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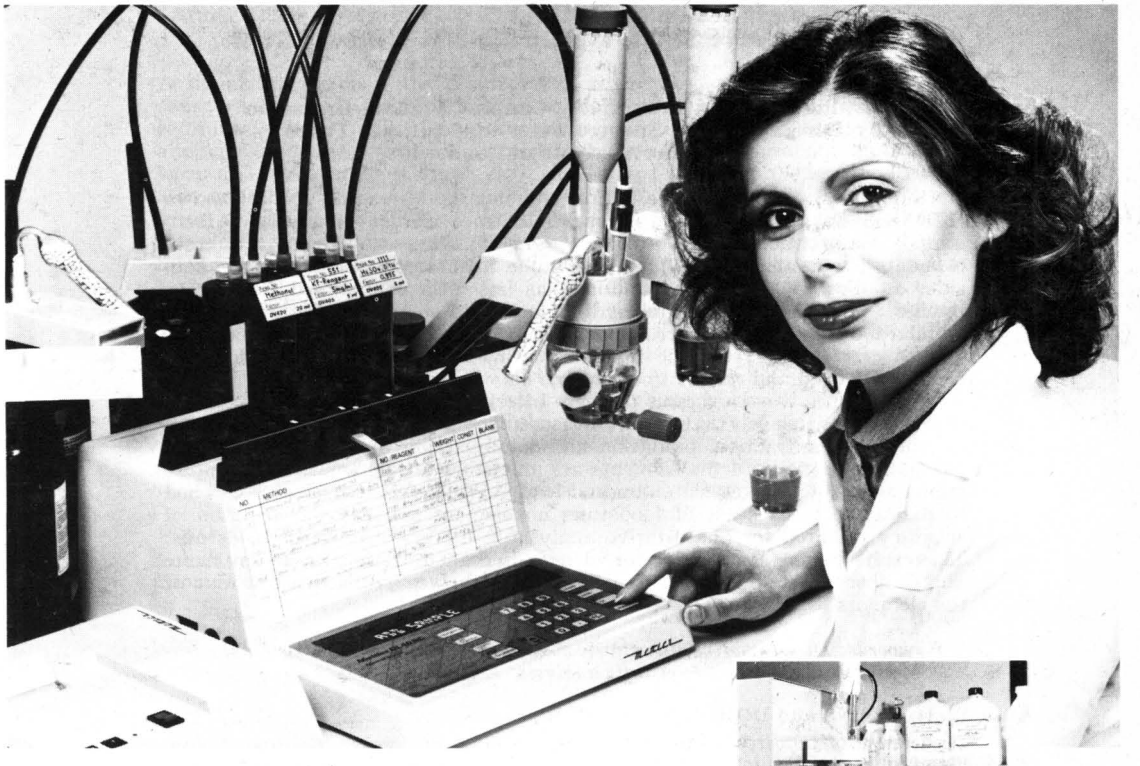

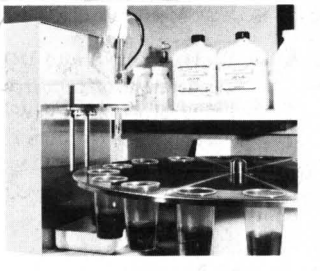

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Evaluation of an Inductively Coupled Plasma Emission Direct-reading Spectrometer for Multiple Trace Element Analysis of Foodstuffs

An inductively coupled plasma optical emission direct-reading polychromator (ICP-OES) has been evaluated for multiple trace element analysis of foodstuffs. Major trace elements (copper, iron, manganese and zinc) and trace elements (cadmium, lead, nickel, chromium, arsenic and tin) have been considered. For each element, random bias has been exemplified for routine practice, in both sulphuric and hydrochloric acid standard solutions, by the standard deviations for instrument noise, repeatability and reproducibility of measurement. Systematic bias originating from direct interferences has been categorised and relevant corrections have been calculated.

To avoid the worst excesses of these interferences and to concentrate the trace elements to levels that permit measurement, a method involving isolation by chelation, extraction, oxidation and solubilisation in fixed acid solution has been used. Six of the elements are amenable to this treatment: copper, manganese, zinc, nickel, cadmium and lead. When this treatment is applied to digests of a selection of foodstuffs no element satisfies the criterion of accuracy required for quantitative analysis in dietary surveys of foodstuffs. The results display comparable confidence intervals to those obtained by flame atomic-absorption spectrometric measurement, however, for the first four of the elements listed.

Keywords: *Direct-reading inductively coupled plasma*; *optical emission spectrometer*; *trace elements*; *foodstuffs analysis*

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Analyst, 1982, **107**, 977–993.

Direct Determination of Lead in Used Engine Oils by Atomic-absorption Spectrophotometry

An improved method has been developed for measuring lead in used lubricating oils. The procedure utilises a mixture of acid and a liquid anion exchanger (Aliquat 336) to dissolve the lead particles in 4-methylpentan-2-one and measurement is made by atomic-absorption spectrophotometry. The method is quantitative in the range 0.1–2.5% *m/m* and is independent of effects caused by variations in particle size, lead species or oil additives. The method is rapid and has good repeatability.

Keywords: *Lead determination*; *used lubricating oils*; *liquid anion exchangers*; *atomic-absorption spectrophotometry*

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Analyst, 1982, **107**, 994–999.

Study of an Automatically Triggered Digital Integrator for Atomic Spectrometry of 15 Elements Using Discrete Nebulisation

A signal integrator, with an automatic trigger and a voltage to frequency counter, was used in the atomic-absorption spectrometry of silver, calcium, cadmium, cobalt, copper, iron, magnesium, manganese, nickel, lead, strontium and zinc and the atomic-emission spectrometry of sodium and potassium with an air - acetylene flame and the atomic-absorption spectrometry of aluminium, calcium and magnesium with a dinitrogen oxide - acetylene flame using a discrete nebulisation technique. The effects of injection volumes, sample flow-rates and metal concentrations on the variables (peak height, integrated value, aspiration time and the ratio of the integrated value to the aspiration time) were investigated. Each of the integrated values for silver, copper, iron, magnesium, manganese and lead is proportional to the absolute amount of each metal irrespective of the metal concentration and injection volume. The calibration graphs for these six elements were straight lines passing through the origin. The relative standard deviations of the measurements were less than 4%, even with an injection volume of 20 μl, and less than 1% with an injection of 100 μl.

Keywords: *Automatic integration*; *atomic-absorption and -emission spectrometry*; *discrete nebulisation*; *absolute amount method*

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Analyst, 1982, **107**, 1000–1005.

Low-temperature Oxygen - Fluorine Radiofrequency Ashing of Biological Materials in Poly(tetrafluoroethylene) Dishes Prior to the Determination of Tin, Iron, Lead and Chromium by Atomic-absorption Spectroscopy

Low-temperature radiofrequency ashing utilising plasma-excited oxygen alone requires long ashing times and for this reason has not been generally accepted. In this paper it is shown how ashing time can be reduced considerably by introducing fluorine into the oxygen plasma and a novel and efficient method of achieving this is described. Food products and National Bureau of Standards Standard Reference Materials, both of known trace metal content, are prepared by the method described. Analysis of the ash by atomic-absorption spectroscopy shows good recovery of added tin and agreement with certified values for iron, lead and chromium.

Keywords: *Low-temperature plasma ashing*; *oxygen - fluorine*; *PTFE*; *tin, iron, chromium and lead determination*; *atomic-absorption spectroscopy*

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Analyst, 1982, **107**, 1006–1013.

Determination of Chloride, Sodium and Potassium in Salted Foodstuffs Using Ion-selective Electrodes and the Dry Sample Addition Method

The "dry sample addition" method of ion-selective potentiometry is described. This incremental or spiking method requires the addition of a known mass of solid sample directly to a standard solution containing the ion to be measured. Dissolution of the sample produces a change in electrode potential which is related to the sample composition. Dry sample addition has been applied to the analysis of foodstuffs containing up to 2.8% of salt. The chloride, sodium and potassium concentrations are determined by Philips solid-state and plastic ion-selective electrodes. Comparison of this method with titrimetry and atomic-absorption spectrometry gave satisfactory results. The advantages gained in cost, speed of analysis and reliability using ion-selective measurement are discussed.

Keywords: *Ion-selective electrodes*; *dry sample addition potentiometry*; *determination of chloride, sodium and potassium*; *salted foodstuffs*

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Analyst, 1982, **107**, 1014–1018.

On-line Electrochemical Detection of Oxidisable Organic Molecules of Pharmaceutical Importance

On-line electrochemical detection in the oxidative mode using a wall-jet electrode has been evaluated for 1,4-benzodiazepines and other drug molecules of widely differing structure. The method has been applied satisfactorily to the automated analysis of some 1,4-benzodiazepines, coefficients of variation of *ca.* 1–3% being obtained.

Keywords: *On-line electrochemical analysis*; *1,4-benzodiazepines*

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Analyst, 1982, **107**, 1019–1025.

Electrochemical Determination of Trimethoprim

A differential-pulse polarographic method has been developed for the determination of trimethoprim. The E_p occurs at -1.378 V when the pH of the system is 3.6. Cyclic voltammetry in anhydrous acetonitrile demonstrated that both trimethoprim and sulphamethoxazole could be determined by anodic oxidation at a platinum electrode provided that each was present as a single entity.

Keywords: *Trimethoprim determination*; *differential-pulse polarography*; *controlled-potential coulometry*; *cyclic voltammetry at a rotating platinum electrode*

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Analyst, 1982, **107**, 1026–1031.

Differential Electrolytic Potentiometry with Periodic Polarisation. Part XXV. Direct and Mark-space Biased Periodic Current Polarisation in Acid - Base Titrimetry in Acetic Anhydride - Acetic Acid

The applications of direct current and mark-space bias square wave differential electrolytic potentiometric techniques to acid - base titrations in an acetic anhydride - acetic acid mixed solvent have been examined. Antimony metal - metal oxide electrodes were used as indicating systems. Titration curve shapes are similar to those obtained from aqueous acid - base titrations, but the required current density and percentage bias are much higher. Several compounds that do not exhibit basic properties in water were titrated successfully with perchloric acid. The advantage of using an aprotic solvent in conjunction with acetic anhydride is demonstrated.

Keywords: *Differential electrolytic potentiometry*; *antimony electrodes*; *acetic anhydride solvent*; *non-aqueous acid - base titrimetry*

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Analyst, 1982, **107**, 1032–1039.

Flow Injection Voltammetric Determination of Nitrite by Reduction at a Glassy Carbon Electrode in Acidic Bromide or Chloride Media

Nitrite can be determined by reduction at a glassy carbon electrode held at +0.3 V *versus* a saturated calomel electrode by flow injection voltammetry at concentrations of greater than 1×10^{-6} M by direct injection of sample solution (25 μl) into an eluent 3.2 M in hydrochloric acid and 20% *m/V* in potassium bromide. Alternatively, the nitrite may be pre-reacted with acidic bromide before injection and determined at concentrations above 1×10^{-7} M. The use of an acidic chloride medium is less satisfactory.

Keywords: *Flow injection analysis*; *nitrite determination*; *voltammetry*

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Analyst, 1982, **107**, 1040–1046.

Determination of Chemical Oxygen Demand of Wastewaters Without the Use of Mercury Salts

The use of silver nitrate solution to suppress chloride interference in the chemical oxygen demand test is described. Chlorides in wastewater are precipitated as silver chloride and in this form are only slightly oxidised. The proposed procedure obviates the use of toxic mercury(II) sulphate and is similar to the standard procedure in accuracy and reproducibility of results over a wide range of chloride concentrations.

Keywords: *Chemical oxygen demand without mercury*; *wastewaters*

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Analyst, 1982, **107**, 1047–1053.

Determination of Organic Pharmaceuticals with *N*-Bromosuccinimide. Part III. Some Pyrazolone Derivatives by Direct Titration

Seven members of the pyrazolone group have been studied, namely, 3-methyl-1-phenyl-3-pyrazolin-5-one (MPP), phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), 4-aminophenazone (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), aminophenazone (amidopyrine; 2,3-dimethyl-4-dimethylamino-1-phenyl-3-pyrazolin-5-one), dipyrone [sodium (2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)methylaminomethanesulphonate], morazone [2,3-dimethyl-4-(3-methyl-2-phenylmorpholinomethyl)-1-phenyl-3-pyrazolin-5-one] and propyphenazone (4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one). The direct titration of the pyrazolone group with standard *N*-bromosuccinimide was only successful for MPP and phenazone in glacial acetic acid using methyl orange as the indicator. The concentration of the acetic acid must be not less than 9 N at the end of the titration. MPP can also be titrated successfully in 1 N hydrochloric acid, the end-point being detected either by methyl orange or potentiometrically. The molar ratio of the reaction was found to be always 1:1 (phenazone : *N*-bromosuccinimide) and 1:2 (MPP : *N*-bromosuccinimide).

A reaction mechanism is suggested involving the formation of a 4-bromo derivative during the titration of either MPP or phenazone with *N*-bromosuccinimide. A second molecule of *N*-bromosuccinimide is consumed in brominating the secondary amino group in the case of MPP.

Keywords: *Titrimetry*; *bromination*; *pyrazolone derivative determination*; *N-bromosuccinimide*

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Analyst, 1982, **107**, 1054–1059.

A Study of the Formation and Stability of the Iron(III) - Thiocyanate Complex in Acidic Media

The formation of the iron(III) - thiocyanate complex in sulphuric and perchloric acids has been studied spectrophotometrically. Under no circumstances has any evidence been found that more than one thiocyanate ion enters the complex, contrary to widely held beliefs that up to six ions may be involved. Hydroxyl, sulphate and hydrogen sulphate ions and water molecules complete the co-ordination sphere. Decomposition products have been isolated and identified. Three ranges of acid concentration are identified, each region characterised by the manner of variation of the molar absorptivity and formation constant with change in acid concentration, and an interpretation is offered. Decomposition of both reagent and complex render thiocyanate unsatisfactory as a reagent for the precise determination of iron.

Keywords: *Iron(III) - thiocyanate complex*; *iron determination*; *spectrophotometry*; *reaction mechanisms*

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Analyst, 1982, **107**, 1060–1064.

September 1982 Vol. 107 No. 1278

The Analyst

Evaluation of an Inductively Coupled Plasma Emission Direct-reading Spectrometer for Multiple Trace Element Analysis of Foodstuffs

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An inductively coupled plasma optical emission direct-reading polychromator (ICP-OES) has been evaluated for multiple trace element analysis of foodstuffs. Major trace elements (copper, iron, manganese and zinc) and trace elements (cadmium, lead, nickel, chromium, arsenic and tin) have been considered. For each element, random bias has been exemplified for routine practice, in both sulphuric and hydrochloric acid standard solutions, by the standard deviations for instrument noise, repeatability and reproducibility of measurement. Systematic bias originating from direct interferences has been categorised and relevant corrections have been calculated.

To avoid the worst excesses of these interferences and to concentrate the trace elements to levels that permit measurement, a method involving isolation by chelation, extraction, oxidation and solubilisation in fixed acid solution has been used. Six of the elements are amenable to this treatment: copper, manganese, zinc, nickel, cadmium and lead. When this treatment is applied to digests of a selection of foodstuffs no element satisfies the criterion of accuracy required for quantitative analysis in dietary surveys of foodstuffs. The results display comparable confidence intervals to those obtained by flame atomic-absorption spectrometric measurement, however, for the first four of the elements listed.

Keywords: Direct-reading inductively coupled plasma; optical emission spectrometer; trace elements; foodstuffs analysis

Spectrographic analysis of trace levels of elements with the d.c. arc had been applied over many decades to the analysis of substrates, including plant materials, as solids or in solution,[1] when in 1964 a more stable energy source, the inductively coupled plasma (ICP), was suggested by Greenfield *et al.*[2] and Wendt and Fassel,[3] independently, for the generation of free atoms and excitation of these released atoms. The principle weaknesses of spectrographic analysis were its relative lack of sensitivity, susceptibility to interferences by inorganic species on the analyte element and excessive variation in results. It became apparent, with time, that the inherent stability of the plasma energy source when applied to measurement of trace elements in solution gave improved sensitivity and reduced variation in measurement and could remove well known interferences inherent in traditional elemental analysis obtained from arc or flame optical emission spectroscopic analysis.

For the past two decades, however, elemental analysis has been dominated by flame atomic-absorption spectrometry with sequential measurement in solution. It was suggested that with a simultaneous multi-elemental facility for measurement, the inductively coupled plasma optical emission spectrometer (ICP-OES) could seriously challenge this domination.[4] Subsequent analytical application of the ICP-OES, with its wide linear calibration range, was initially reported for a variety of samples at high analyte concentrations,[5] to major,* major trace and some trace elements in water and seawater,[6–8] and to major and major trace elements in biological materials.[9–11] It is significant that for trace elements in

* For the purposes of this investigation of foodstuffs, sodium, potassium, calcium, magnesium, phosphorus and chloride are termed major elements, copper, iron, manganese and zinc are termed major trace elements and any other elements are termed trace elements.

biological materials there is a dearth of proven application, *e.g.*, for standard reference materials only levels of the major elements and the major trace elements copper, iron, manganese and zinc have been reported. These four elements, together with cadmium, lead, nickel, arsenic, tin, chromium, cobalt and others, are routinely determined in this laboratory by atomic-absorption spectrometry for the United Kingdom total diet survey, in other diet surveys and in retail foodstuffs as occasion demands. This paper presents the results of an investigation on the attempted determination of levels of these elements normally found in foodstuffs or dietary foodstuff homogenates with measurement by an inductively coupled plasma optical emission direct-reading polychromator.

Direct interferences, introducing systematic bias into results, may originate from a number of sources with this instrumental technique. They may be listed under three headings:

1. Interferences on an element by other inorganic species due to compound formation, so prevalent in flame chemistry, appear to be absent.[12,13] As ICP-OES tends to measure atom - ion emission to gain sensitivity, possible similar ionisation interferences are of more importance. By careful control of operating parameters, investigations to date suggest relatively small interferences of this kind, although the mechanisms involved are not clearly understood.[12–14] Kornblum and De Galan,[15] however, advise caution and suggest that such interferences may depend on the particular combinations of analyte and interfering elements.

2. Another type of interference originates from nebulisation and more so from transport of the sample into the plasma. It has been shown that the signal intensity can be seriously affected by changes in viscosity from nebulising mineral acids of different concentration for a high-power plasma source.[16] The ranges of acid strength investigated in this instance were particularly wide and a much smaller range of difference would be expected from measurements on foodstuff digests. Such ranges have been investigated for perchloric acid,[9,10] and it would appear that strict control of acidity is desirable for measurement. Alternatively, the nebuliser may be pumped above the natural rate, although this will not necessarily remove the interference from transport effects.[16]

3. There remain the spectral interferences, which were admitted to be serious as application of the instrumental technique progressed. These have been outlined by several workers and those recognised at present may be classified as (*a*) spectrometer stray light, (*b*) overlap from broadened lines and elemental spectral continuum and (*c*) spectral line coincidence.

(*a*) Normally, the spectral background is a large portion of the total measured signal and, if this background is modified, large systematic errors are inherent in the measurement of low concentrations of solutions. Such modifications can occur from stray light and the intense radiation from calcium and magnesium is a principle cause.[17] Secondary stray light may be caused by reflection and scattering from optical components while primary stray light originates from, for example, imperfections in the diffraction grating. Of the methods for reducing these, band rejection filters fitted to the entrance slit of a polychromator were originally favoured and stray light effects from calcium were considerably reduced, at some expense of detection limits of analyte elements through loss of transmission.[18,19] In more recent instruments the reductions in primary stray light are achieved by replacement of mechanically ruled diffraction gratings with holographically recorded gratings[20] and improved optical design.

(*b*) Similar treatment with band rejection filters of the stray light effects from magnesium delineated true spectral interferences resulting from spectral overlaps and collisional line broadening and recombination continuum from radiative electron - ion recombination processes.[21] Further examples of these phenomena have been described for interferences from the alkaline earth elements and aluminium, to which may be added background variation from atomic and molecular constituents of the plasma.[22] The latter may arise from such species as nitrogen oxide, hydroxyl or atomic argon.

(*c*) To these spectral effects must be added those from spectral line coincidence. A choice of emission line may prevent coincidence and for this purpose a graphics software system has been described.[23] With the remaining spectral effects, for sequential measurement with a monochromator, correction of interference is possible although some difficulty may be encountered with highly structured backgrounds. For simultaneous measurement with a polychromator, mathematical correction has been suggested,[24] and has been applied in practice.[9,10] These corrections are usually found to be non-linear and the extent to which such corrections would be applicable for multi-spectral interference is unclear.

Experimental

The methods used in this investigation do not signify endorsement of the procedure.

Reagents

All reagents should be of the grade specified, with low contents of the elements to be determined. Solutions should be prepared with distilled water.

Nitric acid, sp. gr. 1.42. It is recommended that BDH Chemicals Aristar grade or an equivalent grade of acid be used.

Sulphuric acid, sp. gr. 1.84 *and dilute* (1 + 19). Recommended grade as for nitric acid.

Hydrochloric acid, sp. gr. 1.18 *and dilute* (1 + 3 *and* 1 + 19). Recommended grade as for nitric acid.

Ammonia solution, sp. gr. 0.88. Analytical-reagent grade.

Ammonium tetramethylenedithiocarbamate [*ammonium pyrrolidine dithiocarbamate* (*APDC*)]. This reagent should not be discoloured or have an excessively strong odour of ammonia. If necessary, it can be purified as described previously.[25]

Diethylammonium diethyldithiocarbamate (*DDDC*).

Chelating reagent solution, 1.0% m/V solution of APDC - DDDC. Dissolve 1.00 ± 0.01 g of each reagent in 100 ± 1 ml of water.

Chloroform. Analytical-reagent grade.

Standard solutions

Primary standard solutions, specially prepared for atomic-absorption spectrometry, can be purchased from commercial sources and contain 1.0 g l^{-1} of element in 1 N hydrochloric or nitric acid. Dilute each primary standard solution 1 + 19 with distilled water to give solutions containing 50 mg l^{-1} of the element and store in clean polyethylene bottles. For tin, prepare this standard immediately before use. If used to prepare composite standards containing sulphuric acid, dilute serially solutions of each element to suitable levels, ensuring each solution contains the equivalent of 40 ml of sulphuric acid (1 + 19) per litre. If used to prepare composite standards in hydrochloric acid dilute serially solutions of each element to suitable levels to contain the equivalent of 10 ml of hydrochloric acid (1 + 1) per litre.

