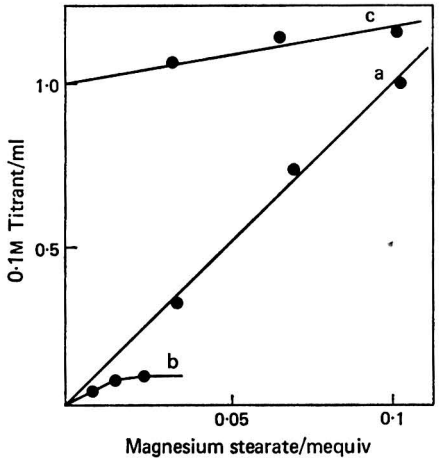

Fig. 4. Calibration graphs for the thermometric titration of magnesium stearate: a, titration of the basic function; b, titration of the basic function after removal of precipitated magnesium oxalate; and c, titration of the acidic function + 1 mequiv of benzoic acid. Details are given in the procedure

Tables II and III show that the precision that can be obtained in the determination of the L-dopa content of formulations is similar to that achieved with the pure catecholamines. When one considers also that the thermometric method could be considerably faster than

the B.P. method, particularly if difficulties arise with indicator adsorption in the latter, then the method reported in this paper might be worthy of consideration as a procedure for routine assays.

With the technique at its present state of development, *i.e.*, with a lower limit of determination of 10 to 20 μg, it cannot yet be considered to be suitable for the determination of catecholamines in body fluids and tissue extracts, unless a concentration step can be introduced into the analytical procedure.

We thank the donors of the test compounds and formulations as indicated above. Mr. M. B. Arthur of Brocades (Great Britain) Ltd. is thanked for helpful advice on assay procedures for L-dopa.

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NOTE—References 7, 8 and 9 are to Parts I, II and III of this series, respectively.

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The Determination of Di-n-alkyl Phthalates in Cosmetic Preparations by Gas - Liquid Chromatography

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An improved gas-chromatographic method for the direct determination of C_1–C_4 di-n-alkyl phthalates in toiletry samples that contain ethanol is described and a range of perfume essential oils and perfume synthetic chemicals is examined for possible interference.

Manufacturers of preparations that contain ethanol are required by H.M. Customs and Excise to include denaturants in order to render the preparations unpotable. The suitability of newly proposed denaturants and their effective concentration are determined according to the recommendations of the Laboratory of the Government Chemist, which examines also the finished products to ensure that the required level of nauseousness is maintained. The list of accepted denaturants is large and increasing and, as the samples examined are drawn from the entire cosmetic range and indeed extend to any retailed preparations (other than beverages) that contain spirit, special analytical problems arise.

Diethyl phthalate is commonly used as a denaturant of toiletries because it is usually compatible with other ingredients and has properties that may be convenient, *e.g.*, as a perfume fixative or plasticiser. Its inclusion at a minimum level of 1 per cent. *V*/*V* in perfumes made with Q-grade industrial methylated spirit to the Statutory Formula II is mandatory.[1] In a previous paper[2] from this laboratory, three methods for the determination of diethyl phthalate were described as follows: by (*i*) direct gas - liquid chromatography with two alternative column systems; (*ii*) isolation of the diethyl phthalate by column chromatography followed by measurement of optical density at 227 nm; and (*iii*) gravimetric determination as phthalanil, which involves alkaline hydrolysis, liberation of the free phthalic acid by acidification and extraction into diethyl ether followed by treatment with aniline to give a precipitate that can be weighed. The acidification stage provides the possibility of determining the phthalic acid by back-titration.

Formerly, the only comparable published gas - liquid chromatographic method for the determination of diethyl phthalate[3] involved the use of 30 per cent. sodium dodecylbenzenesulphonate as the stationary phase and a thermal conductivity detector. Although this system yielded symmetrical peaks, the time required for a single analysis was excessive. By making use of low loadings of polar phases that were stable and non-fugitive at high temperatures, Hancock, Rose and Singer[2] were able to shorten the analysis time and thus provided a useful routine method. Some tailing of peaks was experienced but this was not considered unacceptable. The stationary phases were fluorosilicone oil FS 1265 and silicone gum rubber SE-30, which were used in each instance at a loading of only 1·5 per cent. *m*/*m* on HMDS-treated Chromosorb W. These authors compared results obtained from a range of toiletry samples by the gravimetric method with those obtained by ultraviolet absorption measurements, and those for gas - liquid chromatographic determinations, including also the results calculated from the phthalic acid values obtained by back-titration. Agreement was generally good in both instances but some discrepancies were noted with the comment that the chromatographic method served as a screening technique to overcome the non-specificity inherent in the other methods. It was frustrating that the FS 1265 system, although less prone to tailing, and therefore giving better chromatograms than those obtained with the SE-30 system, failed to resolve diethyl phthalate from isopropyl myristate, a fairly common toiletry ingredient, while, even with SE-30, with which this resolution was complete, it was found that commercial isopropyl myristate contained an impurity that eluted close to diethyl phthalate.

In more recent work in which the determination of diethyl phthalate in small-arms propellents is described,[4] the stationary phase used was 20 per cent. SE-30 on an acid-washed Chromosorb W support at 200 °C, and the published chromatograms show that, even with such high loading of the stationary phase, the problem of tailing had not been entirely eliminated.

The system for the determination of diethyl phthalate now proposed is, as previously claimed,[5] free from the interference complained of and providess ymmetrical peaks at retention times compatible with routine analysis. It is applicable to many other denaturants including dimethyl phthalate and, after slight modification, to di-n-butyl phthalate.

APPARATUS—

A Pye, Model 104, gas chromatograph with a flame-ionisation detector was used.

Columns—Usually glass, 1·52 m × 3 mm i.d. (5 feet × ¼ inch o.d.), but stainless-steel columns have also been used without difficulty.

Packing—This consists of 8 per cent. of nonylphenoxypoly(ethyleneoxy)ethanol on 80 to 100-mesh acid-washed DMCS-treated Chromosorb W. The prepared column contains 5·6 to 5·7 g of packing and should have an efficiency of 1200 to 1800 plates per metre, as measured on the peak obtained from a 1-μl injection of 2 per cent. diphenylamine in ethanol. Columns are packed under gentle suction to within 11 cm of the injection head and both ends are plugged with glass string. The stationary phase, also known as Antarox CO-990 and Igepal CO-990, has been mentioned[6] in connection with the determination of dimethyl phthalate in propellent plasticisers but under slightly different conditions. Its recommended temperature range is 50 to 225 °C.

Column conditioning—Conditioning overnight at 225 °C with nitrogen passing through the column at the rate of 5 ml min^{-1} has proved satisfactory.

Carrier gas—Nitrogen at a flow-rate of 55 to 60 ml min^{-1}.

Column temperature—For dimethyl phthalate and diethyl phthalate, 200 to 210 °C, and for di-n-butyl phthalate, 220 °C.

The temperature of both the injection block and detector oven was 250 °C, and the attenuation was ×5000.

The detector response is measured from a chart recorder as peak height.

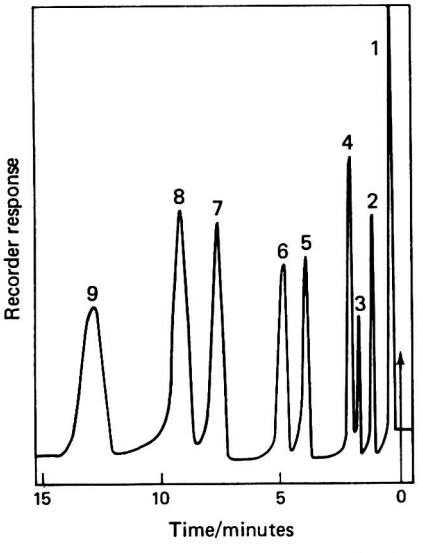

Fig. 1. Gas chromatogram of mixture A. Peaks: 1, ethanol; 2, methyl salicylate; 3, 1-chloro-4-nitrobenzene; 4, isopropyl myristate; 5, dimethyl phthalate; 6, diethyl phthalate; 7, di-n-propyl phthalate; 8, diphenylamine; and 9, di-n-butyl phthalate

Injections were made from a 1-μl syringe fitted with an 11-cm needle.

Unless otherwise stated, puriss. grade chemicals were used; the ethanol used was absolute ethanol.

Fig. 1 shows a chromatogram obtained under these conditions at 205 °C from a 0·2-μl injection of "mixture A," a solution of mixed dialkyl phthalates and some other compounds of interest in ethanol at concentrations ranging from 0·1 to 1·0 g per 100 ml, and in Table I are listed the retention times of the constituents of this mixture in the order of their elution.

TABLE I

ELUTION OF CONSTITUENTS OF "MIXTURE A"

Compound	Retention time/minutes	R_x
Ethanol	0·4	0·08
Methyl salicylate	1·2	0·24
1-Chloro-4-nitrobenzene	1·8	0·37
Isopropyl myristate	2·2	0·45
Dimethyl phthalate..	4·0	0·82
Diethyl phthalate	4·9	(1·00)
Di-n-propyl phthalate	7·5	1·53
Diphenylamine	9·0	1·84
Di-n-butyl phthalate	12·8	2·61

TEST OF LINEARITY OF DETECTOR RESPONSE—

A series of standard solutions containing from 0·5 to 4·0 per cent. V/V of diethyl phthalate in ethanol was prepared and each solution was then mixed with an equal volume of a solution containing 2 g of diphenylamine in 100 ml of ethanol. Each mixture was injected under the conditions described and the peak heights were measured for both components. In Fig. 2, the peak height ratio of diethyl phthalate to diphenylamine is plotted against the initial diethyl phthalate concentration and is clearly linear within the limits of measurement

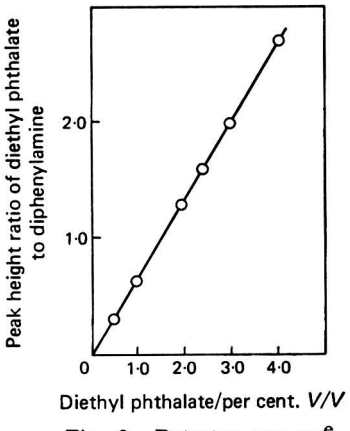

Fig. 2. Detector response over a range of diethyl phthalate concentrations

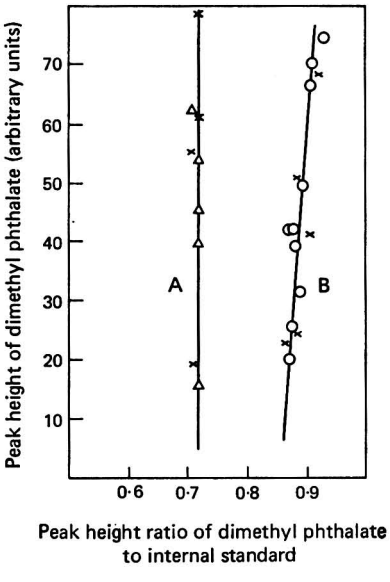

Fig. 3. Calibration graph for determination of dimethyl phthalate. Ratio: A, of dimethyl phthalate to diphenylamine; and B, of dimethyl phthalate to 1-chloro-4-nitrobenzene. ×, Results for first day; and △ and ○, results for second day during continuous running

over the phthalate concentration range 0·2 to 2·0 per cent., which is twice the normal denaturant level.

Analytical methods

Dimethyl phthalate—

Prepare a standard solution containing 0·5 per cent. V/V of dimethyl phthalate and 1·0 g of diphenylamine per 100 ml of ethanol. Make successive injections of this solution over the volume range 0·1 to 1·0 μl under the conditions described above. Measure the peak heights for dimethyl phthalate and diphenylamine and then plot the dimethyl phthalate peak height as ordinate against the peak height ratio of dimethyl phthalate to diphenylamine as abscissa (Fig. 3A). This peak height ratio is 0·6 to 0·7, which enables samples containing excess of dimethyl phthalate up to 50 per cent. above the legal denaturant minimum to be analysed at a single attenuation without dilution.

To a known volume of each sample add an equal volume of a standard solution containing 2 g of diphenylamine per 100 ml of ethanol (for convenience, 2-ml aliquots were dispensed from pipettes into 10-ml McCartney bottles). Inject the mixture and measure the heights of the peaks due to dimethyl phthalate and diphenylamine. Determine the ratio of the former to the latter (x) and read off the ratio (y) corresponding to each dimethyl phthalate peak height from the standard graph. The concentration of dimethyl phthalate in the sample is then x/y per cent. V/V.

If the standard graph is considered to be close enough to the vertical, plotting of ratios can be dispensed with and an average ratio applied to any volume injected; the calculation then becomes one of simple proportion.

The same procedure can be followed with an alternative internal standard. If a solution containing 0·6 g of 1-chloro-4-nitrobenzene in 100 ml of ethanol is substituted throughout for the 2 per cent. diphenylamine solution, analytical results are obtained in exactly the same way. The only differences are that, as 1-chloro-4-nitrobenzene elutes before dimethyl phthalate, the duration of each analysis is reduced while the risk of interference from perfume constituents is increased. A typical standard graph obtained with this internal standard is shown in Fig. 3B.

Diethyl phthalate—

The analytical procedure is exactly as described above for dimethyl phthalate and the same two alternative internal standards are used. Fig. 4 shows a standard graph with diphenylamine as internal standard, to which points were added over a period of 8 days' continuous running.

Di-n-propyl phthalate—

This ester has not yet found use as a denaturant but it has been included in order to complete the C_1–C_4 series. The method is as described for dimethyl phthalate except that the temperature of the column oven is set at 215 °C. Diphenylamine is the appropriate internal standard and, under these conditions, the retention times are 4·9 minutes for di-n-propyl phthalate and 5·6 minutes for diphenylamine.

Di-n-butyl phthalate—

The method is as described for dimethyl and diethyl phthalates but with the following modifications: the temperature of the column-oven is 220 °C; the nitrogen flow-rate is 80 ml min^{-1}; and the two alternative internal standards are diphenylamine and methyl *p*-hydroxybenzoate, each at a concentration of 2 g per 100 ml of ethanol.

Under these conditions, the retention times are diphenylamine 5·4, di-n-butyl phthalate 7·3 and methyl *p*-hydroxybenzoate 11·4 minutes.

Fig. 5A shows a graph of di-n-butyl phthalate peak height against the peak height ratio of di-n-butyl phthalate to diphenylamine over a 2-day period, while Fig. 5B shows the graph obtained over a similar period when the diphenylamine was replaced with methyl *p*-hydroxybenzoate.

Turbidity occasionally results when the sample is mixed with the solution of internal standard. The solution can sometimes be clarified for long enough to permit an injection of homogeneous liquid by gently warming the capped bottle, but if this method fails, dilution

of the mixture with a known excess of ethanol can be combined with appropriate reduction in attenuation. Alternatively, the internal standard can be replaced with the second choice.

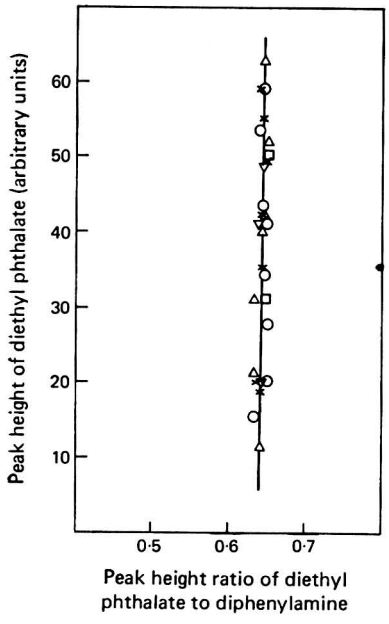

Fig. 4. Calibration graph for determination of diethyl phthalate. Continuous running for 8 days: different symbols indicate results obtained on different days

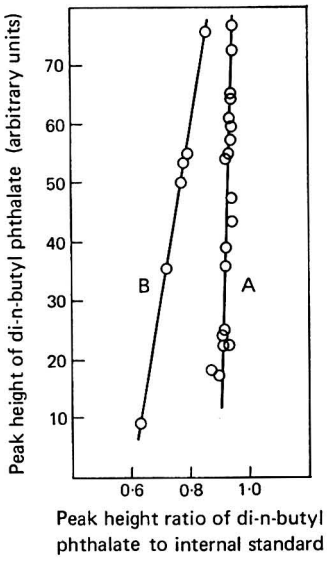

Fig. 5. Calibration graph for determination of di-n-butyl phthalate. Ratio: A, of di-n-butyl phthalate to diphenylamine; and B, of di-n-butyl phthalate to methyl *p*-hydroxybenzoate

Results

Recoveries from solutions of pure phthalates in ethanol—

A range of standard solutions of each ester in ethanol was prepared; with di-n-propyl phthalate a single concentration was considered sufficient. Each ester was weighed, dissolved in and made up to volume with ethanol in calibrated flasks at 20 °C and the solution was then taken for analysis as an unknown. Three to ten determinations were made at each level by the methods described above as appropriate to each ester. Mean recoveries are given in Table II, where the percentage of ester in the prepared solutions has been converted for comparison from grams per 100 ml into per cent. *V*/*V* by means of published density data at 20 °C.

These results provided assurance that the system was functioning correctly and was capable of yielding significant results.

Recoveries from toiletry samples—

For these recovery experiments toiletry samples were chosen to represent various types of goods received for analysis. In each instance the content of the ester concerned had been found to be nil or negligible. Each sample was treated as follows: a 6-ml aliquot was transferred into each of four 14-ml McCartney bottles, 2·0, 1·5, 1·0 and 0·5 ml of ethanol were added to successive bottles followed by 0, 0·5, 1·0 and 1·5 ml, respectively, of the phthalate concerned in ethanolic solution at a concentration of 10 per cent. *V*/*V*, all additions being made by means of graduated pipettes. These mixtures were then analysed by the appropriate procedure as described above. The results are set out in Tables III, IV and V.

TABLE II

RECOVERIES OF PURE ESTERS FROM ETHANOLIC SOLUTION

Ester	Ester dispensed, per cent. *V/V*	Found by analysis, per cent. Against 1-chloro-4-nitrobenzene	Found by analysis, per cent. Against diphenylamine
Dimethyl phthalate	0·18	0·18 ± 0·01	0·19 ± 0·01
	0·45	0·47 ± 0·01	0·45 ± 0·02
	0·75	0·76 ± 0·01	0·74 ± 0·00
	0·99	0·99 ± 0·01	0·99 ± 0·01
	1·38	1·38 ± 0·02	1·37 ± 0·01
	1·77	1·74 ± 0·02	1·76 ± 0·02
	Mean recovery, per cent.	102	99
Diethyl phthalate	0·22	0·21 ± 0·01	0·22 ± 0·00
	0·47	0·47 ± 0·00	0·48 ± 0·00
	0·80	0·80 ± 0·00	0·81 ± 0·01
	1·25	1·25 ± 0·02	1·27 ± 0·01
	1·51	1·52 ± 0·02	1·52 ± 0·01
	1·38	2·02 ± 0·02	1·96 ± 0·01
	Mean recovery, per cent.	100	101
Di-n-propyl phthalate	1·10	—	1·09 ± 0·03
		Against methyl *p*-hydroxybenzoate	Against diphenylamine
Di-n-butyl phthalate	0·20	0·19 ± 0·00	0·19 ± 0·00
	0·40	0·38 ± 0·00	0·38 ± 0·00
	0·79	0·79 ± 0·03	0·79 ± 0·00
	1·19	1·22 ± 0·03	1·21 ± 0·01
	1·98	2·13 ± 0·03	2·14 ± 0·03
	Mean recovery, per cent.	99	100

TABLE III

RECOVERY OF DIMETHYL PHTHALATE

Internal standard	Description of sample	Dimethyl phthalate recovered, per cent. *V/V*, after addition of— Nil	0·5 per cent.	1·0 per cent.	1·5 per cent.
Diphenylamine	Cleansing lotion	Negligible	0·44	1·03	1·48
	After-shave lotion	0	0·52	0·98	1·51
	Toilet water	Negligible	0·51	0·96	1·52
	Eau-de-cologne	0	0·49	1·08	1·50
	Skin tonic	0	0·50	1·02	1·50
	Mean recovery per cent.		98	101	100
1-Chloro-4-nitrobenzene	Perfume	0	0·49	0·95	1·49
	Friction lotion	0	0·50	1·02	1·52
	After-shave lotion	0	0·51	1·10	1·50
	Bay-rum*	0·02	0·51 (0·49)	0·99 (0·97)	1·57 (1·55)
	Mean recovery per cent.		100	100	101

* Figures in parentheses were used in calculation of mean recovery.

EXAMINATION OF PERFUME ESSENTIAL OILS AND PERFUME SYNTHETIC CHEMICALS FOR POSSIBLE INTERFERENCE—

It is clear from the results that the method provides a quick and efficient analytical procedure for the determination of these toiletry denaturants. However, it is appreciated that dependence on a single-column system fails to eliminate the possibility of undetected interference. It was, therefore, a matter of immediate interest to examine a range of typical

TABLE IV

RECOVERY OF DIETHYL PHTHALATE

Internal standard	Description of sample	Diethyl phthalate recovered, per cent. V/V, after addition of— Nil	0·5 per cent.	1·0 per cent.	1·5 per cent.
Diphenylamine	Lavender water	0	0·51	0·99	1·51
	Hair lotion A	0	0·50	1·01	1·50
	Hair lotion B	0	0·50	1·02	1·50
	Spirit shampoo*	0	0·41	0·90	1·45
	After-shave lotion	Negligible	0·50	1·00	1·50
	Vegetable hair tonic	0	0·50	1·00	1·51
	Setting lotion†	0·03	0·50 (0·47)	1·02 (0·99)	1·51 (1·48)
	Tonic cleanser	0	0·48	0·99	1·52
	Toilet water	Negligible	0·50	0·96	1·55
	Skin tests	0	0·51	1·00	1·50
	Mean recovery per cent.		99	100	101
1-Chloro-4-nitrobenzene	Deodorant	0	0·49	1·00	1·48
	Toilet water A	0	0·50	0·99	1·52
	Toilet water B	0	0·51	0·99	1·54
	Skin cleanser	0	0·51	1·08	1·51
	After-shave lotion	Negligible	0·50	1·00	1·51
	Friction lotion	0	0·49	1·03	1·49
	Perfume	Negligible	0·50	1·03	1·49
	Skin tonic	0	0·49	0·98	1·52
	Mean recovery per cent.		100	101	101

* Persistent sediment on mixing.
† Figures in parentheses used in calculation of mean recovery.

perfume synthetic chemicals and some available perfume oils for this possibility. The perfume chemicals were injected as 10 per cent. V/V solutions in ethanol but the perfume oils were injected undiluted with increased attenuation in order to demonstrate trace constituents.

In most instances, elution of all of the compounds was complete within 2 minutes of injection under the conditions of the method. In Table VI the perfume synthetic chemicals are listed alphabetically, giving the retention time of the principal constituent and its R_{DEP} value (diethyl phthalate = 1), together with the number of other constituents; figures in parentheses convey the number of separate peaks that can be ascribed to trace constituents.

TABLE V

RECOVERY OF DI-n-BUTYL PHTHALATE

Internal standard	Description of sample	Di-n-butyl phthalate recovered, per cent. V/V, after addition of— Nil	0·5 per cent.	1·0 per cent.	1·5 per cent.
Diphenylamine	Toilet water	0	0·51	0·98	1·52
	Hair lotion	Negligible	0·47	1·02	1·48
	After-shave lotion	Negligible	0·53	1·02	1·55
	Skin cleanser	0	0·50	1·00	1·52
	Mean recovery per cent.		100	100	101
Methyl *p*-hydroxybenzoate	Skin cleanser	0	0·49	1·04	1·56
	Hair lotion	Negligible	0·53	0·98	1·46
	After-shave lotion	0	0·49	0·99	1·50
	Perfume	0	0·53	1·04	1·53
	Mean recovery per cent.		102	101	101

In some instances spectrographically pure chemicals might have been obtainable but the interest in this survey applied equally to impurities, isomers, etc., that are normally found in commercial-grade products. Comparable results for some perfume essential oils are listed in Table VII, except that no R_{DEP} values are given because, in many instances, no single constituent predominated.