Composite standard solutions

Prepare these solutions to contain the concentrations described in Table I for sulphuric acid (1 + 19) solutions 0–14, and in Table II for hydrochloric acid (1 + 19) solutions 01–N.

Apparatus

All glass apparatus must be kept permanently full of 1 N nitric acid when not in use; glass apparatus used for preparing standard solutions of tin should be kept full of 1 N hydrochloric acid.

TABLE I

COMPOSITION OF SULPHURIC ACID (1 + 19) MULTI-ELEMENTAL STANDARDS (mg l^{-1})

Standard solution	Cu	Mn	Fe	Zn	As	Cd	Co	Cr	Ni	Pb	Sn
0	0	0	0	0	0	0	0	0	0	0	0
1	—	—	—	—	—	—	—	—	0.005	0.005	—
2	—	—	—	—	0.05	0.001	0.005	0.005	0.01	0.01	0.05
3	—	—	—	—	0.1	0.002	0.01	0.01	0.015	0.015	0.1
4	—	—	—	—	0.15	0.003	0.015	0.015	0.02	0.02	0.15
5	—	—	—	—	0.2	0.004	0.02	0.02	0.03	0.03	0.2
6	—	—	—	—	0.25	0.005	0.025	0.025	0.05	0.05	0.25
7	0.01	0.01	0.1	0.05	—	—	—	—	—	—	—
8	0.02	0.02	0.2	0.1	—	—	—	—	—	—	—
9	0.05	0.05	0.5	0.25	—	—	—	—	—	—	—
10	0.1	0.1	1.0	0.50	—	—	—	—	—	—	—
11	0.15	0.15	1.5	0.75	—	—	—	—	—	—	—
12	0.2	0.2	2.0	1.0	—	—	—	—	—	—	—
13	0.3	0.3	3.0	1.5	—	—	—	—	—	—	—
14	0.5	0.5	5.0	2.5	—	—	—	—	—	—	—

TABLE II

COMPOSITION OF HYDROCHLORIC ACID (1 + 19) MULTI-ELEMENTAL STANDARDS (mg l^{-1})

Standard solution	Cu	Mn	Fe	Zn	As	Cd	Co	Cr	Ni	Pb	Sn
01	0	0	0	0	0	0	0	0	0	0	0
A	—	—	—	—	0.05	0.01	0.01	0.05	0.05	0.05	0.25
B	—	—	—	—	0.1	0.02	0.02	0.1	0.1	0.1	0.5
C	—	—	—	—	0.15	0.03	0.03	0.15	0.15	0.15	0.75
D	—	—	—	—	0.2	0.04	0.04	0.2	0.2	0.2	1.0
E	0.05	0.05	0.5	0.25	0.3	0.05	0.05	0.3	0.3	0.3	1.5
F	0.1	0.1	1.0	0.5	0.5	—	—	0.5	0.5	0.5	2.5
G	0.2	0.2	2.0	1.0	—	—	—	—	—	—	—
H	0.5	0.5	5.0	2.5	—	—	—	—	—	—	—
J	1.0	1.0	10.0	5.0	—	—	—	—	—	—	—
K	1.5	1.5	15.0	7.5	—	—	—	—	—	—	—
L	2.0	2.0	20.0	10.0	—	—	—	—	—	—	—
M	3.0	3.0	30.0	15.0	—	—	—	—	—	—	—
N	5.0	5.0	50.0	25.0	—	—	—	—	—	—	—

Inductively coupled plasma optical emission spectrometer

An Applied Research 137 ICPQ instrument was used, consisting of an inductively coupled argon plasma operated at 27.12 MHz with direct reading on a 25-channel 1-m Paschen-Runge quantometer. The normal compromise operating conditions involved an incident power of 1.6 kW, viewing height of 15 mm above the load coil, a plasma argon flow-rate of 10 l min^{-1}, an auxiliary argon flow-rate of 1 l min^{-1} and a sample flow-rate of 1 l min^{-1}. Aspiration was through an all-glass concentric Meinhard-type pneumatic nebuliser without pumping. A rejection filter was used to ensure minimal residual spectral interference from calcium. For magnesium, only secondary stray light was removed from adjacent polychromator channels with a filter over the secondary magnesium slit.[26] Measurements were made at the following analytical wavelengths: Cu I, 324.75; Fe II, 259.94; Mn II, 257.61; Zn II, 202.55; As I, 193.77; Cd II, 226.50; Co I, 345.35; Cr II, 283.56; Ni II, 231.60; Pb II, 220.35; and Sn I, 189.99 nm.

Procedure

Preparation of sample digests

These should be prepared according to method (1)C of the Analytical Methods Committee[27] using precautions described subsequently.[28] The resulting 100 ml of digest, which is in nominally 5% *V/V* sulphuric acid, should be colourless and should not contain any suspended solids. Prepare at the same time two reagent blanks from the volumes of acid used in sample oxidation.

Chelation and concentration

Measure 70 ± 1 ml of sample digest into a 150-ml beaker and using a pH meter adjust the pH to 4 ± 0.2 with ammonia solution. When necessary, a smaller aliquot must be diluted with sulphuric acid (1 + 19) to give a measured concentration within the calibration range. Remove the electrode assembly and rinse. Transfer the solution into a 100-ml separating funnel with minimum rinsing. Add 5 ± 1 ml of chelating reagent solution, shake for 15 s and allow to stand for 3 min. Extract with 10 ± 1 ml of chloroform, shaking for 30 s. Allow the solution to stand until separation is complete, then drain the chloroform layer into a dry 150-ml Pyrex beaker. Repeat the extraction with chloroform after further addition of chelating reagent and finally extract with 10 ml of chloroform. Evaporate the combined chloroform extracts to dryness on a boiling water-bath, add 15 ml of nitric acid (sp. gr. 1.42) and boil to dryness on a hot-plate. Cool the residue and dissolve it in 2 ml of hydrochloric acid (1 + 3), transfer into a 10-ml calibrated flask, washing with several 2-ml volumes of water, and dilute to 10 ml. Reagent blank solutions should be treated similarly.

Measurement

For the trace elements, standardise the sensitivity setting of the polychromator channels to the highest permissible value. Stabilise aspiration with the relevant acid of concentration 1 + 19. Arrange the samples, standards and blank solutions in random order but with a reagent blank in each half of the total series. Note the sequence of the standard solutions and move forward two places in the sequence for subsequent measurement series. Aspirate each solution in turn and return to acid (1 + 19) for 1–2 min between each solution. Record the digital readings for consecutive 10-s integration times and then re-read the series of solutions in reverse order to give a total of four readings for each solution. Calibration may be obtained by computer techniques; for this investigation all calculations were obtained arithmetically.

Results and Discussion

Calibration

For multi-elemental measurement of solutions originating from foodstuffs, the standard concentrations used for calibration must vary considerably between elements. Because of this, care must be exercised in avoiding incompatible combinations of elements in the acid medium of choice which could give losses from solution by precipitation. Cross-contamination of one element at low concentrations by impurities in an element present at high concentrations must similarly be avoided, as it is not unusual to have impurity levels of 10^{-4}% in metals. The multi-elemental standards used for sulphuric acid (1 + 19) and hydrochloric acid (1 + 19) are tabulated in Tables I and II; those in hydrochloric acid are in general 10 times greater to allow for the 7-fold concentration obtained by chelation and extraction. The overlap of low concentrations of the major trace elements upon the trace elements indicated in Table II did not cause cross-contamination or spectral interference; this was proved subsequently.

It must be recognised that quantitative analysis must be shown to be free from systematic bias, obtained from direct interferences, while the random bias must be defined for the measured concentration range. With the ICP-OES system, experience indicates that correction must be made for known direct interferences. To assess these it is initially necessary to obtain good estimates for the variation of measurement on standard solutions at levels relevant to those expected in foodstuffs. The stipulation must be made that the variation is assessed within a series of measurements containing food digests (which need not be recorded) or solutions containing potential interferences, *i.e.*, simulating routine practice.

The first seven series of standard readings obtained, according to the procedure, over a period of 2 months were processed by a previously described system,[29] for each element for both sulphuric acid (1 + 19) and hydrochloric acid (1 + 19) solutions. Each standard net response was expressed in terms of the lowest standard concentration and the average net response for all the standards for each calibration was calculated in terms of this concentration for each element. The ratio of the sum of the net responses for each standard to the sum of the net average response indicated the expected linearity for each element. The ratios of means falling outside 95% confidence limits (95 C.L.) imposed by the reproducibility, s, when practical measurement was possible, numbered 3 out of 122.

The standard deviation representing instrument noise, s_n, can be obtained from the paired consecutive readings for each elemental standard solution for the seven calibration lines and expressed as the coefficient of variation (C.V.) from the average net response for each standard. The resulting values will be representative of the noise levels, in practical use, inherent in a single integration reading for 14 degrees of freedom (d.f.) from which s_n (7 d.f.) for the mean of four readings, used throughout this investigation, may be obtained by dividing by 2.

In practice, it is desirable to measure as many samples as possible with each series of standard solutions and hence the time for measurement will exceed 1 h. During this time the instrument response may drift, or there may be drift because of the differences in the nature of the samples. The standard deviation for within-series measurements, the repeatability, s_o, will consist of such contributions together with that from s_n, and may be calculated by partial analysis of variance for single readings and hence the mean of four readings (7 d.f.).

As linearity has been proved for these calibrations, the standard deviation of between-series variation of measurement (which includes the blank variation), the reproducibility, s,

for the mean of four readings, may be obtained from the difference between net mean individual responses at each concentration with the net average response for individual calibration lines. Each standard deviation, s_n, s_o and s for each elemental standard, when measurement was possible, is recorded in Tables III and IV. Because of the insensitivity of the fixed cobalt emission line, no information at relevant levels of cobalt can be included.

The value for s_n expressed as C.V. decreases with increasing elemental concentration. A constant level is obtained for the major trace elements irrespective of which acid standards are measured, and this constant level is of the order of 0.4%. This applies over a wide range of concentrations, *e.g.*, for a range for iron of 0.5–50 mg l^{-1} and for zinc of 0.5–25 mg l^{-1}. Undoubtedly a similar constant effect would be obtained for the trace elements if measurement was made at elevated levels outside the scope of this investigation. When expressed as con-

Table III

Calibration data—major trace elements

Each coefficient of variation is for 7 degrees of freedom. Sensitivity settings for the ICP-OES were reduced for iron and zinc standards in hydrochloric acid (1 + 19).

	Coefficient of variation of measurement for mean of four integration readings, %											
	Copper			Iron			Manganese			Zinc		
Standard solution	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s
0*	0.9	3.6	—	0.4	1.7	—	0.2	0.4	—	0.8	0.8	—
01*	2.3	3.2	—	2.4	4.5	—	0.3	0.5	—	1.0	1.7	—
7	10	31	29	0.9	1.2	3.8	2.3	3.5	24	1.6	1.7	5.0
8	4.4	13	19	0.6	1.2	1.2	0.9	1.7	7.2	0.8	2.3	12
9	2.1	4.5	4.7	0.4	0.6	0.8	0.5	1.0	2.3	0.7	1.7	7.4
E	3.6	5.6	5.0	0.9	0.8	1.6	1.0	1.7	1.1	0.8	1.3	3.0
10	1.0	2.8	1.4	0.3	0.6	0.7	0.4	0.6	1.5	0.3	1.2	1.6
F	2.1	2.5	1.4	0.4	0.5	1.3	0.7	1.0	1.1	0.5	1.1	1.1
11	0.6	1.2	1.0	0.2	0.9	0.8	0.3	1.0	1.1	0.2	1.2	0.8
12	0.7	1.6	1.1	0.3	0.9	0.7	0.4	0.8	1.6	0.3	1.2	0.9
G	1.3	1.2	1.5	0.3	0.3	2.1	0.7	1.0	1.3	0.8	0.8	1.4
13	0.9	1.4	1.8	0.5	1.0	0.7	0.5	1.1	0.9	0.7	1.5	0.7
14	0.4	0.8	2.0	0.3	0.8	0.6	0.3	0.8	1.6	0.4	0.6	0.7
H	0.6	0.6	0.5	0.3	0.5	0.7	0.4	0.5	0.4	0.4	0.6	0.7
J	0.3	0.3	0.8	0.4	0.3	0.5	0.7	0.8	0.6	0.5	0.6	0.5
K	0.3	0.4	1.7	0.3	0.4	0.5	0.5	1.0	0.8	0.4	0.6	1.1
L	0.3	0.3	1.1	0.3	0.5	0.6	0.3	1.0	0.2	0.5	0.7	1.2
M	0.3	0.4	1.3	0.3	0.5	0.6	0.5	0.9	0.8	0.5	0.7	0.8
N	0.3	0.5	1.3	0.3	0.4	1.1	0.5	0.6	1.1	0.4	0.7	1.2

* Expressed as micrograms per litre.

Table IV

Calibration data—trace elements

Each coefficient of variation is for 7 degrees of freedom.

	Coefficient of variation of measurement for mean of four integration readings, %																	
	Arsenic			Cadmium			Chromium			Nickel			Lead			Tin		
Standard solution	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s	s_n	s_o	s
0*	33	32	—	0.6	1.5	—	0.7	2.4	—	1.8	3.7	—	—	—	—	8.3	7.7	—
01*	51	48	—	1.3	1.6	—	1.4	2.3	—	2.8	2.8	—	28	45	—	18	17	—
1	—	—	—	—	—	—	—	—	—	62	86	150	—	—	—	—	—	—
2	36	44	78	51	90	98	13	27	32	23	32	52	—	—	—	18	21	16
3	23	30	24	40	89	72	6.5	23	18	11	24	20	—	—	—	7.6	6.8	8.1
4	18	29	25	28	38	20	6.7	13	9.0	6.2	17	21	—	—	—	8.5	8.3	4.8
5	14	15	12	29	29	49	4.1	7.3	12	6.5	8.2	14	—	—	—	4.1	4.9	7.3
6	11	13	7.4	14	28	43	4.1	6.5	9.1	6.1	8.2	24	—	—	—	4.9	5.0	7.5
A	—	—	—	9.0	11	10	3.5	3.8	3.7	8.1	10	5.3	27	52	65	6.6	6.7	10
B	44	59	57	6.8	7.6	6.9	1.7	1.8	2.1	5.5	5.5	4.6	22	48	42	3.9	3.6	3.5
C	30	30	25	4.3	5.1	2.3	1.1	1.3	1.1	3.9	3.4	6.2	19	19	15	2.0	1.9	1.9
D	33	30	26	3.4	4.2	2.7	0.7	1.2	1.3	2.6	2.9	3.2	10	11	17	2.6	2.2	2.0
E	21	20	9.8	1.6	3.2	1.9	0.6	0.8	0.9	2.0	2.1	3.7	9	14	25	1.7	1.8	1.5
F	13	11	18	—	—	—	0.3	0.4	1.4	1.5	2.1	4.7	5	6	15	0.6	1.2	1.9

* Expressed as micrograms per litre.

centration (see Tables I and II for conversion), s_n is constant for the standard blanks and a very narrow concentration range, usually below 0.1 mg kg^{-1}, for both trace and major trace elements in either sulphuric or hydrochloric acid solution.

The trends for s_o and s are similar to those for s_n but the values expressed as C.V. tend to be higher for the same elemental concentration. Expressed as concentration, the narrow constant range obtained for s_n is wider and tends to extend over the range 0–0.5 mg l^{-1} for each element. The 95 C.L. for the ratios of standard deviations for 7 d.f. against 7 d.f. is 0.45 to 2.23. Inspection of the ratios of s_o to s_n indicates that these are exceeded in half of the comparisons for the major trace elements in sulphuric acid. Similar inspection of ratios of s to s_o indicate a similar number for manganese and zinc in sulphuric acid and copper and iron in hydrochloric acid. For the trace elements (Table IV) these ratios are seldom exceeded except for the highest standard F. It would appear that as s_n declines to a constant low value, instrument drift becomes significant and this is indicative of indirect interference, which is to be discussed later.

Interferences

It has been previously recorded that for a system of measurement involving flame or flameless atomic-absorption spectrometry, interferences may be of four distinct types[30]:

A. Directly upon the response of a measured element in the presence of an interfering species.

B. Indirectly on the variation of an element response in the presence of interfering species.

C. Indirectly upon the variation of subsequent element response during an extended series of measurements in routine use.

D. Indirectly on the response of subsequent measurement in an extended series of measurements.

For measurement by the direct-reading ICP-OES, direct interference A has to be subdivided into four categories, as follows:

A1. From nebulisation and transport into the plasma of acidic solutions of different concentrations.

A2. From nebulisation and transport into the plasma of solutions containing varying amounts of total dissolved solids.

A3. From compound formation or ionisation effects from other inorganic species.

A4. From spectral effects from other inorganic species.

*Direct interference A*1

The changes in response caused by transport of and by the viscosity of different acid concentrations[9,10,16] were considered for a narrow concentration range of both sulphuric and hydrochloric acids for each of the major trace elements using standard solutions 9, 14, F and H. Net responses were obtained by subtraction of the blank from 1 + 19 sulphuric and hydrochloric acid to reflect actual practice but, as expected, blank responses at different acid concentrations differed in magnitude only slightly. The average ratios for the four major trace elements each at two concentrations for the different acid concentrations are shown in Table V and indicate the systematic bias likely from small changes in acid concentration.

The experimental work described in this account was made at fixed acid concentrations and the extraction procedure similarly measured solutions of constant acid strength. For

TABLE V

CHANGES IN ELEMENTAL RESPONSE FOR DIFFERENT ACID CONCENTRATIONS

Sulphuric acid concentration	Ratio*	Range	Hydrochloric acid concentration	Ratio*	Range
1 + 19	1.00	—	6 + 94	0.98	0.95–0.99
1 + 24	1.04	1.02–1.06	1 + 19	1.00	—
1 + 32	1.09	1.06–1.14	1 + 24	1.02	1.01–1.03
1 + 49	1.13	1.10–1.21	3 + 97	1.04	1.03–1.05

* This refers to the average ratio of eight responses for the major trace elements compared with that in acid (1 + 19).

acid digestions prepared in sulphuric acid some loss would be expected, depending on the time taken for digestion. While measurement by atomic-absorption spectrometry (AAS) has been shown to be stable over the range 3–5% *V*/*V* sulphuric acid, this would not be so for measurement by ICP-OES.

Direct interferences A2 and A3

The major elements (species) in foodstuff digests are sodium, potassium, calcium, magnesium and phosphorus. These were tested for interference individually for the highest amounts likely and, when necessary, at a half and a quarter of these amounts on standards 0, 9 and 14 for the major trace elements in sulphuric acid (1 + 19) and similarly on standards 01, F, H and N in hydrochloric acid (1 + 19). For the trace elements 0, 3, 6 and 01, B and F (E for cadmium) were similarly tested. Phosphorus was added as sodium dihydrogen orthophosphate. Three or four series of solutions were measured with relevant standards within the same series of readings. The elemental responses of solutions 0 or 01 plus added species were subtracted from solutions 9, F, etc., if greater than standards 0 or 01; standards 0 or 01 were subtracted if these were greater. The total dissolved solids (TDS) (maximum 1.1% *m*/*V*) were calculated in terms of the acidic anionic species and as orthophosphoric acid for phosphorus (for the latter, the contribution of sodium in orthophosphate salt was subtracted). The relative differences in response with and without added species are illustrated in Table VI for the major trace elements. The 95 C.L. were calculated from s_0 (Table III) and are restrictive as they do not contain the variation from the blank or contributions from solution preparation.

Similar tests were made for sulphuric acid solutions containing separately added aluminium (0.5 mg), silica (0.5 mg), tin (0.5 and 1.0 mg) and, as relevant, iron (2 mg) and a combination of copper and manganese (0.2 mg) with zinc (1 mg). Also tested were hydrochloric acid solutions containing separately added aluminium (5 mg), silica (5 mg), tin (5 and 10 mg) and, as relevant, iron (20, 10 and 5 mg), copper (1.5 and 0.5 mg), manganese (1.5 and 0.5 mg) and zinc (7.5 and 2.5 mg). None of these added species contributed more than 0.07% *m*/*V* TDS in solution. The 95 C.L. were exceeded only occasionally for these added species and all figures are included in the over-all non-significant averages at the foot of Table VI. Conversely, most of the figures in Table VI exceed the 95 C.L. in a negative direction.

The figures display the random distribution expected from such an exercise, particularly for copper, but closer inspection suggests the following conclusions. (a) There is a tendency for the negative interference for each species to be proportional to the amount tested, expressed as TDS, irrespective of the acid solution measured. (b) The interference from calcium and magnesium is greater per unit amount than from sodium, potassium and phosphorus. (c) The degree of interference from an inorganic species is relatively constant irrespective of the concentration of the major trace element measured. (d) Interferences from mixtures appear to be cumulative of the individual species. If these interferences are expressed as milligrams per kilogram of TDS required to give 1% depression in response, using for each acid solution the highest concentration standard for each major trace element and the highest amount of interfering species (for magnesium, 25 mg were used in hydrochloric acid), the results shown in Table VII are obtained.

It seems likely that a 1% depression is obtained from about 1000 mg kg^{-1} of TDS for sodium and potassium and therefore another source of depression must exist for calcium which applies consistently to each of the major trace elements. It is suggested that this is an ionisation interference, but this seems unlikely for copper as an atom emission line is measured. A similar comment applies to magnesium except that the extent differs for iron and is probably absent for copper. For phosphorus a reduced and variable depression from TDS is apparent and another anomaly is iron, for which the depression is less for sodium and potassium.

Table IV indicates that the extent of these interferences would not be identifiable within the large values likely for 95 C.L. for the trace elements, with the exception of chromium in hydrochloric acid, in which instance the trends appeared similar to those for iron.