TABLE VI

PERFUME SYNTHETIC CHEMICALS

Compound	Retention time of principal constituent/minutes	R_{DEP}*	No. of impurities
Pentyl acetate	0·3	0·05	1
Benzyl benzoate	11·4	2·07	–
Bornyl acetate	0·9	0·17	2 + (2)
Butyl acetate	0·4	0·08	(2)
Cinnamyl acetate	3·1	0·56	1 + (8)
Citronellol	1·0	0·18	(11)
Citronellyl acetate	0·9	0·17	1
Citronellyl butyrate	(14 constituents in 3·7 minutes)		
Citronellyl isobutyrate	1·0	0·18	1 + (13)
Citronellyl valerate	1·6	0·30	4 + 4
Diethyl malonate	0·6	0·12	1
Diethyl phthalate	5·5	1·00	–
Diethyl succinate	0·8	0·15	1 + (1)
Diethyl tartrate	4·4	0·80	2 + (1)
Diethyl sebacate	11·2	2·14	3
Ethyl acetate	0·2	0·05	–
Ethyl butyrate	0·3	0·05	(1)
Ethyl octanoate	0·6	0·71	3 + (6)
Ethyl cinnamate	3·0	0·54	(6)
Ethyl cyanoacetate	0·9	0·18	2 + (2)
Ethyl heptanoate	0·4	0·08	2 + (4)
Ethyl hexylacetate	0·5	0·10	(1)
Ethyl lactate	0·4	0·08	1 + (2)
Ethyl palmitate	4·6	0·84	3 + (5)
Ethyl phenylacetate	1·2	0·23	1 + (1)
Geraniol	1·1	0·21	1 + (2)
Geranyl acetate	0·8; 1·0	0·14; 0·18	3 + (9)
Geranyl propionate	0·2	0·04	1 + (1)
Isopropyl palmitate	4·6	0·83	(2)
D-Limonene	0·4	0·09	5 + (7)
Linalol	0·6	0·81	(1)
Linalyl butyrate	0·9	0·17	(6)
Linalyl propionate	0·8	0·15	2 + (9)
Menthyl acetate	0·7	0·12	1 + (4)
Menthyl salicylate	7·3	1·32	1
Musk (synthetic)	9·7	1·77	1
Nerol	1·0	0·18	(1)
Nerolidyl acetate	0·6	0·11	1 + (3)
Nerolidyl propionate	3·0	0·54	1 + (7)
α-Pinene	0·3	0·06	3 + (2)
Pulegol	0·7	0·14	2 + (3)
Sextol phthalate	14	1·54	(Poor response)
Terebene	0·5	0·10	2 + (5)
α-Terpineol	0·9	0·17	2 + (5)
Terpineol	0·9	0·17	2 + (10)
Terpinoline	0·4	0·07	4
α-Terpinyl acetate	1·1	0·21	2 + (5)
Tetrahydrogeraniol	0·7	0·14	1 + (4)

* DEP denotes diethyl phthalate.

It will be noted that, on this evidence, the risk of interference is small. The first constituent peak obtained from Indian sandalwood oil is the only one of all those examined which would affect the validity of the analysis. The ultraviolet absorption spectrum of a solution of diethyl phthalate in ethanol was compared with that of a solution of Indian sandalwood oil in ethanol at the same concentration. The sandalwood oil was shown to be free from diethyl phthalate and the ultraviolet method would therefore resolve any difficulty with formulations that contain both of these substances.

The authors thank Mr. A. J. Blake and Mr. G. A. Pask for technical assistance and the Government Chemist for permission to publish this paper.

Table VII

Perfume essential oils

Oil (with source, if known)	Retention times of principal constituents/ minutes	Number of subsidiary constituents	Retention time of final constituent/ minutes
Oil of fennel (U.S.S.R.)	0·5; 1·3	9	9·1
Geranium bourbon essence ..	0·7; 0·9; 1·1	14	5·0
Juniper berry oil (U.K.)	0·4; 0·4	12	4·1
Lavender oil	0·5; 0·7; 0·8	8	2·4
Lavender exotic	0·4; 0·6; 0·7	10	2·4
Lavender	0·4; 0·6; 0·8	8	2·3
Lavender abrialis (France) ..	0·2; 0·5; 0·5	9	2·4
Lemon oil (Italy)	0·5	8	1·5
Oil of limes (U.K.)	0·3; 0·4; 0·9; 1·0	4	1·2
Litsea cabeba (China)	0·4; 1·0	15	4·7
Mace oil	0·3; 0·4; 0·7; 4·1	12	4·15
Oil of marjoram	0·5; 0·5; 0·7; 0·9	20	4·9
Myrcene	0·4; 4·1; 4·7	9	4·7
Oil of rosemary	0·3; 0·4; 0·8	6	2·4
Oil of rosemary (Spain)	0·4; 0·5; 0·8	6	2·4
Oil of rosemary (Peru)	0·5; 0·9	14	4·8
Sandalwood oil (India)	5·4; 6·7	13	9·0
Tangerine oil	0·4; 0·5	5	2·7
Oil of thyme, red	0·6; 3·2	14	8·5
Oil of thyme, white	0·6; 3·2	10	3·4
Oil of ylang ylang (U.K.) ..	0·5; 0·6; 1·0; 1·2; 1·3	13	5·2
Oil of ylang ylang (France) ..	0·8; 0·9; 1·1; 1·2	15	5·2
Diethyl phthalate	5·5	—	—

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Residues of Prophylactics in Animal Products

Part III.* The Determination of Carbarsone in Poultry Meat

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A method for the determination of carbarsone in poultry meat is described. The carbarsone is extracted from the sample with methanol and, after clean-up on an ion-exchange column, hydrolysed to arsanilic acid with sodium hydroxide. The arsanilic acid is diazotised, coupled with 2-aminoethyl-1-naphthylamine and determined spectrophotometrically.

Carbarsone (4-ureidophenylarsonic acid) is incorporated into poultry feedingstuffs for growth-promoting or prophylactic purposes, usually at a level of about 375 mg kg^{-1}. The need exists for a method for the determination of residues of carbarsone that could occur in meat derived from poultry treated with this compound, other than by determination of the total arsenic content, which is capable of being used to determine residues of carbarsone down to at least 1 mg kg^{-1}. This level corresponds to 0·29 mg kg^{-1} as elemental arsenic, which is well below the limit of 1 mg kg^{-1} specified in the Arsenic in Food Regulations.[1] Although total arsenic determinations are sufficient to check compliance with the Arsenic in Food Regulations, the proposed method supplies additional information concerning the form in which the arsenic is present.

Weston, Wheals and Kensett[2] have published a method for the determination of carbarsone in animal feedingstuffs. Their method is based on the conversion of carbarsone into arsanilic acid, which is then reduced to aniline and the aniline determined by gas chromatography with flame-ionisation detection. However, flame-ionisation detection was found not to be sufficiently sensitive or selective for the amounts of residues likely to occur in animal tissues and so attempts were made to prepare a derivative of aniline suitable for electron-capture detection. Derivatives were prepared by reaction with bromine, trichloroacetic anhydride, trifluoroacetic anhydride, 1-fluoro-2,4-dinitrobenzene and heptafluorobutanoyl chloride. The heptafluorobutanoyl derivative was found to be the most suitable. The procedure of Weston, Wheals and Kensett was applied to treated chicken tissues and the aniline obtained was converted into the heptafluorobutanoyl derivative. The results were unsatisfactory because variable recoveries resulted and co-extractives gave rise to high blank values. Various clean-up procedures were examined, the most suitable of which was thin-layer chromatography. By use of thin-layer plates coated with cellulose and a mixture of butan-1-ol, glacial acetic acid and water (50 + 25 + 25) as the developing solvent, carbarsone could be separated from most of the co-extracted material, but the interfering material that gave rise to the high blank values was not removed. This failure may occur because the method is prone to interference from compounds that yield aniline or a similar amino compound on hydrolysis and reduction.

To overcome this problem of interference a method was sought in which the phenylarsonic acid moiety of the molecule remained intact; the possibility of using fluorescence spectrophotometry was therefore examined. It was found that an aqueous solution of carbarsone fluoresced at a wavelength of 315 nm when excited at 255 nm. However, an aqueous extract of chicken tissue contained co-extractives that interfered, and none of the clean-up procedures tried was effective in removing this co-extracted material. These difficulties may arise because a wavelength of 255 nm is required for excitation, as Parker[3] has shown that most impurities present in solvents fluoresce when excited at 250 nm.

As the above approaches proved unsuccessful, methods based on colorimetric procedures were investigated. The basis of these methods was conversion of carbarsone into arsanilic acid, diazotisation of the arsanilic acid and coupling with first 2-aminoethyl-1-naphthylamine,[4] secondly 1-naphthol[5] and thirdly resorcinol.[5] The diazotisation of the arsanilic acid was carried out as described by El-Dib[5] as this method was found to be more effective than the procedure of Bratton and Marshall[6] and gave low blank values. The procedure involving

* Part II of this series appeared in *Analyst*, 1972, **97**, 254.

the use of 2-aminoethyl-1-naphthylamine was preferred as the dye produced can be extracted into butan-1-ol and is more sensitive. Accordingly, the method proposed by the Society for Analytical Chemistry Prophylactics in Animal Feeds Sub-Committee[4] has been adapted so as to enable amounts of carbarsone down to 1 μg to be determined.

Before this colorimetric determination could be applied to an extract of chicken tissue, it was necessary to clean up the extract and it was considered desirable that the clean-up should also give specificity to the method. Use has therefore been made of the ionic character of the arsonic acid group. Carbarsone can be held on an anion-exchange resin and, after washing the resin with water to remove most of the interfering material, can be eluted with sodium chloride solution. Sephadex QAE anion exchanger has been found to be the most suitable material.

Method

Apparatus—

Chromatographic columns—These were made of glass, 140 mm in length and of 19 mm i.d., fitted with a PTFE stopcock and with a 100-ml capacity reservoir.

Preparation of chromatographic column—Place sufficient glass helices in the chromatographic column to fill the narrow tube that joins the stopcock to the column. Insert a small plug of cotton-wool on top of the helices and add 3 g of sand to form a level base. Carefully add a prepared slurry of 2 g of Sephadex QAE in water and allow it to settle to give a column about 50 mm in height. Run off the water and wash down any Sephadex adhering to the walls of the glass column, then place more sand on top of the Sephadex to form a layer about 5 mm deep. Prepare a separate column for each sample.

Filtration apparatus—This comprised a Büchner funnel fitted with a glass sinter of porosity 3, an adaptor with a side-arm for connection to a suction pump and a 150-ml conical flask.

Mixer—A high-speed laboratory mixer, made by Silverson Machines Ltd., was used.

Reagents—

All reagents should be of analytical-reagent grade quality.

2-Aminoethyl-1-naphthylamine dihydrochloride solution—Dissolve 50 mg of 2-aminoethyl-1-naphthylamine dihydrochloride in water and dilute to 50 ml. Prepare the solution freshly each day.

Butan-1-ol.

Carbarsone standard solution—Dissolve 10·0 mg of 4-ureidophenylarsonic acid in water and dilute to 100 ml; then dilute 2·5 ml of this solution to 100 ml. The latter solution contains 2·5 μg ml^{-1} of carbarsone.

Celite 545—This was obtained from Koch-Light Laboratories Ltd.

Hydrochloric acid, 5 M.

Methanol.

Sand, acid washed—Heat the sand at 500 °C for 10 hours, cool and store it at room temperature.

Sephadex QAE ion exchanger, Type A-25—Allow 2-g portions of the Sephadex to swell in 25 ml of water, either at room temperature for 1 to 2 days or on a boiling water bath for 2 hours.

Sodium chloride.

Sodium chloride solution, 0·3 M.

Sodium hydroxide, pellets.

Sodium nitrite solution—Dissolve 1 g of sodium nitrite in water and dilute to 50 ml. Prepare this solution immediately before use.

Sulphamic acid solution—Dissolve 2·5 g of sulphamic acid in water and dilute to 25 ml. Prepare the solution freshly each day.

Procedure—

Transfer 25 g of minced poultry meat into a 250-ml beaker, add 70 ml of methanol and macerate the mixture with a high-speed mixer for 2 minutes. Filter the extract through the Büchner funnel, which has previously been covered with a 2-mm layer of Celite. Repeat the extraction twice by macerating the sample with 70-ml portions of methanol and filtering through the Büchner funnel. Combine the filtrates and dilute to 250 ml with methanol.

Transfer by pipette a 10 or 25-ml aliquot of this last solution to a small beaker and evaporate it to about 1 ml on a steam-bath. Add a few millilitres of water and pour the solution on to the top of a prepared Sephadex column. Allow the solution to pass through the column until the liquid level reaches the top of the column and collect the eluate in a receiver that is capable of measuring a volume of 100 ml. Wash out the beaker with small volumes of water and transfer the washings to the column, then pass water through the column at a rate of 1 to 2 ml min^{-1} until 100 ml of eluate have been collected. Discard the eluate. Pass 0·3 M sodium chloride solution through the column at a rate of 1 to 2 ml min^{-1}, reject the first 10 ml of eluate and collect the next 30 ml in a 150-ml flask.

To the contents of the flask add 5 g of sodium hydroxide pellets and, when these have dissolved, boil the solution gently for 1·25 hours under a reflux condenser. Wash down the condenser with a few millilitres of water and cool the flask in a cold water bath. Add 30 ml of 5 M hydrochloric acid, mix, again cool in a cold water bath and transfer the acidified solution to a 100-ml separating funnel with the aid of a small volume of water. Add 1 ml of sodium nitrite solution, mix thoroughly and, after 3 minutes, add 1 ml of sulphamic acid solution. Allow the mixture to stand for 15 minutes, shaking it frequently so as to ensure complete destruction of the excess of sodium nitrite. Add 1 ml of the 2-aminoethyl-1-naphthylamine dihydrochloride solution and mix well. After 10 minutes add 10 ml of butan-1-ol and shake the mixture, then add 10 g of sodium chloride, again shake well and allow the layers to separate. Run the lower, aqueous layer into a second separating funnel and the butan-1-ol into a 25-ml calibrated flask. Extract the aqueous layer twice more, first with 10 ml and then with 5 ml of butan-1-ol, add the extracts to the 25-ml calibrated flask and dilute to the mark with butan-1-ol. Measure the absorption of this solution at a wavelength of 542 nm in a 4-cm cell against butan-1-ol as reference solution. Ascertain the amount of carbarsone present in the sample solution by reference to a standard graph.

Preparation of standard graph—

Transfer 1, 2, 3 and 4-ml portions of the carbarsone standard solution, equivalent to 2·5, 5·0, 7·5 and 10·0 μg of carbarsone, to separate 150-ml flasks. Add 0·3 M sodium chloride solution to the contents of each flask until the volume of solution is 30 ml in each instance, and then proceed as described above, commencing at "... add 5 g of sodium hydroxide pellets." The standard solutions should be run through the procedure at the same time as the sample with use of the same reagents.

Results and discussion

Carbarsone is soluble in water and methanol but is only sparingly soluble in other organic solvents. Methanol was found to be the most suitable solvent for the extraction of carbarsone from chicken tissue because with water or dilute acid increased problems were encountered in the filtration step, and no other suitable extraction procedure could be found.

The recovery of carbarsone from chicken tissue was simulated by adding known volumes of a solution of carbarsone in methanol to weighed amounts of minced tissue and leaving them overnight before extraction as described under Procedure. The results are shown in Table I. The recovery appears to depend on the procedure used for spiking the samples, because recoveries varied with the length of time that elapsed between spiking and extraction. Recoveries of over 90 per cent. were obtained when carbarsone was added to a methanolic extract of chicken tissue or when the sample of tissue was extracted immediately after spiking, whereas, if the spiked sample was kept for 3 to 4 days before extraction, the recoveries were about 60 per cent. Consequently, it is important that samples are extracted immediately after being minced and the carbarsone content of the extract determined without delay. As is usual in residue work, it is difficult to obtain standard samples with known amounts of the compound under investigation incorporated in the tissue so as to enable absolute determinations to be made.

Most chicken samples tested gave a small blank value, corresponding to 0·4 mg kg^{-1} of carbarsone. Total arsenic determinations were carried out on chicken sample B (Table I) and the arsenic content was found to be less than 0·1 mg kg^{-1}. The history of these samples was not fully known, so it is possible that the blank value may be the result of an additive fed to the birds at some time during their lifetime.

TABLE I

RECOVERY OF CARBARSONE FROM CHICKEN TISSUE

Sample	Carbarsone added/mg kg^{-1}	Carbarsone found/mg kg^{-1}	Recovery, per cent.
A	0	0	—
	1·0	0·75	75
	1·0	0·7	70
	1·0	0·7	70
	1·0	0·75	75
	1·0	0·7	70
	1·0	0·7	70
	1·0	0·7	70
	1·0	0·7	70
	1·0	0·75	75
		Mean	72
B	0	0·45	—
	3·0	2·5	68
	3·0	2·5	68
	3·0	2·6	72
	3·0	2·6	72
	3·0	2·6	72
	3·0	2·6	72
		Mean	71
C	0	0·4	—
	3·0	2·8	80
	3·0	2·6	73
	3·0	2·35	65
		Mean	73
B	0	0·45	—
	6·0	4·75	72
	6·0	4·5	68
	6·0	4·75	72
	6·0	4·5	68
	6·0	4·75	72
	6·0	4·5	68
		Mean	70
D	0	0·4	—
	6·0	4·4	67
	6·0	4·7	72
	6·0	4·4	67
		Mean	69

The yield of arsanilic acid from carbarsone is dependent on the concentration of sodium hydroxide and the hydrolysis time. Conversion into arsanilic acid was complete when 5 g of sodium hydroxide were added to the eluate from the Sephadex column and the solution heated for 1·25 hours. After formation of the dye by the coupling of diazotised arsanilic acid with 2-aminoethyl-1-naphthylamine, it was found necessary to extract the aqueous phase more than once with butan-1-ol in order to ensure complete extraction of the dye. Once the dye had been extracted into butan-1-ol it was stable for at least 3 hours.

There was no interference from amprolium, dinitolmide, ethopabate or sulphaquinoxaline. Of the other arsenicals used in animal feedingstuffs, nitarsone (4-nitrophenylarsonic acid) and roxarsone (4-hydroxy-3-nitrophenylarsonic acid) did not interfere. However, arsanilic acid is eluted with carbarsone from the ion-exchange column and is determined by the procedure, but the interference from this source can be overcome by applying the method to a second aliquot of the sample extract with which the hydrolysis stage has been omitted. The carbarsone content of the sample can then be calculated by subtracting the absorbance obtained without hydrolysis from the value obtained after hydrolysis.

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The Determination of Microgram Amounts of Sulphate by Emission Spectroscopy of Barium with a Nitrous Oxide - Acetylene Flame

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The sulphate content of aqueous solutions has been determined indirectly, in the ranges 0·5 to 5·0 and 1·0 to 10·0 p.p.m., from barium emission measurements. By using a slight excess of barium, the sulphate is precipitated in a 50 per cent. solution of propan-2-ol and its concentration is calculated from the decrease in the barium content of the solution. The amount of barium in solution is determined from its emission at 553·55 nm in a nitrous oxide - acetylene flame. The only major flame interference detected is that from the band emission of calcium; 410 p.p.m. of calcium gave an emission intensity equal to that of 1 p.p.m. of barium. Sulphate has been determined in both pure solutions and in synthetic sample solutions containing other electrolytes. Major interferences were noted for potassium and ammonium oxalate, sodium orthovanadate, nickel chloride and to a lesser extent for sodium fluoride and perchloric acid. The sulphur content of biological material, digested by oxygen-flask combustion, has been determined satisfactorily by using this method.

The determination of barium as a method of indirectly measuring microgram amounts of sulphate has, in the past, had limited application owing to interferences and the low sensitivity of the techniques[1–7] available for determining barium, which have made the method relatively unattractive.

Some procedures,[8,9] while possessing sensitivity, have not been amenable to rapid routine analysis because of the need to use radioisotopes. However, recent work[10] with the nitrous oxide - acetylene flame has enabled a sensitive, non-radiochemical method for measuring barium to be produced, which is comparatively free from interferences.

A simple procedure is described here, which depends on the precipitation of barium sulphate in a 50 per cent. solution of propan-2-ol. This medium was chosen because it reduces the solubility of barium sulphate to an acceptable level, and because it can be sprayed into a nitrous oxide - acetylene flame without causing instability.

A particular range of sulphate concentrations can be measured by selecting an amount of barium slightly in excess of that which is equivalent to the maximum amount of sulphate. Following precipitation and centrifugation, the unreacted barium content of the solution can be determined from its emission intensity at 553·55 nm in the nitrous oxide - acetylene flame. It has been found that this method can be used for the measurement of sulphate concentrations in the presence of an excess of many electrolytes.

Experimental

Instrumental—

A Techtron AA4 spectrophotometer, with a reciprocal dispersion of 3·3 nm mm^{-1}, was operated with a flame-emission accessory and a slit width of 50 μm. The nitrous oxide - acetylene flame was formed on a Techtron AB50 burner. The nitrous oxide pressure was maintained at 26 p.s.i. while the acetylene flow was adjusted to give maximum barium emission.

Measurement of emission—After a 5-minute warm-up period the test solutions were introduced into the flame through a Techtron nebuliser and spray chamber. The flame was viewed lengthwise and the emission intensity of the atomic resonance line at 553·55 nm was measured without noise suppression. The emission signal from barium in a 50 per cent. solution of propan-2-ol was as stable as that originating from aqueous solution. However, the approach to a steady reading following sample changes was considerably slower than that for an aqueous solution.

REAGENTS—

Analytical-reagent grade chemicals were used throughout.

Standard barium solution—Dissolve 0·9782 g of barium chloride dihydrate in distilled water and dilute to 1 litre to give a stock solution containing 550·0 p.p.m. of barium. Dilute 5 and 10-ml volumes of the stock solution to 100 ml so as to give working solutions containing 27·5 and 55·0 p.p.m. of barium, respectively.

Stock sulphate solution, 1000 p.p.m.—Dissolve 1·814 g of potassium sulphate in distilled water and dilute the solution to 1 litre. Prepare working solutions in the concentration range 0·5 to 10 p.p.m. of sulphate by appropriately diluting the stock solution.

Chloroacetic acid - potassium hydroxide solution—Dissolve 47·25 g of chloroacetic acid in distilled water. Add 3·40 g of potassium hydroxide and dilute the solution to 250 ml. Filter the solution through Whatman No. 542 filter-paper before use.

Propan-2-ol.

RECOMMENDED PROCEDURE—

Quickfit conical flasks with ground-glass stoppers were used as reaction vessels. Reaction volumes were prepared by mixing 10 ml of aqueous sulphate solution, 15 ml of propan-2-ol, 2 ml of chloroacetic acid - potassium hydroxide solution and 3 ml of standard barium chloride solution. (The barium concentration of the standard solution is chosen so as to accommodate the required sulphate range.) For the ranges 0·5 to 5·0 and 1·0 to 10·0 p.p.m. of sulphate it is recommended that solutions containing 27·5 and 55·0 p.p.m. of barium, respectively, be used.

The flasks were agitated for 15 hours, and the barium sulphate suspension was centrifuged at 3500 *g* for 20 minutes. Aliquots of the supernatant liquid were then taken for emission analysis.

Although the two ranges of sulphate concentration given above overlap considerably, the use of the weaker barium solution is advantageous for smaller sulphate concentrations, particularly at and below the 1 p.p.m. level, because of the nature of the difference method used.

DEVELOPMENT OF THE METHOD

ENHANCEMENT OF BARIUM EMISSION BY POTASSIUM—

Results similar to those reported[10] for 1 p.p.m. of barium in aqueous solution were obtained for 1 p.p.m. of barium in a 50 per cent. solution of propan-2-ol. The barium emission intensity obtained in the presence of 600 p.p.m. of potassium (the concentration of potassium obtained from the chloroacetic acid - potassium hydroxide solution) was 97 per cent. of that obtained in the presence of 1000 p.p.m. of potassium.

EMISSION INTENSITY AS RELATED TO BARIUM CONCENTRATION—

The relative emission intensity was found to be linearly related to barium concentration over a wide concentration range (Table I).

The background emission at 553·55 nm originating from the flame and reagents is shown for a zero addition of barium, relative to 2·42 and 22·3 p.p.m. of barium.

TABLE I

VARIATION OF EMISSION INTENSITY WITH BARIUM CONCENTRATION

Barium concentration, p.p.m.	Relative emission intensity*	Barium concentration, p.p.m.	Relative emission intensity†
0	6	0	0·8
0·17	13·5	1·72	9·8
0·34	20·2	3·44	17·5
0·81	37·5	5·15	26
1·20	54·5	8·58	43
1·61	70	12·0	58
2·42	100	17·2	80
		22·3	100

* Relative to 2·42 p.p.m. of barium = 100.
† Relative to 22·3 p.p.m. of barium = 100.

ENHANCEMENT OF BARIUM EMISSION BY PHOSPHATE—

The effect of phosphate (as potassium dihydrogen orthophosphate) on the emission intensity produced by 2·7 and 5·3 p.p.m. of barium was measured for amounts containing 0 to 10 mg of phosphorus. The presence of 4 mg of phosphorus in solution produced an enhancement of the order of 2 per cent. for both barium concentrations. A maximum enhancement of 8 per cent. was obtained from 10 mg of phosphorus in a solution containing 2·7 p.p.m. of barium. The total volume of each solution was 30 ml. The results given later in Table VII for added potassium chloride (22 mg) show that this enhancement is not due to the suppression of barium ionisation by potassium.

CALCIUM EMISSION AT 553·55 nm IN THE NITROUS OXIDE - ACETYLENE FLAME—

The emission intensity from calcium in a 50 per cent. solution of propan-2-ol in the presence of potassium was linearly related to concentration, but the intensity was reduced by a factor of 2·6 when the Techtron burner head AB40 was replaced with the Model AB50. While using the latter the net emission from 410 p.p.m. of calcium was identical with that produced by 1 p.p.m. of barium.

EFFECT OF REACTION TIME ON PRECIPITATION—

A factor that greatly affects the measurement of sulphate concentration is the rate of growth of barium sulphate crystals during precipitation. By using sulphate labelled with sulphur-35 it was possible to measure the efficiency of removal of sulphate from solution as barium sulphate. The efficiencies obtained by centrifuging at 3500 *g* are given in Table II as functions of sulphate concentration, reaction time and degree of agitation of the solution.

It is evident from Table II that, by agitating the solutions during precipitation, the efficiency of sulphate separation at low concentrations was increased. A high efficiency of separation at all concentrations in the range 0·5 to 5·0 p.p.m. of sulphate was obtained when the reactants were agitated for 15 hours. Maximum efficiency was achieved within 15 hours for all concentrations in the range 1·0 to 10·0 p.p.m. of sulphate.

TABLE II

PERCENTAGE OF SULPHUR-35 ACTIVITY REMOVED BY CENTRIFUGATION FOLLOWING PRECIPITATION

	Unstirred solution		Agitated solution		
Reaction time/hours	14	90	0·5	2·5	15
Sulphate concentration, p.p.m.					
0·5	75	92	44	67	94
1·01	88	95	58	84	96
2·01	91	97	74	93	98
4·03	95	97	—*	—*	97
5·04	97	97	77	94	96

* Not determined.

RESULTS

SULPHATE DETERMINATION IN STANDARD SOLUTIONS—

The sulphate contents of dilute potassium sulphate solutions were measured by using standard barium chloride solutions containing 24·9 and 49·7 p.p.m. of barium. The more dilute barium solution was used for the range 0·5 to 5·0 p.p.m. of sulphate.

Table III gives the emission from the unreacted barium for various sulphate concentrations. The theoretical emission intensities, calculated for stoicheiometric reactions in which all of the sulphate is precipitated, are given in Table III for comparison.

As barium sulphate has an acceptably small but nevertheless finite solubility in a 50 per cent. solution of propan-2-ol, the measured emission values (adjusted for flame background) should, in principle, be slightly greater than the theoretical values given in Table III.

EFFECT OF PHOSPHATE ON PRECIPITATION—

The presence of phosphate (as potassium dihydrogen orthophosphate) during the precipitation reaction caused, in some instances, a reduction in the residual emission intensity.

TABLE III

RESIDUAL EMISSION INTENSITY AS A FUNCTION OF SULPHATE CONCENTRATION

Sulphate* concentration, p.p.m.	Emission		Sulphate† concentration, p.p.m.	Emission	
	Measured (adjusted)	Theoretical		Measured (adjusted)	Theoretical
0	100	100	0	100	100
0·81	84·5	84·5	1·22	89·0	88·3
1·22	76·5	76·6	2·03	82·0	80·5
2·03	60·0	61·1	4·05	62·5	61·1
4·05	19·5	22·4	4·87	54·5	53·3
4·87	6·2	6·7	6·07	42·5	41·7
			9·33	11·0	10·4

* Precipitated with a solution containing 24·9 p.p.m. of barium.
† Precipitated with a solution containing 49·7 p.p.m. of barium.

By precipitating in the presence of 2·5 mg of phosphorus, the effect of the phosphate on the determination of sulphate in the ranges 0·5 to 5·0 and 1·0 to 10·0 p.p.m. of sulphate, by using solutions containing 26·6 and 53·2 p.p.m. of barium, respectively, was ascertained. The results are given in Table IV.

TABLE IV

EFFECT OF 2·5 mg OF PHOSPHORUS AS PHOSPHATE ON RESIDUAL EMISSION INTENSITY

Sulphate* concentration, p.p.m.	Residual emission intensity		Sulphate† concentration, p.p.m.	Residual emission intensity	
	Measured (adjusted)	Theoretical		Measured (adjusted)	Theoretical
0	100	100	0	100	100
2·5	76·0	77·6	0·5	90·3	91·0
5·0	51·0	55·2	1·0	81·2	82·1
6·0	41·3	46·2	2·0	62·8	64·1
7·5	27·3	32·7	3·0	45·6	46·2
9·0	16·9	19·3	5·0	12·5	10·3
10·0	9·9	10·3			

* Precipitated with a solution containing 53·2 p.p.m. of barium.
† Precipitated with a solution containing 26·6 p.p.m. of barium.

The presence of 2·5 mg of phosphorus reduced the residual barium emission intensity obtained for the concentration range 1 to 10 p.p.m. of sulphate, the maximum reduction in intensity occurring at 6·0 p.p.m. of sulphate. In contrast, a negligible effect was observed for the range 0·5 to 5 p.p.m. of sulphate.

The reduction in residual barium emission following a precipitation is in contrast with the enhanced barium emission in the presence of phosphate referred to earlier. It is believed that phosphate becomes involved in the precipitation process and that a small amount of barium, in addition to that removed stoicheiometrically with sulphate, is lost from solution.

The influence of different amounts of phosphate on the residual emission intensity obtained for a single sulphate concentration in the range 1 to 10 p.p.m. of sulphate is given in Table V. In order to obtain the maximum variation in results an aliquot containing 6·15 p.p.m. of sulphate was allowed to react with 3 ml of a solution containing 55·21 p.p.m. of barium (see Table IV).

Table V shows that 0·125 mg of phosphorus produces an effect of about 2 per cent. An increase from 0·125 to 1·00 mg of phosphorus resulted in a further reduction in emission intensity, while the change from 1·00 to 2·63 mg had no additional effect. By reducing the residual barium emission intensity, phosphate causes an overestimation of the sulphate concentration.

At the sulphate concentration that gives maximum phosphate interference the presence of as much as 0·125 mg of phosphorus as phosphate can be tolerated. Even when 2·63 mg of phosphorus as phosphate is present the sulphate concentration is overestimated by only 9 per cent. The effect of phosphate on the determination of sulphate can be partially eliminated by preparing a standard graph from sulphate solutions containing phosphate. However, this standard graph will not be linear.

TABLE V

VARIATION OF RESIDUAL EMISSION INTENSITY WITH PHOSPHATE CONTENT

Phosphate content/mg of phosphorus ..	0	0·125	0·25	0·50	1·00	2·63
Residual emission intensity	50·3	48·7	47·9	46·9	45·7	45·7

INFLUENCE OF OTHER ELECTROLYTES ON PRECIPITATION—

Microgram amounts of sulphate were precipitated in the presence of different electrolytes in order to determine the possible effect of each on the measurement of sulphate concentrations by this procedure. The effect of each salt, with the exception of calcium nitrate, was examined for only one sulphate concentration (see Table VI).

In Table VII the effect of several other salts, at a single concentration, is given so as to indicate interferences arising from reaction with barium alone or from an effect on the precipitation reaction.

TABLE VI

EFFECT OF ELECTROLYTES ON RESIDUAL BARIUM EMISSION INTENSITIES OBTAINED FROM THE PRECIPITATION OF 4·62 p.p.m. OF SULPHATE

Sodium fluoride/mg	Residual barium emission intensity	Perchloric acid/mmol	Residual barium emission intensity	Potassium oxalate/mg	Residual barium emission intensity
0	62·8	0	61·4	0	61·4
1·5	61·7	0·24	60·4	1·0	59·4
3	62·3	0·47	61·4	2·1	57·9
6	56·6	0·93	61·9	4·1	55·8
9	51·5	1·39	67·5	6·15	54·3
15	40·8	2·32	75·6	10·25	51·8

Calcium nitrate—The effect of 2 mg of calcium [as $Ca(NO_3)_2.4H_2O$] on the precipitation was determined for several sulphate concentrations in the range 1 to 10 p.p.m. of sulphate. It was found that, after subtracting the calcium component of the emission, the residual barium emission intensity obtained for each sulphate concentration was identical, within experimental error, with that given in Table III.

TABLE VII

INDICATIONS OF THE EFFECT OF OTHER SALTS ON SULPHATE DETERMINATIONS

Salt	Amount/mg	Relative effect on barium alone* (emission units)	Relative effect on sulphate precipitation† (emission units)
None	—	50	62·3
Magnesium chloride hexahydrate	16	49	61·7
Cobalt chloride hexahydrate ..	20	49·5	61·2
Nickel chloride hexahydrate ..	20	44	50·0
Sodium orthovanadate	6 (approx.)	46	51·0
Sodium acetate trihydrate ..	24	48·5	61·2
Sodium citrate dihydrate.. ..	8	48	58·7
Potassium chloride	22	50	62·8
Potassium iodide	6	49·7	59·7
Potassium carbonate	10	51	61·7
Potassium hydrogen tartrate ..	6	50·5	60·2
Ammonium chloride	15	49	59·7
Ammonium molybdate	6	49	60·7
Ammonium oxalate monohydrate	20	48	47·5
Aluminium chloride hexahydrate	45	44·5	54·1
Citric acid	22	48·5	57·6

* The salts were present in 50 per cent. solutions of propan-2-ol containing chloroacetic acid - potassium hydroxide and 5·52 p.p.m. of barium. The solutions were agitated for 14 hours and centrifuged at 3500 *g*. Aliquots of the supernatant solution were then analysed.

† The emission intensity values were obtained from the analysis of a 10-ml aliquot of a 4·62 p.p.m. sulphate solution by using 3 ml of 55·21 p.p.m. barium solution. The same procedure as that described in the above footnote was used.

Sodium fluoride—Amounts of sodium fluoride up to 3 mg had no effect on the determination of 4·62 p.p.m. of sulphate in the range 1 to 10 p.p.m. of sulphate. With amounts

of sodium fluoride above 3 mg the residual barium emission intensity decreased linearly with concentration (see Table VI).

Perchloric acid—The residual barium emission intensity was unaffected by the presence of 0·93 mmol of perchloric acid during the precipitation reaction. Greater amounts of perchloric acid apparently increase the solubility of barium sulphate in the medium (see Table VI).

Potassium oxalate—A progressive decrease in the residual barium emission intensity was obtained for amounts of potassium oxalate increasing from 1 to 10 mg (see Table VI).

Sodium chloride and sodium nitrate—Amounts of these salts up to 20 mg of each had no effect on the residual barium emission intensity obtained for 4·62 p.p.m. of sulphate.

Analysis of biological material—

The sulphur content of sixteen samples of biological material including mixed pasture, lucerne, grass, onion, fruit tree leaves, dried blood and faeces has been determined. The samples were digested by oxygen-flask combustion followed by absorption in dilute sodium hydroxide solution. Aliquots of this solution were analysed by the above procedure.

Eight samples were analysed in duplicate, six in triplicate and two in quadruplicate. The replicates were prepared from fresh sub-samples of material (not from the same solution) and at least one replicate was produced by a different analyst. The sulphur content of the dry matter ranged from 0·135 to 0·804 per cent. The over-all coefficient of variance was 3·3 per cent. and the standard deviation was 0·0126.

Conclusion

Barium emission in the nitrous oxide - acetylene flame provides a satisfactory means of determining microgram amounts of sulphate indirectly. Smaller solution volumes can be analysed by this procedure simply by reducing by a constant factor the sample volume and the reagent volumes given in this paper. The minimum volume is limited only by the amount required for an accurate emission reading.

The sulphur content of biological material, digested by oxygen-flask combustion, has been satisfactorily determined by using this method. An alternative digestion procedure, which involves the use of nitric and perchloric acids, can be used provided the perchloric acid concentration in the digest is reduced sufficiently by dilution prior to the precipitation of barium sulphate.

In principle, atomic-absorption spectroscopy could be applied in a similar way to determine the residual barium in solution after precipitation of the sulphate, but this possibility has not been explored in the present work.

The author gratefully acknowledges the assistance given by the late Mr. J. E. Allan, who also suggested the idea on which this paper is based. The author also thanks Dr. J. H. Watkinson for providing the results for the determination of sulphur in biological material.

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Pyridylazonaphthols (PANs) and Pyridylazophenols (PAPs) as Analytical Reagents

Part III.* Formation of Copper(II) Complexes and Their Determination in Alloys

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The reactions between copper(II) and 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN) and 4-(2-pyridylazo)phenol (*p*-PAP) have been studied by spectrophotometry and graphical analysis, and their equilibrium constants have been determined. A rapid and simple method for the spectrophotometric determination of copper in aluminium alloys has been devised. A solution of the alloy with pH 2 is extracted with an equal volume of a 5×10^{-3} M solution of *o*-α-PAN in carbon tetrachloride and the absorbance of the extract is measured at 590 nm. The method has been checked by the analysis of standard alloys. The effect of interferences is discussed. The extraction behaviour of several cations with *o*-α-PAN is discussed and the sequential separation of copper(II), zinc(II), manganese(II) and lead(II) is outlined.

In Part II of this series[1] and in unpublished studies (D. Betteridge and H. Freiser), the chelates of manganese(II), zinc(II) and lanthanum(III) with PANs and PAPs were considered. In this paper, the study of the chelates of copper(II) with 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN) and 4-(2-pyridylazo)phenol (*p*-PAP) is reported. The results provide guidance on the optimum use of these reagents and show that *o*-α-PAN can be used for the rapid selective spectrophotometric determination of copper in aluminium alloys.

Experimental

Solutions—

Copper(II) solutions were prepared and standardised with EDTA. The buffer solution used in the solvent-extraction procedure had a pH of 2·05 and was prepared by diluting 25 ml of 0·2 M potassium chloride solution *plus* 26 ml of 0·2 M hydrochloric acid to 100 ml with de-ionised water. Other solutions were prepared as described previously.[1]

Apparatus—

The apparatus used was identical with that described previously.[1]

Procedures—

Spectrophotometric determination of formation constants—The methods used were identical with those described previously.[1] Absorbance curves of the chelates of *o*-α-PAN and *p*-PAP were obtained. Absorbance measurements were made at 546 and 580 nm for the *p*-PAP and *o*-α-PAN chelates, respectively.

Solvent extraction—The procedure used was identical with that described previously.[1] For extraction of the *o*-α-PAN chelate, the spectrophotometer was set at 590 nm. A calibration graph of absorbance *versus* copper(II) concentration in parts per million was also obtained by this method.

Preparation of samples of alloys for analysis—The alloy was dissolved in a 1 + 1 mixture of 8 M nitric acid and 6 M hydrochloric acid (40 ml). The acidic solution was added dropwise because of the vigorous reaction. When all of the acid had been added, the solution was boiled for 1 hour so as to ensure complete dissolution of the alloy and to remove the oxides of nitrogen.

Duplicate results were obtained in all instances.

* For particulars of Part II of this series, see reference list, p. 519. For Part IV of this series, see p. 520.

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Determination of copper in aluminium alloys—Adjust the pH of an aliquot of the alloy solution containing 0·4 to 4 p.p.m. of copper(II) to approximately 2. Add an aliquot of buffer of pH 2. Add a sufficient amount of a 5×10^{-3} M solution of *o*-α-PAN in carbon tetrachloride so that the volumes of the aqueous and organic phases are equal and are convenient for manipulation. Extract the mixture for a fixed period of time (to be determined by the operator, as described below), and, after the extraction is completed and the layers have separated, measure the absorbance of the organic phase at 590 nm against a blank of *o*-α-PAN solution.

Theoretical

Copper(II) - *o*-α-PAN system in the presence of excess of reagent—

The absorbance curve is shown in Fig. 1. The magnitude of the absorbances suggest that in the lower pH range a 1:1 chelate is formed, whereas a 1:2 chelate is formed in the higher pH range. The Sommer method as outlined in Part II of this series[1] is used to confirm or disprove these postulates.

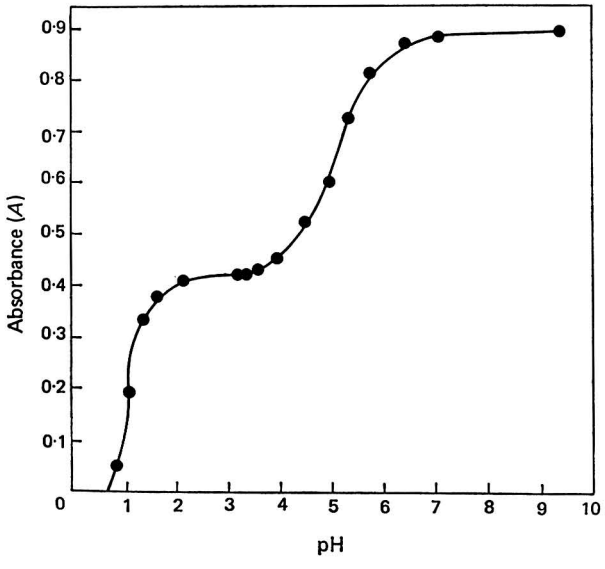

Fig. 1. Absorbance *versus* pH for the copper - *o*-α-PAN system in the presence of excess of reagent

Lower pH range—The formation of a 1:1 chelate is suggested according to the following reaction scheme:

$$Cu^{2+} + HR \overset{{}^{*}K_{CuR}}{\rightleftharpoons} CuR^{+} + H^{+}$$

$$
\begin{aligned}
{}^{*}K_{CuR} &= \frac{[CuR][H]}{[Cu][HR]} \\
&= \frac{[CuR][H]}{[Cu]C_{R\,Tot}\alpha_1} \\
&= \frac{[CuR]}{[Cu]} \cdot \frac{1}{C_{R\,Tot}} \cdot \frac{[H]}{\alpha_1} \qquad \ldots\ \ldots\ \ldots\ \ldots \quad (1)
\end{aligned}
$$

where

$$\alpha_1 = \frac{[HR]}{C_{R\,Tot}} = \frac{[HR]}{[HR] + [H_2R]}$$

and the symbols used are as defined previously.[1]

Therefore,

$$\frac{1}{\alpha_1} = 1 + \frac{[H]}{K_{a_1}}$$

The total absorbance, A, is given by

$$A = \epsilon_{CuR}\,[CuR] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

and the total metal-ion concentration, C_{Cu}, by

$$C_{Cu} = [Cu] + [CuR] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

so that

$$[Cu] = \frac{C_{Cu}\epsilon_{CuR} - A}{A} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

From equations (1), (2) and (4) it can be shown that

$$\frac{C_{Cu}}{A} = \frac{1}{\epsilon_{CuR}} + \frac{1}{{}^*K_{CuR}\,\epsilon_{CuR}\,C_R} \cdot \frac{[H]}{\alpha_1} \quad .. \quad .. \quad .. \quad (5)$$

and that

$$\log {}^*K_{CuR} + pH = \log\left[\frac{A}{(C_{Cu}\epsilon_{CuR} - A)} \cdot C_{R\,Tot} \cdot \frac{1}{K_{a_2}\alpha_1}\right] \quad .. \quad .. \quad (6)$$

Higher pH range—For the formation of a 1:2 chelate, it is postulated that

$$Cu^{2+} + 2HR \rightleftharpoons CuR_2 + 2H^+$$

The algebra used is the same as that derived for the zinc(II) and manganese(II) chelates in the previous paper,[1] *i.e.*, transformation IV.

$$\frac{C_R}{A} = \frac{1}{\epsilon_{CuR_2}} + \frac{1}{{}^*K_{CuR_2}\,\epsilon_{CuR_2}\,C_R^2} \cdot [H]^2 \quad .. \quad .. \quad .. \quad (7)$$

and

$$\log {}^*K_{MR_2} + 2pH = \log\left(\frac{A}{C_{Cu}\epsilon_{CuR_2} - A} \cdot \frac{1}{C_R^2 K_{a_2}^2}\right) \quad .. \quad .. \quad (8)$$

If the reactions postulated are correct, these functions should give straight-line graphs that yield values for the molar absorptivity and the stability constants.

COPPER - *p*-PAP SYSTEM—

The absorbance curves are shown in Fig. 2. In this instance, the possibilities of the formation of a 1:1 or a 1:2 complex in the presence of excess of either metal ions or of reagent must be considered. It was found, by working through the algebra and plotting the experi-

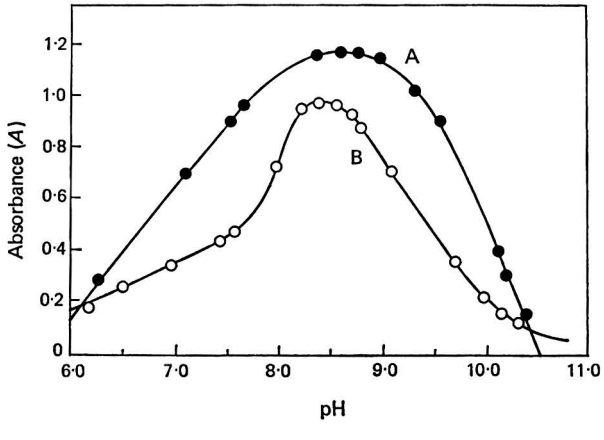

Fig. 2. Absorbance *versus* pH for copper - *p*-PAP systems: (A) in the presence of excess of reagent; and (B) in the presence of excess of metal ions

mental results for the transformations, that the 1:2 complex was not formed in the presence of excess of metal ions.

Excess of metal ions—1:1 *complex*—The proposed chelation reaction is

$$Cu^{2+} + R^- + OH^- \rightleftharpoons CuROH$$

i.e., only R^- reacts as indicated by the maximum formation of the chelate at high pH values. It is also assumed that only HR and R^- species are present in significant concentrations.

$$K_{CuROH} = \frac{[CuROH]}{[R]} \cdot \frac{1}{[Cu][OH]}$$

The total metal-ion concentration is given by

$$[Cu] = C_{Cu}$$

The total absorbance, A, is given by

$$A = \epsilon_R[R] + \epsilon_{HR}[HR] + \epsilon_{CuROH}[CuROH]$$

The total reagent concentration, C_R, is given by

$$\begin{aligned} C_R &= [R] + [HR] + [CuROH] \\ &= [CuROH] + [R]\{1 + [H]/K_{a_2}\} \end{aligned}$$

By utilising the same type of algebraic manipulations as described previously, it is possible to obtain the following equations:

$$\frac{C_R}{A} = \frac{1}{\epsilon_{CuROH}} + \frac{Ay - C_R(\epsilon_R + \epsilon_{HR}[H]/K_{a_2})}{A[OH]K_{CuROH}C_{Cu}\epsilon_{CuROH}} \quad .. \quad .. \quad .. \quad (9)$$

where

$$y = 1 + [H]/K_{a_2}$$

and

$$\log\left\{\frac{Ay - C_R(\epsilon_R + \epsilon_{HR}[H]/K_{a_2})}{C_R\epsilon_{CuROH} - A}\right\} = \log K_{CuROH} + \log C_{Cu} - pOH \quad .. \quad (10)$$

Excess of reagent—1:1 *complex*—The proposed reaction is

$$Cu^{2+} + R^- + OH^- \rightleftharpoons CuROH$$

The total metal-ion concentration, C_{Cu}, is given by

$$C_{Cu} = [Cu] + [CuROH]$$

The total absorbance, A, is given by

$$A = \epsilon_{CuROH}[CuROH]$$

It can be shown that

$$\frac{C_{Cu}}{A} = \frac{1}{\epsilon_{CuROH}} + \frac{A}{K_{CuROH}\epsilon_{CuROH}[R][OH]} \quad .. \quad .. \quad .. \quad (11)$$

and

$$\log K_{CuROH} + \log C_R + (pH - pOH) = \log\left(\frac{A}{C_{Cu}\epsilon_{CuROH} - A} \cdot \frac{1}{K_{a_2}}\right) \quad .. \quad (12)$$

Excess of reagent—1:2 *complex*—The proposed chelation reaction is

$$Cu^{2+} + 2R^- \rightleftharpoons CuR_2$$

As before, it can be shown that

$$\frac{C_{Cu}}{A} = \frac{1}{\epsilon_{CuR_2}} + \frac{[H]^2}{K_{CuR_2}K_{a_2}^2C_R^2\epsilon_{CuR_2}} \quad .. \quad .. \quad .. \quad .. \quad (13)$$

and

$$\log K_{CuR_2} + 2\log C_R + 2pH = \log\left[\frac{A}{(C_{Cu}\epsilon_{CuR_2} - A)K_{a_2}}\right] \quad .. \quad .. \quad (14)$$

Again, graphs of equations (9) to (14) should yield straight lines if the reactions postulated are correct.

Results and discussion

Analysis of absorbance *versus* pH curves—

Copper - o-α-PAN system—The absorbance curve (Fig. 1) can be divided into two main regions, the lower, steep portion corresponding to a 1:1 chelate and the upper portion possibly to a 1:2 chelate. Equations (5) and (6) gave linear graphs and a value of log K_{CuR} of $14{\cdot}30 \pm 0{\cdot}20$ and a molar absorptivity of $2{\cdot}00 \times 10^4$. This value of log K_{CuR} is in good agreement with the value of 14·05 obtained by the method of continuous variations (D. Betteridge and H. Freiser, unpublished studies), and also agrees with the observations of Shun'ichiro, Carter and Fernando,[2] who confirmed the formation of such a species by X-ray diffraction.

The linearity of equations (7) and (8) was less good and suggested that a mixture of 1:1 and 1:2 chelates is formed and that the reaction is strongly dependent upon conditions.

Copper - p-PAP system—The absorbance curves are shown in Fig. 2. Preliminary investigations by continuous variation,[3] slope-ratio[4,5] and mole-ratio[6] methods demonstrated that chelates with either a 1:1 or a 1:2 ratio of copper to ligand may be formed. Straight-line graphs are obtained for equations (9) to (14), indicating that the equilibria suggested are reasonable. The values for the molar absorptivity and stability constants are shown in Table I.

Table I

Molar absorptivities and stability constants for the copper(II) - *p*-PAP system

Condition	ϵ_{CuROH}	K_{CuROH}	ϵ_{CuR_2}	K_{CuR_2}
Excess of metal ions ..	$4{\cdot}19 \times 10^4$	$10{\cdot}64 \pm 0{\cdot}07$		
Excess of reagent	$3{\cdot}93 \times 10^4$	$11{\cdot}22 \pm 0{\cdot}02$	$2{\cdot}00 \times 10^4$	$11{\cdot}01 \pm 0{\cdot}02$

The results of these investigations suggest that the 1:1 chelate is more favoured and that the formation of the 1:2 chelate is most likely to occur in the presence of excess of reagent.