Although these effects are often mentioned in the literature, no attempt is usually made to quantify them. The results recorded here may be singular to the nebuliser and instrument used. For the purposes of this investigation, when required, the combined direct inter-

TABLE VI

INTERFERENCE EFFECTS A2 AND A3 ON MEASUREMENT OF COPPER, IRON, MANGANESE AND ZINC

Element			Relative difference in response with and without added elemental species, %*																	
			Copper					Iron					Manganese					Zinc				
Acid†			1		2			1		2			1		2			1		2		
Concentration/mg l^{-1}			0.05	0.5	0.1	0.5	5.0	0.5	5.0	1.0	5.0	50.0	0.05	0.5	0.1	0.5	5.0	0.25	2.5	0.5	2.5	25.0
95% confidence limits, %			±10.6	±1.9	±6.0	±1.4	±1.2	±1.4	±1.9	±1.2	±1.2	±1.0	±2.4	±1.9	±2.4	±1.2	±1.4	±3.9	±1.4	±2.6	±1.4	±1.7
Added ion	Amount/ mg‡	TDS§ × 10^3/mg l^{-1}																				
Na^+	200	6.2	−12	−4.5¶	—	—	—	−1.0	−4.2¶	—	—	—	−7.6	−5.3¶	—	—	—	−6.9	−5.3¶	—	—	—
	100	2.5	—	—	−10	−3.8	−3.9¶	—	—	−3.7	−2.1	−2.4¶	—	—	−4.8	−3.1	−3.1¶	—	—	−2.8	−2.3	−1.7¶
K^+	200	4.5	−5.9	−3.9¶	—	—	—	−4.3	−2.6¶	—	—	—	−7.8	−4.4¶	—	—	—	−7.7	−3.9¶	—	—	—
	100	1.9	—	—	(−16)	−2.4	−2.5¶	—	—	(−3.5)	−1.1	−0.9	—	—	(−4.9)	−1.7	−2.3¶	—	—	(−3.9)	−1.9	−2.5¶
Ca^{2+}	40	1.4	−15	−2.9¶	—	—	—	−3.6	−3.3¶	—	—	—	−7.1	−4.4¶	—	—	—	−11	−5.3¶	—	—	—
	20	0.6	—	—	(−8.0)	−0.8	−2.4¶	—	—	(−2.6)	−1.2	−2.0¶	—	—	(−3.9)	−0.8	−1.7¶	—	—	(−6.8)	−2.5	−1.9¶
Mg^{2+}	50	2.5	−5.1	−2.7¶	(−1.0)	−0.4	+1.9	−6.7	−4.4¶	(+0.4)	−1.2	−1.1	−8.5	−6.0¶	(−6.8)	−2.7	−2.3¶	−3.1	−5.8¶	(−1.4)	−3.6	−5.0¶
	25	1.2	−8.6	−1.5	−3.2	−0.8	−1.4	−3.8	−2.9¶	−1.9	+0.2	−1.6	−12	−1.6¶	−6.5	−1.6	−2.7¶	−6.6	−1.9¶	−2.4	−1.8	−2.6¶
	12.5	0.6	+7.4	−2.1	−6.0	+0.5	+0.2	−0.7	−1.7	+0.4	+0.3	+0.3	−2.0	−1.4	−2.7	−0.4	−0.7	−2.9	−6.2¶	0	+0.2	+0.5
$P(PO_4^{3-})$	200	6.1	−1.1	−3.2¶	—	—	—	−3.9	−3.6¶	—	—	—	−5.0	−4.7¶	—	—	—	−1.2	−3.0¶	—	—	—
	100	3.1	—	—	+1.5	−0.6	−0.8	—	—	−1.1	−1.6	−1.2	—	—	−0.5	−1.7	−1.0	—	—	−1.5	−0.1	+0.4
Ca^{2+} + Mg^{2+} + $P(PO_4^{3-})$	40 + 50 + 100	6.9	−7.5	−4.5¶	—	—	—	−9.6	−8.4¶	—	—	—	−13	−10¶	—	—	—	−12	−11¶	—	—	—
Ca^{2+} + Mg^{2+} + $P(PO_4^{3-})$	40 + 50 + 50	5.4	−6.1	−5.5¶	—	—	—	−9.0	−9.2¶	—	—	—	−8.6	−11¶	—	—	—	−13	−13¶	—	—	—
Ca^{2+} + Mg^{2+} + $P(PO_4^{3-})$	20 + 25 + 50	3.1	—	—	−11	−1.1	−1.6¶	—	—	−5.0	−3.1	−3.5¶	—	—	−8.2	−3.3	−4.1¶	—	—	−5.8	−6.2	−6.2¶
Over-all average non-significant differences‖			+2.8	−0.6	−2.5	0.0	+0.1	+0.5	−0.1	+0.1	−0.1	−0.5	0.0	−0.1	−0.2	−0.1	−0.6	−1.5	−1.4	+0.4	0.0	0.0

* ( ) Implies a duplicated set of readings.
† Acid 1 is sulphuric acid (1 + 19); acid 2 hydrochloric acid (1 + 19).
‡ For 100 ml of digest prepared from 10 g of foodstuff, the concentration of interfering species as mg kg^{-1} can be obtained by multiplying by 100.
§ TDS equals total dissolved solids calculated in terms of the anion of the measured acid solution with phosphorus calculated as orthophosphoric acid. The TDS shown for magnesium is for sulphuric acid; in hydrochloric acid the TDS is 2.0, 1.0 and 0.5 × 10^3 mg l^{-1} for 50, 25 and 12.5 mg of added magnesium, respectively.
¶ ≤ 5% significance.
‖ This average includes differences when aluminium, silica, tin and relevant major trace elements are added at levels described in the text.

TABLE VII

INTERFERENCE EFFECTS A2 AND A3 ON COPPER, IRON, MANGANESE AND ZINC EXPRESSED AS TOTAL DISSOLVED SOLIDS

Added ion	Total dissolved solids which gives 1% depression/mg l^{-1}			
	Copper	Iron	Manganese	Zinc
Na^+	1010	1260	990	1320
K^+	960	1900	930	960
P (PO_4^{3-})	2900	2140	2200	—
P (Na^+)*	600	650	520	1380
Ca^{2+}	360	360	340	290
Mg^{2+}	820	600	400	400
Ca^{2+} + Mg^{2+} + P (PO_4^{3-})	1480	770	650	520

* Calculated on the total dissolved solids from sodium included with the phosphorus and the total depression from sodium and phosphorus.

ferences A2 and A3 were used to correct for each of the major elemental species at a constant percentage level, irrespective of the major trace element concentration present. In practice, for digests from foodstuffs, this positive correction should not exceed 10%.

Direct interference A4

In the testing of the above interferences, measurement of solutions 0 and 01 with concomitant elemental species permits an assessment of any direct spectral interferences A4. When the difference between these solutions and standards 0 and 01 exceeded 95 C.L. positively for a trace or major trace element, the content of the elemental species was checked independently by flame atomic-absorption spectrometry for impurities. These could be of the order of 1×10^{-5}–1×10^{-6} mg kg^{-1} per mg kg^{-1} and after subtraction the remainder, if present, was considered to be defined by direct interference A4. The occasions satisfying this criterion are listed in Table VIII. In general, these factors were relatively constant for the different levels of interfering species but more reliance would be placed on the higher levels tested; there was no significant difference for the factors calculated from 0 in sulphuric and 01 in hydrochloric acid solution.

Both interferences for zinc have been previously defined as spectral line coincidence at 202.55 nm, but that from magnesium for the remainder of the elements may be caused by primary stray light in the instrument used for this investigation. For the low wavelength of measurement of arsenic and tin, broad band continua are probably the cause of interference, while the interference from the crowded iron emission is not surprising. Interference from manganese has not been previously recognised. The list in Table VIII does not reflect an exhaustive trial of elemental species or combinations of these species in digests from foodstuffs. It may be noted, however, that the interference from magnesium is unaltered in the

TABLE VIII

SPECTRAL INTERFERENCES A4

Results are milligram per kilogram of element per milligram per kilogram of interfering element.

Element	Interfering element				
	Magnesium	Iron	Manganese	Aluminium	Copper
Copper	—	3.5×10^{-5}	—	—	—
Iron	—	—	1.0×10^{-3}	—	—
Zinc	2.1×10^{-4}	—	—	—	5.4×10^{-3}
Cadmium	2.6×10^{-5}	1.4×10^{-4}	2.0×10^{-4}	9.0×10^{-5}	—
Chromium	1.6×10^{-4}	1.6×10^{-3}	3.0×10^{-4}	—	—
Nickel	1.1×10^{-4}	—	—	—	—
Lead	4.4×10^{-4}	—	—	—	—
Arsenic	2.8×10^{-3}	2.0×10^{-3}	6.8×10^{-3}	1.2×10^{-2}	—
Tin	1.2×10^{-3}	4.4×10^{-4}	3.8×10^{-3}	1.3×10^{-3}	—

presence of calcium and phosphorus. These spectral interferences will have much greater effect for trace elements such as arsenic and cadmium present at very low concentrations in foodstuffs. The corrections indicated in Table VIII are negative concentration corrections and when required should be applied prior to and separately from that of direct interferences A2 and A3.

Indirect interferences

Indirect interference B may be assessed from the calculation of s_n and s_o for solutions 9, F, etc., containing high levels of species which cause direct interference A2 and A3. The 95 C.L. of the ratio of standard deviations for these solutions with those of standard solutions in hydrochloric acid were not exceeded, but in sulphuric acid they were exceeded for zinc and tin measurements. This was traced to solutions containing calcium and would be caused by intermittent coating of the plasma torch injector tip. The main source of interference B is, however, more subtle.

The randomness of results in Table VI has been noted for the lowest concentrations of the major trace elements, *e.g.*, copper at 0.05 and 0.10 mg l^{-1}. For solutions 0 and 01 with added elemental species, occasions when 95 C.L. were exceeded positively have been dealt with by direct interference A4. There were also several occasions when the 95 C.L. were exceeded negatively. In sulphuric acid this occurred 5 out of 39 times for the major trace elements and once out of 47 times for the trace elements; no interfering species was unduly represented. For hydrochloric acid the incidence was 8 out of 106 for the major trace elements and 17 out of 147 for the trace elements; potassium was unduly represented in the latter (12 times). It has been noted that changes in acid strength do not alter the spectral background. The presence of some major elemental species would seem sometimes to increase the noise in the spectral background. This indirect interference B, while having a diminishing effect at higher measured levels, will increase sharply variation, *i.e.*, random bias, as measured levels decrease towards the detection limit.

Indirect interference D, and hence C, or C alone is always present in a measuring system involving solution aspiration with existing nebulisers into atomic-absorption flames,[29] or in electrothermal atomic-absorption spectrometry, and will increase the variation of measurement at all concentration levels. These interferences are also apparent in solution nebulisation into the plasma, as the calibration data have indicated. In the measurement of sample solutions against such calibration solutions, these indirect interferences may be doubly represented. Corresponding increases in derived functions such as confidence intervals and detection limits will ensue (such indirect interferences are absent in many analytical techniques involving discontinuous measurement). The cumulative effects of these indirect interferences together with any direct interferences, as yet unrecognised, will become apparent only during application of the direct-reading ICP-OES to the measurement of foodstuff digests.

Application to Analysis of Foodstuffs

The reproducibilities, s, obtained for standard blank and standard solutions up to 0.5 mg kg^{-1} are constant and permit derivation of elemental limits of detection according to a previously described system based on the formula $t_{0.005}s$.[31] These will be only an indication of what may be achieved in practice and may be compared with those actually achieved in application to foodstuff digests by atomic-absorption spectrometric measurement (Table IX). The latter are desired practical limits of detection (for 100 ml of digest from 10 g of foodstuff), but for cadmium, chromium, lead and nickel allow for a concentration stage prior to measurement. Clearly for levels present in foodstuffs only major trace elements and, for canned goods, tin could be measured directly by the direct-reading ICP-OES.

Measurements were made on sulphuric acid digests from reference materials for the major trace elements against multi-elemental standards 0–14 (Table I) as a matter of interest. These values were corrected for positive spectral interference A4 and then for negative direct interferences A2 and A3. The resulting values should reflect direct interference A1, and from previous experience for the food matrices concerned should not give ratios to the actual contents greater than 1.09 (Table V). The results of this comparison are shown in Table X. Copper cannot be measured but for the other three elements the expected range of ratios is

TABLE IX

COMPARISON OF LIMITS OF DETECTION

Values by AAS are in application to food digests; for cadmium, chromium, lead and nickel they include a 7-fold concentration stage and for tin a 5-fold dilution.[28–30] For 100 ml of digest prepared from 10 g of foodstuff, used generally in this laboratory, concentration as milligrams per kilogram in original foodstuff may be obtained by dividing by 10.

	Amount per 100 ml solution/μg		
		ICP-OES	
Element	AAS	Sulphuric acid (1 + 19)	Hydrochloric acid (1 + 19)
Copper	1.0	0.8	0.7
Iron	10	1.0	3.0
Manganese	2.0	0.5	0.5
Zinc	4.0	2.7	2.0
Cadmium	0.08	0.4	0.3
Chromium	0.2	0.6	0.6
Lead	0.6	—	12
Nickel	0.6	1.8	2.1
Arsenic	0.2	8	17
Tin	1.2	3.4	7.3

acceptable only for flour and NBS liver. The rapid destruction of organic matter in 1 g of dried kale and spinach could hardly result in loss of half of the sulphuric acid in the resulting digest. The conclusion must be that even after correction of known direct interferences, the values obtained continue to reflect considerable positive systematic bias (dilution of these digests would yield solutions beyond useful detectable levels for copper and zinc).

Although it has been suggested that effects from direct interferences A1 may be minimised by addition of high concentrations of say sodium, it is evident that the trace and major trace elements should be removed from interfering species available in foodstuff digests and solubilised at a constant acid concentration, thereby removing direct interferences A1–A3. This can be achieved with a chelating ion-exchange column,[32] or by chelation and extraction into solvent.[33] Each has the added merit of concentrating trace elements to levels permitting measurement by the ICP-OES. Chelation and extraction were selected for trial.

It was established that organic solvents unbalanced the background continuum of the plasma. Back-extraction into a fixed acid concentration was therefore not permissible and it was necessary after chelation and extraction to remove the solvent, oxidise with nitric acid to dryness and solubilise in acid to give a final hydrochloric acid (1 + 19) solution. This pre-treatment for a sample series took one man-working day and involved considerable manipulation. Chelation and extraction of standard solutions 0, 3, 6, 9 and 14 (Table I) in sulphuric acid (1 + 19) followed by measurement with standards 01–N indicated that manganese and zinc were not separated. Adjustment of solutions to pH 4 and similar trials were more successful and the following average recoveries were obtained for standard solutions 0–14 (Table I): Cu, 101; Fe, 99; Mn, 102; Zn, 104; Cd, 105; Ni, 97; Pb, 83; Sn, 69; Cr, 0; and As, 0%.

TABLE X

RATIO OF RESULTS OBTAINED BY DIRECT MEASUREMENT WITH ICP-OES TO THOSE OBTAINED BY FLAME AAS ON FOODSTUFF DIGESTS

Foodstuff material	Copper	Iron	Manganese	Zinc
Dried milk	6.7	1.36	0.91	1.08
Flour	1.43	1.05	1.04	1.09
NBS liver	1.05	1.01	0.99	1.04
Bowen's kale	6.7	1.18	1.29	1.21
NBS tuna	1.15	1.11	1.20	1.12
NBS spinach	1.64	1.07	1.09	1.09

The fixed insensitive line used in the ICP-OES precluded measurement of cobalt. Chromium(VI) may be chelated with dithiocarbamates but it is not possible to obtain and retain this state in foodstuff digests; reduction to chromium(III) followed by a separate chelation with pentane-2,4-dione is necessary.[34] Digestion with nitric acid yields arsenic(V), and this valence state is incompatible with the described procedure[35]; further, the detection limit would preclude the determination of arsenic in most foodstuffs. The efficiency of the procedure for lead and tin was disappointing and, when applied in practice to foodstuff digests, iron was only partly recovered because of precipitation at pH 4 or reduction to iron(II) by the dithiocarbamates. This leaves five of the original eleven elements plus lead, which was persevered with. The comment must be made that possession of a multi-elemental measuring facility does not automatically ensure measurement for a wide range of elements; in practical use, it may differ little from a sequential measuring facility.

The recovery of an analyte from substrates will only prove the accuracy of a method. The accuracy of results can only be proved by obtaining agreement with certified levels of standard reference materials. For the ICP-OES, spectral interference, in particular, would not effect elemental recovery and hence an exercise to establish the recovery of added elements in discrete form from foodstuffs was not carried out. Two reference materials used for inter-analyst assessment (dried milk and white plain flour), Bowen's kale and three NBS reference materials (bovine liver, tuna and spinach) were used as standard reference materials. Each has been used extensively for the development of methods using atomic-absorption spectrometric measurement and subsequently to prove the accuracy of results during the monitoring of foodstuff homogenates. Digests were prepared using masses of dry materials simulating corresponding wet masses, and total analyses were made in triplicate by the two analysts concerned. Each result was corrected for relevant known direct interferences A4 according to the factors in Table VIII. Analyst significance was present in 4 out of 48 element material combinations, two of which involved lead (the range of cadmium is elevated to that normally found in foodstuffs). Means and 95% confidence intervals (95 C.I.) for the means obtained by this procedure are displayed in Table XI and compared with means and 95 C.I. for 6–10 total analyses obtained by existing procedures with measurement by atomic-absorption spectrometry.[28,29] Table XII displays in greater detail the results by the present procedure, but inclusion of this table does not endorse the method.

Comparison of the two sets of means in Table XI indicates the number of occasions disagreement exists, 18 out of 36. Enhanced means using ICP-OES measurement are rare, *e.g.*, cadmium and lead in bovine liver. That for cadmium is singular and it would appear that this material may contribute spectral interference at the ion-emission line for cadmium. Those for lead are not surprising at a first impression because of the lengthy pre-treatment and the ubiquitous nature of lead (see later). Most of the disagreements are negative and again the lengthy pre-treatment must be questioned. The remnants of the extracts were therefore measured by flame atomic-absorption spectrometry for three reference materials. For zinc in kale and NBS tuna the values were in agreement with measurement by ICP-OES but that for NBS spinach was increased to 46 mg kg^{-1}, while the copper values increased to agree with those previously obtained for kale and NBS tuna but only to a value of 11.0 mg kg^{-1} for spinach. While manganese and cadmium in NBS spinach are in agreement, it would appear that copper, zinc and nickel are incompletely chelated for this material. For copper it would appear that the measurement is also at fault. The general lack of agreement with established levels in these reference materials given by the present procedure suggests that it is inaccurate and hence unacceptable.

Nevertheless, more information may be gathered from consideration of variation. The decreasing gradation of repeatability, s_o, and reproducibility, s, with increasing amount may be judged from Table XII for each element; copper is particularly uneven. Comparison of s_o and s of results, expressed as C.V. (relevant concentrations may be obtained by multiplying amount by 0.07), with that for measurement of standard solutions (Tables III and IV) at similar concentrations indicate that the 95 C.L. of ratios of standard deviations are invariably exceeded. While the lengthy pre-treatment could contribute to these increases in s_o and s, indirect interferences D and C during measurement are probably the main contributors. Lead is an exception to the above and a high C.V. is involved with measurement; this is a contributory cause to the disagreements with accepted values previously noted. Both procedures using measurement by ICP-OES and AAS give similar variations, reflected

TABLE XI

EVALUATION OF ACCURACY OF METHOD IN APPLICATION TO REFERENCE MATERIALS

Reference material	Concentration/mg kg^{-1}					
	Dried milk	Flour	NBS bovine liver*	NBS tuna	Bowen's kale	NBS spinach*
Sample mass/g	2.5	10	2	5	1	1
Copper—						
Mean by ICP	0.37†	1.32†	194	3.07†	4.25†	10.2†
±95 C.I. of mean	0.04	0.04	6	0.16	0.18	1.0
Mean by AAS‡..	0.63	1.36	198	3.27	4.99	12.1
±95 C.I. of mean	0.07	0.02	7	0.08	0.33	0.4
Manganese—						
Mean by ICP	0.32†	5.17	10.2†	0.51	15.4	167§
±95 C.I. of mean	0.02	0.08	0.4	0.01	1.0	7
Mean by AAS	0.76	5.25	11.0	0.51	15.6	170
±95 C.I. of mean	0.10	0.08	0.4	0.12	0.8	4
Zinc—						
Mean by ICP	43.4†	5.25†	139	14.2	32.0	43.1†
±95 C.I. of mean	1.8	0.17	5	0.2	3.0	4.0
Mean by AAS	45.8	5.38	142	14.7	33.1	50.8
±95 C.I. of mean	1.2	0.08	4	0.5	1.4	1.9
Cadmium—						
Mean by ICP	0.005	0.034	0.36†	0.042	1.02	1.39
±95 C.I. of mean	0.005	0.007	0.028	0.007	0.09	0.11
Mean by AAS	0.003	0.037	0.25	0.046	1.01	1.40
±95 C.I. of mean	0.004	0.003	0.025	0.010	0.12	0.14
Lead—						
Mean by ICP	0.22†	0.08†	0.43†	0.53	2.36	1.75†
±95 C.I. of mean	0.25	0.09	0.53	0.06	0.56	1.33
Mean by AAS	0.02	0.03	0.25	0.50	2.23	1.10
±95 C.I. of mean	0.03	0.03	0.04	0.07	0.19	0.06
Nickel—						
Mean by ICP	0.03	0.03†	0.05	1.01	0.94†	4.8†
±95 C.I. of mean	0.05	0.03	0.05	0.10	0.10	0.7
Mean by AAS	0.03	0.01	0.05	1.10	0.78	5.8
±95 C.I. of mean	0.04	0.01	0.06	0.13	0.10	0.2

* Certified and non-certified (in parentheses) levels for NBS bovine liver and spinach are as follows: bovine liver, Cu 193, Mn 10.3, Zn 130, Cd 0.27, Pb 0.34 mg kg^{-1}; spinach, Cu 12, Mn 165, Zn 50, Cd (1.5), Pb 1.2 and Ni (6) mg kg^{-1}. Disagreements with the means by AAS for bovine liver for Mn and Zn have been discussed in reference 29.

† These values are outside expected levels imposed by 95 C.I.s of procedures using AAS measurement.

‡ All results are calculated on a dry-mass basis. With the exception of dried milk, flour and in part spinach, for which means were obtained during this investigation using AAS measurement, the remainder of the means for AAS measurement are those quoted in references 28 and 29. Cadmium measurement for bovine liver, kale and spinach was made on an aliquot of that normally taken and calculation of 95 C.I. based on amount would be reduced accordingly, *e.g.*, to 0.03, 0.03 and 0.05 μg, respectively, compared with 0.06, 0.09 and 0.11 μg by ICP measurement.

§ This result was obtained by applying the procedure to a 10-ml aliquot of digest.

by the 95 C.I. of means displayed in Table XI for copper, manganese and zinc. A 7-fold concentration for measurement by ICP-OES is compared with no concentration for measurement by AAS. It may seem strange that the former measurement does not give improved variations, but the standard deviation of measurement is constant over an extremely wide range. Concentration of elements classified as major trace elements, present well above detection limit levels, therefore does not confer any improvement in the variation of measurement or the derived confidence intervals. For cadmium, lead and nickel comparison is for approximately the same concentration factor (for cadmium the footnotes to Table XI apply for AAS measurement). Detection limits for results for these three elements may be derived from the three reference materials having the lowest s, in terms of amount, via the variance, for 15 d.f. (materials returning zero results must be excluded). These derived values are cadmium 0.15, lead 2.4 and nickel 0.5 μg, and may be compared with the detection limits

TABLE XII

REPLICATE ANALYSES FOR TRACE AND MAJOR TRACE ELEMENTS IN REFERENCE MATERIALS

Each array represents six results obtained by two analysts in triplicate.

Element	Reference material	Sample mass/g	Mean content/ mg kg^{-1}	Range of content/ mg kg^{-1}	Amount/ μg	Repeatability		Reproducibility	
						%	μg	%	μg
Copper	Dried milk	2.5	0.37	0.32–0.43	0.92	11	0.10	11	0.10
	NBS spinach*	1	11.0	8.0–13.7	1.58	15	0.24	18	0.28
	Bowen's kale	1	4.25	3.96–4.42	4.25	3.3	0.14	4.0	0.17
	NBS spinach	1	10.2	9.0–11.6	10.2	9.1	0.93	9.1	0.93
	Flour	10	1.32	1.28–1.38	13.2	2.5	0.33	2.8	0.37
	NBS tuna	5	3.07	2.82–3.17	15.3	3.3	0.50	5.0	0.75
	NBS liver*	2	171	139–194	48.8	11	5.4	16	7.8
	NBS liver	2	194	187–202	388	2.9	11.2	2.9	11.2
Manganese	Dried milk	2.5	0.32	0.30–0.34	0.79	4.9	0.039	5.6	0.045
	NBS tuna	5	0.51	0.49–0.52	2.53	2.2	0.055	2.3	0.059
	NBS liver*	2	10.3	9.7–10.8	2.95	3.5	0.10	4.2	0.12
	Bowen's kale	1	15.4	14.5–16.5	15.4	3.9	0.60	6.0	0.93
	NBS liver	2	10.2	9.8–10.6	20.3	3.3	0.67	3.3	0.67
	NBS spinach*	1	167	156–173	23.9	3.8	0.91	3.8	0.91
	Flour	10	5.17	5.08–5.25	51.7	1.4	0.73	1.4	0.73
Zinc	NBS spinach*	1	43.3	36.2–49.7	6.2	12	0.8	12	0.8
	Bowen's kale	1	32.0	27.7–35.6	32.0	7.4	2.4	9.0	2.9
	NBS liver*	2	126	106–140	35.9	9.3	3.3	12	4.3
	NBS spinach	1	43.1	38.7–48.4	43.1	8.9	3.8	8.9	3.8
	Flour†	10	5.25	5.10–5.43	52.5	1.6	0.9	3.0	1.6
	NBS tuna	5	14.2	14.1–14.4	71.5	0.7	0.5	1.0	0.7
	Dried milk	2.5	43.4	40.9–45.7	108	3.7	4.0	3.9	4.3
	NBS liver	2	139	134–147	277	3.5	9.6	3.5	9.6
Cadmium	Dried milk	2.5	0.005	0–0.011	0.013	—	0.012	—	0.012
	NBS liver*	2	0.39	0.19–0.67	0.11	45	0.050	57	0.063
	NBS tuna	5	0.042	0.037–0.048	0.21	15	0.031	15	0.031
	NBS spinach*†	1	1.55	1.16–2.49	0.22	17	0.037	46	0.100
	Flour	10	0.034	0.026–0.042	0.34	20	0.067	20	0.067
	NBS liver	2	0.36	0.31–0.39	0.72	7.4	0.053	7.4	0.053
	Bowen's kale	1	1.02	0.90–1.13	1.02	8.2	0.084	8.2	0.084
	NBS spinach	1	1.39	1.24–1.52	1.39	7.3	0.100	7.3	0.100
Lead	Dried milk	2.5	0.22	0–0.63	0.55	—	0.60	—	0.60
	Flour	10	0.084	0–0.16	0.84	—	0.81	—	0.81
	NBS liver†	2	0.43	0–1.13	0.86	—	0.48	—	1.00
	NBS spinach†	1	1.75	0.44–3.32	1.75	30	0.53	73	1.27
	Bowen's kale	1	2.36	1.6–2.9	2.36	17	0.39	22	0.51
	NBS tuna	5	0.53	0.48–0.62	2.65	11	0.28	11	0.28
Nickel	Dried milk	2.5	0.03	0–0.11	0.07	—	0.11	—	0.11
	NBS liver	2	0.05	0.02–0.12	0.11	61	0.07	80	0.09
	Flour	10	0.03	0–0.06	0.29	—	0.17	—	0.23
	NBS spinach*	1	5.40	2.97–7.49	0.77	29	0.23	40	0.31
	Bowen's kale	1	0.94	0.85–1.09	0.94	9.8	0.10	9.8	0.10
	NBS spinach	1	4.83	3.86–5.71	4.83	14	0.69	14	0.69
	NBS tuna	5	1.01	0.91–1.16	5.05	9.5	0.48	9.5	0.48

* These results were obtained by applying the procedure to a 10-ml aliquot of digest.
† 5% analyst significance.

shown in Table IX. That for nickel is comparable to measurement by AAS, but for lead and cadmium they do not permit the determination of the levels normally found in dietary foodstuff homogenates.

Conclusion

The attempted application of an inductively coupled plasma optical emission direct-reading polychromator has been described for the simultaneous measurement of the major trace elements, copper, iron, manganese and zinc, and the trace elements cadmium, lead, nickel, chromium, arsenic and tin. The measurement of multi-elemental standards has been assessed, for a situation simulating routine practice, for linearity and variation. The latter has been defined by the standard deviations representing instrument noise, the repeatability and the reproducibility. Each is as low as or lower than literature values, where these exist, for the instrument used. From the reproducibility, limits of detection have been derived that permit an assessment of the application of measurement. From both repeatability and reproducibility, confidence limits have been calculated that permit the assessment of interferences likely to be encountered in measurement.

These interferences have been categorised into seven distinct classes. Direct interference A1 is that from nebulisation and transport of different acid concentrations; A2, similarly, is

that for solutions containing different levels of total dissolved solids; A3 is that from ionisation and compound formation effects with other inorganic species; and A4 is that from spectral effects from other inorganic species. Direct interference A1 may be avoided by using a constant acid strength; A4 may be corrected by a direct concentration factor for each interfering species; A2 and A3 combined may be corrected for each interfering species by means of a percentage correction applied separately to and after correction for A4. The three categories of indirect interferences will enhance the variation of results at all levels of analyte element concentration, but in particular at low measured concentrations.

Chelation and extraction followed by oxidation and solubilisation at a fixed hydrochloric acid concentration have been used to avoid the worst excesses of these interferences and to concentrate the trace elements to levels that permit measurement. Of the elements studied, only copper, manganese, zinc, cadmium, nickel (and lead) are amenable to this treatment. When the total procedure is applied to standard foodstuff reference materials no element satisfies the criterion of accuracy required for quantitative analysis, necessary for dietary survey exercises. The variation of measurement by the ICP-OES is extremely low, but in application is sharply increased. Comparable confidence intervals to those for results by AAS measurement, however, may be attained for copper, manganese, zinc and nickel. For cadmium and lead the enhanced variation of measurement does not permit determinations at the levels normally found in foodstuffs.

While both measurement by ICP-OES and the concentration technique (which is widely used) may have been at fault in this exercise, simultaneous measurement for the quantitative analyses of low levels of elements in foodstuffs does not appear to confer the advantages sought. It is possible that optimisation of the conditions of measurement with a monochromator ICP-OES would remove some of the anomalies described, *i.e.*, hitherto unrecognised spectral interferences and interferences due to ionisation or compound formation from particular combinations of inorganic species, permitting sequential measurement of some of the elemental levels required.

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Direct Determination of Lead in Used Engine Oils by Atomic-absorption Spectrophotometry

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An improved method has been developed for measuring lead in used lubricating oils. The procedure utilises a mixture of acid and a liquid anion exchanger (Aliquat 336) to dissolve the lead particles in 4-methylpentan-2-one and measurement is made by atomic-absorption spectrophotometry. The method is quantitative in the range 0.1–2.5% *m/m* and is independent of effects caused by variations in particle size, lead species or oil additives. The method is rapid and has good repeatability.

Keywords: Lead determination; used lubricating oils; liquid anion exchangers; atomic-absorption spectrophotometry

Used lubricating oils are frequently analysed for their total lead content to assist in engineering studies conducted on fuels and lubricants. Oil analysis is also used to monitor the levels of wear metals, including lead, to indicate the mechanical condition of moving parts of engines before wear is visually evident.[1,2] Similarly, metal contents are measured before waste oils are burnt, with or without heat recovery, as a method of disposal[3,4] and also prior to recycling (re-refining).[5,6]

The lead found in used engine oils occurs mainly as decomposition products resulting from combustion of the fuel. The lead is in the form of finely divided particles essentially suspended in the oil. It occurs as complex mixtures[7] of simple inorganic compounds such as lead(II) bromide, chloride, oxide and sulphate with possibly some lead(II) orthophosphate. The lead contents of used oils occur in the range 0.1–2.5% *m/m*, depending on factors such as the length of time in service, the age and condition of the engine and the lead content of the fuel.

The method for the determination of lead used at present in our laboratory is based on a classical procedure developed by the Institute of Petroleum.[8] The oil sample is completely destroyed by wet ashing with mineral acids to produce a precipitate of lead sulphate, which is dissolved in sodium hydroxide solution and the lead is determined by either polarography or atomic-absorption spectrophotometry (AAS). This method is very time consuming and prone to losses of lead due to spattering during the ashing stage.

A more rapid AAS procedure that avoids the wet-chemical treatment is widely used for wear-metal monitoring.[1,2] The oil samples are diluted with 4-methylpentan-2-one and the metals are determined directly by AAS. Standards for calibration are prepared from organometallic compounds dissolved in the same matrix. This method, however, is known to be dependent on particle size and not quantitative for some elements. Saba and Eisentraut[9,10] modified the method to overcome this problem for measuring titanium and molybdenum. Initially they reacted the oil samples with a mixture of acids such as hydrofluoric acid plus either hydrochloric or nitric acid to dissolve the metal particles. This they achieved quickly and simply by shaking the mixture for 2 min before dilution with 4-methylpentan-2-one and measurement. Standard solutions were prepared from finely divided powders of the metals dissolved in the acid - oil mixtures.

Application of a similar technique for the determination of lead was thought likely to be simple, rapid and quantitative, and such a method is described in this paper.

Experimental

Scope of Method

The method is satisfactory for used oils having metallic additives containing barium, calcium, zinc, phosphorus, etc. The concentration range is 0.1–2.5% *m/m*.

Outline of Method

The sample containing a uniform dispersion of finely divided inorganic lead particles is

reacted with hydrochloric acid and Aliquat 336 by shaking, to dissolve the lead, in 4-methylpentan-2-one. The mixture is centrifuged to remove any insoluble precipitate. The lead is measured in the clear supernatant liquid by atomic-absorption spectrophotometry. The analyser is calibrated with a standard solution of lead(II) chloride in a similar matrix.

Reagents

Hydrochloric acid, 35.4% *m/m*. AnalaR grade.
4-Methylpentan-2-one. AAS grade.
Aliquat 336 (*trioctylmethylammonium chloride*), 9% *m/V solution in 4-methylpentan-2-one*. Aliquat 336 is obtainable from Eastman-Kodak Ltd., Kirkby, Lancashire.
Lubricating oil. Lead-free base oil.
Standard lead solution. Dry some laboratory reagent-grade lead(II) chloride in an oven at 105 °C for 3 h and cool in a desiccator. Weigh accurately 1.3426 g into a beaker and add 90 g of Aliquat 336. Dilute to about 200 ml with 4-methylpentan-2-one and swirl to dissolve the lead chloride. Transfer quantitatively into a calibrated flask and dilute to 1 l with 4-methylpentan-2-one.

1.00 ml of solution ≡ 1.00 mg of lead.

The solution should be placed in a tightly stoppered borosilicate glass bottle and stored at room temperature. Immediately any precipitation or change in concentration of the standard is suspected, discard it. These standard solutions are stable under the above storage conditions for at least 6 months.

Apparatus

Stirrer (*homogeniser*). Turbo-agitator L60, Moritz Chemical Engineering.
Centrifuge.
Mechanical shaker.
Atomic-absorption spectrophotometer. Air - acetylene flame.
Analytical balance.
Calibrated flasks, grade B. 100 ml and 1 l.
Pipettes, grade B. 5, 10 and 15 ml.

Calibration (Full Range)

For the preparation of reference solutions, pipette into successive 100-ml calibrated flasks 0.0, 5.0, 10.0 and 15.0 ml of standard lead solution. Add 15, 10, 5 and 0 ml of Aliquat 336 solution, respectively, to the successive flasks, then add 4.0 g of base oil; mix and dilute to 100 ml with 4-methylpentan-2-one. This gives reference solutions containing 0, 5.0, 10.0 and 15.0 mg of lead.

Over-all Calibration Procedure

To check the over-all response and to establish a calibration graph (if needed), set up the analyser according to the manufacturer's general instructions. Ignite the burner and allow it to stabilise whilst aspirating 4-methylpentan-2-one for a few minutes. During this time the acetylene flow-rate should be adjusted (reduced) to minimise luminosity in the flame without causing flame lift-off from the burner head. Then aspirate the top standard and make adjustments to the nebuliser, to the burner height, burner alignment and impact bead (if fitted) to give maximum absorbance (about 0.150).

Aspirate the reference solutions in order of decreasing lead content and measure the absorbances at a wavelength of 261.4 nm, recording the values to 0.001 absorbance unit. Prepare a calibration graph by plotting net absorbance on the ordinate against mass of lead on the abscissa. The line should pass through the origin.

Routine Procedure

1. Measure the total mass of oil by using pre-weighed sample cans.
2. Warm the oil in the can to 50–60 °C and stir vigorously with the turbo-agitator until all of the sediment is homogeneously suspended in the oil.
3. Using a pipette, transfer sufficient oil in duplicate to contain about 10–15 mg of lead (Table I), into pre-weighed 100-ml calibrated mixing flasks and weigh to the nearest 0.001 g.

4. Add 0.25 ml of hydrochloric acid and 15 ml of Aliquat 336 to each flask and shake for 5 min. Add base oil to adjust the total to 4.0 g, add 4-methylpentan-2-one, mix and dilute to the calibration mark with the solvent.
5. Prepare a standard containing 15.0 ml of lead solution, 4.0 g of base oil and 0.25 ml of hydrochloric acid in a 100-ml calibrated flask, mix and dilute to the mark with the solvent.
6. Transfer portions of the samples and standard into centrifuge tubes and spin for 5 min at 3000 rev min^{-1}.
7. Set up the analyser and optimise the response with the standard solution (supernatant liquid) as specified in the calibration procedure. Re-set at zero with 4-methylpentan-2-one.
8. "Spray" the samples (supernatant liquid) using identical settings and record the absorbances.

TABLE I

RECOMMENDED SAMPLE SIZE

Expected lead content, % *m/m*	Sample size: Approx. volume/ml	Sample size: Mass/g
0.1–0.4	5	4
0.4–0.8	2.5	2
0.8–1.6	1.3	1
1.6–2.5	0.6	0.5

Calculation

$$\text{Lead content (\% } m/m) = \frac{\text{absorbance of sample} \times 1.5}{\text{absorbance of standard} \times \text{sample mass (g)}}$$

Analytical range: 0–15 mg of lead.
Absorbance: 0–0.150.
Wavelength: 261.4 nm.

Results and Discussion

Initially we applied the technique of the previous workers[9,10] using hydrochloric acid alone, hydrochloric acid - nitric acid or hydrochloric acid - perchloric acid mixtures with oils of known lead content. All of the test solutions gave poor and variable analyser responses and seemed to indicate that the lead was incompletely dissolved. The operating parameters of the analyser are given in Table II.

Other workers[11,12] had shown that it was possible to dissolve inorganic compounds such as lead(II) chloride in 4-methylpentan-2-one by using liquid anion exchangers, such as the quaternary ammonium compound Aliquat 336 (trioctylmethylammonium chloride).

Solubility tests (10 min, mechanical shaker) with an Aliquat 336 - 4-methylpentan-2-one mixture showed that lead(II) chloride and lead(II) bromide were completely soluble, lead(II) oxide and lead(II) orthophosphate were slightly soluble and lead(II) sulphate was virtually insoluble.

The experiments were repeated but with the addition of a small volume (0.5 ml) of concentrated hydrochloric acid to each test solution. The results showed that all of the lead compounds were soluble in this mixture and the analyser gave an identical response to lead irrespective of the compound present.

The optimum amounts of hydrochloric acid and Aliquat 336 were determined in further tests. In separate experiments, the volume of hydrochloric acid (35.4% *m/m*) was varied from 0 to 0.5 ml and the Aliquat 336 concentration from 3 to 24 g l^{-1}. It was found that the optimum practical amounts were 0.25 ml of hydrochloric acid (2.5 ml l^{-1} of concentrated hydrochloric acid) and 13.5 g l^{-1} of Aliquat 336. Both amounts represented about a 20-fold excess of reagent over the maximum amount of lead expected to be present in sample aliquots. Variations in the concentration of hydrochloric acid of $\pm 50\%$ and of Aliquat 336 of -25% to $+70\%$ were not deleterious.

So far the experiments had been carried out with pure chemicals but without the base oil

TABLE II

OPERATING PARAMETERS FOR ANALYSER

Instrument	Perkin-Elmer 306
Burner control box	Perkin-Elmer, Model 303–0240
Wavelength	261.4 nm
Slit width	0.7 nm
Grating	Ultraviolet
Lead lamp	Juniper 7.5 mA, or Perkin-Elmer 10 mA
Air—	
Cylinder regulator	50 p.s.i.g.
Burner control	30 p.s.i.g.
Flow-meter setting	6
Flow-rate	15 l min^{-1}
Acetylene—	
Cylinder regulator	9 p.s.i.g.
Burner control	8 p.s.i.g.
Flow-meter setting	3
Flow-rate	4.2 l min^{-1}

present. Some of these tests were repeated using the optimised reaction mixture but with the addition of amounts of lead-free base oils. A series of tests was made by varying the amount of oil from 0 to 5 g per 100 ml of solution using seven commercially available and one prototype oil. Here it was necessary to centrifuge the solutions after shaking because of precipitate formation and to analyse the clear supernatant liquids. The analyser response for lead was then constant irrespective of the amount or type of oil present.

The effect of variations in reaction time (shaking time) was studied in the range 0–14 min using authentic engine oils containing lead (previously assayed by a wet-chemical method). After reaction each test solution was observed to have thrown down a black, gelatinous precipitate, so measurements were again made on centrifuged solutions. The results showed that the lead particles had dissolved almost instantaneously during hand mixing of the reagents and before the prescribed shaking period. Even so, a shaking time of 5 min (mechanical shaker) was subsequently chosen for the test method.

A calibration graph was constructed with results obtained from test solutions containing 0–25 mg of lead ($PbCl_2$) per 100 ml of the diluted reaction - oil mixture. The compositions of the test solutions and the results are given in Table III. A typical calibration graph is shown in Fig. 1. We found that the analyser response to lead was almost linear up to 15 mg of lead, but that significant curvature was evident at the higher concentrations. For practical reasons, therefore, a linear working range of 0–15 mg of lead (0–150 mg l^{-1} of lead) was selected for the test method.

The stability and shelf-life of a standard solution of lead(II) chloride (1.0 mg ml^{-1} of lead) containing Aliquat 336 (9% *m*/*V*) was assessed when stored (tightly stoppered) at room temperature. The concentration of this solution was determined periodically by AAS against a freshly prepared standard solution on each occasion. The results showed that the

TABLE III

CALIBRATION DATA

	Flask contents					
No.	Oil/g	HCl/ml	Aliquat 336/ mg	4-Methylpentan-2-one/ ml	Lead/ mg	Relative absorbance
1	2.5	0.25	1350		0	0.003
2	2.5	0.25	1350		5.0	0.047
3	2.5	0.25	2250		5.0	0.047
4	2.5	0.25	1350		10.0	0.091
5	2.5	0.25	2250	Balance to 100	10.0	0.091
6	2.5	0.25	1350		15.0	0.130
7	2.5	0.25	2250		15.0	0.130
8	2.5	0.25	1800		20.0	0.169
9	2.5	0.25	2250		25.0	0.201

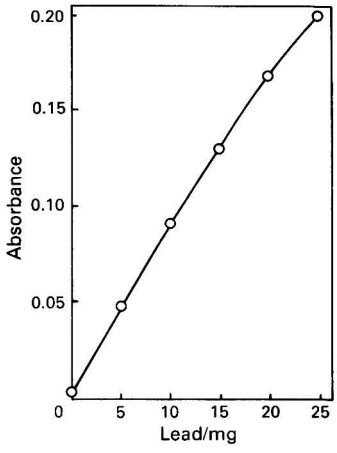

Fig. 1. Calibration graph for lead in oil determination. Perkin-Elmer 306 instrument with air - acetylene flame. Wavelength, 261.4 nm; slit width, 0.7 nm; and sample volume, 100 ml.