Solvent extraction—

As the volumes of the organic and aqueous phases equilibrated were equal, then by using the established theory,[7–9] the distribution ratio, D, is given by

$$D = \frac{A_{obs.}}{A_{max.} - A_{obs.}}$$

where $A_{max.}$ is the maximum absorbance and $A_{obs.}$ is the observed absorbance, and the percentage extracted, E, is given by

$$E = \frac{D}{D+1} \cdot 100$$

The extraction of copper(II) with *o*-α-PAN has been studied previously.[1] The pH dependence of the extraction of copper with this reagent has been published[10] and it has also been used as an extractive indicator in titrations that involve the formation of complexes.[11] This work serves to extend this application into a practicable analytical method for the determination of copper.

Extraction curves for the copper(II), nickel(II), zinc(II) and manganese(II) chelates of *o*-α-PAN are shown in Fig. 3. No extraction of lead(II), iron(II), iron(III) or titanium(IV) was observed in the pH range in which the extraction of copper(II) occurred. Equilibrium was attained quickly (2 minutes) but the vials were shaken for 30 minutes so as to ensure complete extraction. The lowest pH for complete extraction as indicated by Fig. 3 is 2·05. The colour stability of the chelate was checked by absorbance measurements, when a constant value was obtained over a period of 24 hours. The calibration graph is shown in Fig. 4. A straight-line graph was obtained, which showed that Beer's law was obeyed. The chelate has a molar absorptivity of $4{\cdot}96 \times 10^4$. The chelate was probably extracted as $Cu(o\text{-}\alpha\text{-}PAN)_2$, and no evidence was found to support the view that the extracted species was CuR(OH) or, in the presence of ammonium thiocyanate, CuR(CNS), as have been proposed for the extraction of copper with *o*-β-PAN.[12,13]

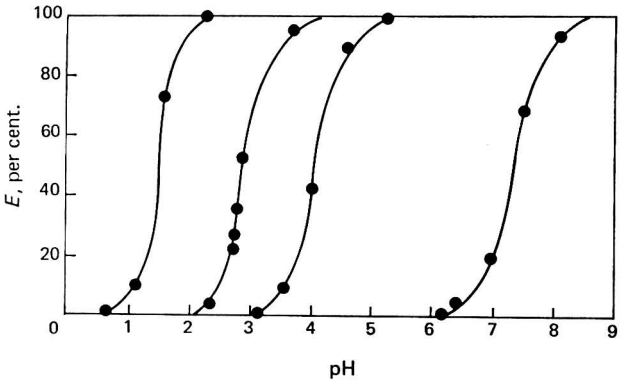

Fig. 3. Extraction curves for the chelates of *o*-α-PAN (5×10^{-3} M) in carbon tetrachloride. Left to right: copper(II), nickel(II), zinc(II) and manganese(II) chelates

INTERFERENCES—

The extent of interference depends on pH and on whether the reaction is carried out in aqueous methanol or an extraction procedure is used. Fig. 5 shows the absorbance of various ions as a function of pH. Comparison with Fig. 3 shows that considerable improvement in selectivity is gained by extraction of the copper(II) complex. Nevertheless, in the absence of nickel(II) and iron(II), the simpler non-extractive procedure would be satisfactory. We consider that the determination of copper is normally performed in the presence of these ions and accordingly we investigated the effect of interferences in the extraction procedure.

The precise definition of the limits of interference is prevented because two important parameters are likely to be varied by individual workers, *viz.*, pH and the rate of extraction. Fig. 3 shows that while it is feasible to use a pH of 2, the control of pH is less critical if the extraction can be carried out at pH 3. If the concentrations of nickel and zinc in a given sample are low in comparison with that of copper, then extraction at pH 3 is permissible and would be preferable to the procedure given. A guideline is provided by the simplified extraction equation for the system

$$n\mathrm{HR}_{(o)} + \mathrm{M}^{n+} \overset{K_{\mathrm{extract}}}{\rightleftharpoons} \mathrm{MR}_{n(o)} + n\mathrm{H}^{+}$$

$$D = \frac{K_{\mathrm{extract}}[\mathrm{HR}]_o^n}{[\mathrm{H}^+]^n}$$

where the subscript (o) refers to the organic phase.

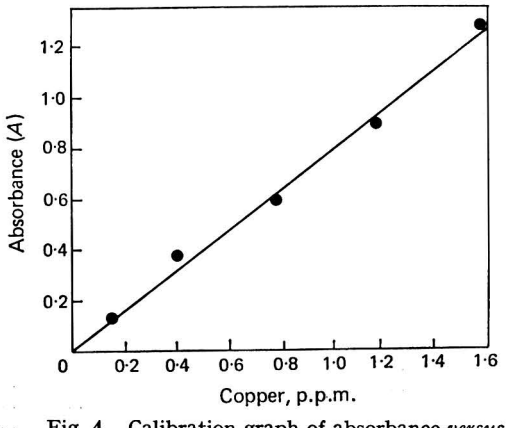

Fig. 4. Calibration graph of absorbance *versus* concentration of copper

Hence, for a bivalent ion, the extent of interference decreases 100-fold for a unit decrease in pH and increases 100-fold for every unit increase in pH, and for a tervalent ion there is a 1000-fold change for every unit change in pH. (These considerations are applicable, of course, only over the pH range for which $D < 10^2$.)

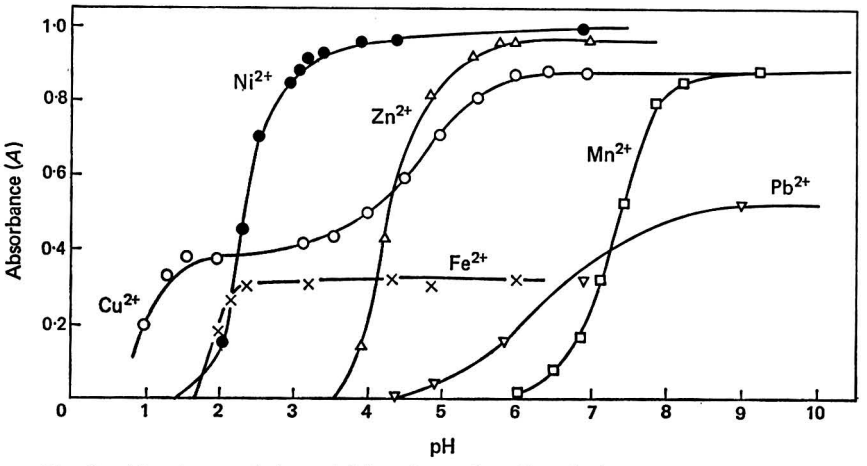

Fig. 5. Absorbance of six metal ions in methanolic solutions of *o*-α-PAN (10^{-4} M)

The variation in the rate of extraction is dependent upon the mode of shaking adopted by the operator. The extraction curves shown in Fig. 3 are for equilibrium conditions. Our studies showed that considerable improvement in selectivity can be gained by carrying out the extraction in the minimum time possible. It has been noted that a few minutes are necessary for the extraction of copper and a longer period of time is required to extract nickel(II), cobalt(II) and zinc(II) completely.

A somewhat gloomy view of the extent of interferences was taken, and shaking was carried out for twice as long as necessary and a pH of 2·5 was used rather than 2. At a copper concentration of 10^{-5} M, the following molar excesses gave an interference of 10 per cent. or less: cobalt(II), 15; nickel(II), 20; zinc(II) and iron(III), 150; lead(II), 200; and manganese(II), chromium(III), vanadium(V) and vanadium(IV), above 1000. Most important, aluminnium(III) and iron(II) did not give any interference over any practicable range of concentrations. Hence, it would seem possible to determine copper in iron or steel if the sample were dissolved in such a way as to result in the iron being present as iron(II). This possibility has not been explored.

DETERMINATION OF COPPER IN STANDARD ALUMINIUM ALLOYS—

The copper content of two British Chemical Standard aluminium alloys was determined by the extraction and spectrophotometric procedure.

Aluminium alloy "A" had the following composition (per cent.)—

Copper	4·68	Iron	0·51
Zinc	2·37	Silicon	0·39
Nickel	1·85	Tin	0·05
Lead	1·51	Aluminium	87·30
Magnesium	1·34		

The average copper content found by extraction and spectrophotometric determination was 4·68 ± 0·05 per cent.

A 10 per cent. magnesium - aluminium alloy had the following composition (per cent.)—

Copper	0·03	Zinc	0·05
Magnesium	10·57	Titanium	0·10
Silicon	0·10	Chromium	0·06
Iron	0·19	Aluminium	88·84
Manganese	0·06		

The average copper content found by extraction and spectrophotometric determination was 0·030 ± 0·001 per cent.

These results are in exact agreement with those quoted on the certificate of analysis, and confirm that this procedure for the determination of copper in aluminium is precise, sensitive and selective. The method is superior to the existing colorimetric procedure involving the use of sodium diethyldithiocarbamate[14] because it is less time consuming, involves fewer chemical manipulations and is therefore less likely to lead to error. A further advantage of the method is that copper can be quantitatively determined in the presence of a large excess of metals other than aluminium, *e.g.*, lead, nickel, zinc and manganese, which react at higher pH. The low pH value used in the procedure is not only advantageous for promoting selectivity but it also minimises the occurrence of side-reactions such as hydrolysis.

COUNTER-CURRENT EXTRACTION—

The good extraction and spectrophotometric characteristics of several of the *o*-α-PAN_2 metal chelates suggest that the reagent might be useful for the sequential extraction of metal ions from a mixture. This possibility was explored by carrying out successive extractions and adjustments to the pH in a fourteen-tube, hand-operated, counter-current extraction apparatus. The mixture taken contained copper(II), zinc(II), manganese(II) and lead(II) ions. Lead ions remained in the aqueous phase but the other ions were extracted in the order copper, zinc, manganese at pH 4, 6·9 and 9·1, respectively, when the concentration of *o*-α-PAN in carbon tetrachloride was 5×10^{-3} M.

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

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Pyridylazonaphthols (PANs) and Pyridylazophenols (PAPs) as Analytical Reagents

Part IV.* Formation of Complexes with Titanium(IV)

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The formation of complexes of titanium(IV) with PANs and PAPs has been investigated in order to ascertain the types of complexes that are formed and to elucidate the optical and stability constants of such complexes. The competing effect of hydrolysis on these reactions has also been considered. The reaction with 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN) has been examined in order to determine the analytical utility of this complex formation.

PREVIOUS papers[1–3] have considered both theoretical and practical applications of pyridylazonaphthols (PANs) and pyridylazophenols (PAPs) as analytical reagents. This paper presents the results of similar investigations with titanium(IV).

EXPERIMENTAL

SOLUTIONS—

A standard solution of titanium(IV) was prepared by the method of Roseman and Thornton[4] and standardised gravimetrically with cupferron.[5] The solution was prepared shortly before use. Solutions that were more than 1 day old did not react with the reagents. Other solutions were prepared as described previously.[1,2]

APPARATUS—

The apparatus used was identical with that described previously.[1,2]

PROCEDURES—

Spectrophotometric determination of formation constants—The methods used were identical with those described previously.[2] Absorbance curves of the chelates of 2-(2-pyridylazo)-1-naphthol (*o*-α-PAN), 2-(2-pyridylazo)phenol (*o*-PAP) and 1-(2-pyridylazo)-2-naphthol (*o*-β-PAN) were obtained. Absorbance measurements were made at 582, 560 and 536 nm for *o*-α-PAN, *o*-β-PAN and *o*-PAP, respectively.

Solvent extraction—The procedure used was identical with that described previously.[2] Only the extraction of the *o*-α-PAN chelate was considered, the absorbance measurements being made at 590 nm.

THEORETICAL

The predominant titanium species present[6] in the pH range 0 to 5·5 is TiO^{2+}, and the predominant reagent species in the pH range corresponding to the maxima of the absorbance curves is the neutral species HR (the protonated form H_2R^+ is of significance at lower pH values). The absorbance *versus* pH curves shown in Fig. 1 indicate a stoicheiometry of 1:2 for each chelate.

Following through the Sommer method, as in the previous papers, the suggested chelation reaction is

$$TiO^{2+} + 2HR \overset{{}^*K_{MR_2}}{\rightleftharpoons} TiOR_2 + 2H^+ \quad .. \quad .. \quad .. \quad (1)$$

where

$${}^*K_{MR_2} = \frac{[MR_2][H]^2}{[M]\,[HR]^2} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

* For Part III of this series, see p. 512.

† Present address: BP Chemicals (U.K.) Ltd., Llandarcy, Swansea.

‡ Present address: Bausch and Lomb Co. Ltd., Toronto, Canada.

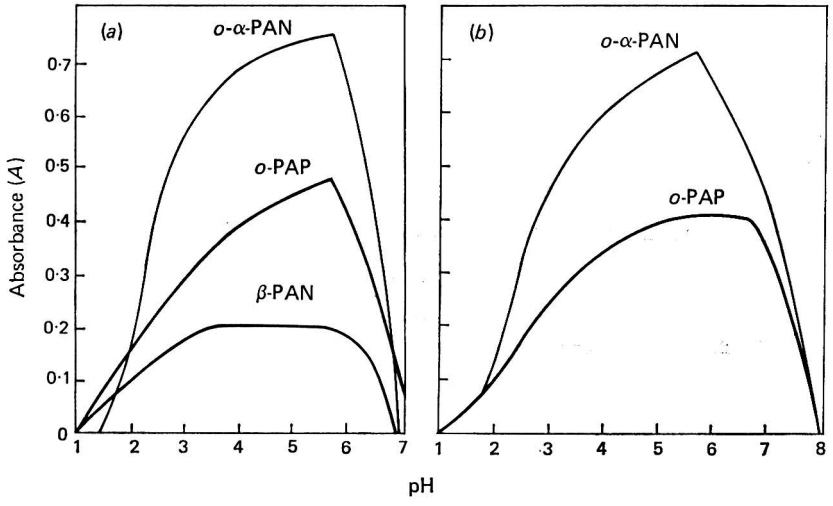

Fig. 1. Absorbance *versus* pH graphs for titanium(IV) chelates: (*a*) in the presence of excess of metal ions; and (*b*) in the presence of excess of reagent

IN THE PRESENCE OF EXCESS OF TITANIUM(IV)—

The symbols adopted in the previous papers are used. If it is assumed that $[M] = C_M$, the total absorbance, A, is given by

$$A = \epsilon_{MR_2}[MR_2] \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and

$$C_R = 2[MR_2] + [HR] + [H_2R^+] \quad .. \quad .. \quad .. \quad (4)$$

then, by arguments similar to those used in Part II[2] [transformation (III)]—

$$\frac{C_R}{A} = \frac{2}{\epsilon_{MR_2}} + \frac{y[H]^2}{{}^*K_{MR_2}C_M\epsilon_{MR_2}[HR]} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where $y = 2 + K_{a_2}/[H]$, or in terms of the stability constant, K_{MR_2},

$$K_{MR_2} = \frac{[MR_2]}{[M][R]^2}$$

As $[M] = C_M$ and $[MR_2]/[HR] = Ay(C_R\epsilon_{MR_2} - 2A)$,

$$\log K_{MR_2} + 2pH = \log\left\{\frac{Ay}{(C_R\epsilon_{MR_2} - 2A)[HR]\ K_{a_2}^2\ C_M}\right\} \quad .. \quad .. \quad (6)$$

IN THE PRESENCE OF EXCESS OF REAGENT—

Under these conditions,

$$[HR] = C_R$$

$$C_M = [M] + [MR_2]$$

$$\frac{C_M}{A} = \frac{1}{\epsilon_{MR_2}} + \frac{[H]^2}{{}^*K_{MR_2}C_R^2\epsilon_{MR_2}} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

and

$$\log K_{MR_2} + 2pH = \log\left[\frac{A}{(C_M\epsilon_{MR_2} - A)\ K_{a_2}C_R^2}\right] \quad .. \quad .. \quad (8)$$

Graphs of equations (5), (6), (7) and (8) should, if the initial assumptions are valid, yield straight lines, from which the molar absorptivities and stability constants can be calculated.

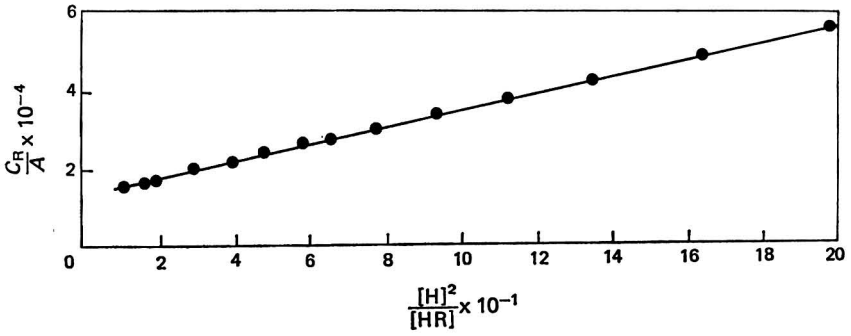

Fig 2. Graph of equation (5) in the presence of excess of metal ions

Results and discussion

Analysis of the absorbance *versus* pH curves—

The reaction of *o*-β-PAN with titanium is very much weaker than that of the other two reagents, and consequently no further investigation of the reaction was carried out. The absorbance curves of the chelates of *o*-α-PAN and *o*-PAP were considered. Figs. 2 and 3 show the graphs of equations (5) and (8), which are straight lines, indicating that the initial assumptions made were correct. The molar absorptivities and stability constants are shown in Table I. If one assumes that a chelate species with a ratio of metal to reagent of 1:1 is

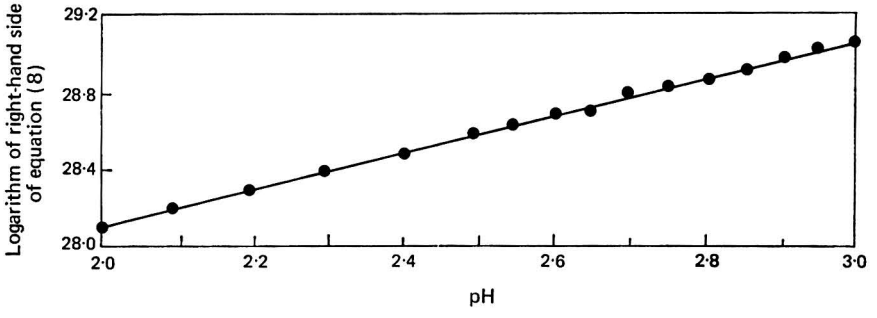

Fig. 3. Graph of equation (8) in the presence of excess of reagent

formed instead of a 1:2 complex, it is possible to carry out similar algebraic manipulations so as to derive equations equivalent to equations (5) to (8) [*cf.* transformations (VIII) and (IX) in Part II[2]]. Fig. 4, which is the graph of one of these equations, is not linear, thus showing that the initial assumption of a 1:1 complex is not correct. This example demonstrates the usefulness of this method for identifying possible species.

Table I

Molar absorptivities and stability constants for chelates of titanium (IV) with *o*-α-PAN and *o*-PAP

	Excess of reagent		Excess of metal ions	
Reagent	ϵ_{MR_2}	Log K_{MR_2}	ϵ_{MR_2}	Log K_{MR_2}
o-α-PAN	$1{\cdot}74 \times 10^4$	$23{\cdot}44 \pm 0{\cdot}08$	$1{\cdot}72 \times 10^4$	$23{\cdot}46 \pm 0{\cdot}02$
o-PAP	$9{\cdot}89 \times 10^3$	$20{\cdot}50 \pm 0{\cdot}08$	$1{\cdot}04 \times 10^4$	$20{\cdot}21 \pm 0{\cdot}02$

The results were obtained from different studies that involved the use of different reagent and metal-ion concentrations. The method of continuous variations[7,8] confirmed that the stoicheiometry of the chelates is 1:2, and gave values for the stability constants that were in good agreement with those given above.

At the upper pH levels, the absorbance decreases with increasing pH. This behaviour

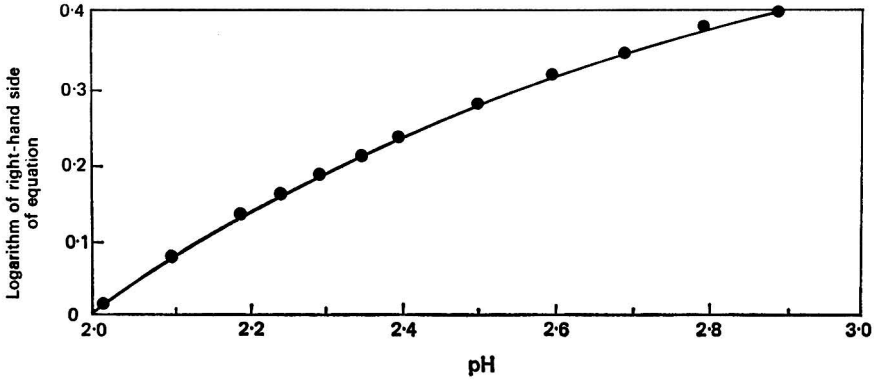

Fig. 4. Graph of equation to test for the 1:1 complex in the presence of excess of metal ions: $\log K_{MR} + pH = \log\{A/[(C_R\epsilon_{MR} - A)C_M K_{a_2}] - \log \alpha_1\}$

is similar to that observed for manganese(II) and zinc(II) chelates[2] and can be attributed to the occurrence of hydrolysis reactions. It is well known that the titanium species hydrolyse easily, and the decrease in absorbance may be caused by the preferential hydrolysis of the TiO^{2+} species according to the following equilibria—

$$TiOR_2 \overset{K_{MR_2}}{\rightleftharpoons} 2R^+ + TiO^{2+} \underset{H_2O}{\overset{2H_2O}{\rightleftharpoons}} \begin{matrix} HTiO_3^- + 3H^+ \\ TiO_2 + 2H^+ \end{matrix}$$

The existence of these equilibria can be verified by calculating the theoretical absorbance curves obtained by using the conditional coefficients α and β as described for lanthanum in Part II.[2]

The values of the equilibrium constants involving the titanium species are[6]

$$TiO^{2+} + 2H_2O \rightleftharpoons HTiO_3^- + 3H^+$$

$$\frac{\log [HTiO_3]}{[TiO^{2+}]} = -16{\cdot}82 + 3pH$$

and

$$TiO^{2+} + H_2O \rightleftharpoons TiO_2 + 2H^+$$

$$\log [TiO^{2+}] = -1{\cdot}18 - 2pH$$

It is now possible, by using these constants, to substitute in order to find values for β, then C_R and then the absorbance. The results for these calculations in the presence of excess of metal ions and excess of reagent are shown in Fig. 5.

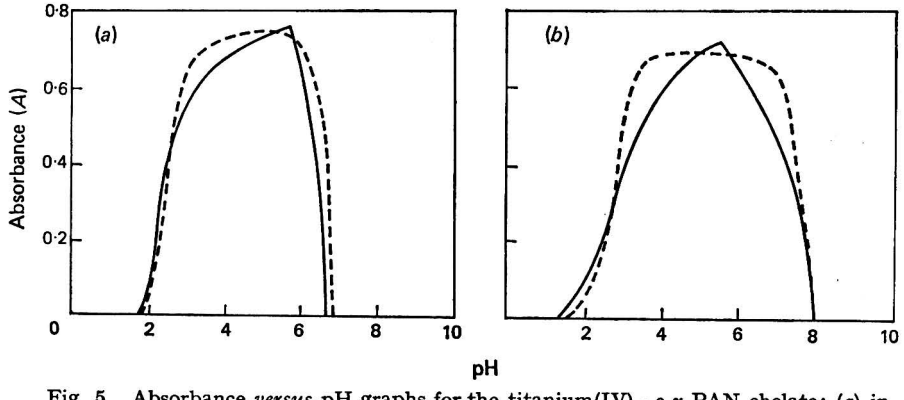

Fig. 5. Absorbance *versus* pH graphs for the titanium(IV) - *o*-α-PAN chelate: (*a*) in the presence of excess of metal ions; and (*b*) in the presence of excess of reagent. Solid lines, experimental; and broken lines, theoretical

The agreement between the curves is evidence that the suggested equilibria are reasonably correct, but the discrepancies suggest that other equilibria may also be significant over part of the pH range investigated. We have tried the most likely permutations, *e.g.*, $TiOR_2OH^-$, without success.

Analytical utility—

The optical and stability constants indicate the possible use of *o*-α-PAN for the spectrophotometric determination of titanium(IV), but also that some ions will interfere, especially Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+} and Fe^{3+}. The interference of iron could be overcome if the titanium complex could be extracted. Application of the methods described in Part II[2] [equation (20)] and assuming a partition coefficient of 10^4 for $TiOR_2$ and a reagent concentration of 10^{-3} M gives the pH range for extraction of 4 to 6. Copper(II), nickel(II) and zinc(II) can be extracted over this range and will interfere. In practice, the extraction of titanium was incomplete over the pH range 2 to 6 with *o*-α-PAN in n-butanol, carbon tetrachloride, n-octanol, isobutyl methyl ketone and chloroform as solvents.