TABLE IV

ANALYSIS OF TYPICAL USED OILS: COMPARISON OF METHODS

Sample No.	Lead content, % *m/m*	
	Wet-chemical method	AAS method
1	0.61, 0.58	0.60, 0.60
2	0.48, 0.51	0.55, 0.55
3	0.54, 0.54	0.56, 0.56
4	0.50, 0.51	0.50, 0.51
5	0.63, 0.62	0.64, 0.64
6	0.55, 0.55	0.57, 0.57
7	0.48, 0.49	0.50, 0.49
8	0.32, 0.30	0.36, 0.37
9	0.31, 0.31	0.38, 0.38
10	0.48, 0.50	0.54, 0.55
11	0.40, 0.39	0.53, 0.52
12	0.61, 0.59	0.71, 0.71
13	0.73, 0.77	0.86, 0.88
14	0.74, 0.70	0.81, 0.81
15	0.74, 0.70	0.70, 0.69
16	0.77, 0.83	0.82, 0.81
17	0.44, 0.42	0.44, 0.43
18	0.60, 0.62	0.60, 0.60
19	0.54, 0.55	0.64, 0.62
20	0.49, 0.48	0.54, 0.52
21	0.32, 0.38	0.46, 0.46
22	1.50, 1.46	1.49, 1.48
23	0.46, 0.45	0.41, 0.41
24	0.50, 0.49	0.45, 0.45
25	0.49, 0.49	0.55, 0.54
26	1.03, 0.97	1.04, 1.10
27	0.25, 0.26	0.26, 0.30
28	0.95, 0.85	0.94, 0.95
29	0.78,* 1.06*	0.98,* 0.98*
30	0.24, 0.24	0.26, 0.26
F calculated	—	1.05
F tabulated	—	1.84
t calculated	—	4.00
t tabulated	—	2.05
Repeatability, %	0.07	0.04

* Sample 29 not used in statistical analysis.

solution was stable under these conditions, with no change in concentration, for at least 6 months.

Because of the nature of the oil samples it was not possible to provide synthetic test samples with absolute concentration figures. Checks on the accuracy and precision of the AAS method were made, therefore, by comparing the results with those obtained by the original wet-chemical method. The AAS procedure used is described in detail under Experimental, and both methods were applied to the analysis of 30 typical used oils. The results obtained are given in Table IV. The data were evaluated statistically and the *F*-test variance ratio and Student's *t*-test were calculated.[13,14] The results showed that on average the AAS method had a positive bias, giving, therefore, slightly higher results compared with those obtained by the original procedure. The lower results with the original method were, however, thought likely to be due to losses of lead during the ashing stage, as mentioned earlier. An over-all estimate of the repeatability[15] of the AAS method gave a result of 0.04% *m/m*.

The particle size range of lead compounds suspended in used oils and the possible effect on the determination of lead by the "wear-metal method"[1,2] was not known. Final tests were made on five used oils, which were analysed by the wear-metal and the proposed AAS procedure. The results are given in Table V and showed that the former method gave lower results than those obtained with the proposed procedure, and on standing they were even lower. This was thought to show that the wear-metal method, as applied here to the determination of lead, was dependent on particle size, whereas the AAS procedure was quantitative and, as expected, was independent of the particle size of suspended lead.

TABLE V

PARTICLE SIZE EFFECTS

	Lead content, % *m/m*		
	Wear-metal method		
Sample	Immediate measurement	Standing time 1 h	AAS method (immediate measurement)
1	0.96	0.55	1.10
2	0.39	0.18	0.43
3	0.63	0.46	0.86
4	0.76	0.68	0.87
5	0.21	0.14	0.22

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Study of an Automatically Triggered Digital Integrator for Atomic Spectrometry of 15 Elements Using Discrete Nebulisation

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A signal integrator, with an automatic trigger and a voltage to frequency counter, was used in the atomic-absorption spectrometry of silver, calcium, cadmium, cobalt, copper, iron, magnesium, manganese, nickel, lead, strontium and zinc and the atomic-emission spectrometry of sodium and potassium with an air - acetylene flame and the atomic-absorption spectrometry of aluminium, calcium and magnesium with a dinitrogen oxide - acetylene flame using a discrete nebulisation technique. The effects of injection volumes, sample flow-rates and metal concentrations on the variables (peak height, integrated value, aspiration time and the ratio of the integrated value to the aspiration time) were investigated. Each of the integrated values for silver, copper, iron, magnesium, manganese and lead is proportional to the absolute amount of each metal irrespective of the metal concentration and injection volume. The calibration graphs for these six elements were straight lines passing through the origin. The relative standard deviations of the measurements were less than 4%, even with an injection volume of 20 μl, and less than 1% with an injection of 100 μl.

Keywords: *Automatic integration*; *atomic-absorption and -emission spectrometry*; *discrete nebulisation*; *absolute amount method*

Flame atomic-absorption spectrometry has been studied in detail by use of discrete nebulisation of a small volume of sample[1] and applied successfully to the analyses of some elements in various samples.[1,2] The sensitivity obtained by the measurement of peak height was, however, too low to enable the determination of the concentration of trace metals.

A digital integrator, automatically triggered by the electrical conductance of the nebulised sample solution, was recently proved to be applicable to the flame atomic-absorption spectrometry of copper by a discrete nebulisation technique.[3] The copper contents in some NBS standard biological samples were determined accurately.[4]

This paper deals with the fundamental study of the application of the automatically triggered digital integrator to flame atomic-absorption and flame atomic-emission spectrometry by the discrete nebulisation of solutions of 15 elements with an air - acetylene and/or a dinitrogen oxide - acetylene flame. The effects of injection volume and sample flow-rate on the variables (peak height, integrated value, aspiration time and the ratio of the integrated value to the aspiration time) were investigated.

Experimental

Apparatus

All of the apparatus and equipment, except for the home-made burner head with a slit 6 cm long and 0.5 mm wide for the dinitrogen oxide - acetylene flame, were the same as those reported in a previous paper.[4] A small PTFE funnel with a platinum electrode is coupled directly to the nebuliser needle (platinum pipe), which is the counter electrode. When the channel between the platinum electrodes is filled with the injected sample solution, the electrical conductivity between both the electrodes acts as an automatic trigger and the signal is integrated for a pre-set time. Details of the injection system of sample solution and the related materials are also given in references 3 and 4.

Operating Conditions

The absorbances of aluminium, calcium and magnesium were measured under the same operating conditions with a dinitrogen oxide - acetylene flame: the flow-rate of the acetylene

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was 5.5 l min^{-1} (0.5 kg cm^{-2}); the flow-rate of the dinitrogen oxide was 4.4 l min^{-1} (1.5 kg cm^{-2}) for the nebuliser and 2.0 l min^{-1} (1.5 kg cm^{-2}) auxiliary; burner height, position 2.5; and sample flow-rate, 5.4 ml min^{-1}.

The wavelengths were: silver, 328.1; aluminium, 309.3; calcium, 422.7; cadmium, 228.8; cobalt, 240.7; copper, 324.8; iron, 248.3; potassium, 766.5; magnesium, 285.2; manganese, 279.5; sodium, 589.0; nickel, 232.0; lead, 283.3; strontium, 460.7; and zinc, 213.9 nm.

Contrary to a previous paper,[1] in this study the response for emission is faster than that for absorption, *i.e.*, the condenser capacitance of the emission was smaller than that of the absorption. The integration time (5 s) and the other operating conditions are the same as those given in the previous paper.[4]

Reagents

The stock solutions of each element (2000 p.p.m. in 0.5 M nitric or hydrochloric acid) were prepared by dissolving analytical-reagent grade compounds in nitric or hydrochloric acid and diluting with nitric or hydrochloric acid and doubly distilled water to give a mass of 100 g.

The working standard solutions of each element were prepared by diluting the stock solutions to appropriate concentrations by mass in 10-ml polypropylene bottles with doubly distilled water and nitric acid using micropipettes. All these solutions contain 0.1 M nitric acid.

Results and Discussion

Effect of Injection Volume on Peak Height of Signal

As with copper,[1] the peak height for the atomic-absorption signal increases with an increase in the injection volume, up to a volume of about 100 μl, under a given sample flow-rate and then it remains constant, the height being the same as that obtained by the continuous nebulisation. The results are exemplified in Fig. 1, together with those of the other variables. All the data are summarised in Table I. The minimum injection volume giving a constant peak height and the peak height itself increases with an increase in the sample flow-rate. The minimum volume is about 50 μl for the emission (potassium and sodium in Table I), as the response of the electrical circuitry is faster than that for absorption.

Effect of Injection Volume on the Integrated Value

The area under the spike-like signal was integrated satisfactorily by the combined use of the automatic trigger and digital counter. The procedure has been described previously.[3]

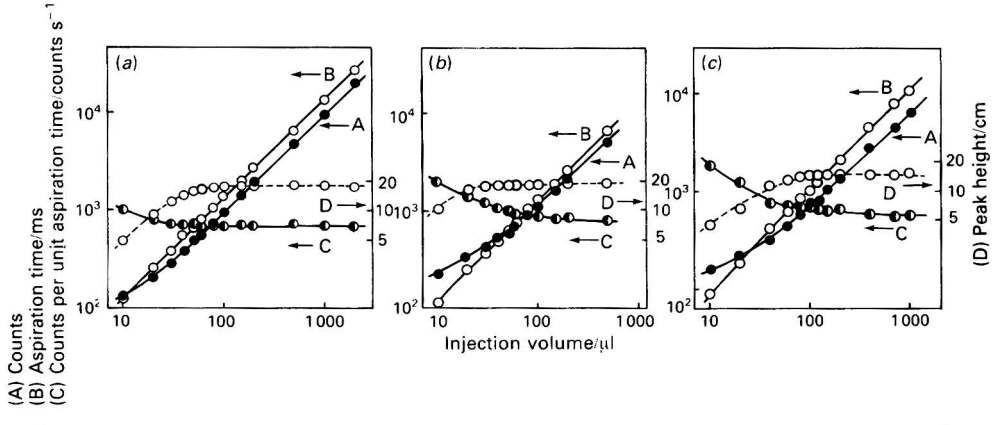

Fig. 1. Effect of injection volume on four variables. Sample flow-rate, 4.5 ml min^{-1}. Concentration: (*a*) manganese, 2.3 μg ml^{-1}; (*b*) sodium, 5 μg ml^{-1}; and (*c*) magnesium, 0.6 μg ml^{-1}. (*a*) and (*b*), air - acetylene flame; and (*c*), dinitrogen oxide - acetylene flame.

TABLE I

RELATIONSHIP BETWEEN INJECTION VOLUME AND FOUR VARIABLES

Element	Calibration graph	Injection volume/μl*				Extrapolation	Calibration range, p.p.m.
		Aspiration time	Count	Count/ aspiration time	Peak height		
Ag	Linear	30	30	40	100	A†	0–0.5
Ca	Curve	30	NL‡	NL‡	100	NL‡	0–1.2
Cd	Curve	30	50	50	100	NL‡	0–1.0
Co	Curve	30	60	60	100	NL‡	0–2.0
Cu	Linear	30	40	40	100	A†	0–0.5
Fe	Linear	30	40	40	100	A†	0–2.0
Mg	Linear	30	40	40	80	A†	0–0.03
Mn	Linear	30	40	50	100	A†	0–0.4
Ni	Curve	30	60	60	100	NL‡	0–2.0
Pb	Linear	30	50	50	100	A†	0–4.0
Sr	Curve	30	150	150	80	NL‡	0–0.5
Zn	Curve	30	40	40	80	NL‡	0–0.8
K	Curve	30	NL‡	NL‡	50	NL‡	0–5§
Na	Linear	30	NL‡	NL‡	50	NL‡	0–4¶
Al	Curve	30	NL‡	NL‡	100	NL‡	0–180‖
Ca	Curve	30	NL‡	NL‡	120	NL‡	0–1.0‖
Mg	Linear	30	NL‡	NL‡	120	NL‡	0.0.3‖

* At larger than the figures tabulated (in microlitres) the relationship between injection volume and variables is linear.
† A, The absolute amount method is possible.
‡ NL, Relationship between injection volume and variables is not linear.
§ Burner parallel.
¶ Burner at right angles.
‖ Dinitrogen oxide - acetylene flame.

With a constant concentration, the integrated values (counts) for silver, cadmium, cobalt, copper, iron, magnesium, manganese, nickel, lead and zinc increase linearly with an increase in the injection volume above 50 μl. Fig. 1(*a*) shows the result for manganese as a typical example. This linearity begins only at a volume of larger than 150 μl for strontium (column 4, Table I). Non-linear increment is observed for calcium, potassium and sodium with an air - acetylene flame and for aluminium, calcium and magnesium with a dinitrogen oxide - acetylene flame. Figs. 1(*b*) and 1(*c*) show the results for sodium and magnesium. A linear relationship is observed between the integrated value and the concentration of silver, copper, iron, magnesium, manganese and lead with a constant injection volume (columns 2 and 4, Table I). A non-linear relationship is observed for the other elements (Table I and Fig. 2). These phenomena are the same as those observed in the continuous nebulisation method so far. It is clear from Fig. 2 that the slopes of the calibration graphs increase with an increase in the injection volume. Thus the integrated values for silver, copper, iron, magnesium, manganese and lead seem to be proportional to the absolute amount of the analyte.

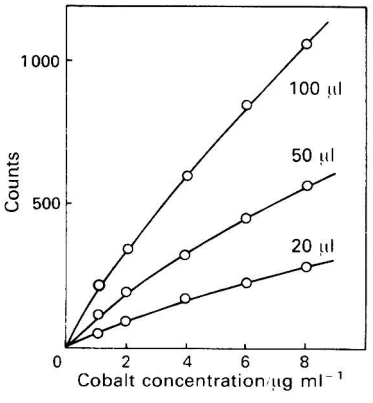

Fig. 2. Calibration graphs for cobalt for different injection volumes. Air - acetylene flame.

Effect of Injection Volume on Aspiration Time

The aspiration time increases linearly with an increase in the injection volume of more than 30 μl under a given sample flow-rate (Table I), although the precise feature is not very clear in Fig. 1. The aspiration time is defined as the time required for the injected sample solution to pass through the channel between both electrodes. The aspiration time depends mainly upon the viscosity of the sample solution.

Effect of Injection Volume on the Ratio of the Integrated Value to the Aspiration Time

The ratio of the integrated value to the aspiration time is constant irrespective of the injection volume when it is more than about 40 μl for silver, copper, iron, magnesium and zinc, more than about 50 μl for cadmium, manganese and lead, more than about 60 μl for cobalt and nickel and more than about 150 μl for strontium (column 5, Table I). However, such a relationship does not hold for calcium, potassium and sodium with an air - acetylene flame and for aluminium, calcium and magnesium with a dinitrogen oxide - acetylene flame because of the non-linearity between the integrated value and the injection volume. Typical examples are given in Fig. 1 (*b*) and (*c*). This ratio is dependent only upon the concentration of analyte in the sample solution, as in the peak-height method. Thus, the calibration graph of this ratio against the concentration of analyte is either linear or non-linear, depending upon the elements. Table I indicates that a straight line calibration graph passing through the origin of the coordinates is obtained with an appropriate injection volume of more than about 50 μl for silver, cadmium, copper, iron, magnesium, manganese, lead and zinc, more than about 60 μl for cobalt and nickel, and more than about 150 μl for strontium. A typical example is shown in Fig. 3. A simple calibration graph was not obtained, even by use of a large sample volume, for the other elements (aluminium, calcium, potassium and sodium), because of the non-linearlity between the integrated value and the injection volume. Sensitive calibration graphs passing through the origin are also obtained with given injection volumes of less than the volumes mentioned above, irrespective of a linear or non-linear relationship between the integrated value and the aspiration time and the injection volume.

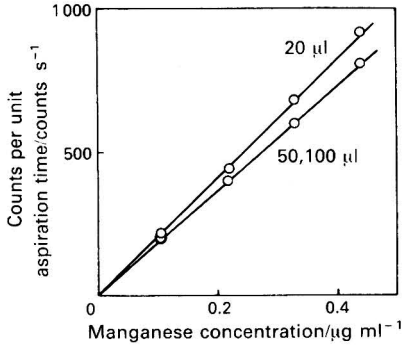

Fig. 3. Calibration graphs for manganese for different injection volumes. Air - acetylene flame.

Reproducibility

The over-all averages of the relative standard deviations of the variables obtained with 20-, 50- and 100-μl sample solutions of an appropriate concentration of the 15 elements are given in Table II. The relative standard deviation of each variable obtained with 50- and 100-μl sample solutions is very small and superior to that obtained by the continuous nebulisation method. The relative standard deviation of each variable is rather small even with an injection volume of 20 μl. Thus, the reproducibility of this method is satisfactory. The use of an integrated value or the ratio of the integrated value to the aspiration time instead of using the peak height is especially useful for the determination of the given elements. This is discussed further below.

TABLE II

REPRODUCIBILITY (%) OF FOUR VARIABLES ($n = 10$)

Variable	Injection volume/μl		
	20	50	100
Aspiration time	1–3	1–2	~1
Peak height	2–3	1–2	0.5–1
Count	~2	1–2	0.5–1.5
Count/aspiration time	2–5	1–1.5	0.5–1

Effect of the Sample Flow-rate on Variables

The effect of sample flow-rate was investigated with an injection volume of 100 μl, except for strontium (150 μl) and sodium (50 μl), under the constant flame conditions. A typical example is shown in Fig. 4. The aspiration time decreases with increase in the sample flow-rate. The integrated values obtained with an air - acetylene flame are the highest and essentially constant at the given range of sample flow-rates, between 4.4 and 5.5 ml min^{-1} for all the elements except calcium, strontium and cadmium and between 3.6 and 4.4 ml min^{-1} for cadmium. Such a result is expected because the same absolute amount of analyte is nebulised. Thus, the ratio of the integrated value to the aspiration time increases with the sample flow-rate. However, the integrated value decreases with increase in the sample flow-rate for calcium and strontium although the reason remains unsolved.

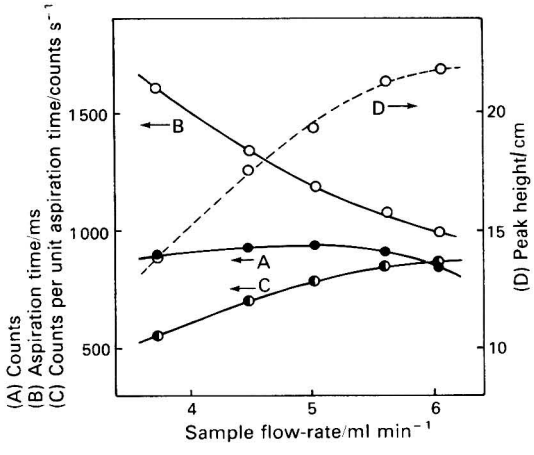

Fig. 4. Effect of sample flow-rate on four variables. Concentration of manganese, 2.06 μg ml^{-1}. Injection volume, 100 μl. Air - acetylene flame.

Application of the Absolute Amount Method

As linear relationships are obtained between the integrated value and the concentration of the analyte at a constant injection volume, and also between the integrated value and the injection volume at the constant concentration, the integrated value is proportional to the product of the concentration of the analyte and the injection volume, *i.e.*, the absolute amount of analyte in the sample solution. Thus, the absolute amount method is applicable, as it is with copper.[4] The results for the six elements obtained with an air - acetylene flame and for the two elements with a dinitrogen oxide - acetylene flame are given in Table III. The concentrations of copper, iron and lead, which are too low to be determined on the straight line calibration graphs in the normal concentration ranges, can be determined on these calibration graphs by the injection of a large volume of the sample solution. The results obtained by this absolute amount method are summarised in Table IV for nine elements. The results for copper, iron, magnesium, manganese and lead with different

TABLE III

COUNTS AT CONSTANT ABSOLUTE AMOUNTS OF METAL

Injection volume/μl	Cu (156 ng)*	Fe (200 ng)*	Pb (300 ng)*	Ni (300 ng)*	Ca (200 ng)*	Na (200 ng)*	Mg (50 ng)†	Ca (270 ng)†
2000	678	530	473	885	731	582	262	402
1000	672	523	470	852	731	587	284	441
600	672	—	468	822	—	—	287	473
300	675	—	473	774	—	—	300	—
200	678	530	470	725	682	613	312	490
150	676	—	466	703	—	—	—	—
100	675	526	467	671	632	636	332	516
75	669	—	482	683	—	—	333	525
60	665	—	456	677	—	—	349	540
50	659	514	462	671	629	671	344	538
Mean	671.9	524.6	468.7	746.3	681.0	617.8	311.4	490.6
Standard deviation	6.082	6.618	6.945	81.23	50.26	36.79	30.17	49.48
Relative standard deviation, %	0.90	1.26	1.48	10.9	7.38	5.96	9.69	10.1

* Air - acetylene flame.
† Dinitrogen oxide - acetylene flame.

injection volumes are in good agreement with results from standard solutions containing known amounts of the metal ions. However, Tables I, III and IV indicate that calcium, cadmium, cobalt, nickel, strontium, zinc, potassium and sodium with an air - acetylene flame and aluminium, calcium and magnesium with a dinitrogen oxide - acetylene flame cannot be determined by the absolute amount method. Thus, this method can be applied to the determination of very low concentrations of silver, copper, iron, magnesium, manganese and lead in various samples by use of a large volume for injection. Application of this method to standard rocks, glasses and serum will be described elsewhere.

TABLE IV

DETERMINATION OF LOWER LEVELS OF METALS WITH LARGE SAMPLE VOLUMES

Element	Concentration, p.p.m.	Injection volume/μl	Measured, p.p.m.	Standard solutions, p.p.m.
Cu	0.0155	2000	0.0157	0–0.8
		1000	0.0149	
Fe	0.053	2000	0.050	0–4.0
		1000	0.051	
	0.103	2000	0.102	
		1000	0.101	
Mg	0.021	1000	0.022	0–0.25
Mn	0.053	2000	0.056	0–2.0
		1000	0.054	
Pb	0.052	2000	0.053	0–6
	0.155	1000	0.151	
Ca	0.105	2000	0.113	0–4
		1000	0.107	
Cd	0.213	1000	0.30	0–2.5
		500	0.25	
Ni	0.159	2000	0.202	0–6
		1000	0.188	
Co	0.225	1000	0.36	0–8

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Low-temperature Oxygen - Fluorine Radiofrequency Ashing of Biological Materials in Poly(tetrafluoroethylene) Dishes Prior to the Determination of Tin, Iron, Lead and Chromium by Atomic-absorption Spectroscopy

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Low-temperature radiofrequency ashing utilising plasma-excited oxygen alone requires long ashing times and for this reason has not been generally accepted. In this paper it is shown how ashing time can be reduced considerably by introducing fluorine into the oxygen plasma and a novel and efficient method of achieving this is described. Food products and National Bureau of Standards Standard Reference Materials, both of known trace metal content, are prepared by the method described. Analysis of the ash by atomic-absorption spectroscopy shows good recovery of added tin and agreement with certified values for iron, lead and chromium.

Keywords: Low-temperature plasma ashing; oxygen - fluorine; PTFE; tin, iron, chromium and lead determination; atomic-absorption spectroscopy

When oxygen is passed into a vacuum chamber held at less than 1.0 mmHg by a two-stage vacuum pump and subjected to a radiofrequency field (RF) oscillating at 13.56 MHz, an excited species of oxygen is formed consisting of atoms, ions and electrons with limited lifetimes of the order of 1 s or less.[1] These vibrational and atomic states of oxygen were investigated by Gleit and Holland[2] who found that oxidation of organic matter was possible at temperatures of less than 200 °C within the influence of an oxygen plasma. The technique is often referred to as low-temperature ashing (LTA).

These plasma chemistry oxidation reactions have been utilised in the preparation of food samples prior to the analysis of the ash for tin, iron, lead and chromium by atomic-absorption spectroscopy.

The primary objective of these studies was to develop a method to replace existing methods of sample preparation using wet or dry high-temperature oxidations,[3] with more refined procedures capable of retaining the very low levels (less than 0.1 μg ml^{-1}) of some metals found in some foods. The procedure is intended to support and become a potential reference method for more rapid systems of analysis previously published.[4]

These alternative technologies are, for reasons of economy, safety and energy conservation, worthy of much closer examination. They have not been widely accepted mainly because of difficulties in completely oxidising, within acceptable time limits, some organic materials when oxygen is used alone. Some manufacturers of plasma-ashing equipment recognised these limitations and added agitators or heaters to assist oxidation, but with only limited success. Few manufacturers recognised that the route to successful and efficient ashing for some metals was by means of a plasma chemistry reaction using atomic fluorine. Investigations at these laboratories were prompted by the fact that ashing was always significantly more efficient and more rapid when fluorine in the form of hydrofluoric acid (2% m/V) was added to the food product before drying. The same effect was found if poly(tetrafluoroethylene) was placed in an adjacent aluminium or silica crucible, during the ashing procedure. After 10–15 min in the oxygen plasma, a greenish glow indicating emission due to the presence of fluorine became evident. In the absence of fluorine, the plasma discharge was pink for oxygen alone or blue owing to carbon dioxide being formed as a by-product of sample oxidation.

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These techniques were acceptable for some elements, but for others, unacceptably high blank values arose. When ashing was carried out in silica crucibles, it was found that volatile silicon tetrafluoride (SiF_4) was formed. Impurities such as lead and iron were left on the surface and these were dissolved during acid or alkali dissolution of the ash. These contaminated the sample and gave grossly inflated values for lead and iron. The silica crucibles lost weight during exposure to the oxygen - fluorine plasma and the inside walls of the silicon - quartz reactor chamber became slightly etched. Some manufacturers use aluminium reactor chambers and one of the reasons for selecting aluminium is given below.

When aluminium dishes were used for food samples containing chromium, it was found that a protective film of aluminium oxyfluoride, as measured by X-ray photoelectron spectroscopy, was formed on the surface, but during subsequent acid dissolution of the ash, chromium(VI) reacted with the aluminium surface to form a passive film containing chromium. These preliminary experiments were instrumental in directing studies towards using poly(tetrafluoroethylene) (PTFE) crucibles or dishes. The method described is based on the accelerating effect of atomic fluorine on oxidation by atomic oxygen. The novelty lies in the use of PTFE as both the source of fluorine and the sample container.

Oxygen - fluorine plasma chemistry is not new; tetrafluoromethane (CF_4, Freon 14) is recommended by the Branson - International Plasma Corporation[5] and they justifiably claim that CF_4 will also accelerate the rate of oxidation. More recently Carter and Yeoman[6] have given clear indications of the advantages of CF_4 plasma chemistry reactions for the determination of cadmium in blood. In the procedure to be described, there are many advantages in using PTFE dishes as the source of fluorine and there are equally good reasons why the technique is to be preferred to conventional classical wet or dry oxidations for some elements.

Plasma Chemistry Reactions

A plasma is often referred to as the fourth state of matter, but it will only exist so long as energy is sustained. A chemical reaction inside a plasma reactor will immediately stop when power is switched off; there is no thermal lag.

Under the conditions described and inside the plasma reactor, two important reactions occur: firstly, atomic oxygen (O*) and other vibrational states of oxygen are formed by capacitative or inductive coupling of radiofrequency energy to oxygen; and secondly, atomic oxygen attacks the structure of PTFE[7] to produce fragmented CF_2, which further reacts with atomic oxygen to produce atomic fluorine (F*) as follows:

$$2O^* + CF_4 \rightarrow C + 4F \rightarrow :CF_2 + 2F \rightarrow CO_2 + 4F^*$$

The accelerated oxidation rate can be attributed to the smaller diameter of the fluorine atom and hence its greater penetrating power. Also, the closer proximity of the source of atomic fluorine, which has a short lifetime, to the material to be ashed enables high concentrations of atomic fluorine to accumulate exactly where they are required.

The factors which affect the oxidation rate in an oxygen - fluorine plasma chemistry reactor are as follows. (*i*) Exposed surface area: the larger the surface area and the smaller the particle size, the faster is the ashing rate. (*ii*) Sample load: ashing time is generally proportional to sample mass. (*iii*) Temperature: high temperatures increase oxidation rate but may promote volatilisation of certain elements. High radiofrequency power input increases temperature but only an optimum radiofrequency power level or perfectly tuned matching of parameters will produce the maximum useful population of activated atomic oxygen - fluorine species for the task. (*iv*) Chemical structure and physical nature: silicone rubber is considerably more resistant to attack than PTFE because of its structure and silicone content. It is for this reason that equipment manufacturers use silicone rubber as seals and gasket materials on radiofrequency reactors. (*v*) Concentration of activated species: input power to the discharge and oxygen pressure control the concentration of activated species. The oxygen pressure controls the amount of power that can be accepted in the efficient production of activated states. (*vi*) Decomposition products: CO, CO_2 produced during oxidation retard the oxidation rate, but these diminish because they are exhausted from the system by the vacuum pump.

Most of the factors given above are to a large extent interdependent. It is possible that other gases which dissociate and ionise in the plasma will also influence the rate of oxidation.

The advantages of using PTFE compared with CF_4 are as follows. (*i*) Poly(tetrafluoroethylene), following exposure to atomic oxygen, develops unique hydrophobic properties enabling the analyst to manipulate the dissolved ash in ways not possible by other means, such as quantitative transfer of the dissolved ash without washing because the PTFE surface is not wetted. (*ii*) The crucible or dish is not affected by most acids and may be heated to 250 °C. (*iii*) The material is self cleaning during reactor exposure and the loss in weight is small enough to allow many hundreds of determinations to be performed before the dish/crucible becomes unserviceable. (*iv*) Tetrafluoromethane (CF_4) is expensive and does not perform a dual function. (*v*) The impurity levels found in PTFE are very low and for most applications beyond the detection limit of the method of analysis used.

It seems therefore, that even if CF_4 is used, a resistive material in which to carry out the ashing procedure would be required and this material should be of high purity and immune from reaction with the metals present during dissolution.

The advantages of plasma ashing over wet or dry oxidation procedures are as follows: (*i*) the economic aspects are today of major importance in terms of materials, chemicals, energy and applied man hours; (*ii*) automatically tuned reactors require little attention and fitted with suitable fail-safe devices can be operated overnight; (*iii*) the technique is cleaner (lower blanks, less contamination) and less manipulation is required; (*iv*) most of the hazards associated with conventional procedures do not arise; (*v*) there is no reaction between the container and metals in solution; and (*vi*) it is possible to monitor the progress of a plasma chemistry reaction by measuring emission intensities indicating the presence of a particular element, *e.g.*, a nitrogen emission line at 674 nm is used in silicon chip manufacture to indicate when silicon nitride has been removed in a CF_4 plasma, and photodiodes are used to measure the intensity of the emission line.[7]

The disadvantages of using the procedure are relatively few: (*i*) it is not acceptable over as wide a range of elements (unless low-temperature traps are used) because of the formation of volatile fluorides of, for example, boron, phosphorus, sulphur, silicon, titanium, uranium and tungsten; (*ii*) the capital cost is high; (*iii*) there is a need to dry samples before treatment; (*iv*) static electricity effects necessitate precautionary delays before retrieval of the sample from the reactor chamber; and (*v*) most oxygen supply lines already installed are metal and there may be a need to replace these with nylon to avoid contamination by metal oxides.

Experimental

Apparatus

Reactors. Instruments were supplied by each of the following manufacturers: Tracerlab Ltd., Division of Electrónics, Richmond, CA, USA, Model LTA 600 (UK Agents, Laboratory Impex Ltd., Lion Road, Twickenham); Branson - International Plasma Corporation (IPC), Hayward, CA, USA, Model IPC 4000/104B (UK Agents, Teledyne-Tac, Bath Road, Cranford, Middlesex); and Nonotech (Thin Films) Ltd., Sedgley Park Trading Estate, Prestwich, Manchester, Model P100.

When in use each reactor must be equipped with a silicon or aluminium reactor chamber, silicone rubber gaskets and tube connectors and a vacuum system using a halocarbon oil.

As a safety precaution, all units should be fitted with a trap filled with disodium tetraborate crystals to neutralise fluorine emissions and should be vented to the external atmosphere. The manufacturers will supply data on radiofrequency shielding and the current leakage levels permitted.

Homogeniser. A Kenwood Chef using a liquidiser attachment was used to prepare all foods to a slurry. The original liquidiser blades were replaced by titanium blades made in the laboratory.

Electronic balance. A Mettler, Model P1200, electronic balance was used for all weighing operations and was also used to measure (by mass) precise dilution volumes using the tare facility on the balance. The mass of ash remaining after oxidation was sufficiently small (0.02–0.06 g) to enable solutions to be prepared by simply adding fixed volumes of acid within the range 5–25 ml, without correction.

Atomic-absorption spectrometer. A Varian-Techtron atomic-absorption spectrometer, Model A.A.5, was used for flame analysis (tin and iron) and a carbon rod attachment (CRA), Model 63, was used for electrothermal atomisation (lead and chromium).

Poly(tetrafluoroethylene) crucibles. These were machined from 15 mm thick PTFE sheet ("Fluon," Imperial Chemical Industries, Plastics Division, Welwyn Garden City). The usual precautions were observed in machining this material.[8] The external dimensions were as follows: diameter 60 mm; depth 10 mm; and wall 3 mm. After cleaning in 1 + 1 nitric acid, they were placed in an oxygen plasma to etch and clean the sufrace for 15 min at 100 W. Non-volatile impurities, which accumulated as a result of machining on the surface, were dissolved away by immersing the entire surface in a solution of hydrochloric acid containing hydrogen peroxide. Under the test conditions in a Branson - International Plasma Chemistry Reactor, mass losses of 0.06 g h^{-1} at 100 W were typical, but depended on the type of reactor and to some extent on the position of the PTFE crucible inside the reactor.

Reagents

All reagents were of Aristar grade (BDH Chemicals Ltd., Poole, Dorset).

Hydrochloric acid - hydrogen peroxide. A 40-ml aliquot of hydrochloric acid (sp. gr. 1.17) was diluted with water and 2 ml of hydrogen peroxide (30% *m/V*) were added, giving a total volume of 100 ml.

Nitric acid. Nitric acid (40 ml) was diluted with water to 100 ml.

Standard solutions. Multi-element solutions of chromium, iron and tin were prepared from single element stock solutions (1000 μg ml^{-1}) by appropriate dilution in 20% *m/V* hydrochloric acid. Lead solutions were prepared in 20% *m/V* nitric acid from a separate stock solution (1000 μg ml^{-1}). High-purity salts or metals were used to prepare all stock solutions.

Procedure

Weigh 1.0–5.0 $\pm$ 0.02 g of a representative homogenised sample in a PTFE crucible of known mass using an electronic balance. Dry the sample for 2 h at 120 °C.

For the spiked samples, add the appropriate amount of standard solution to the PTFE crucibles and dry at 100 °C in an oven to remove any excess of acid. When dry, add the sample and dry at 120 °C for 2 h.

Use the reactor conditions recommended by the manufacturer for the reactor model being used, *e.g.*, for an IPC, Model 4000–104B: oxygen pressure 14–34 kN m^{-2}; oxygen flow-rate 300 ml min^{-1}; vacuum 0.5 mmHg; and radiofrequency power 100 W.

Treat single 5.0-g samples for 4–8 h and increase the time proportionately for up to 3 $\times$ 5-g samples.

Test the treated samples with 1–2 drops of distilled water to establish complete ashing, which will be evident by the absence of any black particles. If ashing is incomplete return to the reactor for further treatment. To avoid ash loss by static electricity, allow the charge to dissipate with the radiofrequency power switched off before sample withdrawal from the reactor.

Dissolve the sample ash in an acid or alkali suitable for the analytical technique to be used.

Tin and iron can be determined by atomic-absorption spectroscopy because they are usually present in sufficient amounts. A dinitrogen oxide - acetylene flame is used for tin and an air - acetylene flame for iron. The solvent used is hydrochloric acid - hydrogen peroxide at the concentration recommended under Reagents. The same solvent is used for chromium determinations, but the instrumental method relies on carbon rod analyses. The determination of lead requires nitric acid and carbon rod analysis but it is possible to use a flame technique where the concentration is sufficiently high.

Results and Discussion

The significant acceleration of oxidation due to the presence of fluorine is shown in Fig. 1 in which 5.0-g samples of boysenberries were dried at 120 °C to the same initial dry mass before low-temperature ashing treatment. The same effect was shown by a number of other

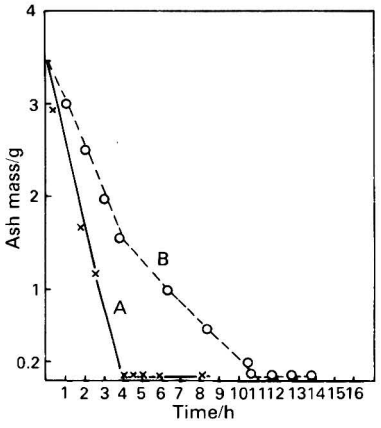

Fig. 1. Comparative ashing rates for 5.0 g of boysenberries in syrup using radiofrequency-excited oxygen with aluminium crucibles (B) and oxygen - fluorine with PTFE crucibles (A). Samples dried for 1 h at 120 °C to the same dry mass of 3.5 g. Values plotted have been corrected for crucible weight loss and each plotted point is a separate sample. Radiofrequency power, 100 W; and oxygen flow-rate, 300 ml min^{-1}.

products but for the sake of clarity results have not been included. It should also be mentioned that the mechanism by which fluorine increases the oxidation rate is dominated by an induction period (not shown). The period is probably controlled by the time it takes the atomic oxygen to attack and fragment the PTFE to form activated fluorine.

Tin in the ash residue is apparently present as SnO (grey ash residue) and is acid soluble. It may also be present as a soluble fluoride, SnF_2 or SnF_4. If SnO_2 is present, which is intractable, then it will be necessary to resort to lithium metaborate fusion in platinum at 900 °C and dissolution of the cooled melt in 20% *m/V* hydrochloric acid. In practice this has not arisen and it is considered that if SnO_2 is present it is highly dispersed and would dissociate in a dinitrogen oxide - acetylene flame.

Tables I and II show results obtained using the procedure described with PTFE crucibles.

The results agree well with the certified values (Standard Reference Materials, National Bureau of Standards, USA), and good recoveries of added tin are also obtained.

For lead and chromium determinations, sample mass to dilution volume ratios were

Table I

Determination of tin, lead, iron and chromium in National Bureau of Standards Reference Materials (SRMs)

The crucibles were PTFE, the sample mass was 1.0 g and all results are in micrograms per millilitre.

	Tin		Iron		Lead		Chromium	
Material	Found	Certified	Found	Certified	Found	Certified	Found	Certified
SRM 1575, pine needles + 20 μg of tin	21.5, 20.3	? (+20)	203, 215	200 ± 10	10.8	10.8 ± 0.5	2.6	2.6 ± 0.2
SRM 1571, orchard leaves + 20 μg of tin	20	0.34–4.1 (+20)	284	300 ± 20	ND*	45 ± 3	ND*	2.48 ± 0.34
SRM 1577, bovine liver + 200 μg of tin	200	0.18 (+200)	280	268 ± 8	0.37	0.34 ± 0.08	ND*	0.088 ± 0.012

* ND = not determined.

TABLE II

COMPARATIVE DETERMINATIONS OF TIN, LEAD AND CHROMIUM IN CANNED BOYSENBERRIES (LABORATORY PREPARED SAMPLES)

The crucibles were PTFE, the sample mass was 5.0 g and all results are in micrograms per millilitre. Values in parentheses are those obtained by a procedure published previously.[4]

Reference No.	Tin	Iron	Lead	Chromium
21/1	19 (19)	121 (128.8)	0.25 (0.19)	0.034 (0.035)
21/1	19 (19)	129 (128.8)	—	0.034 (0.035)
22/5	2.0 (<5)	31 (27)	1.2 (1.19)	0.038 —
22/5	2.0 (<5)	31 (27)	1.2 (1.19)	0.040 —

deliberately reduced so that a flame atomic-absorption result could be obtained. A gain in sensitivity thus obtained, however, resulted in a small volume of solution sufficient for the determination of only two elements. There are a number of atomic-absorption techniques that will overcome this disadvantage, *e.g.*, a micro-sampling accessory.

In Table II, some results on boysenberries are given; these show good agreement with values obtained by a previously published procedure, which uses pressure decomposition in polystyrene containers.

In Tables III and IV, results obtained in both aluminium and vitreosil (silica) dishes are given. Chromium values in Table III are excluded for the reasons already explained.

TABLE III

DETERMINATION OF TIN, IRON AND LEAD IN CANNED FOODS

Aluminium dishes were used and the sample mass was 5.0 g.

Reference No.	Material	Mass of ash/g	Volume for analysis/ml	Tin found,* p.p.m.	Iron found,* p.p.m.	Lead found,* p.p.m.
22	Boysenberries	0.01	25	5 (5)	29.2 (27.6)	1.18 (1.18)
22	Boysenberries	0.02	25	5 (2)	26.8 (27.6)	1.04 (1.18)
22	Boysenberries	0.06	25	5 (2)	26.0 (27.6)	1.05 (1.18)
9	Pulped tomato	0.02	10	28 (34)	— —	0.15 (0.18)
24	Strawberries	0.04	10	182 (180)	7.2 (5.9)	— —

* Values in parentheses were obtained by a procedure reported in reference 4.

TABLE IV

COMPARISON OF PLASMA ASH AND PRESSURE DECOMPOSITION FOR THE DETERMINATION OF TIN USING VITREOSIL (SILICA) DISHES

Material	Tin found/μg	
	Plasma ash	Pressure decomposition
Plums	5	5
Plums + 200 μg of tin	205	205
Carrots	9	10
Carrots + 200 μg of tin	200	200
Grapefruit	65	73
Grapefruit	191	196
Grapefruit	43	46
Full cream evaporated milk	7	7

In Table IV plums and carrots are duplicate samples and show the total tin present before addition. Recoveries are based on a procedure described in reference 4.

Table V gives an indication of the range of food products and other materials ashed by low-temperature ashing.

TABLE V

LOW-TEMPERATURE ASHING OF A RANGE OF ORGANIC MATERIALS OF PLANT ORIGIN

Sample	Initial mass/g	Residue/g	Time/h	Radiofrequency power/W	Plasma type	Sample ashed, %
Pine needles*	1.9836	0.0719	17	300	O_2	96.3
Plant tissue*	5.0544	0.6996	193	150	O_2	86.1
Boysenberries	5.00	0.01	9	100	O_2 - F	99.8
Raw sugar*	2.001	0.0022	16	263	O_2	99.8
Orange juice*	17.4447	0.2218	30	300	O_2	99.8
Tomato	5.0000	0.04	7	100	O_2 - F	99.6
Strawberries	5.00	0.04	7	100	O_2 - F	99.2
Boysenberries	5.00	0.06	4	100	O_2 - F	98.8
Boysenberries†	5.00	1.60	4	100	O_2	68
Boysenberries	5.00	0.02	4	100	O_2 - F	99.6

* Data obtained and published by permission of LFE Corporation, Waltham, MA, USA, using one of their range of reactors.

† Incomplete ashing.

Justification for the validity of this technique can only be applied to the elements so far examined, *i.e.*, tin, iron, lead and chromium. Some elements may form volatile fluorides under the test conditions, *e.g.*, boron, uranium, titanium, molybdenum, tungsten, sulphur and silicon, but experimental work to establish this needs to be undertaken.

There is also some evidence in the literature of the loss of certain elements in an oxygen plasma, *e.g.*, arsenic, selenium, gold and silver.[9] The mechanism for such losses is not clear. There is in fact some advantage to be gained in the losses of some volatile fluorides; silicon appears to be a troublesome element in reacting with or retaining some of the elements of interest in SRMs. It is considered that this is one of the reasons why good recoveries are obtained for lead and iron in pine needles and bovine liver, SRMs 1575 and 1577, respectively.

Most of the elements found in biological materials are oxidised to their higher oxidation states by atomic oxygen and further react with atomic fluorine to form oxyfluorides. It has been established by chemical tests that chromium is in the hexavalent form. Aluminium metal reacts to form aluminium oxyfluoride as established by X-ray photoelectron spectroscopy and silica volatilises as silicon tetrafluoride.

The colour of the ash from biological materials containing added metals as their nitrates or chlorides at the 200 μg level are as follows: chromium, orange; tin, grey; iron, rust brown; lead, white; and aluminium, white.

Conclusions

Standard Reference Materials (SRMs) ashed by the procedure described and analysed for tin, lead, iron and chromium show good recoveries. These good recovery values are to be expected because losses due to retention by silica residues would not arise. Silica is volatilised as SiF_4.

The comparative results show good agreement for tin, lead, iron and chromium in canned foods and added tin is fully recovered.

In view of increasing material, chemical and energy costs and in spite of high initial capital cost there is strong evidence in favour of adopting this technique not only for reference work but also to support more rapid routine procedures.

The advantages are real: (*i*) reduced applied man-hours; (*ii*) reduced chemical costs; (*iii*) fewer hazards compared with both dry and wet conventional oxidation; and (*iv*) less contamination, lower blanks, higher sensitivity, less interference are all related to the purity of the ash obtained and the absence of certain elements such as silicon.