Calibration graphs for titanium concentrations in the range 5×10^{-6} to 10^{-4} M were prepared by adding 10 ml of a $2{\cdot}0 \times 10^{-4}$ M solution of *o*-α-PAN in 40 per cent. ethanol to an aliquot of titanium solution, adding 5 ml of phthalate buffer (pH 4 to 6) and making the volume up to 25 ml with water. The colour, which developed instantly, was stable for several hours and was measured at 582 nm in 1-cm cells. The order of addition of reagents was not important, but the buffer was added last so as to minimise the possibility of hydrolysis of titanium. The presence of equal amounts of Fe^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions made the determination impossible. The effect of masking reagents on this reaction was difficult to predict as values for stability constants for titanium complexes, other than for EDTA, are not available, but several reagents were tried in an effort to eliminate the interference of the above ions.

EDTA, as predicted, reacted preferentially with titanium(IV). Citrate, which has two donor oxygen atoms, and ethane-1,2-dithiol, which has two donor sulphur atoms, failed to mask either the interferences or titanium. Reducing agents, such as hydroxylammonium chloride, did not produce the required result. The possibility of producing mixed ligand complexes with different absorption maxima, such as $TiR_2O_2^{2-}$, by using hydrogen peroxide was also tried, but without success.

Conclusions

The optical and stability constants of the chelates of titanium(IV) with *o*-α-PAN and *o*-PAP have been determined and the results suggest that an analytical method for titanium based upon them would be sensitive. Furthermore, the colours, once they have been formed, are stable. Unfortunately the method is subject to interference from several common ions, which would have to be removed prior to the determination.

We are grateful to the T. and E. Williams Scholarship Fund and to the S.R.C. for maintenance grants to two of us (D.J. and F.S., respectively).

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Note—References 1, 2 and 3 are to Parts I, II and III, respectively, of this series.

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An Ultramicro-scale Method for the Determination of the Uranyl Cation

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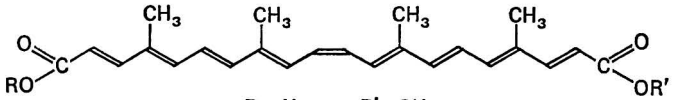
A method that requires the use of very simple equipment has been developed whereby the uranyl cation can be determined at the micro-scale and ultramicro-scale levels with high precision. The method involves the use of the catalytic effect shown by the uranyl cation on the photo-decolorisation of a naturally occurring carotenoid-type pigment. The interfering effects of several common anions and cations are also discussed.

Annatto is the red colouring matter of the seed of the plant *Bixa Orellana* L.[1] and is a mixture of natural pigments that have similar chemical structures, the principal constituent being labile bixin—



R = H; R' = CH_3

Solutions of bixin in acetic acid undergo photochemical decolorisation when irradiated with short-wave ultraviolet light. As nitrogen-purged solutions of bixin do not lose their colour when exposed to ultraviolet light, the reaction is thought to be based on its photochemical oxidation. As the catalytic oxidation of naturally occurring pigments has been used[2] to determine ultramicro-scale amounts of cations, it was decided to investigate the possibility of discovering a cation that might catalyse the photochemical oxidation of labile bixin. About twenty cations were tested but only three had a noticeable effect on the velocity of the reaction, and of these three the uranyl cation had a much more pronounced effect than the other two cations. Investigation of this catalytic effect revealed that the reaction could be used to determine uranyl ions in the range 0·01 to 10 p.p.m.

Several good techniques have been published for the determination of trace amounts of uranium by activation analysis,[3] fluorimetry,[4,5] fission-track counting[6] and alpha-counting,[7] and although these methods offer good sensitivity at low concentrations, all of them suffer from the disadvantage that they require the use of equipment or techniques, or both, not normally encountered in most chemical laboratories. Atomic-absorption spectroscopy is attractive but Perkin-Elmer only claim a lower detection limit of 30 p.p.m. for their Model 303 instrument. Florence and Farrar[8] described an excellent spectrophotometric method for the determination of uranium in ores that involves the use of the 2-(2-pyridylazo)-5-diethylaminophenol - zinc complex but their lower detection limit is 15 p.p.m. of uranium oxide (U_3O_8) in an ore.

In our method, a standard graph of the bixin decolorisation gradients is prepared for the range of uranyl-ion concentrations of interest. Once this graph has been obtained a routine analysis can be completed in approximately 30 minutes (the time required to observe suitable decolorisation of the solution under ultraviolet irradiation); however, it should be realised that any number of solutions can be irradiated simultaneously according to the lamp facilities available. In the present work, one lamp was used to irradiate ten solutions simultaneously and their final transmittance values were read consecutively. Each transmittance reading required approximately 30 s.

Experimental

cis-Bixin, in the form of the sodium salt, can conveniently be obtained by separation on a Sephadex column[9] and then precipitated as the free acid with dilute hydrochloric acid.

In the present experiments the whole of the colouring matter from 40 g of Bolivian annatto was extracted into chloroform at room temperature and the filtered extract was then mixed with anhydrous alumina (Merck, Extra Pure). The dyed alumina was then washed with chloroform until no further colouring matter could be removed. The only pigment that remains adsorbed on to the alumina is the *cis*-bixin, which was removed by placing the stained alumina on top of a chromatographic column containing clean alumina suspended in benzene. The column was then eluted with an acetic acid - ethanol mixture (1 + 9 *V*/*V*). The eluted product was recrystallised from glacial acetic acid (m.p. 185 to 186 °C and λ_{max} 468 nm). The yield was 400 mg, which was a sufficient amount to enable the entire set of experiments to be completed.

PREPARATION OF STANDARD DECOLORISATION CURVE—

Previous experiments had shown that concentrations of water greater than 1 per cent. in the solutions to be decolorised reduced the rate of decolorisation and hence the sensitivity of the test, and so chromatographic acetic acid (99 to 100 per cent.) was used without further purification to prepare the bixin solutions containing uranyl ions in the following concentrations: 0, 0·5, 0·1, 0·3, 0·7, 1, 2, 3, 4, 5, 7 and 10 p.p.m.

In order to prepare these solutions, 0·00930 g of high-purity uranyl nitrate was weighed on a Mettler microbalance and then dissolved in 50 ml of the acetic acid, thus giving a standard solution containing 100 p.p.m. of uranyl ions. For the solutions containing from 1 to 10 p.p.m. of uranyl ions, aliquots of the standard solution (from 0·1 to 1 ml) were made up to 5 ml with the acetic acid and the solutions were then made up to 10 ml with a 2000 p.p.m. solution of bixin in acetic acid. In this way, an initial concentration of 1000 p.p.m. of bixin was obtained for each solution, which ensured the presence of a large excess of the pigment throughout the irradiations.

The more dilute solutions containing from 0·05 to 0·7 p.p.m. of uranyl ions were prepared in the same way but by using aliquots of standard solutions containing 10 and 1 p.p.m. of uranyl ions. Kimax and Pyrex glassware (grade A) was used throughout; the temperature of the solutions was stabilised at 20 °C so as to ensure accuracy in volume measurement and all solutions were freshly prepared prior to exposure.

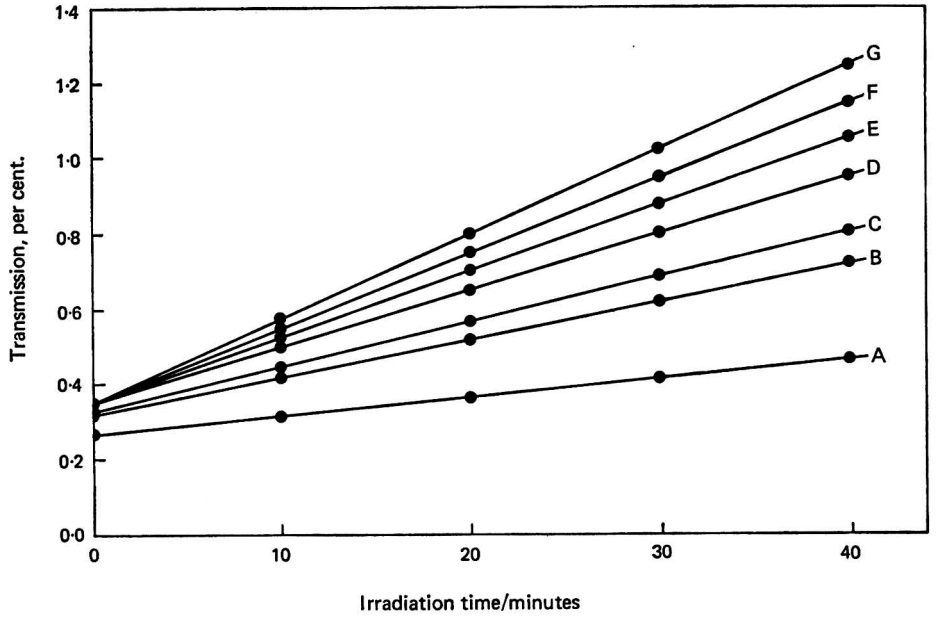


Fig. 1. Decolorisation graphs for bixin at different uranyl concentrations: A, 0; B, 0·5; C, 1; D, 2; E, 3; F, 4; and G, 5 p.p.m.

Short-wave irradiations of the solutions were made with a Cromato-Vue 257·3-nm mercury lamp, which is operated at 0·36 A and 220 V; 2·7 ml of each solution were transferred by pipette into matched 1-cm quartz cells (Beckman), which were then irradiated at a distance of 25 cm from the lamp. After each 10-minute irradiation, the lamp was switched off and the cells were transferred into a Beckman DU-2 spectrophotometer, with which transmittance readings were taken at 468 nm. For the concentration range of uranyl ions above 7 p.p.m., the time of each irradiation was reduced to 1 minute or less. This step was found to be necessary in order to ensure that the corresponding transmittance - time graph was a straight line. For each concentration of uranyl ions the decolorisation graph of transmittance against irradiation time was plotted and found to be linear in each instance (Fig. 1).

In order to facilitate the use of these results for the practical determination of uranyl ions, it was decided simply to plot the gradient of each graph as a function of the uranyl-ion concentration.

Four complete decolorisation series were run for each concentration of uranyl ions and the values shown in Table I are the mean values with the maximum divergence shown as the error. As expected, the greatest divergence was observed at low uranyl-ion concentrations. For the 0·05 p.p.m. solution the divergence was 3 per cent. and this error reduced to 2·7 per cent. in the 10 p.p.m. solution, although the error in the middle of the range was more frequently of the order of 1 per cent.

TABLE I

RELATIONSHIP OF URANYL-ION CONCENTRATION TO GRAPH GRADIENT

$[UO_2^{2+}]$, p.p.m.	Gradient × 10^3	$[UO_2^{2+}]$, p.p.m.	Gradient × 10^3
0·0	5·0 ± 0·05	2·0	15·0 ± 0·15
0·05	6·0 ± 0·10	3·0	17·5 ± 0·17
0·1	7·0 ± 0·15	4·0	20·0 ± 0·16
0·3	8·8 ± 0·15	5·0	22·5 ± 0·2
0·7	11·0 ± 0·15	7·0	27·0 ± 0·9
1·0	12·0 ± 0·16	10	37·3 ± 1·0

By converting these errors into errors in uranyl-ion concentration the following conclusions can be drawn: in the region of 10 p.p.m. an error of 2·7 per cent. in the decolorisation gradient indicates that the uranyl-ion concentration could be between 9·6 and 10·4 p.p.m., the deviation from the mean being of the order of 4 per cent.; and in the region of 0·05 p.p.m.

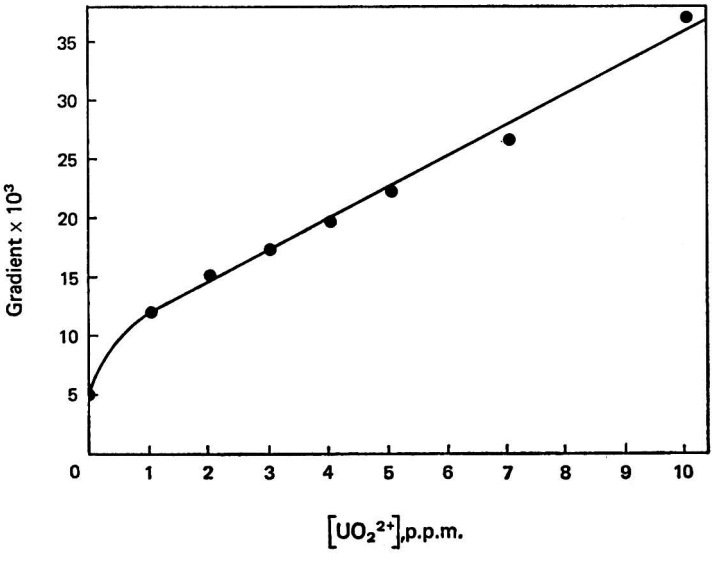


Fig. 2. Uranyl concentration (0 to 10 p.p.m.) as a function of decolorisation gradient

an error of 3 per cent. indicates that the concentration could be between 0·045 and 0·055 p.p.m., with a deviation of 10 per cent.

These results are shown in Figs. 2 and 3, from which it can be seen that the relationship of gradient to concentration is linear between 1 and 10 p.p.m. of uranyl ions.

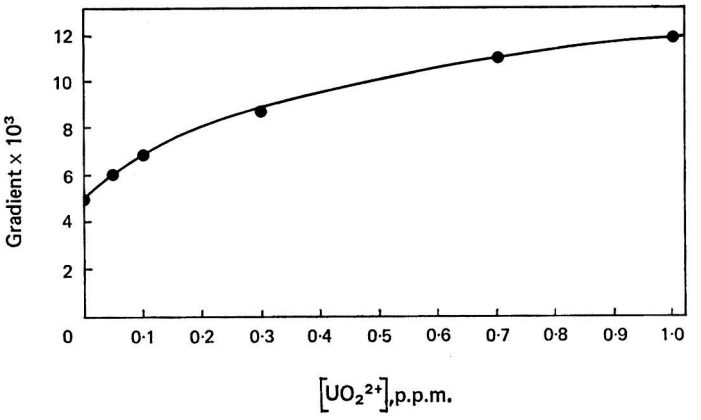


Fig. 3. Uranyl concentration (0 to 1 p.p.m.) as a function of decolorisation gradient

In order to test for possible interfering effects of other ions, we carried out irradiations on a series of solutions, all of which contained 1 p.p.m. of uranyl ions, together with 500 p.p.m. of any of the following cations: Fe^{2+}, Cd^{2+}, Pb^{2+}, Ca^{2+}, Na^{+}, Al^{3+}, Mn^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+} and Sn^{4+}. As these cations were tested as the nitrate, sulphate, chloride, acetate or carbonate and no deviation was observed in any instance, we conclude that none of the above cations or anions interfere in the determination. Hg^{2+} and Mg^{2+}, as the chlorides, caused an increase in the rate of decolorisation of about 5 per cent., but as they were present in a concentration 500 times greater than that of uranyl ions, we do not regard this increase as a serious interference.

Discussion

The results show that uranyl ions have a catalytic effect on the photo-oxidation of labile bixin and that this effect can be used in the quantitative determination of the ions in micro-scale and ultramicro-scale concentrations. Although the relationship of decolorisation gradient to uranyl-ion concentration is non-linear at the parts per billion (10^9) level, each point on the graph can be obtained simply and with a precision such that determinations of uranyl ions down to 0·05 p.p.m. can be made with a maximum error of 10 per cent.

Four test decolorisations on a standard solution containing 0·01 p.p.m. of uranyl ions revealed that their concentration could be determined to an accuracy within 15 per cent. by using this method.

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An Improved Plasma Jet System for Spectrochemical Analysis

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The stability of a plasma jet has been improved by replacing the direct-injection nebuliser with a pre-mixed chamber - nebuliser arrangement of the type used in flame spectrometry. This arrangement has alleviated many of the operational problems that were experienced with the original system. As a result of the improved performance, the unit can be operated in conjunction with instantaneous photoelectric read-out. Details of the modification are given and the stability obtained is demonstrated. The sensitivity of the modified plasma jet has been investigated and detection limits for the most sensitive lines of sixty-seven elements are presented. These detection limits are compared with those obtained by using a nitrous oxide - acetylene flame with the same optical arrangement and detection equipment.

THE plasma jet described by Margoshes and Scribner[1] and later by Owen[2] and Mittledorf and Landon[3] has proved to be a significant development in the spectrochemical determination of elements in solution. The high-temperature environment provides favourable conditions for exciting most elements. Unlike the lower temperature chemical combustion flames, the source is free from some chemical interference effects[4] and is capable of exciting those elements which tend to form stable oxides in flames.[5] The plasma jet lacks the stability of a flame and in most applications either photographic recording[6,7] or electronic integration of a photomultiplier output[4] is used. Photographic recording is tedious and time consuming and requires repetitive exposures in order to obtain precision data. Photoelectric read-out is a more rapid and convenient method of monitoring signals and the signal can be displayed as either instantaneous intensity or time-integrated intensity. In general, the former is preferred because it permits continuous monitoring of the emission stability of the discharge. The stability of the plasma jet as indicated by Lerner[8] suggests that it is suitable for use with instantaneous read-out, and this aspect was investigated further.

EXPERIMENTAL

A plasma jet, which is commercially available from Spex Industries Inc. and is described as a spectrograph accessory, was operated in conjunction with a grating monochromator with non-integrated photoelectric detection similar to that used in flame spectrometry. We found, however, that the stability of the device was unsatisfactory for operation in this way. Lack of stability was attributed to the deposition of solids on the tip of the nebuliser, which resulted in rapid decay of the signal. Because the nebuliser nozzle was close to the discharge, it corroded rapidly and had to be replaced frequently. Other problems experienced with this sytem were the need to shut down the equipment in order to clean the capillary when blockages occurred and the need to use aqueous - organic solvent mixtures so as to prevent the build-up of solution on the upper graphite control ring. This latter problem indicated that the evaporation of the droplets was inefficient because of the unsatisfactory method of introducing aerosol into the discharge.

In order to overcome these problems of introduction of the sample, and in an attempt to achieve adequate stability, the plasma jet was modified by replacing the direct-injection nebuliser with a pre-mixed aerosol chamber.

The modification is simple and results in longer periods of continuous operation. The improved stability is satisfactory for operation with instantaneous photoelectric detection by using the equipment described. The improved performance of the modified system resulted in higher sensitivity and the detection limits for sixty-seven elements were obtained in order to assess the value of the system for spectrochemical analysis. These detection limits were, in general, an order of magnitude better than those previously obtained with

the direct-injection system. In order to assess further the value of the system for spectrochemical analysis, the detection limits were compared with those obtained with a nitrous oxide - acetylene flame by using the same optical arrangement and detection system.

APPARATUS—

A full description of the direct-injection plasma jet has been reported previously,[2,3] and a sectional diagram of the modified system is shown in Fig. 1. An EEL nebuliser (Evans Electroselenium Ltd.) was mounted on a Perspex screw-cap, which replaced the normal press-fit cap of an EEL spray chamber. The drain tube of the chamber was connected to a U-tube so as to maintain a positive pressure of 3 to 4 cm (water gauge) inside. The spray from the nebuliser was directed past a baffle-plate and then through a 90° angle into the discharge via a glass tube of 3·5 mm i.d. The glass tube was positioned so that the lip was in contact with the lower graphite ring. A poly(vinyl chloride) tube between the ground-glass joint and the glass tube provided a flexible coupling between the spray chamber and the plasma jet.

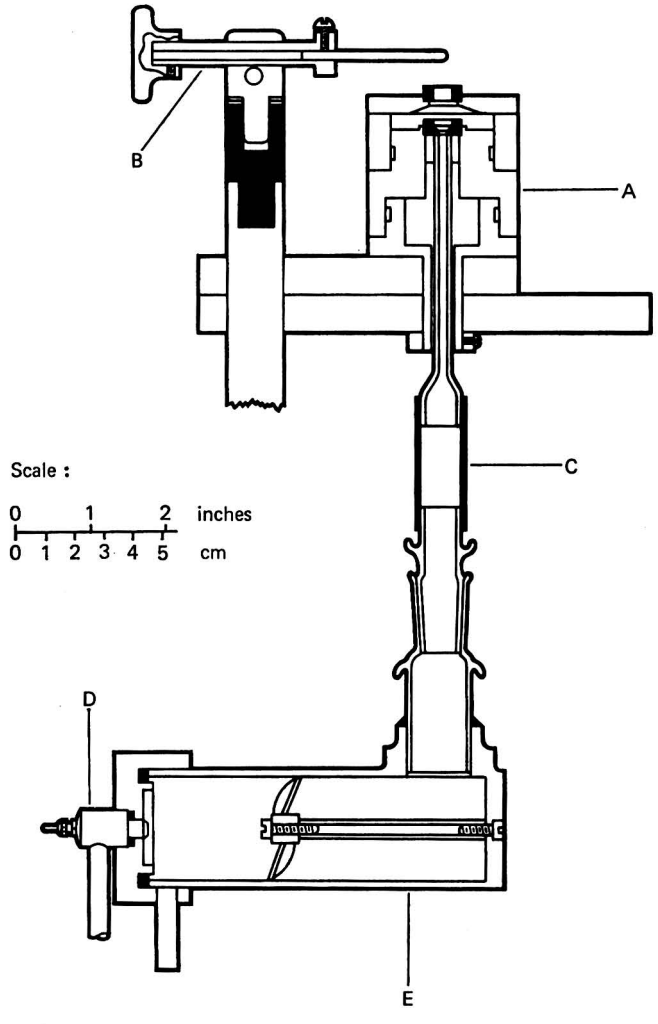

Fig. 1. Sectional diagram of modified plasma jet. A, plasma jet; B, adjustable transfer electrode; C, flexible coupling; D, nebuliser; and E, spray chamber

The unit was mounted on an optical bar and light from the source (up to 4 mm above the upper graphite ring) was focused by a spherical lens on to the entrance slit of a 0·5-m plane grating monochromator (Jarrell-Ash Co.). The grating had 1180 lines per millimetre, giving a reciprocal linear dispersion of 1·6 nm mm^{-1} in the first order. The slits were 30 μm wide. The water-cooled transfer electrode holder was adjustable and permitted the electrode (a 3 mm diameter thoriated tungsten rod) to be advanced manually into the discharge so as to compensate for erosion of the electrode. This system permitted continuous operation for up to 2 hours. Emission signals were detected on an E.M.I. 6256S photomultiplier operating at 700 to 1000 V (variable) and were measured on a d.c. microvoltmeter connected to a 10-mV strip-chart recorder. Recently, a Keithley 414S picoammeter has replaced the microvoltmeter and permits considerable back-off of the high emission background of the source.

The three-phase 415-V mains supply was rectified so as to supply direct current and a ballast network of 1-kW radiator bars in parallel was used so as to give a variable output of up to 30 A. The potential drop across the gap was 50 V and a Tesla coil coupling provided the spark ignition for the arc. It was essential to incorporate a small inductance in the ballast circuit so as to maintain stability of the current.

The flows of the helium tangential gas and argon nebulising gas were controlled by pressure reducers and needle valves; the flow-rates of the gases were monitored by using precision-bore flow-rate meters.

For the assessment of sensitivity, the plasma jet was operated under the conditions described below. The comparison with the nitrous oxide - acetylene flame was carried out by replacing the plasma jet with the burner and fog chamber from a Unicam SP900 flame spectrophotometer; the same optical layout was thus retained. The emission from the red "feather" of the flame immediately above the primary reaction zone was monitored.

Results

Optimisation of operating conditions—

In order to achieve satisfactory emission stability, the current, tangential flow-rate of the gas and flow-rate of the solution had to be optimised. Currents in excess of 15 A did not result in improvement but accelerated the rate of erosion of the transfer electrode, which limited the long-term stability and the length of the operating period. Tangential flow-rates in excess of 12 l min^{-1} (supply pressure 30 p.s.i.) caused deterioration of the intensity of the signal and increased the audible noise level of the discharge. The nebuliser was set to give a sample consumption of 4 ml min^{-1}. With the baffle-plate in the spray chamber, the efficiency was found to be 5 per cent. The rate of spraying into the discharge was therefore about 0·2 ml min^{-1}, which was considerably less than that obtained with the direct-injection system (1 to 1·5 ml min^{-1}). The rate of spraying chosen was a compromise between the amount of solution reaching the discharge and the size of the droplets in the aerosol. The absence of build-up of solution on the upper graphite ring indicated that most of the droplets entered the discharge.

Under these conditions (current, 15 A; tangential flow-rate of gas, 12 l min^{-1}; and flow-rate of solution, 4 ml min^{-1}), the pitch of the audible noise of the discharge did not change when the aerosol was introduced, thus indicating that there was no significant disturbance in the behaviour of the arc. However, different results were obtained with the direct-injection nebuliser. The rate of erosion of the transfer electrode was very much reduced in the modified system and a three-fold increase in continuous operating time was achieved.

Emission stability—

The stability of the new system is demonstrated in Fig. 2, which shows a series of signals recorded for an aqueous solution of zinc of concentration 150 μg ml^{-1} at a wavelength of 213·9 nm. The coefficient of variation for the average values for each of the signals was 1·2 per cent. The average deviation between consecutive readings based on an estimate of the mean value for each individual signal was 0·05 μA. The direct-injection system was less stable: the average deviation between consecutive readings was approximately 0·2 μA for a set of signals of similar strength. The long-term stability was difficult to assess quantitatively because the transfer electrode had to be adjusted frequently.

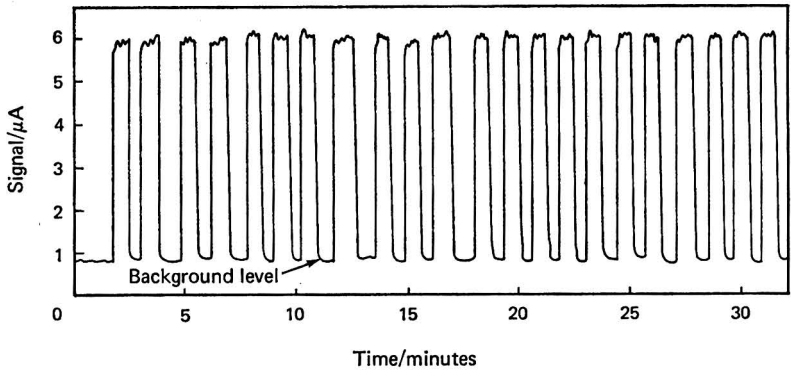

Fig. 2. Repetitive recordings of intensity of signals for zinc. Wavelength, 213·9 nm; concentration of zinc solution, 150 μg ml^{-1}

SENSITIVITY—

Detection limits obtained for sixty-seven elements are given in Table I. These limits are expressed as micrograms per millilitre in aqueous solution and are defined as that concentration of an element which gives rise to a signal equal to twice the standard deviation of the background noise level at the appropriate wavelength. For comparison, the detection limits obtained for a number of elements with the nitrous oxide - acetylene flame are also given. These limits are also based on twice the standard deviation of the background noise level and were determined by using the same optical arrangement and detection equipment.

DISCUSSION

The incorporation of the pre-mixed aerosol chamber does not require any significant modification to the plasma jet itself. With simple workshop facilities, the remainder of the equipment can be manufactured at low cost. The system described has been in operation for about 2 years and the only occasional replacement has been of the glass tube that is in contact with the graphite ring. The life of these tubes is about 6 months in regular operation.

The operation and performance of the new system have several advantages over the direct-injection system, namely:

(*a*) elimination of corrosion of the nebuliser;
(*b*) control over the rate of spraying;
(*c*) production of a more uniform mist;
(*d*) ease of cleaning the nebuliser capillary; and
(*e*) longer operating periods with more stable emission.

The stability achieved with the modified system is suitable for use with instantaneous photoelectric read-out. As well as giving higher sensitivity, this system permits continuous visible monitoring of the stability of the emission.

A survey of detection limits with the modified system showed that the sensitivity was, in general, an order of magnitude better than that previously obtained with the direct-injection system. For example, the detection limits were aluminium, 3; barium, 0·1; boron, 0·5; copper, 1; gadolinium, 4; scandium, 0·2; strontium, 0·1; silicon, 2; titanium, 0·6; and vanadium, 2 μg ml^{-1}. These detection limits, however, were obtained by using argon as both the tangential and the nebulising gas. The argon - argon system gives a much higher background than helium - argon, which is partly responsible for the reduced sensitivity.[4] Attempts to operate the original system with the helium - argon system were unsuccessful so that a strict comparison could not be undertaken. However, the major contributing factor in achieving the higher sensitivity is the greater stability of the emission in the modified system. Table I shows that the detection limits obtained with the modified system compare very favourably with those obtained with the nitrous oxide - acetylene flame. A major advantage of the plasma jet, however, is its ability to excite those elements which tend to form stable oxides in the high-temperature flame. This group of elements includes zirconium,

TABLE I

DETECTION LIMITS FOR THE MOST SENSITIVE LINE OF THE ELEMENT IN THE MODIFIED PLASMA JET AND IN THE NITROUS OXIDE - ACETYLENE FLAME

Element	Plasma-jet detection limit/μg ml^{-1}	Wave-length/nm	N_2O - C_2H_2 flame detection limit/μg ml^{-1}	Wave-length/nm
Aluminium	0·3	396·2	0·06	369·2
Antimony	0·2	259·8	—*	—
Arsenic	0·5	235·0	—	—
Barium	0·2	553·5	0·03	553·5
Beryllium	0·003	313·0†	—	—
Bismuth	5	306·8†	—	—
Boron	0·05	249·8	—	—
Calcium	0·008	393·4	0·002	422·7
Carbon	15	247·9	—	—
Cerium	2	415·0	—	—
Chromium	0·4	267·7	0·01	425·4
Cobalt	0·8	228·6	0·8	345·4
Copper	0·2	324·8	0·2	327·4
Dysprosium	0·3	353·2	0·05	404·6
Erbium	0·6	400·8	—	—
Europium	0·1	382·0	0·005	459·4
Gadolinium	0·7	342·2	0·5	440·2
Gallium	0·1	417·2	0·2	417·2
Germanium	0·2	265·1	0·5	265·1
Gold	0·2	242·8	4	267·6
Hafnium	0·6	264·1	—	—
Holmium	0·3	345·6	0·03	405·4
Indium	0·3	451·1	0·01	451·1
Iridium	1·5	254·4	—	—
Iron	0·2	259·9	0·3	372·0
Lanthanum	0·5	394·9	0·8	441·7
Lead	0·2	283·3	2	405·8
Lithium	0·08	670·8	0·002	670·8
Lutetium	0·06	261·5	1	451·9
Magnesium	0·02	279·7	0·06	403·1
Manganese	0·04	257·6	0·04	—
Mercury	0·3	254·6	—	—
Molybdenum	0·1	281·6	0·6	390·3
Neodymium	3	401·2	—	—
Niobium	0·4	405·9	—	—
Nickel	0·3	221·6	0·4	341·4
Osmium	0·7	225·6	—	—
Palladium	0·5	361·0	1	363·5
Phosphorus	0·6	253·6	—	—
Platinum	0·5	265·9	12	265·9
Praseodymium	4	414·3	—	—
Rhenium	0·5	221·4	0·5	346·1
Rhodium	0·5	343·5	0·3	369·2
Ruthenium	0·6	372·8	—	—
Samarium	0·3	360·9	—	—
Scandium	0·03	361·4	0·06	402·0
Selenium	2	204·0	—	—
Silicon	0·2	251·6	—	—
Silver	0·2	338·3	0·1	328·1
Sodium	0·02	589·0	—	—
Strontium	0·01	407·8	0·003	460·7
Tantalum	1·5	296·5	7	481·3
Tellurium	0·7	214·3	—	—
Terbium	0·07	369·4	0·1	431·9
Thallium	0·4	535·0	—	—
Thorium	2	401·9	—	—
Thulium	0·5	346·2	0·1	371·8
Tin	0·6	284·0	—	—
Titanium	0·08	334·9	0·2	399·8
Tungsten	2	400·9	—	—
Uranium	7	386·0	—	—
Vanadium	0·2	309·3	0·05	437·9
Ytterbium	0·07	328·9	0·0004	398·8
Yttrium	0·08	371·0	1	362·1
Zinc	0·1	213·9	—	—
Zirconium	0·3	339·2	6	360·1

* Not observed.
† Second order.

beryllium, silicon, boron, titanium and some rare earths, and reference to Table I and other published detection limits[9] indicates the greater sensitivity for these elements in the plasma jet. Phosphorus, uranium, thorium, iridium, cerium and osmium also have high sensitivities and are not appreciably excited in chemical combustion flames.

The excitation of lines of high energy, such as that of phosphorus at 253·6 nm (7·2 eV), indicates the high temperature of the source, which results in a high degree of ionisation, and it is generally found that the most sensitive lines are those which originate from ionised species. This effect is the reason for the poor sensitivity of the alkali metals sodium and lithium, for which ionic lines are inaccessible.

The detection limits given in Table I for both tungsten and carbon were determined in the presence of a high background emission level for these elements in the discharge owing to the erosion of both the transfer electrode and the graphite electrodes. By replacing the graphite electrodes with copper electrodes, the detection limit for carbon is reduced to 2 μg ml^{-1}, taking into account the blank level that arises from contributions from carbon dioxide present in the water and gases. The plasma jet operates very satisfactorily with copper electrodes; less erosion occurs than with graphite electrodes and only occasional replacement is required.

The discharge is also characterised by a fairly high hydroxyl band emission (band head at 306·4 nm), and owing to this emission the emission lines of both bismuth and beryllium had to be recorded in the second order of the grating so as to minimise spectral interference.

By using the system described, analyses that are not applicable to or readily accomplished by flame spectrometry have been carried out. Some typical examples are the determinations of phosphorus in uranium compounds at the 100 p.p.m. level; low levels of rare-earth elements in association with solvent-extraction studies; hafnium in reactor-grade zirconium; beryllium below the parts per million level; and total soluble carbon in aqueous effluent streams.

These applications exemplify the usefulness of the plasma jet in the spectrochemical analysis of solutions. As the modified system can be operated in a manner similar to that with chemical combustion flames with the optical arrangement described and without the need to use integrated output, it can be conveniently interchanged with an air - acetylene or nitrous oxide - acetylene burner, thus permitting a wide range of elements to be determined with high sensitivity by selecting the appropriate excitation source.

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A Method for Determining Free Azide Ions by Automatic Analysis in the Presence of a Covalent Cephalosporin Azide*

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An assay was devised in order to follow quantitatively the release of azide as free ions, N_3^-, from a cephalosporin azide when attacked by β-lactamase enzymes produced by specific strains of bacteria. Experiments were arranged to ascertain whether or not the azide cleavage occurred at the same rate as the rupture of the β-lactam ring. The assay was required to determine free azide at concentrations between 2 and 20 μg ml^{-1}; other experimental limitations were imposed by the requirements of enzymolysis.

The procedure adopted was based on the complete oxidation of azide ions to nitrogen by an excess of a standard aqueous solution of nitrite ions at pH 4·6. The residual nitrite was removed by diazotisation with 4-aminosalicylic acid, followed by coupling of the product with a second molecule of the 4-aminosalicylic acid. The reaction mixture was then rendered alkaline by the addition of tetramethylammonium hydroxide solution. The final yellow colour was stable and had an absorption maximum at 440 nm. It was not possible, however, to achieve sufficient operational reproducibility manually, and an AutoAnalyzer system was therefore used.

The results show that cleavage of azide from the parent molecule occurs at the same rate as the rupture of the β-lactam ring and parallels the decline in microbiological potency.

The antibiotic activity of cephalosporins is known to decline when they are attacked by β-lactamase enzymes produced by organisms that infect the body. This decline is accompanied by a decrease in ultraviolet absorption at a wavelength of 440 nm. An examination of the effect of such enzymes on derivatives of cephalosporin azides was undertaken as part of an investigation into the breakdown routes followed by these antibiotics. It was necessary to determine the free azide ions as they were produced in test preparations that contained both covalent cephalosporin azide and an enzyme inhibitor. Moreover, test solutions were also to be sampled for microbiological assay of their antibiotic potency and, simultaneously, for measurement of their ultraviolet absorption at 260 nm. An analytical method for the determination of free azide was therefore sought that was sensitive, reliable, rapid and practicable for use with a small amount of material.

Azide is labile in the 3-position of the cephalosporin nucleus; it can readily accept an electron and leave the nucleus by the mechanism illustrated (Fig. 1). β-Lactamase would sever the carbonyl - nitrogen bond and release the lone pair of electrons into the dihydrothiazine ring. Further electron displacement would result in the release of azide as N_3^- ions.

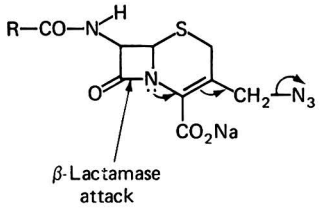

Fig. 1. Attack by β-lactamase on the sodium salt of 3-azidomethyl-7β-benzylthioacetamidoceph-3-em-4-carboxylic acid

* Based on a paper presented at the Third SAC Conference, Durham, July 12th to 16th, 1971.

An aqueous solution containing 400 μg ml^{-1} of covalent cephalosporin azide could be expected to yield 40 μg ml^{-1} of free azide on complete degradation. The analytical method was needed for concentrations of this order and, so that the earlier part of the breakdown could be followed meticulously, sensitivity had to be high, *i.e.*, between 2 and 10 μg ml^{-1}.

At high enzyme concentrations, only 4 ml of the reaction solution could be spared. Additional limiting factors included buffer concentration, enzymolysis temperature and inhibitor efficacy.

The concentrations of the β-lactamase preparations used were chosen so as to afford complete degradation in a reasonable time (Fig. 2). At the highest concentration (*E*) they were solutions in 0·1 M phosphate buffer at pH 7. Quantitative differences in enzymolysis and in azide release rates at temperatures of 4 and 37 °C were anticipated and were determined.

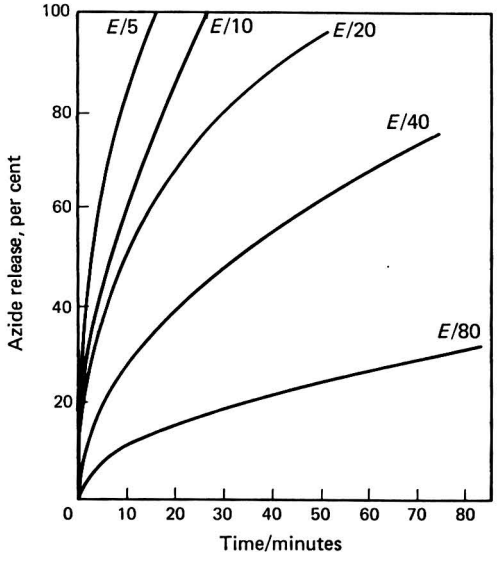

Fig. 2. Azide release at different enzyme concentrations (*E*)

An enzyme inhibitor was added in order to arrest the production of azide ions during the assay. This addition limited the assay time to 10 minutes, as the 7α- or 7β-naphthoic derivative of cephalosporin or penicillin finally used as inhibitor would not arrest the enzyme attack for a longer period of time. Many otherwise suitable inhibitors, such as phenylmercury(II) salts, were discounted on grounds of solubility. Thus, adjustments to the temperture and pH of the sample in order to bring them within the time limits suitable for the assay had to be undertaken without delay.

Various methods were considered, and included gasometric analysis, which required measurement of liberated nitrogen after the sample had been oxidised with sodium iodide and trichloroacetic acid[1]; distillation as hydrazoic acid and subsequent titration with standard alkali; anodic polarography[2]; and precipitation of free azide as silver azide (AgN_3), measured potentiometrically.[3] None of these methods was sufficiently sensitive. Titrimetric methods, based on the reaction between cerium(IV) and azide ions, were not attempted as they were already known to be very slow at low concentrations.[4] Attention was therefore focused on colorimetry.

Reagents—

Potassium nitrite solution—A 0·03 per cent. *m*/*V* aqueous solution of AnalaR grade material is used (2 to 3 drops of Tween 20 are added to 500 ml of reagent so as to prevent any tendency to surge).

Sodium 4-aminosalicylate solution—A 2 per cent. *m/V* aqueous solution of laboratory-reagent grade material is made up.

Acetate buffer solution, 2 M—Equal volumes of 4 M acetic acid and 4 M sodium acetate solution are mixed. The pH is adjusted to between pH 4·50 and 4·70 by using, if necessary, acetic acid or sodium hydroxide solution.

Tetramethylammonium hydroxide solution—A 25 per cent. aqueous solution of laboratory-reagent grade material is diluted with an equal volume of distilled water.

All of the above reagents should be filtered through a fluted Whatman No. 541 filter-paper.

Standard azide solution—A standard solution of sodium azide containing 1000 p.p.m. of free azide (N_3) (1·548 g l^{-1}) is freshly prepared in 0·005 M phosphate buffer solution at pH 7·5. Suitable dilutions are made by using the same buffer.

Experimental

Methods of forming the red complex $(FeN_3)^{2+}$ by adding solutions containing azide ions to acidified iron(III) nitrate[4] or to perchlorate solution[5] have been described, but cephalosporin derivatives, and especially their azides, interfere in the reaction. An otherwise promising alternative involving the use of diazotised sulphanilic acid coupled with 2-naphthylamine[3] was considered to be unsafe for routine analysis.

The procedure ultimately adopted was based on the complete oxidation of azide ions to nitrogen, by addition of excess of a standard solution of nitrite ions, in an aqueous solution strongly buffered at pH 4·6. The residual nitrite reacted with 4-aminosalicylic acid to form a diazo compound that coupled with another molecule of the 4-aminosalicylic acid. The reaction mixture was made alkaline with tetramethylammonium hydroxide solution so as to give a stable yellow - brown solution that had an absorption maximum at 440 nm.

A number of critical factors suggested that manual analysis would not be very satisfactory. Accordingly, an assay was developed that made use of an AutoAnalyzer system into which the required adjustments to pH and temperature could be incorporated. Each assay was completed in 7 minutes. The AutoAnalyzer accepted samples at the rate of twenty per hour and analysed solutions in the concentration range 2 to 20 μg ml^{-1}.

The enzyme purification process necessitated elution of the β-lactamase from a chromatographic column with 0·1 M phosphate buffer at pH 7·0 because the enzymes were insoluble in distilled water. A consequent advantage, however, was that the enzymolysis solution needed no further buffering to avoid loss of azide as hydrazoic acid.

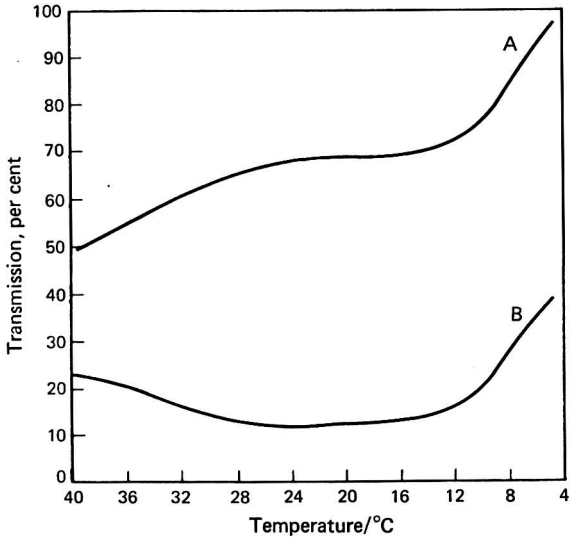

Fig. 3. Transmission at 440 nm with continuous aspiration of a solution containing (A) 10 and (B) 0 μg ml^{-1} of azide

The subsequent determination of azide required samples to be at pH 4·6, and because the diazotising and coupling reactions proceeded at a faster rate in an acetic acid - acetate medium than in phosphate or phthalate-buffered conditions, a strong acetate buffer (2 M) was used. The presence of this buffer also reduced the temperature dependence of the assay and interference from inorganic ions.

Uniformity of temperature was achieved by immersing all the AutoAnalyzer coils in a water-bath that was capable of controlling temperatures to within $\pm 0{\cdot}1$ °C. In addition, all of the reagents were brought to the azide assay temperature by passing them through suitable temperature-regulating coils also immersed in the constant-temperature bath.

Fig. 3 illustrates the temperature dependence of the assay at concentrations of 0 and 10 μg ml^{-1} of azide. This dependence is smallest between 18 and 26 °C and consequently represents a stable working temperature range while also ensuring almost maximum sensitivity.

Problems arose at the final stage, when the product was made alkaline so as to stabilise the final colour. When, in the interests of speed and sensitivity, the assay was conducted below pH 4, excessive amounts of tetramethylammonium hydroxide were needed to neutralise the acetate buffer, thus causing solubility problems. Above pH 4·6, the reaction became markedly slower; accordingly, 4·6 was selected as being the optimum pH. The most suitable temperature range was found to be between 16 and 22 °C. Lower temperatures, in the region of 4 to 10 °C, protracted the assay time beyond the working limit of the inhibitor and caused difficulties through the condensation of atmospheric water vapour on the colorimeter cell.

To ensure a rapid reaction at pH 4·6, a large excess of sodium 4-aminosalicylate was used. In practice, a concentration of about 5 per cent. m/V was found to be the upper limit for this reagent; beyond that, absorption by excess of reagent at 440 nm became unacceptable. Further, above 5 per cent. m/V the sensitivity was too low for small concentrations of azide to be measured.

There was no interference from non-oxidising or non-reducing ions below concentrations of azide of 2 mg ml^{-1}. A 40 per cent. reduction in sensitivity was observed with cephalosporin azide at 10 mg ml^{-1}, but this level was twenty-five times greater than the normal working concentration.

THE MANIFOLD—

Initially, the air aspirated between the sample and wash solutions caused noisy recordings. To overcome this defect, a re-sample loop was incorporated in the AutoAnalyzer manifold (Fig. 4). The sample was segmented with air and brought to the reaction temperature by means of a small mixing coil immersed in the constant-temperature water-bath. The nitrite reagent, introduced into the sample stream at point A, was prepared and its temperature was regulated continuously by mixing equal volumes of potassium nitrite solution and acetate buffer (2 M) in another coil.

When the buffer solution and sample were mixed, and the nitrite was then added, the results were not entirely reproducible. This imprecision was attributed to a varying loss of hydrazoic acid into the air bubbles. It was also noted that any segmentation of an acidic nitrite stream at the preferred temperature led to a similar lack of reproducibility. Hence, the diazotising mixture was prepared and added in a continuous stream.

Sodium 4-aminosalicylate was added at point B after appropriate temperature regulation. Difficulties were incurred when the viscous tetramethylammonium hydroxide reagent was added to the liquid stream. Mixing became difficult and, as a result, the irregular proportioning due to pump pulsing became critical. This problem was solved by including a pulse suppressor [Fig. 5 (*a*)], a chamber mixer [Fig. 5 (*b*)] and a capillary side-arm T-piece of i.d. 0·05 mm at point C. The temperature of the tetramethylammonium hydroxide reagent was not critical.

The whole manifold was assembled in a small thin-layer chromatographic tank (9 × 9 × 3 inches) filled with coolant. Greater sensitivity was obtained when the final reaction time was increased by using additional delay coils, and in order to eliminate any tendency to surge under these conditions the extra coils had successive turns of increasing diameter.

The range and sensitivity of the method can be seen in Fig. 6, which shows peaks from eight standards within the concentration range 0 to 20 μg ml^{-1}. The degree of discrimination between high and low peaks shows the negligible carry-over between sample and wash solutions. The reproducibility of the method is illustrated by peaks from replicate samples at

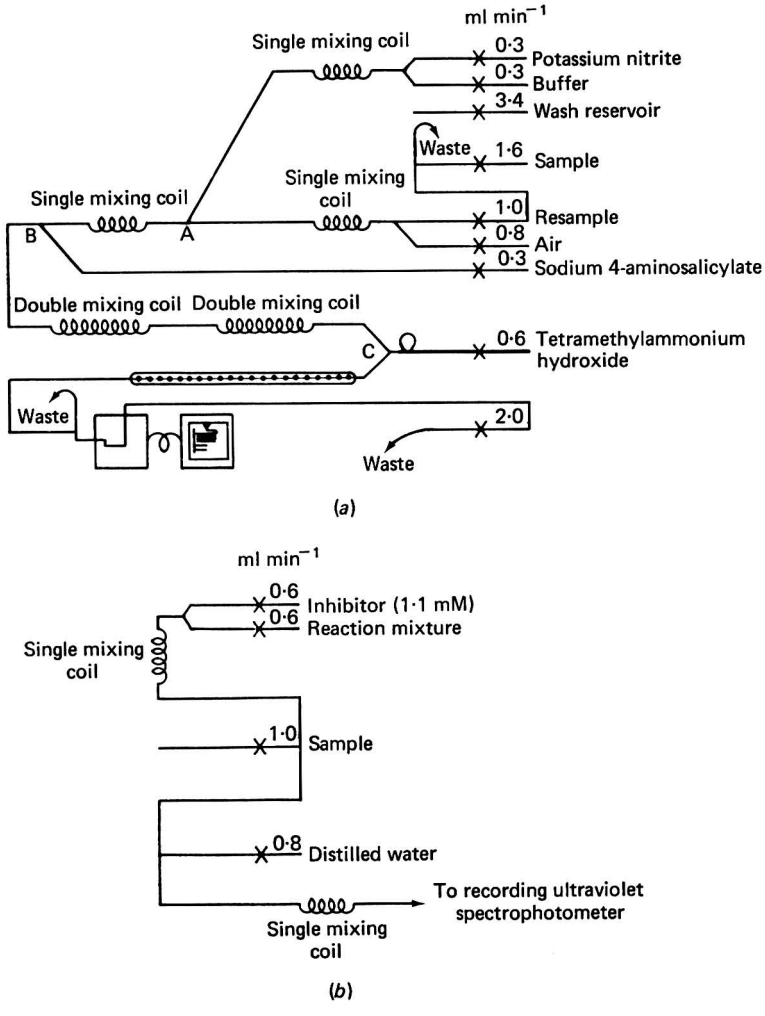

Fig. 4. (*a*) AutoAnalyzer manifold; and (*b*) diluent manifold

concentrations of 7·5 and 15 μg ml^{-1} of free azide, while a steady state is indicated by the peaks resulting from continuous aspiration of a 15 μg ml^{-1} solution of azide.