There is a need to explore the influence of other gases in their vibrational states, *e.g.*, helium, argon, hydrogen, nitrogen and neon, on oxidation by oxygen - fluorine plasma chemistry reactions because they may significantly increase the concentration of excited oxygen or fluorine atoms.

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Determination of Chloride, Sodium and Potassium in Salted Foodstuffs Using Ion-selective Electrodes and the Dry Sample Addition Method

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The "dry sample addition" method of ion-selective potentiometry is described. This incremental or spiking method requires the addition of a known mass of solid sample directly to a standard solution containing the ion to be measured. Dissolution of the sample produces a change in electrode potential which is related to the sample composition. Dry sample addition has been applied to the analysis of foodstuffs containing up to 2.8% of salt. The chloride, sodium and potassium concentrations are determined by Philips solid-state and plastic ion-selective electrodes. Comparison of this method with titrimetry and atomic-absorption spectrometry gave satisfactory results. The advantages gained in cost, speed of analysis and reliability using ion-selective measurement are discussed.

Keywords: *Ion-selective electrodes*; *dry sample addition potentiometry*; *determination of chloride, sodium and potassium*; *salted foodstuffs*

The traditional methods for the determination of salt in foodstuffs have been titrimetric and photometric. Chloride has been determined principally by the Mohr argentometric titration method,[1] and either atomic-absorption spectrometry or flame photometry have been applied to the alkali metals. Both methods are for many foodstuffs either expensive, insensitive or time consuming. The Mohr titration procedure using coloured indicators is, in particular, an insensitive method for foodstuffs containing colouring agents or insoluble fibrous material. In recent years, ion-selective electrodes have been used for the determination of chloride in a number of foodstuffs, for example cheese[2] and corn syrup.[3] However, in practice these measurements have required the dispersal of the food in water followed by direct potentiometry or titration with a silver salt. The application of incremental or spiking methods of ion-selective measurement, with the advantages of reliability and time saving, has not been tested on foodstuffs.

This paper describes a dry sample addition method of ion-selective potentiometry. This little known incremental method involves the addition of a known mass of solid sample directly to a standard solution containing the ion to be measured. The electrode potential change arising from the dissolution of the soluble salts from the sample is related to its original composition. Dry sample addition has been applied to the determination of chloride, sodium and potassium in salted foodstuffs. A variety of foods were tested ranging from powdered solids to oil-based pastes and containing up to 2.8% *m/m* of salt. The dry sample addition method effectively combines the sample dispersion and ion-selective measurement procedures into one time-saving step. Comparison of the results from ion-selective measurement with those from titrimetric and atomic-absorption spectrometric methods gave satisfactory results.

Experimental

Apparatus

Three Philips ion-selective electrodes, a solid-state chloride (IS550–Cl), a plastic sodium (IS561–Na) and a plastic potassium (IS561–K), were used. Ion-selective electrodes were stored in a 10^{-3} M solution of the appropriate ion between measurements and overnight. A double-junction calomel reference electrode (Philips, Type R44/2–SD/1) contained salt bridge electrolytes of 0.1 mol l^{-1} ammonium nitrate for sodium and potassium ions and 1 mol l^{-1} potassium nitrate for chloride ion measurements. All the electrode potential

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measurements were made with a Philips digital pH - millivolt meter, Model PW9409, connected to a single pen chart recorder (Model PM 8251). All solutions were stirred continuously using a magnetic stirrer with small PTFE stirring bars.

A Pye Unicam SP9–800 atomic-absorption spectrometer and an SP9 computer were used for the confirmatory tests for sodium and potassium.

Reagents

All solutions were prepared in de-ionised water from analytical-reagent grade materials.

Chloride stock solution, 1000 mg l^{-1}. Prepared by dissolving 2.102 g of potassium chloride in water and diluting the solution to 1 l.

Sodium stock solution, 1000 mg l^{-1}. Prepared by dissolving 2.544 g of sodium chloride in water and diluting the solution to 1 l.

Potassium stock solution, 1000 mg l^{-1}. Prepared by dissolving 1.908 g of potassium chloride in water and diluting the solution to 1 l.

Standard solutions of chloride, sodium and potassium, 10 *and* 100 mg l^{-1}. Prepared by sequential volume dilution of the appropriate stock solution.

Reference electrode salt bridge electrolytes, 0.1 mol l^{-1} *ammonium nitrate solution and* 1 mol l^{-1} *potassium nitrate solution*. Prepared by dissolving 8 g of ammonium nitrate or 101.1 g of potassium nitrate in water and diluting the solution to 1 l.

Ionisation buffers for atomic-absorption spectrometry, 10% *m/m sodium and potassium sulphate solutions*. Prepared by dissolving either 10 g of sodium sulphate decahydrate or potassium sulphate in water and diluting to 100 ml.

Silver nitrate for argentometric titration, 5×10^{-3} mol l^{-1}. Prepared by dissolving 0.849 g of silver nitrate in water and diluting to 1 l.

Procedure

Determination of the electrode sensitivity

A preliminary calibration of the three ion-selective electrodes was made by measuring the electrode potential of 100-ml portions of the appropriate 10 and 100 mg l^{-1} standard solutions of chloride, sodium or potassium. The electrode sensitivity (millivolt per decade change in concentration) was determined from the difference between the two values of electrode potential for each standard solution. This was carried out before each analysis of a fresh portion of sample using the dry sample addition procedure.

Dry Sample Addition Procedure

A plastic boat containing a portion of the food sample was weighed accurately. A 100.00-ml portion of the 10 mg l^{-1} chloride, sodium or potassium standard solution was pipetted into a 150-ml beaker. The appropriate electrode pair was immersed in the solution and the electrode potential allowed to equilibrate for about 2–3 min and recorded. A portion of the untreated food sample was immediately transferred into the standard 10 mg l^{-1} solution. The stirring speed was normally increased to encourage dissolution of the solid sample. The plastic boat was re-weighed and the mass added determined by difference. The final electrode potential after addition of the sample was recorded and the change in electrode potential obtained by difference.

Determination of chloride, sodium and potassium by dry sample addition

The dry sample addition method requires the addition of a known mass of solid sample directly to a standard solution of the ion to be measured. The salt is leached from the sample, a process encouraged by vigorous stirring of the standard solution. A subsequent increase in the salt concentration of the standard solution is sensed by the ion-selective electrode and a change in the electrode potential incurred. The attainment of a constant potential change confirms that all of the soluble salt has been leached from the sample. This change in electrode potential (ΔE) is proportional to the increase in ion concentration induced by the sample, C_Δ (gram ion per litre) and described by equation (1) which was derived from the basic Nernst expression for the ion-selective electrode,

$$\Delta E = \pm S \log \left[1 + \frac{C_\Delta}{C_{ST}} \right] \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where S is the electrode sensitivity or slope (millivolts per decade change in concentration) obtained from the electrode calibration before measurement and C_{ST} is the concentration of initial standard solution (moles per litre). The $\pm$ refers to the sign of the electrode slope and is positive for cations and negative for anions. Rearrangement of equation (1) enables the measured ion content of the solid sample to be calculated from equation 2:

$$\text{Ion content of sample (\% } m/m) = \frac{C_{ST}V_{ST}A_r}{m}\left[10^{\pm\Delta E/S}-1\right] \times 100 \quad .. \quad (2)$$

where V_{ST} (litres) is the volume of initial standard solution containing the ion to be measured, A_r is the relative atomic mass of the measured ion and m is the mass of solid sample added (grams).

Confirmatory tests for determination of chloride, sodium and potassium

Chloride measurement using the Mohr[1] titration method. After each dry sample addition measurement, a 20.00-ml portion of the standard solution containing dissolved foodstuff was taken and titrated with standard silver nitrate solution (5×10^{-3} mol l^{-1}) using a mixture of potassium chromate and potassium dichromate as an end-point indicator. The background level of 10 p.p.m. of chloride already present in the sample solution was subtracted from the measured concentration.

Sodium and potassium measurement by atomic-absorption spectrometry. After each dry sample addition measurement, a portion (5–15 ml) of the standard solution containing dissolved foodstuff was diluted and an ionisation buffer incorporated in the final solution at a level of 2%. Similarly a 2% buffer was incorporated into both standard sodium and potassium solutions. Ionisation buffers of 10% sodium sulphate decahydrate and potassium sulphate solution were used for potassium and sodium measurements, respectively. Flame atomic absorption was used with a stoicheiometric acetylene - air mixture. Wavelength settings of 589.0 and 766.5 nm were chosen for sodium and potassium, respectively, with a band pass of 0.5 nm. The background level of 10 p.p.m. of sodium and potassium already contained in the sample solutions was subtracted from the measured concentration.

Results and Discussion

The dry sample addition method was applied to the determination of different salted food products commonly found on the British food market. The foods varied from dried crispbread, to powders, pastes and sauces containing up to 2.8% of salt.

An accurate determination of electrode sensitivity was made before each fresh portion of sample was tested by dry sample addition. This procedure helped to establish the best accuracy of measurement and was used to examine any detrimental effects from contaminants (*i.e.*, oils) in the foods on the characteristics of the chloride, sodium and potassium ion-selective electrode membranes. Typical values of electrode sensitivity recorded during a series of six successive replicate chloride analyses of salad cream are included in Table I. Only small changes in the chloride electrode sensitivity were noted during the sequence of measurements. No significant changes in sensitivity were found for the chloride, sodium

TABLE I

CHANGE IN ELECTRODE SENSITIVITY DURING THE DETERMINATION OF CHLORIDE IN SALAD CREAM

Sample portion No.	Mass added/g	Chloride in sample/ g per 100 g	Electrode sensitivity before analysis/ mV decade^{-1}
1	0.3073	1.61	53.3
2	0.2338	1.66	53.6
3	0.2609	1.64	53.0
4	0.2118	1.63	53.0
5	0.2149	1.54	53.2
6	0.3115	1.51	53.9

and potassium electrodes with any of the foods tested by dry sample addition. The dispersal of the food sample in a large volume of standard solution effectively dilutes the level of any contaminants impinging upon the ion-selective membrane surface.

The accuracy and reproducibility of the dry sample addition technique is affected significantly by the magnitude and stability of the change in electrode potential after addition of the sample. In general, the monovalent electrodes of chloride, sodium and potassium should be subject to a potential change in the range 20–40 mV, and this was achieved for the foods tested. Clearly, small changes in electrode potential would incur significant errors of measurement, whereas changes in excess of a decade of concentration, *i.e.*, greater than 50 mV, would affect the over-all ion concentration of the initial standard solution significantly, thus invoking the need for some form of ionic strength adjustment. Typical potential changes of between 20 and 40 mV were attained by using a 100-ml portion of the appropriate initial 10 p.p.m. standard solution; the sample masses were in the range 0.1–1 g. A constant potential change after sample addition was an indication of the complete dissolution of all unbound soluble salts in the food sample. Waiting times for dissolution varied from 2 to 10 min using the fast stirring speed, the time allowed being dependent on the nature and consistency of the foodstuff. All electrode potential readings were taken as constant when a change of 0.1 mV or less was observed over a 30-s period.

Results for the determination of chloride in piccalilli sauce, cream of chicken soup and salad cream are given in Table II. Further results for the determination of sodium and potassium in salad cream, dried milk powder, peanut butter, dried crispbread and tomato ketchup are included in Tables III and IV, respectively. The average ion content of six

Table II

Determination of chloride in salad cream, piccalilli sauce and cream of chicken soup by dry sample addition

Sample	Range of sample mass added/g	Range of chloride electrode sensitivity (S)/mV decade^{-1}	Range of potential change after addition (ΔE)/mV	Chloride in sample*/ g per 100 g	Coefficient of variation, %
Salad cream	0.2118–0.3115	53.0–53.9	33.7–41.3	1.60	3.7
Piccalilli sauce	0.1164–0.2519	55.2–59.3	27.8–41.3	1.68	5.5
Cream of chicken soup (ready to serve)	0.1746–0.3242	54.7–58.5	23.8–34.4	0.89	3.8

* Average of six replicate analyses.

Table III

Determination of sodium in dried crispbread and peanut butter by dry sample addition

Sample	Range of sample mass added/g	Range of sodium electrode sensitivity (S)/mV decade^{-1}	Range of potential change after addition (ΔE)/mV	Sodium in sample*/ g per 100 g	Coefficient of variation, %
Dried crispbread	1.0206–1.2618	49.4–51.6	28.4–31.1	0.25	2.0
Peanut butter	0.4092–0.7561	50.0–52.3	18.8–30.2	0.37	8.7

* Average of six replicate analyses.

Table IV

Determination of potassium in milk powder, salad cream, dried crispbread and tomato ketchup by dry sample addition

Sample	Range of sample mass added/g	Range of potassium electrode sensitivity (S)/mV decade^{-1}	Range of potential change after addition (ΔE)/mV	Potassium in sample*/ g per 100 g	Coefficient of variation, %
Milk powder	0.1785–0.4087	50.2–53.1	32.8–45.9	1.78	2.8
Salad cream	0.3961–0.9028	48.6–50.3	4.5–8.6	0.06	6.6
Dried crispbread	0.9915–1.6333	47.5–51.1	34.7–43.0	0.38	4.7
Tomato ketchup	0.5633–0.8914	51.2–52.3	25.2–31.4	0.37	5.5

* Average of six replicate analyses.

replicate complete analyses is presented for each sample. The ranges of electrode sensitivity, sample mass and change in electrode potential are included with the coefficient of variation for the six replicate analyses. The reproducibility of the method is good as shown by coefficients of variation ranging from 2.0 to 8.7% ($n = 6$) for the foodstuffs tested.

A comparison of the potentiometric results with those from a Mohr titration for chloride and atomic-absorption spectrometry for sodium and potassium is made in Table V. Satisfactory agreement was found for the chloride, sodium and potassium results using the different methods with the majority of the food samples.

The results show that ion-selective potentiometry using a dry sample addition procedure is a satisfactory method for the determination of chloride, sodium and potassium in a range of salted foodstuffs. Dry sample addition is a particularly sensitive technique for the determination of chloride in samples containing colouring agents or fibrous material which often mask or distort the coloured indicator end-point of an argentometric titration. In addition, ion-selective potentiometry secures a reduction in the cost per analysis with the elimination of expensive silver salts. A significant improvement in analysis time using dry sample addition rather than direct potentiometry is achieved in two ways. Firstly, the combination of sample dissolution and ion-selective measurement steps and, secondly, a simplified procedure for calculation of results directly from equation (2). A further improvement in analysis time is envisaged with some foodstuffs if the vigorous mechanical mixing methods, traditionally used in food analysis, were applied to the dissolution step of the sample, for example dried crispbread. Further, microprocessor-based ion-selective instrumentation, *e.g.*, the Philips PW 9416 Ion-Selective Analyser, provides for the direct read-out of sample concentration in percent. by mass by the dry sample addition method. This incremental or spiking procedure may now be applied to the determination of other ions contained in a solid as a soluble species.

TABLE V

COMPARISON OF DRY SAMPLE ADDITION METHOD WITH MOHR TITRIMETRIC AND ATOMIC-ABSORPTION SPECTROMETRIC METHODS FOR SALTED FOODSTUFFS

		Ion content of sample*/g per 100 g		
Sample	Ion	Ion-selective measurement using dry sample addition method	Mohr titration method	Atomic-absorption spectrometry
Salad cream	Chloride	1.60	1.64	
Piccalilli sauce	Chloride	1.68	1.63	
Cream of chicken soup (ready to serve)	Chloride	0.89	0.94	
Dried milk powder	Potassium	1.78		1.65
Salad cream	Potassium	0.06		0.02
Dried crispbread	Potassium	0.38		0.35
Tomato ketchup	Potassium	0.37		0.32
Dried crispbread	Sodium	0.25		0.16
Peanut butter	Sodium	0.37		0.31

* Average of six replicate analyses, per sample and method.

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On-line Electrochemical Detection of Oxidisable Organic Molecules of Pharmaceutical Importance

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On-line electrochemical detection in the oxidative mode using a wall-jet electrode has been evaluated for 1,4-benzodiazepines and other drug molecules of widely differing structure. The method has been applied satisfactorily to the automated analysis of some 1,4-benzodiazepines, coefficients of variation of *ca.* 1–3% being obtained.

Keywords: *On-line electrochemical analysis*; *1,4-benzodiazepines*

The need to develop rapid and inexpensive methods of analysis for compounds of clinical and pharmaceutical importance has resulted in some polarographic and voltammetric on-line methods for their analysis. In these methods either the stream containing the electroactive material is continuously monitored by an electrochemical detector[1,2] or a large number of samples are analysed by passing them repetitively past the detector and recording the current produced by each sample.[3–8] These results are collected in Table I.

The great selection of electrochemical detectors that have been developed for high-performance liquid chromatography (HPLC) can naturally be used in instances when chromatographic separation is not needed,[9–15] as is the case in this paper.

TABLE I

SUMMARY OF ON-LINE ANALYSES OF ORGANIC COMPOUNDS

Reference No.	Sample rate/h^{-1}	Compounds studied	Working electrode*	Mode†	Concentration range studied	Relative standard deviation, %
3	60	Selected drugs	DME	D.c., a.c.	0.4–10 mg ml^{-1}	—
4	15	Benzodiazepines	DME	S.d.c.	5–25 ng ml^{-1}	1.4
5	20	Phenols	Pt, C	N.p.	4×10^{-6}–5×10^{-3} g ml^{-1}	1.5
6	—	Great variety	C	D.c.	1×10^{-3}–1×10^{-6} M	—
7	30	Benzodiazepines	DME	D.c.	0.1 mg ml^{-1}	0.3
8	120	Proteins	DME	D.p.	5–50 μg ml^{-1}	1

* DME = dropping-mercury electrode.
† D.c. = direct current; a.c. = alternating current; s.d.c. = sampled direct current; n.p. = normal pulse; d.p. = differential pulse.

These electrochemical detectors can also be used in flow injection analysis where a small volume of the analyte is aspirated into a flowing background electrolyte. This method has been successfully applied to the analysis of organic compounds.[12,16] The usable potential range of the indicator electrode used in this study, namely the solid glassy carbon electrode, is approximately from +1.5 to −1.2 V. The large positive potential range allows for the study of the oxidation of a great number of compounds that do not reduce and hence cannot be analysed by the DME or the thin mercury film electrode (TMFE). High flow-rates can also be used with solid electrodes, increasing the sensitivity of the technique owing to increased mass transport of the compounds to the electrode surface. With the DME, the flow-rate is critical because the form of the mercury drop is influenced by the flow-rate so

that the drop formation is not always uniform. Solid electrodes are also robust and easy to handle. Their disadvantage is that the surface is not renewed and if the products of the electrode reaction are adsorbed on the surface the signal can be decreased. The effect of fouling can be reduced by selection of a suitable pulse mode so that the potential is applied only for a small fraction of the time required for amperometric detection. Choice of supporting electrolyte (*e.g.*, to include methanol), washing periods between individual samples and electrochemical cleaning of the surface can also be employed in this context.

The oxidative behaviour of many organic molecules at various stationary and rotating solid electrodes has been studied.[17] The oxidative behaviour of 1,4-benzodiazepines, in particular, at rotating platinum and gold disc electrodes has been investigated by Volke *et al.*,[18] who found responses at the electrode for oxazepam, diazepam and flurazepam in a supporting electrolyte of 0.1 M tetraethylammonium perchlorate in acetonitrile. They also found responses at the electrode for amitryptylene, diothiepin and dithiaden, which gave well defined waves in the potential region from +1.2 to +1.3 V.

This paper is concerned with an investigation of the link-up of a glassy carbon wall-jet electrode contained in a flow cell with a Carlo Erba automatic analyser for the detection of oxidisable organic molecules of pharmaceutical importance and, in particular, an investigation of its application to the automated determination of 1,4-benzodiazepines.

Experimental

Reagents

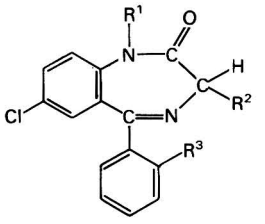
All chemicals used were of analytical-reagent grade and the pure benzodiazepines and their formulations were obtained from Hoffmann La Roche, Nutley, NJ, USA. A Britton - Robinson (BR) buffer solution of pH 4 was found to be optimum for the on-line analysis of 1,4-benzodiazepines. All solutions were 10% with respect to the methanol concentration intended to keep the organic molecules in solution and to aid dissolution of the reactants and products of electrode reactions from the surface to the indicator electrode. The 1,4-benzodiazepines and other nitrogen-containing drug molecules were prepared in their solutions at a concentration of 1×10^{-4} M. These were then placed in their respective compartments in the sample turntable prior to on-line electrochemical detection.

Instrumentation

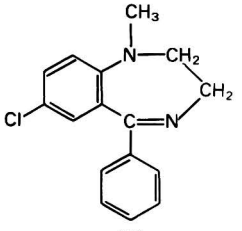
The electrochemical cell was of the wall-jet type[19] and is a slight modification of this design.[20] The incoming solution impinged on the surface of the working glassy carbon electrode and when leaving the cell it passed the tip of the reference electrode. A platinum auxiliary electrode was placed near the working electrode. A PAR 174A polarograph was used for the voltammetric measurements and all potentials were measured against the saturated calomel electrode (S.C.E.). Solutions were pumped to the cell using a Carlo Erba automatic analyser, as shown in Fig. 1, which involved a turntable distributor, peristaltic pump and mixing coils. The currents were recorded with a strip-chart recorder and the measurements were performed at 22 °C. The connections were made to the PAR 174A via the colour-coded wires illustrated in Fig. 1. The pulse time was set at 0.5 s and the differential pulse mode of operation was investigated. The time constant was set at 0.3 s and the modulation amplitude at 100 mV. Nitrogen was also introduced at the front end of the mixing coil as illustrated in Fig. 1 so as not to allow too great a dilution of the analyte, whilst allowing satisfactory mixing with the supporting electrolyte.

Results and Discussion

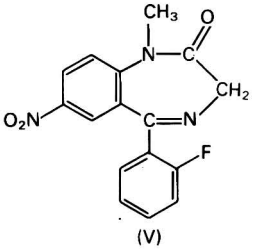
The 1,4-benzodiazepines flurazepam (**I**), oxazepam (**II**), medazepam (**III**), lorazepam (**IV**), flunitrazepam (**V**), prazepam (**VI**), potassium chlorazepate (**VII**), nitrazepam (**VIII**), amino-nitrazepam (**IX**) and chlordiazepoxide (**X**) represent a wide range of molecular structures and, in quiescent solution, give rise to the d.c. voltammetric data illustrated in Table II when subjected to electrooxidation at the glassy carbon electrode in BR buffer of pH 4.0.[21] Results from a recent study[22] using BR buffer of pH 12.0 are also included in Table II.