PROCEDURE FOR DISCRETE SAMPLING—

The manifold is assembled as illustrated. The coolant is circulated at 16 to 22 °C with a tolerance of not more than $\pm$0·1 °C. The filtered reagents are pumped into the manifold

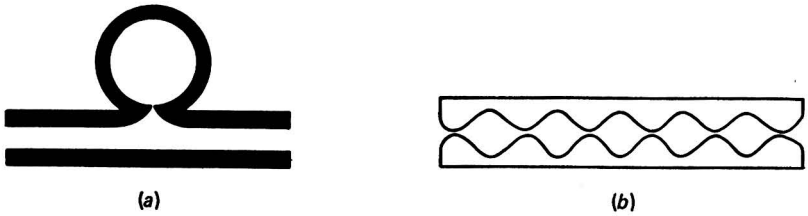

Fig. 5. (*a*) Pulse suppressor; and (*b*) chamber mixer

and a level base-line is established. The sampler unit operates at a rate of 20 per hour (2:1 sample to wash ratio), starting with two high and two low standards. The concentrations of the potassium nitrite solutions are adjusted until these preliminary standards give suitable transmissions at 440 nm. Further standards are prepared in order to establish a calibration graph. Daily calibration is necessary because the response curve is altered significantly by the inevitable small variations that occur in the reagents and the pump tubing. The necessary amount of inhibitor is added to samples that have been withdrawn from the experimental solutions, the solutions are mixed well and placed in a sample cup on the sampler module. They are immediately analysed for ionic azide. The concentration of free azide in each sample is then calculated by reference to the calibration graph.

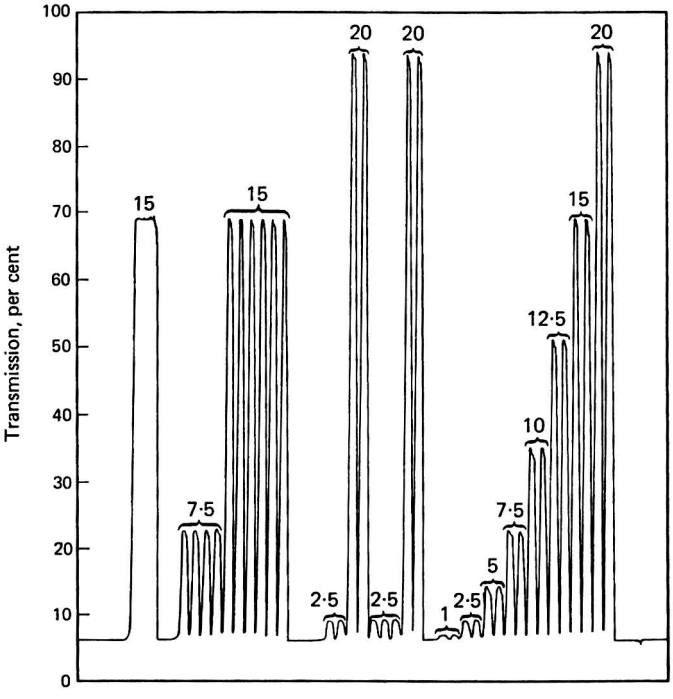

Fig. 6. AutoAnalyzer recording of azide solutions. Values above peaks are azide concentrations in micrograms per millilitre

PROCEDURE FOR CONTINUOUS SAMPLING—

The logical method is to monitor the reaction continuously. However, this can be effected only when a sufficiently large amount of solution is available. The manifold is assembled as in Fig. 4 (*a*), but omitting the re-sample loop and introducing an equal volume of the inhibitor (1·1 mM) into the sample line before splitting the stream [see Fig. 4 (*b*)]. For circulating the coolant, pumping in the filtered reagents and establishing a base-line the method described under Procedure for discrete sampling is followed. A calibration graph is constructed by using six standards, taken over the complete working range, and sampling each until a steady state is achieved. Samples are taken from the reaction vessel in order to establish a base-line for the individual antibiotic, the enzyme preparation is added and mixed well, and the reaction is monitored, taking into account the delay time of the AutoAnalyzer system.

RESULTS

Accuracy to within ±1 per cent. was demonstrated by recovery of the theoretical amount of azide from the covalent cephalosporin azide in six separate experiments. These experiments involved three different enzymes. The reproducibility was better than ±0·5 per cent.

The majority of the enzymolysis results are published elsewhere,[6] and are considered in relation to other biological factors. However, the rate of enzyme attack at 37 °C was only 25 per cent. greater than that at 4 °C, with a similar temperature dependence for the rate of azide release. A typical graph, obtained with an enzyme produced from one specific strain of gram-negative bacteria to illustrate the above features, is given in Fig. 7. The changes in ultraviolet absorption, in azide release, and in microbiological activity against *Staphylococcus aureus*, are plotted against time. The breakdown rates are identical within the limits of experimental error.

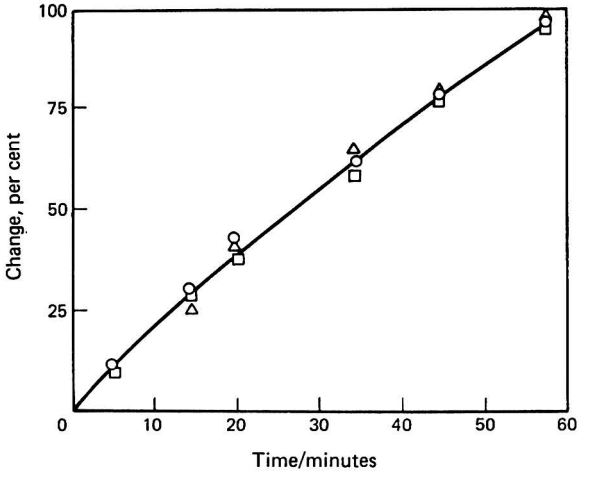

Fig. 7. Comparison of release of azide (○), loss of biological activity (□) and decrease in absorption at 260 nm (△)

Conclusions

The decline in antibiotic activity and the cleavage of azide from the parent molecule occur at the same rate and to the same extent, and are temperature dependent to the same degree, as the hydrolysis of the β-lactam ring. However, other semi-synthetic cephalosporins that have less labile groups than azide in the 3-position are also easily attacked by β-lactamase. In these instances rupture of the β-lactam ring takes place, and no loss of 3-terminal groups occurs, whereas the azide loss is directly dependent upon β-lactam ring hydrolysis. This fully supports the mechanism outlined in Fig. 1.

I thank Mr. J. L. Martin and Mr. R. E. Duncombe for much helpful discussion.

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The Histochemical Detection of Soya "Novel Proteins" in Comminuted Meat Products

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The enforcement of the regulations governing meat and meat products requires the determination of meat content. Meat content is assessed from the total nitrogen content, from which suitable deductions are made for the nitrogen contributed by the other ingredients of significant nitrogen content present in meat and meat products. The availability of "novel proteins" and the possibility of the addition of these proteins to meat products necessitates the detection and determination of "novel proteins" in such products for the true assessment of their meat content. A microscopical method that indicates the presence of "novel protein" of soya origin in meat products has been examined. This method involves the use of a specific technique to demonstrate the presence of carbohydrate material and is diagnostic for the cellular fraction of many processed soya products.

Protein has received much attention during the last two decades and the protein needs and supplies of the world have been the subject of much discussion. The Protein Advisory Group[1] of the United Nations has concluded that the world protein deficit would be about 20 million tons per year in the early 1970s. The awareness of this great deficit, and the view that traditional sources, however developed, would be unable to meet the increasing shortage, has resulted in the search for non-traditional sources of protein, generally termed "novel proteins."

"Novel protein" research has been mainly directed towards oil-seed proteins, leaf protein isolate, field-bean (*Vicia faba* L.) protein isolate and single-cell proteins. Of the four oil-seed proteins (soya bean, groundnut, cotton seed and coconut), the first two are now available in commercial forms for human consumption: groundnut as protein concentrate and protein isolate[2] and soya bean as full fat flour, defatted flakes, grits and flour, protein concentrate and protein isolate.[3,4] The approximate protein percentage contents of the soya bean forms are: full fat flour, 42; defatted flakes, grits and flour, 50 to 55; concentrate, 65 to 70; and isolate, 90 to 95.[3,4] Field-bean isolate is also commercially available for human consumption with a protein content of 85 per cent. These plant proteins have been presented as protein-rich foods, dried meat preparations, hydrolysed vegetable protein, protein drinks[3] and textured vegetable proteins, which include extruded soya protein, spun soya protein[5] and spun field-bean protein.[6] It would appear that, for human consumption, only the soya-bean proteins are available in substantial and rapidly increasing amounts every year. These commercial products are available in the U.S.A., Europe and, more recently, in Great Britain.

British food regulations[7–10] require the quality of both meat and meat products to be assessed by their meat content. The meat content is calculated by using an experimentally determined value for total nitrogen from which appropriate deductions are made for the nitrogen contributed by other ingredients of significant nitrogen content.[11] The presence of significant amounts of "novel protein" in meat products would increase the total nitrogen content value, unless its presence is detected, determined quantitatively and a correction made for its nitrogen contribution. The detection and determination of these "novel proteins," whether declared or undeclared, intentionally or otherwise added to meat products, is becoming increasingly necessary, not only to control the addition of "novel protein" as a substitute for skeletal muscle protein, but also to aid the implementation of the regulations relating to meat products.

A microscopical method for detecting "novel protein" in meat products has been examined in this investigation. The technique involves the controlled oxidation of the

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carbohydrate material present in plant protein products and demonstration of the presence of the resulting aldehydes. The method is capable of distinguishing between the carbohydrate material associated with plant proteins and that from soya beans and could be made semi-quantitative.

Method

Reagents—

Buffered formalin fixative—Dissolve 4·5 g of sodium dihydrogen orthophosphate dihydrate and 6·5 g of disodium hydrogen orthophosphate in about 700 ml of distilled water. To this solution add 100 ml of 40 per cent. formaldehyde solution. Dilute to 1000 ml with distilled water (the pH of this buffer is about 6·8).

Periodic acid solution, 1 per cent.—Dilute 0·6 ml of 50 per cent. periodic acid solution to 50 ml with distilled water.

Schiff's reagent, stock solution—Dissolve 0·5 g of basic fuchsin (C.I. No. 42510) in 100 ml of distilled water and decolorise with a stream of sulphur dioxide. The solution is stable for a few weeks at 4 °C.

Schiff's reagent, working solution—Dilute 1 ml of stock solution to 50 ml with distilled water.

Protein counterstain—Prepare a 1 per cent. *m/V* solution of Procion Brilliant blue (C.I. No. Reactive Blue 4) in distilled water or a 0·5 per cent. *m/V* solution of Light green (C.I. No. 42095) in distilled water.

Sample preparation procedure—

Roll the comminuted meat product into balls approximately 1 cm in diameter or hydrate the dried protein products by soaking in distilled water and place them in buffered formalin fixative for a minimum of 48 hours. Cut cryostat sections by washing the fixed specimens in running water, rapidly freezing, and cutting 10-μm sections in a cryostat cabinet at −18 °C. Alternatively, cut 10-μm wax sections. Details of the wax embedding and sectioning technique are available in standard textbooks on microscopy.

Two distinct extruded soya protein products and one spun field-bean protein isolate were sectioned and stained as described above. As all the samples showed some orientation due to manufacture, sections were cut both parallel to this orientation (longitudinal sections) and at right angles to it (transverse sections). In addition, sections were prepared of a blend of raw pork sausage meat with firstly 10 per cent. *m/m* of defatted soya-bean flour and secondly 10 per cent. *m/m* of moist extruded soya protein.

Staining procedure—

Place the sections in water. (Wax sections should be treated for 10 minutes in xylene followed by absolute ethanol, 90 per cent. ethanol and 70 per cent ethanol.) Oxidise the sections in periodic acid solution for 5 minutes, except the control sections, for which this step should be omitted, and wash them in running water for a further 5 minutes. Treat the sections with Schiff's reagent for 20 minutes and again wash them in running water for 5 minutes. Counterstain for 5 minutes in protein counterstain containing 3 to 4 drops of 1 N sulphuric acid per 50 ml of stain and rinse in water. Then, rinse the sections successively in 70 and 90 per cent. ethanol and dehydrate them in absolute ethanol for 1 minute. Clear in xylene, also for 1 minute, and finally mount the sections in neutral Canada balsam or DPX (refractive index 1·524).

Carbohydrate material appears magenta on staining, *i.e.*, periodic acid Schiff (PAS) positive, while protein appears blue or green, according to the counterstain used. The control shows only the colour due to the counterstain.

Results and observations

All sections of extruded soya products showed regions of PAS positive material when viewed through a 16 mm (×10) objective. The carbohydrate was present both in a characteristic cellular form consistent with being derived from the cells of the soya bean, and also as large areas of amorphous material. Soya-bean cells were clearly observed in the blends of sausage meat with soya flour and sausage meat with soya extrudate.

In contrast, the protein isolate derived from field bean contained no distinct plant cells or discrete areas of carbohydrate material. When the PAS preparation was counterstained,

even lightly, only protein was observed. By omitting the counterstain it was seen that the whole preparation was weakly PAS positive. Photographs showing these results comprise Figs. 1 to 6, all of which are magnified 125 times.

Discussion

Protein products of soya origin, although high in protein content, continue to be associated with some of the carbohydrate constituents of the soya bean. The soya bean is entirely different from other legumes and from cereals in its carbohydrate constituents. With the exception of a few strains, the bean has virtually no starch reserve and the carbohydrate present is composed of other polysaccharides, notably hemicelluloses and cellulose. These carbohydrates contain the 1,2-glycol grouping (CHOH–CHOH), which can be selectively oxidised with dilute periodic acid to yield a dialdehyde (CHO–CHO).

Periodic acid does not oxidise the aldehydes further, so that insoluble carbohydrates remain *in situ* and their presence can be demonstrated by use of Schiff's reagent.[12] It is claimed that the red colour produced is due to the combination of the basic fuchsin with the dialdehyde rather than a simple re-oxidation of the fuchsin sulphurous acid.[13] Materials that give this reaction are known as PAS (periodic acid Schiff) positive and include compounds of carbohydrates with protein or lipids, so that a wide range of materials is involved. It was found with soya flour and soya extrudates that the strongly PAS positive materials occurred in a characteristic cellular form, easily recognised despite any processing that the material had undergone. In the samples examined, cells of the cotyledon were widespread and easy to identify, but other cells, including the hour-glass and endosperm cells, were also present. When the soya products were mixed with commercial sausage meat the plant cells were easily distinguished from other, less structured, carbohydrate material present, *e.g.*, gelatinised starch derived from the sausage rusk.

As many chemical groups oxidise Schiff's reagent, control slides in which Schiff's reagent is allowed to act but with the oxidation stage with periodic acid omitted, are essential, *e.g.*, lignin or residual fixative both re-colour the Schiff's reagent and it is desirable to be aware of such non-specific staining. Each photograph showing PAS positive material is therefore accompanied by a control. For contrast, and for ease of identification, the protein material present was counterstained. Light green and Procion Brilliant blue were both found to be effective for this purpose. They stain all of the protein present; in sausage sections, muscle tissue and flour protein (as well as soya protein) were coloured but it was observed that the soya protein was less intensely coloured than the other proteins. This differentiation is most useful in assessing the proportion of soya material present in comminuted meat products.

The field-bean isolate (Figs. 5 and 6) contained only trace amounts of carbohydrate material, structured cellular carbohydrates being completely absent. This fact suggests that it might be difficult to detect soya isolates as distinct from flours, grits and extrudates. Spun soya contains more than 90 per cent. of protein, and hence much less carbohydrate than other soya products, and, in addition, the carbohydrate that is present is likely to be less structured. The high cost of production of the spun isolate has led to its use in declared forms of presentation, such as whole soya turkey in which the fibrous character is exploited, rather than to its declared or undeclared addition to comminuted meat products, in which the expense is not justified. This limited usage of the isolate suggests that it is the detection and determination of soya flour and extrudate that is currently the more pressing problem for the analyst.

The authors thank Dr. J. E. McKay and Mr. R. A. Dalley, City Analyst, Leeds, for helpful discussions during the work, and Mrs. A. Sharples for technical assistance.

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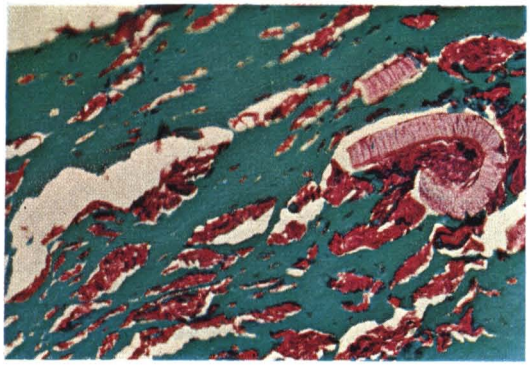

Fig. 1. Transverse section of a cylindrical extrudate showing PAS positive palisade cells and areas of amorphous carbohydrate (magenta). Protein stained with Light green

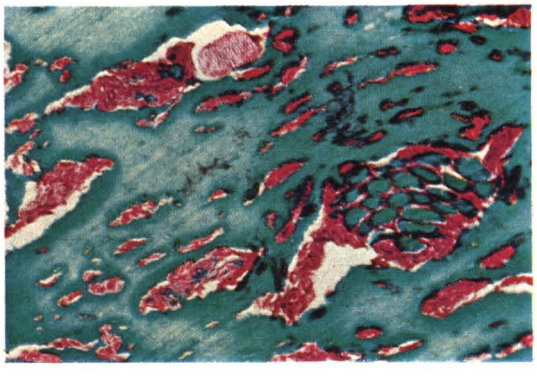

Fig. 2. Longitudinal section of a textured extrudate which macroscopically resembled meat chunks. A group of PAS positive cotyledon cells containing protein are surrounded by amorphous PAS positive material. Protein stained with Light green

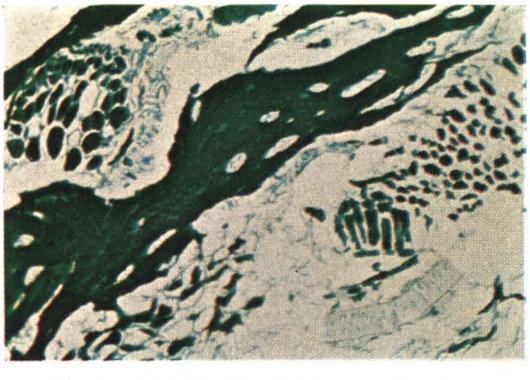

Fig. 3. Control slide to Fig. 2. The textured extrudate has been stained as for Fig. 2 with omission of periodic acid oxidation. The only colour is the protein, stained with Light green

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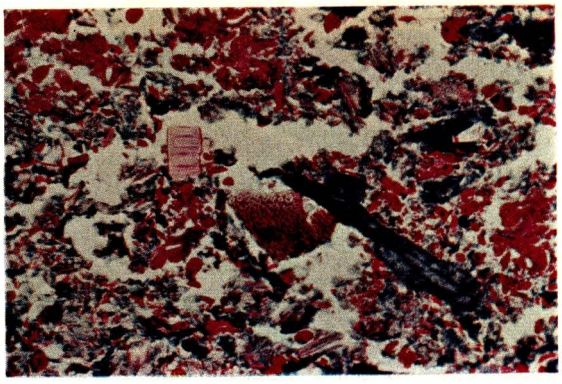

Fig. 4. Defatted soya flour present in pork sausage. Groups of palisade cells (PAS positive) sectioned longitudinally and transversely can be seen. Note PAS positive nature of the baked starch granules present in the rusk of the sausage. These are easily distinguished from soya carbohydrates. Protein counterstained with Procion Brilliant blue

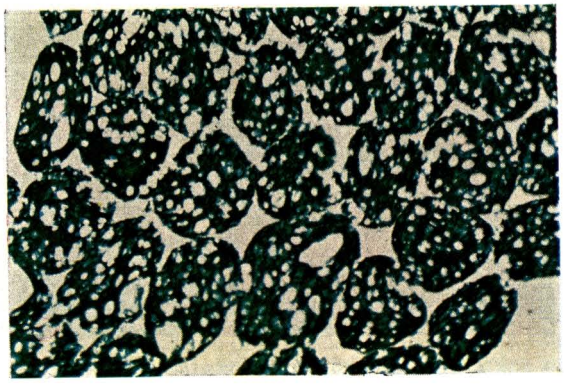

Fig. 5. Spun protein stained with PAS and Light green. Only the protein fraction appears coloured and is identical with the control. Material cut to show fibres in transverse section

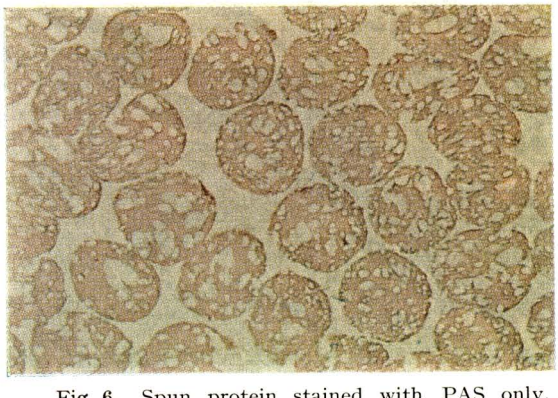

Fig. 6. Spun protein stained with PAS only. This shows a weak, uniform, diffuse, PAS positive fraction, which is masked in the counterstain in Fig. 5. Material cut to show fibres in transverse section

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Interference of Carbon Dioxide, Resulting from the Schöniger Flask Combustion of Organofluorine Compounds, in the Titrimetric Determination of Fluorine

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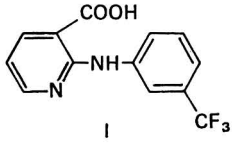
Carbon dioxide produced during the Schöniger flask combustion of organofluorine compounds has been found to interfere in the titrimetric determination of fluorine. When thorium nitrate that had been standardised against sodium fluoride solution was used, the fluorine content found was consistently 93 per cent. of theory. This interference was overcome by adding sodium carbonate to the solution used for standardisation before titration. For detection of the titration end-point, an improved indicator, methylthymol blue, was used.

By using this procedure, compounds containing between 4 and 30 per cent. of fluorine, either in a CF_3 or CF group, have been assayed satisfactorily. No interference from nitrogen, sulphur or chlorine contained in some of the compounds was observed.

In assays of halogens bonded in organic molecules, the Schöniger oxidation method can be used[1,2] to convert halogen atoms into ions, which can then be assayed by various techniques, *e.g.*, colorimetry or titrimetry. In these procedures, it is customary to use as a reference standard an organohalogen compound of high purity. However, in this laboratory we required a method that did not rely upon the combustion of an organic compound as standard but, instead, involved the use of a true standard that did not require combustion, such as the sodium salt of the halogen.

When this oxidative method, followed by colorimetry, was used to determine the amount of fluorine in niflumic acid, **I**, it was found to be only 93 per cent. of theory. In the colorimetric procedure use was made of the coloured complex formed between fluoride ion and the cerium(III) complex of alizarin complexan, 3-[di-(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone, in acetate buffer. When a succinate buffer was substituted for the acetate buffer,[3] the fluorine content found was still only 93 per cent. of theory.



I

It was then decided to examine the possibility of using the thorium nitrate titration procedure. Soep[4] had described the use of thorium nitrate solution, standardised against sodium fluoride solution, for the determination of the fluoride resulting from the combustion of an organofluorine compound. He found that use of this technique gave consistently low values for the fluorine content of the sample, thus requiring the use of a correction factor. In order to avoid the need to use this correction factor, we attempted to determine the cause of this apparent loss of fluorine.

Experimental

The determination of fluorine by titration with thorium nitrate solution is a well known analytical procedure for which a number of different end-point indicators have been used.[5–7] An improved indicator was described by Selig,[7] who used methylthymol blue at a solution pH of 3·35. Because the colour change of this indicator was much more definite than that of the commonly used alizarin red S, we adopted methylthymol blue for use in the titration.

The result of the application of this titrimetric method to the analysis of niflumic acid showed an apparent loss of 7 per cent. of the fluorine, as was expected from the work of Soep.

ATTEMPTS TO TRACE THE APPARENT LOSS OF FLUORINE—

It seemed most likely that loss of fluorine occurred during the Schöniger combustion, so the factors that affected this combustion were studied as follows.

(1) Replacement of the distilled water used to absorb the combustion products with sodium hydroxide solution failed to improve the assay results, which continued to indicate a fluorine content of about 93 per cent. of theory.

(2) The addition of oxidising agents (in this instance, sodium peroxide or potassium perchlorate) to samples before combustion, especially when fluorine is present as a CF_3 group (as in niflumic acid), had been suggested by some workers[8] as a way of avoiding incomplete oxidation. Neither of these oxidising agents, when added, led to an increase in assay values for fluorine content above 93 per cent. of theory.

(3) Silica flasks were substituted for those made of borosilicate glass, because Johnson and Leonard[9] had shown that fluorine determinations by the Schöniger combustion technique carried out in borosilicate glass showed an apparent loss in fluorine content. The fluorine content found was still 93 per cent. of theory, but the assay results appeared to be much more consistent than those previously obtained with borosilicate glass apparatus.

When an aliquot of sodium fluoride solution was used as the absorbing liquid in a blank combustion by the Schöniger method (*i.e.*, by using only the paper-strip carrier and no sample), its titre of fluoride was lower than that of the sodium fluoride solution that was not subjected to the combustion procedure. The difference was attributed to carbon dioxide produced in the combustion; titres for carbon dioxide saturated and carbon dioxide free sodium fluoride solutions differed by about 9 per cent.

Interference by carbon dioxide in the titration of fluoride was first reported by Banerjee[10] and Schöniger.[2] Banerjee showed that carbonate ions seriously affected the colour change of the indicator SPADNS [the trisodium salt of 2-(*p*-sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid], but neither he nor Schöniger reported a decrease in titration value due to the presence of carbon dioxide.

EFFECT OF DIFFERENT CONCENTRATIONS OF DISSOLVED CARBON DIOXIDE ON FLUORIDE TITRES—

In order to determine whether the fluoride titre was dependent on the amount of carbon dioxide dissolved in the solution, various amounts of sodium carbonate were added to aliquots of sodium fluoride solution, which were equivalent to 2 mg of fluoride. After the pH had been adjusted to 3·35, the solutions were titrated with thorium nitrate solution, with methylthymol blue as indicator. The results obtained are shown in Table I.

TABLE I

EFFECT OF THE CONCENTRATION OF DISSOLVED CARBON DIOXIDE ON FLUORIDE TITRES

Amount of sodium carbonate added/mg	Titre for 2 mg of fluoride/ml
0	5·40
100	5·02
300	4·97
500	4·97
700	4·99
900	4·96

The amount of carbon dioxide produced by combustion of the paper carrier was equivalent to about 500 mg of sodium carbonate, and the effect on the titration value may be considered to be constant. Consequently, interference by carbon dioxide can be overcome by addition of sodium carbonate to the sodium fluoride solution before standardisation of the thorium nitrate solution. Schöniger[2] avoided this interference by boiling the solution to expel carbon dioxide before titration. However, we have found the time of boiling to be extremely critical and therefore adopted the alternative procedure described above.

The over-all combining ratio of fluoride to thorium in the presence of carbon dioxide was calculated to be 4·35:1 when using the constant titration value given in Table I.

Method

Apparatus—

Silica flask, 500-ml capacity—This flask (Jobling Ltd.) was fitted with a glass stopper into which was fused a platinum basket on a platinum wire.

Paper-strip carriers—These were cut from Whatman No. 42 ashless paper.

Reagents—

All materials were of AnalaR grade, and distilled water was used throughout.

Sodium fluoride solution—Dissolve 0·11 g (accurately weighed) of oven-dried (110 °C) sodium fluoride in water and dilute the solution to 50 ml.

Thorium nitrate solution, 0·005 M—Weigh 2·76 g of thorium nitrate tetrahydrate into a 1-litre calibrated flask. Dissolve it in and dilute to volume with water.

Methylthymol blue (pentasodium salt) indicator solution, 0·2 per cent. m/V, aqueous—Eastman-Kodak.

Buffer solution, pH 3·35—Dissolve 67 g of glycine and 110 g of sodium perchlorate in about 500 ml of water. Adjust the pH to 3·35 with 60 per cent. perchloric acid. Finally, dilute the solution to 1 litre with water.

Sodium carbonate solution, 5 per cent. m/V, aqueous—Prepare by using the anhydrous salt.

Perchloric acid, approximately 1·0 N—Dilute 105 ml of 60 per cent. perchloric acid to 1 litre with water.

Standardisation of thorium nitrate solution—

With a pipette, transfer 2 ml of sodium fluoride solution into a 100-ml beaker and add, with a measuring cylinder, 10 ml of sodium carbonate solution. Adjust the pH to 3·4 with 1 N perchloric acid. Transfer the solution to a 500-ml silica flask (inconsistent titration values were obtained when using borosilicate glass conical flasks) and add 25 ml of buffer solution (pH 3·35). Add 1 ml of indicator solution and titrate with thorium nitrate solution to the first appearance of a blue colour. Repeat this procedure, omitting the sodium fluoride solution; this is the titration blank.

Procedure—

Determination of fluorine in a sample—Weigh on to a paper-strip carrier enough sample to contain about 2 mg of fluorine. Carefully fold the paper and place it in the platinum basket of the combustion apparatus.

Introduce 20 ml of water into the silica flask, wet the ground-glass joint and flush the flask with oxygen. Ignite the paper strip and immediately insert it into the flask. Tilt the flask so that there is water in the neck, thus forming a barrier between the combustion products and the external atmosphere. When combustion has been completed, shake the flask vigorously for 5 minutes, then allow it to stand, with occasional shaking, for about 30 minutes. Wash down the stopper and platinum basket with distilled water and add 25 ml of buffer solution (pH 3·35) to the flask. Titrate the solution with thorium nitrate solution, using 1 ml of indicator solution. For the titration blank, repeat the above procedure omitting the sample, *i.e.*, by using a paper-strip carrier only.

Results and discussion

Seventeen organofluorine compounds, mainly pharmaceuticals, were assayed for fluorine content by the above method. The compounds chosen represent different types of organofluorine groups that occur in pharmacologically active molecules; some of them contain atoms other than carbon, hydrogen and oxygen, such as nitrogen or sulphur, which, on combustion, may form oxides that would interfere with the assay procedure, as does carbon dioxide.

From Table II, it can be seen that no difficulty was experienced in obtaining complete oxidation of the trifluoromethyl group, as had been reported by some workers,[9] and that no interference in the assay resulted from the presence of nitrogen, sulphur or chlorine.

TABLE II

FLUORINE CONTENT OF ORGANOFLUORINE COMPOUNDS

Compound	Type of organo-fluorine group present	Elements present other than C, H and O	Theoretical fluorine content, per cent.	Actual fluorine found, per cent.	Standard deviation	99 per cent. confidence limits
4-Fluorobenzoic acid	C–F	—	13·56	13·81, 13·61	—	—
Trifluoroacetanilide	CF_3	N	30·14	29·80, 30·50	—	—
Fluphenazine hydrochloride	CF_3	N, S, Cl	11·17	11·10, 11·32	—	—
Triflupromazine hydrochloride	CF_3	N, S, Cl	14·66	14·71, 15·02	—	—
Trifluoperazine dihydrochloride	CF_3	N, S, Cl	11·86	11·52, 11·66	—	—
Hydroflumethiazide	CF_3	N, S	17·21	17·38, 17·72, 17·82	—	—
Bendroflumethiazide	CF_3	N, S	13·53	13·38, 13·69	—	—
Niflumic acid	CF_3	N	20·20	20·10, 20·15, 20·05 20·10, 20·00, 20·15 19·75, 19·85, 19·72	0·17	±0·95%
Flufenamic acid	CF_3	N	20·27	20·25, 20·05	—	—
Triamcinolone	C–F	—	4·82	4·94, 4·92	—	—
Triamcinolone acetonide	C–F	—	4·37	4·26, 4·31	—	—
Fluocinolone acetonide	C–F (two)	—	8·40	8·21, 8·20	—	—
Fluoxymesterone	C–F	—	5·65	5·50, 5·64	—	—
9α-Fluorohydrocortisone acetate	C–F	—	4·50	4·39, 4·43	—	—
Research compound $C_{26}H_{35}FO_6$ (SQ 15102)	C–F	—	4·11	4·04, 4·02, 4·18 4·15	—	—
Research compound $C_{29}H_{33}FO_6$ (SQ 15112)	C–F	—	3·83	3·82, 3·73, 3·78 3·81, 3·88, 3·76 3·84, 3·82, 3·74 3·79	0·046	±1·24%
m-Trifluoromethylbenzoic acid	CF_3	—	29·98	29·50, 30·15, 29·60 29·50, 30·20, 29·60 30·00, 29·60, 30·10	0·25	±1·09%

The 99 per cent. confidence limits were calculated from the assay results obtained for niflumic acid, *m*-trifluoromethylbenzoic acid (the recommended standard for the determination of fluorine[11]) and the research compound $C_{29}H_{33}FO_6$. The confidence limits indicate the precision of the method to be ±1 per cent.

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Book Reviews

QUANTITATIVE CHEMISTRY: AN EXPERIMENTAL APPROACH. By JOHN T. DONOGHUE. Pp. viii + 136. New York and Belmont: Bogden & Quigley Inc. 1972. Price £3.

This book represents a collection of laboratory exercises used in a course of quantitative chemistry at the University of Arkansas. They are deemed to be suitable for a one-year course or, by selection of appropriate experiments, they may be the content of a half-year course. There are approximately thirty-five experiments in the book, ranging from polarography and amperometric titrations, through the colorimetric determination of formamide to the standardisation of aqueous sodium hydroxide solution against potassium hydrogen phthalate. Exercises in flame photometry and fluorimetry compete for space with sketches of Buchner funnels, magnetic stirrers and condensers and yet the book, and its implicit philosophy, are exciting.

The author includes, for several of the experiments, additional exercises that require the student to show, through the calculations, that he has thoroughly understood the basic principles of the practical exercise. These calculations and problems also serve to indicate some of the scope of the practical techniques.

It is time that modern teaching methods used modern analytical techniques and perhaps we may learn something from such an experimental approach.

I do not agree with all of the author's experiments and their arrangement, but it is a worthwhile book and teachers of analytical chemistry should know of it. It presents perhaps a slightly unorthodox viewpoint but one which is nevertheless very acceptable. L. S. BARK

SPOT TESTS IN INORGANIC ANALYSIS. By FRITZ FEIGL and VINZENZ ANGER; Translated by RALPH E. OESPER. Sixth Edition. Pp. xxx + 669. Amsterdam, London and New York: Elsevier Publishing Company. 1972. Price Dfl.125; $39 (approximately).

In these enlightened days of sophisticated instrumental methods of analysis, it is an indisputable fact that good use is still being made of conventional chemical reactions (spot tests) in qualitative analysis.

Solid-source mass spectrometry and emission and atomic-absorption spectroscopy are examples of the powerful analytical tools used for resolving inorganic problems, but they have their limitations and, understandably, are not always to hand. Spot tests are simple to apply; they usually involve small samples, and often provide more information than can be obtained by instrumental methods. An outstanding advantage of these chemical reactions is that they are more likely to provide information on the "state" of the element in question, *e.g.*, the presence of chlorine as the free element, chlorate or hypochlorite, etc.

A typical example of the versatile nature of these reactions, and the simple innovations utilised and described in this book, is the test outlined on page 304 for detecting down to 0·3 μg of potassium permanganate in a saturated solution of alkali chromate. A drop of the test solution is placed on a filter-paper, the cellulosic paper is oxidised by the permanganate, and the manganese dioxide formed is deposited in the capillaries of the paper; the unaffected chromate is washed out. It is difficult to envisage an alternative instrumental or simpler chemical method for dealing with this problem.

This brief introduction is not intended for the many analysts throughout the world who need no introduction to Feigl's publications, and who will have eagerly awaited the appearance of this sixth edition. Since the previous edition of the book was published in 1958, many new analytical reagents and reactions have been mentioned in published papers; these have been assessed by the present authors and, when appropriate, the essential details have been included in this latest, completely revised and enlarged edition of "Spot Tests in Inorganic Analysis."

Compared with the fifth edition of the book, more tests (899 instead of 649) and several new chapters have been added. Chapter 1 retains the title "Development, Present State and Prospects of Inorganic Spot Test Analysis." Chapter 2, "Methodology of Spot Test Analysis," has been completely revised and enlarged by Dr. G. Skalos. This chapter includes sections on, *e.g.*, "Handling of Vapors and Separation of Gases," and "Conduct of Spot Test Analysis." Chapter 3 has the title "Preliminary Orientational Tests." Chapters 3, 4 and 5 of the earlier edition now appear as Chapter 4, "Tests for the Elements, Their Ions and Compounds," which, with 430 pages, is the largest of the book's six chapters. This chapter is divided into sections in which the elements

are dealt with individually and in alphabetical order. These sections are further divided, the sub-sections corresponding to the form in which the element is present, *e.g.*, in the free state, as an anion or cation, in a non-ionic form or organically bound. Numerous references are given in each of these sub-sections, and they are supported by a bibliography of published procedures in which the reactions referred to in the text have been applied on a quantitative basis.

The chapter on "Application of Spot Reactions in Tests of Purity, Examination of Technical Materials, Studies of Minerals," now Chapter 5, contains an additional thirty-four sub-sections (now 129) under such headings, chosen at random, as "Detection of Traces of Nitrate in Alkali Molybdate, Tungstate and Vanadate," and "Detection of Free and Polysulphide Sulphur in Mixtures and Minerals." Chapter 6 is a "Tabular Summary," largely devoted to the limits of identification attained by the spot tests recommended throughout the book.

Regrettably, Professor Feigl did not live to see the release of this sixth edition; he died in January, 1971, and it is evident from the foreword to the book that the bulk of the revision was carried out by Dr. Anger. Perhaps, therefore, it is timely to modify an old cliché and ask, as the occasion demands, "What has Feigl - Anger to say on the subject?"

Apart from giving full marks to Dr. Ralph E. Oesper for the excellence of his translation of this book into the English language, little more remains to be said in praise of the up-dating of a book that is already a recognised classic in its field of application. W. T. ELWELL

INTRODUCTION TO MASS SPECTROMETRY. Second Edition. By H. C. HILL; revised by A. G. LOUDON. Pp. viii + 116. London, New York and Rheine: Heyden & Son Ltd. 1972. Price £2·75; $7.25; DM25 (hardback); £1·50; $3.90; DM13.50 (softback).

This deservedly popular work has now appeared in a second edition. It includes chapters on instrumentation and sample handling, basic aspects of organic mass spectrometry, fragmentation of positive ions and interpretation of the mass spectrum. It also includes a selection of unnamed mass spectra upon which the reader may try identification methods. There are also a selection of references, a subject index and two prefaces.

The book has been skilfully revised by A. G. Loudon, who has kept to the spirit of the original text. Moreover, the publishers have taken this opportunity to modify the section on instrumentation as well as altering the format of the work.

Although the book is deservedly popular, as is evidenced by its translation into German, Italian and Japanese, it is, alas, not without flaws. In particular, certain statements are made that should not be made without further qualification; ranking high amongst these are the more or less casual references to Stevenson. Stevenson's rule lays down certain conditions that may lead to the production of a fragment ion in its ground state, but this is not made clear in the text. Equally important in an introductory text is the need to be precise about simple details, *e.g.* (page 5, line 4), E should be 21·21 eV. Another error appears on the same page: if in a single-focusing mass spectrometer the voltage varies while the field strength remains fixed, the field is represented by H, if the reverse is true the magnetic term is represented by B, the magnetic induction. A somewhat perplexing English phrase has also been noted: "at one time" (page 8, line 8) should surely be "one at a time." Surprisingly, there is also the failure to use the symbol $\rightarrow$ to indicate a rearrangement process. Finally, the reviewer regrets that there is no table to relate SI units, which are now being taught to students, with the traditional units used by their forebears.

One looks forward to the correction of these rather trivial but irritating errors in the next edition.

The book is well produced, eminently readable and is to be thoroughly recommended.

R. I. REED

ANALYTICAL CHEMISTRY OF PHOSPHORUS COMPOUNDS. Edited by M. HALMANN. *Chemical Analysis: A Series of Monographs on Analytical Chemistry and its Applications, Volume* 37. Pp. xii + 850. New York, London, Sydney and Toronto: Wiley-Interscience. 1972. Price £16·80.

This book commences with an introductory chapter on the rôle of phosphorus in the world, which is followed by chapters on methods of determining total phosphorus and the analysis of phosphorus compounds by separation and identification methods. Determination of compound groups and phosphorus in specific materials completes the remainder of the book.

It is obviously difficult to cover such a wide range of topics in detail in only approximately 800 pages. One could, for instance, write a book on the analytical chemistry of the condensed phosphates whereas this, and other, subjects are treated very sketchily in this particular book.

In Chapter 6, after a brief section on general considerations, the authors deal with the mass spectra of various functional types of phosphorus compounds. There is an abundance of examples, but in some cases they degenerate into little more than a description of the mass spectrum obtained. Errors such as Ph_3 for phosphine and $[(CH_3)_3SiO]_3PO_4$ for tristrimethylsilyl phosphate are made more noticeable by the lack of readable material.

Without questioning the accuracy of the data in the chapter concerned with vibrational spectroscopy, it can be said that some stamina is required in order to find the information sought, and spectroscopic data are scattered elsewhere in the book with consequent duplication. The presentation of the data is poor and the tables are, in some instances, far too long and consequently tedious to use. The authors could have used simple molecules in order to show the appearance of typical organophosphorus compounds. There are a number of printing errors in the notes to the spectra.

The chapters on chromatography form a good reference source, provided that one has the patience to find what one is looking for. Why on earth did the editor not do some editing?

On the credit side, the chapter on nuclear magnetic resonance spectroscopy is well balanced and up to date. Comments on the relevance of the latest advances in the field are particularly helpful. On page 157, $P(OCH_3)_3$ is called trimethylphosphine and the coupling range for $CH_3CP(O)<$ is surprisingly absent from the table of coupling constants on page 181.

The sections on classical analysis are adequate, considering the space available. The gas - liquid chromatography of pesticides is very well covered with a full description of the various specific phosphorus detectors. Overall, of its type, this is a useful reference book for non-phosphate chemists and for those phosphate chemists who can afford to buy it for occasional use.

S. GREENFIELD

A PROGRAMMED INTRODUCTION TO INFRARED SPECTROSCOPY. By B. W. COOK and K. JONES. Pp. xvi + 192. London, New York and Rheine: Heyden & Sons Ltd. 1972. Price £1·50; $3.90; DM13.50.

This book is intended for the absolute beginner. It presents the basic principles of infrared spectroscopy, its practice, and its use in routine analytical work in about the clearest and most elementary way possible.

The book opens with a "Criterion Test" of twenty questions that the student can attempt to answer before and after reading the text; a "Validation Report" in the Preface claims that, in terms of this test, the efficiency of the book as a teaching programme is 67 per cent., 62 per cent. of a random group of students who scored less than 20 per cent. in the pre-test scoring 75 per cent. or more after reading the book.

After reading the text carefully, a student should certainly be able to understand the terms used in infrared spectroscopy and the reasons why compounds exhibit infrared absorption; understand the operating principles of the components of a commercial spectrometer; identify and correct the faults that produce spectra of poor quality; prepare samples and obtain spectra for all the different physical forms of sample that commonly occur; calculate the thickness of cells; apply Beer's law, make quantitative measurements and use internal standards; use correlation tables of frequencies; and be able "to start building a working knowledge" of how to interpret spectra —a section that is particularly well presented, with many illustrative examples of reproductions of spectra and quiet inculcation of the basic fact that experience is essential.

The price is very reasonable, but the purchaser must realise that the construction of this type of book leads to his having to buy a lot of plain paper: there are over fifty pages on which there are six or less lines of print saying, in effect, "sorry, but you are wrong, re-read page 123 then try again"; the basic information offered by this book is contained in about ninety pages. Those teachers and students who like to use modern programmed learning texts will nevertheless welcome the appearance of this one. It is a good example of its kind.

D. M. W. ANDERSON

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An Ultramicro-scale Method for the Determination of the Uranyl Cation

A method that requires the use of very simple equipment has been developed whereby the uranyl cation can be determined at the micro-scale and ultramicro-scale levels with high precision. The method involves the use of the catalytic effect shown by the uranyl cation on the photo-decolorisation of a naturally occurring carotenoid-type pigment. The interfering effects of several common anions and cations are also discussed.

GLENN PETER WOOD

Department of Chemistry, University of San Andres, La Paz, Bolivia.

Analyst, 1973, **98**, 525–528.

An Improved Plasma Jet System for Spectrochemical Analysis

The stability of a plasma jet has been improved by replacing the direct-injection nebuliser with a pre-mixed chamber - nebuliser arrangement of the type used in flame spectrometry. This arrangement has alleviated many of the operational problems that were experienced with the original system. As a result of the improved performance, the unit can be operated in conjunction with instantaneous photoelectric read-out. Details of the modification are given and the stability obtained is demonstrated. The sensitivity of the modified plasma jet has been investigated and detection limits for the most sensitive lines of sixty-seven elements are presented. These detection limits are compared with those obtained by using a nitrous oxide - acetylene flame with the same optical arrangement and detection equipment.

J. F. CHAPMAN, L. S. DALE and R. N. WHITTEM

Australian Atomic Energy Commission, Research Establishment, Lucas Heights, New South Wales, Australia.

Analyst, 1973, **98**, 529–534.

A Method for Determining Free Azide Ions by Automatic Analysis in the Presence of a Covalent Cephalosporin Azide

An assay was devised in order to follow quantitatively the release of azide as free ions, N_3^-, from a cephalosporin azide when attacked by β-lactamase enzymes produced by specific strains of bacteria. Experiments were arranged to ascertain whether or not the azide cleavage occurred at the same rate as the rupture of the β-lactam ring. The assay was required to determine free azide at concentrations between 2 and 20 $\mu g\ ml^{-1}$; other experimental limitations were imposed by the requirements of enzymolysis.

The procedure adopted was based on the complete oxidation of azide ions to nitrogen by an excess of a standard aqueous solution of nitrite ions at pH 4·6. The residual nitrite was removed by diazotisation with 4-aminosalicylic acid, followed by coupling of the product with a second molecule of the 4-aminosalicylic acid. The reaction mixture was then rendered alkaline by the addition of tetramethylammonium hydroxide solution. The final yellow colour was stable and had an absorption maximum at 440 nm. It was not possible, however, to achieve sufficient operational reproducibility manually, and an AutoAnalyzer system was therefore used.

The results show that cleavage of azide from the parent molecule occurs at the same rate as the rupture of the β-lactam ring and parallels the decline in microbiological potency.

R. E. WALLER

Glaxo Laboratories Ltd., Greenford, Middlesex.

Analyst, 1973, **98**, 535–541.

The Histochemical Detection of Soya "Novel Proteins" in Comminuted Meat Products

The enforcement of the regulations governing meat and meat products requires the determination of meat content. Meat content is assessed from the total nitrogen content, from which suitable deductions are made for the nitrogen contributed by the other ingredients of significant nitrogen content present in meat and meat products. The availability of "novel proteins" and the possibility of the addition of these proteins to meat products necessitates the detection and determination of "novel proteins" in such products for the true assessment of their meat content. A microscopical method that indicates the presence of "novel protein" of soya origin in meat products has been examined. This method involves the use of a specific technique to demonstrate the presence of carbohydrate material and is diagnostic for the cellular fraction of many processed soya products.

M. COOMARASWAMY and F. OLGA FLINT

Procter Department of Food and Leather Science, The University, Leeds 2.

Analyst, 1973, **98**, 542–545.

Interference of Carbon Dioxide, Resulting from the Schöniger Flask Combustion of Organofluorine Compounds, in the Titrimetric Determination of Fluorine

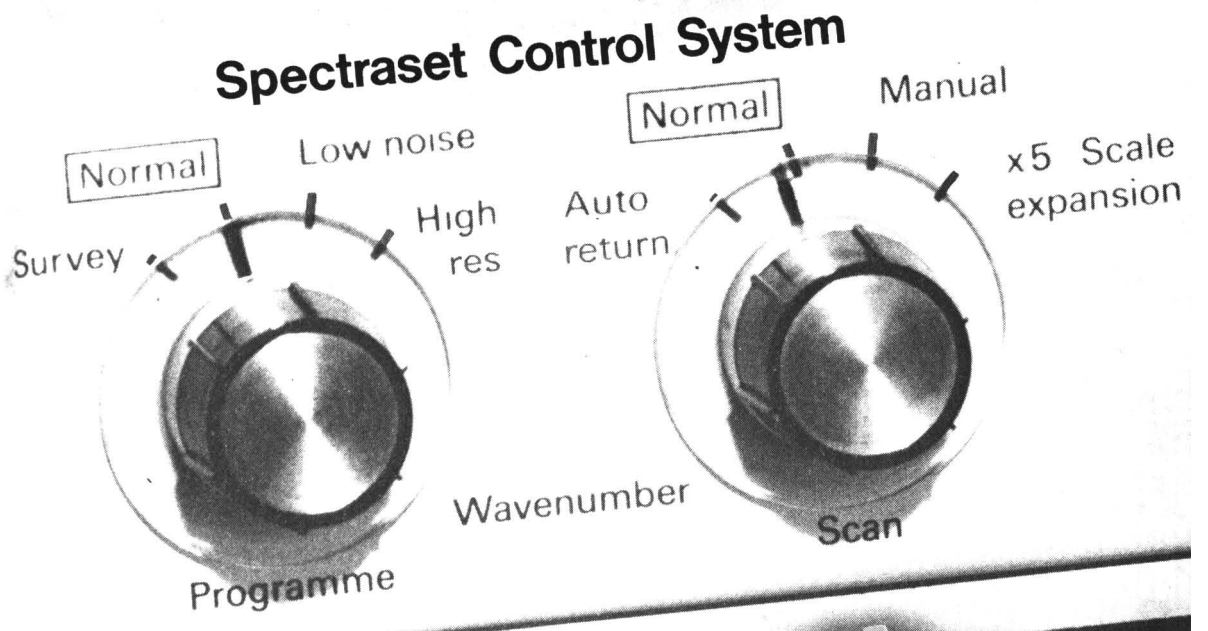
Carbon dioxide produced during the Schöniger flask combustion of organofluorine compounds has been found to interfere in the titrimetric determination of fluorine. When thorium nitrate that had been standardised against sodium fluoride solution was used, the fluorine content found was consistently 93 per cent. of theory. This interference was overcome by adding sodium carbonate to the solution used for standardisation before titration. For detection of the titration end-point, an improved indicator, methylthymol blue, was used.

By using this procedure, compounds containing between 4 and 30 per cent. of fluorine, either in a CF_3 or CF group, have been assayed satisfactorily. No interference from nitrogen, sulphur or chlorine contained in some of the compounds was observed.

WILLIAM F. HEYES

International Development Laboratory, E. R. Squibb & Sons Ltd., Moreton, Cheshire.

Analyst, 1973, **98**, 546–549.



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