(I) $R^1 = -(CH_2)_2N(C_2H_5)_2$; R^2 = H; R^3 = F
(II) R^1 = H; R^2 = OH; R^3 = H
(IV) R^1 = H; R^2 = OH; R^3 = Cl
(VI) R^1 = cyclopropyl; R^2 = H; R^3 = H



(III)



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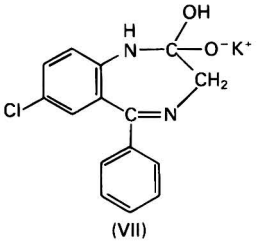

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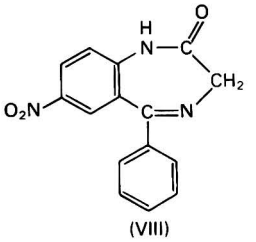

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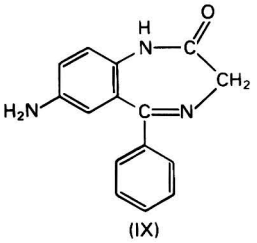

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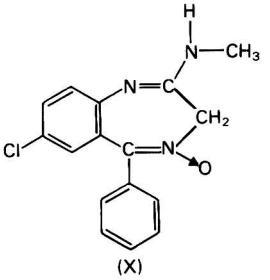


(X)

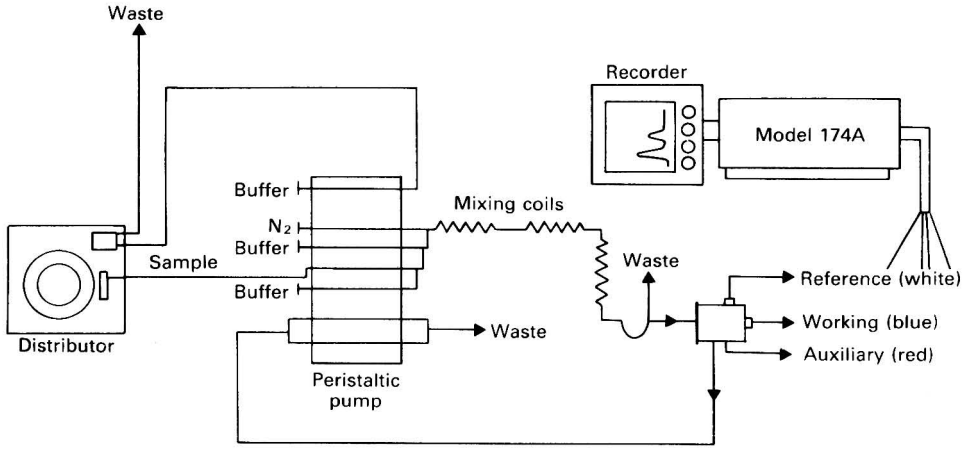


Fig. 1. Link-up of automatic analyser with the wall-jet electrochemical detector.

TABLE II

VOLTAMMETRIC DATA FOR 1×10^{-4} M CONCENTRATIONS OF 1,4-BENZODIAZEPINES (I)–(X) AT THE GLASSY CARBON ELECTRODE

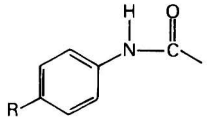
1,4-Benzodiazepine	pH 4		pH 12	
	E_p/V *vs.* S.C.E.	$i_p/\mu A$	E_p/V *vs.* S.C.E.	$i_p/\mu A$
Flurazepam (I)	+1.05	5.0	+0.66	3.2
Oxazepam (II)	+1.33	4	+0.72	2
Medazepam (III)	+0.88, +1.10	4.3, 4.0	+0.68	10.1
Lorazepam (IV)	+1.38	4	+0.8	2
Flunitrazepam (V)	n.e.*	n.e.*	n.e.*	n.e.*
Prazepam (VI)	n.e.*	n.e.*	n.e.*	n.e.*
Potassium chlorazepate (VII)	+1.49	4	+1.04, +1.2	4, i.d.p.†
Nitrazepam (VIII)	n.e.*	n.e.*	+1.14	7.1
Aminonitrazepam (IX)	+0.77, +1.23	3.8, i.d.p.†	+0.45	3.3
Chlordiazepoxide (X)	+1.31	6	+0.9	3

* n.e. = no electrooxidation occurs in the pH range investigated.
† I.d.p. = ill-defined peak.

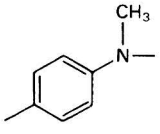
Certain trends in the mechanisms of the electrooxidation of these 1,4-benzodiazepines can be realised for the purposes of this paper.

Oxidation of the 1-N-substituted molecule flurazepam (**I**) takes place on the nitrogen atom of the 1-N side-chain as its E_p value in pH 12 buffer of +0.66 V compares exactly with the E_p value for the tertiary amine $(CH_3)_2N(C_2H_5)$ in the same buffer. Presumably N-oxidation is the mechanistic route in aqueous supporting electrolytes. It is not expected that the 4-N atom is involved in electrooxidation, as the 1-*N*-hydroxyethyl metabolite of flurazepam is not electrooxidisable in the pH range 4–12.[22] In any case, the electron-withdrawing effect of the fluorine atom would not facilitate oxidation of the $\rangle C = N\langle$ group. Volke *et al.*[18] also observed well defined peaks for **I** in acetonitrile with 0.1 M tetrabutylammonium perchlorate supporting electrolyte at a rotating platinum electrode (E_p = +1.05 V). Chlordiazepoxide (**X**) has its azomethine group "blocked" to electrooxidation, as it is already N-oxidised, but has the electrooxidisable $\rangle N = C\langle^{NHCH_3}$ entity present. It is probably N-oxidised at one or other of these nitrogen atoms.

The mechanisms of electrooxidation of the remaining 1,4-benzodiazepines can be grouped under the following functional group subdivisions:

(*i*)  containing molecules, *i.e.*, oxazepam (**II**), lorazepam (**IV**)

and potassium chlorazepate (**VII**),* where R = Cl, are reasonably difficult to oxidise. This is in agreement with the observations of Volke *et al.*,[18] who found **II** to be oxidised at +1.55 V and that **IV** gave an ill-defined wave at the rotating platinum electrode. The mechanism probably involves initial formation of a radical cation $-\dot{\overset{+}{N}}H-$ followed by coupling reactions. *N*-Acetyl-*p*-chloroaniline behaves similarly in that an E_p value of +1.3 V (i_p = 5.2 μA) is observed in BR buffer of pH 4 and +0.83 V (i_p = 3.7 μA) in BR buffer of pH 12. When R = NO_2, as in nitrazepam (**VIII**), resonance delocalisation of the lone pair on the 1-N atom is extensive and precludes electrooxidation in BR buffer of pH 4. Its metabolite, amino-nitrazepam (**IX**), is, however, relatively easy to oxidise, as illustrated in Table II. In this instance it is probable that electrooxidation takes place at both the 1-N atom and the N atom in the 7-position.

(*ii*)  containing molecules, such as flunitrazepam (**V**) and

prazepam (**VI**), are not electrooxidisable, primarily owing to the steric effect of the CH_3 group and the electron-withdrawing effect of the carbonyl group in position 2. When this latter group is absent, as in medazepam (**III**), the electron density is sufficient at the 1-N atom to allow electrooxidation.

The results in Table II were then applied to the on-line analysis of the 1,4-benzodiazepines **I**–**X**, individually at the 10^{-4} M concentration level (Fig. 2). The settings on the PAR 174A were set as follows: detector potential, +1.2 V; current setting, 0.05 mA (d.p.); pulse time, 0.5 s; modulation amplitude, 100 mV; and low pass filter (τ), 0.3 s.

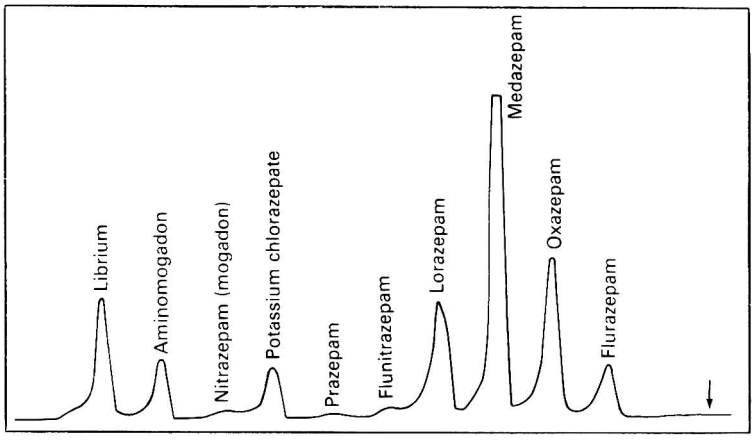


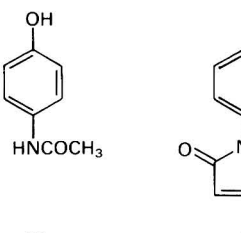
Fig. 2. Response of certain 1,4-benzodiazepines at the glassy carbon in the on-line mode. Conditions: medium, pH 4 Britton - Robinson buffer + 10% methanol; detector potential, +1.2 V; current, 0.05 mA (d.p.); pulse time, 0.5 s; and initial concentration, 10^{-4} M (in methanol).

* This molecule possesses $-\underset{|}{C}(OH)-O^-K^+$ adjacent to the 1–N atom.

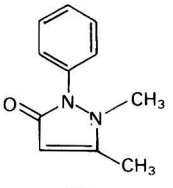
At first, problems with adsorption were encountered, with only three compounds giving reproducible results. The adsorption problem was overcome by the addition of methanol to the buffer (10% by volume) and by extending the wash time slightly. Approximately 30 samples per hour are capable of being analysed using this system (Fig. 1) without poisoning of the electrode. However, after the processing of about 30 samples, the electrode was cleaned with chamois leather as a general precaution.

Tablet contents were also analysed by this on-line method and the results are shown in Fig. 3. The tablet contents analysed were flurazepam (Dalmane, 30 mg in 10 ml of methanol), medazepam (Nobrium, 5 mg in 50 ml of methanol and 10 mg in 50 ml of methanol) and chlordiazepoxide (Librium, 5 mg in 50 ml of methanol). The conditions for the experiment were the same as for that shown in Fig. 2. As an indication of the reproducibility of results, six successive on-line analyses of the flurazepam formulation yielded a coefficient of variation of 2.57%, values of 0.85% and 1.25% being obtained for pure samples of oxazepam and lorazepam.

In addition to the 1,4-benzodiazepines, the following nitrogen-containing drug compounds were found to exhibit oxidative behaviour at the glassy carbon electrode using BR buffer of pH 4 as the supporting electrolyte: paracetamol (**XI**), phenazone (**XII**), nicotine (**XIII**), adrenaline (**XIV**), dibenzepine (**XV**), nortryptyline (**XVI**) and diphenoxylate (**XVII**).



XI



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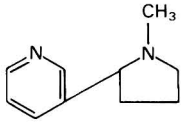
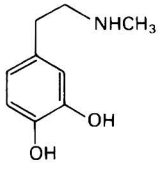
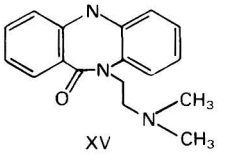


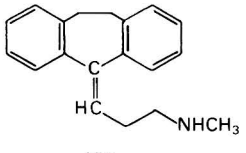
XIII



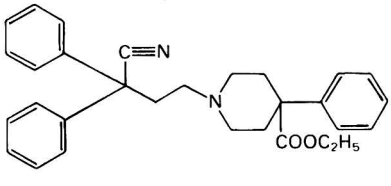
XIV



XV



XVI



XVII

It would therefore appear that on-line electrochemical detection could be applied to the determination of many nitrogen-containing drug molecules as either pure substances or as they are found in drug formulations. Routine tablet quality control analysis can be taken over by this type of detector system, in conjunction with the automatic analyser outlined in this section. The analysis of 30 benzodiazepine samples by reductive polarography using a DME would take approximately 3–4 h (*i.e.*, 6–8 min per sample), whereas one batch of 30 samples could be handled in 1 h by this system (2 min per sample). This detector, in addition, can obviously be used for the determination of many nitrogen-containing drugs at the low concentrations encountered in body fluids following extraction and HPLC separation.

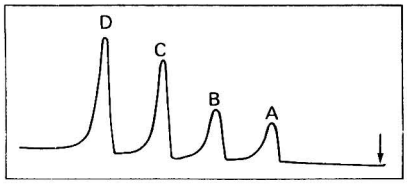


Fig. 3. Electrochemical detection applied to the "on-line" analysis of 1,4-benzodiazepine formulations. Tablet sizes: A, flurazepam (Dalmane, 30 mg in 10 ml of methanol); B, medazepam (Nobrium, 5 mg in 50 ml of methanol); C, medazepam (Nobrium, 10 mg in 50 ml of methanol); and D, Librium (5 mg in 50 ml of methanol).

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Electrochemical Determination of Trimethoprim

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A differential-pulse polarographic method has been developed for the determination of trimethoprim. The E_p occurs at -1.378 V when the pH of the system is 3.6. Cyclic voltammetry in anhydrous acetonitrile demonstrated that both trimethoprim and sulphamethoxazole could be determined by anodic oxidation at a platinum electrode provided that each was present as a single entity.

Keywords: *Trimethoprim determination*; *differential-pulse polarography*; *controlled-potential coulometry*; *cyclic voltammetry at a rotating platinum electrode*

Trimethoprim, an aminopyrimidine, is used widely in the treatment of urinary tract infections and is frequently combined with sulphamethoxazole in the ratio of 1 : 5 for increased effectiveness. In the BP,[1] the trimethoprim content of pharmaceutical dosage forms is determined by ultraviolet spectrophotometry, whereas the USP[2] employs high-performance liquid chromatography (HPLC). Both compendia[1,2] use non-aqueous titration in glacial acetic acid for the assay of the raw material. Other methods of analysis for the dosage forms include gas chromatography,[3] observance of ion-ion pair formation with an indicator,[4] nuclear magnetic resonance spectroscopy,[5,6] HPLC[7,8] and spectrophotometry at 560 nm.[9]

In 1973, Brooks *et al.*[10,11] presented two reports on the determination of trimethoprim in biological specimens in which they employed differential-pulse polarography with a supporting electrolyte of 0.1 N sulphuric acid. The sensitivity and specificity led us to investigate the experimental conditions that would permit the polarographic determination of trimethoprim in pharmaceuticals.

Experimental

Apparatus and Conditions for Polarographic Analysis

A Fisher, Model 320, pH meter fitted with a glass - calomel electrode system was employed to measure the pH of the solutions.

A PAR, Model 174, polarograph equipped with a drop timer (Model 172A), and a Houston Omnigraphic recorder, Model 2000, were used in the investigations. A three-electrode combination was used, consisting of a saturated calomel electrode, a dropping-mercury electrode and a platinum wire as the auxiliary electrode. A PAR, Model 9301, cell was maintained at 24 $\pm$ 1 °C and all sweeps utilised a drop-time of 2 s and a scan rate of 2 mV s^{-1}. Other instrumental parameters were: applied potential range, -1.00 to -1.75 V; current, 100 μA; height of mercury column, 75 cm; flow-rate of mercury, 1.222 mg s^{-1}; modulation amplitude, set at 50 mV; and low pass filter, set at a time constant of 1 s. The instrument was operated in the differential-pulse mode.

Controlled-potential Coulometry

A Hi-Tek digital integrator and digital voltmeter was equipped with a PAR, Model 377A, three-compartment coulometric cell system and connected to a PAR, Model 173, potentiostat - galvanostat. A 5-ml volume of triple distilled mercury was used per sample.

Two buffer systems were employed:

Walpole's acetate buffer. A blank was determined on 19 ml of buffer and 1 ml of 1 M acetic acid, the cell was then emptied, cleaned and dried and a sample was placed in it that consisted of 19 ml of buffer and 1 ml of 10^{-2} M trimethoprim in 1 M acetic acid.

Sorensen's citrate buffer. In this instance, the blank was 19 ml of buffer and 1 ml of methanol and the sample contained 1 ml of 10^{-2} M trimethoprim in methanol.

* Department of Chemistry.

Cyclic Voltammetry

Cyclic voltammetric experiments at a platinum-wire electrode were performed with a Hi-Tek electrochemical station which included a DT–2101 potentiostat and PPR1 waveform generator. Plots were recorded on a Bradford SR–1 x - y recorder. The cell used was of conventional design with a Luggin capillary inserted for accurate probing of the working electrode potential. iR compensation was performed at each sweep speed by the control on the potentiostat. The same experiments were repeated with both gold and vitreous carbon as working electrodes.

Solutions were prepared in anhydrous (less than 0.001% of water) acetonitrile, using tetrabutylammonium tetrafluoroborate (0.1 M) as supporting electrolyte. The reference electrode was a silver wire in 0.01 M silver nitrate in the same electrolyte solution.

Stock solutions of 10.0 mM sulphamethoxazole (99.9%) and 1.0 mM trimethoprim (99.7%) were prepared. Working solutions were made by dilution of aliquots of the stock solution delivered from a microburette. The solutions were purged with dry argon just prior to recording the voltammogram. The electrodes were cleaned and polished prior to the recording and calibration graphs were prepared by obtaining five cyclic voltammograms for each of five standard concentrations of each of the materials.

Reagents

The following reagents were used, all of analytical-reagent grade: acetonitrile, chloroform, citric acid, glacial acetic acid, methanol, sodium acetate, sodium hydroxide, tetrabutylammonium tetrafluoroborate, 1 M acetic acid, 1% gelatin solution, Sorensen's citrate buffer prepared at eight selected pH values over the range 2.2–4.6 and Walpole's acetate buffer (pH 4.0).

pH Dependence Studies

These studies were carried out by using 1 ml of a methanolic stock solution of trimethoprim and Sorensen's citrate buffer at intervals of 0.4 pH unit over the pH range 2.2–4.6.

Preparation of Calibration Graphs

Trimethoprim (99.7%) was obtained from Burroughs Wellcome, Canada, and used without further purification.

A stock solution of trimethoprim (10^{-2} M) was prepared in 1 M acetic acid. Solutions containing various concentrations of trimethoprim were prepared by dilution of the stock solution with Sorensen's citrate buffer at pH 4.0. In the total sample volume of exactly 20 ml, the amount of 1 M acetic acid was always maintained at 1 ml and the final pH of the system at 3.6. Sample concentrations for the preparation of the calibration graph by differential-pulse polarography ranged from 10^{-4} to 5×10^{-4} M.

All samples were purged with oxygen-free nitrogen for 10 min prior to each run and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of 0.9980.

Analysis of Pharmaceutical Dosage Forms

Two methods were employed for tablets because the products of one manufacturer contained an interfering excipient that could not be removed by the usual extraction methods.

Tablets—method 1

Twenty tablets were weighed and finely powdered. An amount of tablet mass was taken that, according to the label, would result in an approximately 10^{-2} M solution of trimethoprim in 25 ml of 1 M acetic acid. The weighed sample was stirred magnetically for 15 min with 15 ml of 1 M acetic acid and then suction filtered. The residue and beaker were washed with 5 ml of 1 M acetic acid and transferred quantitatively into a 25-ml calibrated flask and adjusted to volume with 1 M acetic acid. A 0.6-ml sample of filtrate was pipetted into the polarographic cell and 0.4 ml of 1 M acetic acid and 19 ml of Sorensen's citrate buffer (pH 4.0) were added. All samples were purged with oxygen-free nitrogen, as described previously.

Tablets—method 2

The procedure was modified so that in addition to the 0.6 ml of sample that was pipetted into the polarographic cell, 0.4 ml of 1 M acetic acid, 0.6 ml of 1% gelatin solution and 18.4 ml of Sorensen's citrate buffer (pH 4) were also added.

Suspensions

To a 5-ml sample of the suspension (rinse the pipette well with distilled water), add 25 ml of 0.1 N sodium hydroxide solution and extract the trimethoprim with four 10-ml portions of chloroform. Combine the chloroform extracts and wash with 10 ml of 0.1 N sodium hydroxide solution. Extract the drug from the chloroform with four 5-ml volumes of 1 M acetic acid. Wash the combined extracts with 3 ml of chloroform, then transfer the acetic acid extracts into a 25-ml calibrated flask and adjust to volume with 1 M acetic acid. Transfer 1 ml of this solution into the polarographic cell and add 19 ml of Sorensen's citrate buffer (pH 4.0). Purge the samples with oxygen-free nitrogen, as described previously.

Results and Discussion

In their voltammetric study of trimethoprim, Ellaithy and Volke[12] stated that they obtained clear waves in 0.1 N sulphuric acid, in citrate - hydrochloric acid buffers over the pH range 3–4 and in acetate buffers. The diagram of their waves, however, shows no limiting current plateau for the d.c. waves. In this work, the d.c. wave gave a limiting current plateau in the vicinity of pH 4.

Fig. 1 shows a comparison between the d.c. and differential-pulse waves for trimethoprim in 1 M acetic acid - Sorensen's citrate buffer (pH 4.0). The d.c. wave could have been utilised for analytical purposes but the differential-pulse wave was selected because of its symmetry and increased sensitivity.

The effect of pH on shape and height of the differential-pulse wave was studied. In 0.1 N sulphuric acid (pH $\approx$ 1), two steps were evident by the appearance of a significant bulge on the ascending portion of the differential-pulse polarogram. The existence of this bulge persisted in the Sorensen's citrate buffer system but with decreasing prominence as the pH was increased until the pH was above 2.0. By pH 2.6, only a smooth symmetrical wave appeared, which persisted until pH 4.0. Above pH 4.0, the process again appeared to involve two steps. A plot of i_d against $h^1_{(corr)}$ at pH 3.6 revealed a diffusion-controlled process.

Trimethoprim is adequately soluble in both dimethylformamide and methanol and, consequently, both of these solvents were tested as extraction media for the drug as it occurred in dosage forms. Each of them, however, extracted some constituent of the formulation that interfered with the analysis. One of the most serious offenders was sulphamethoxazole, because of its significant amount. The final extraction procedure that was chosen for the

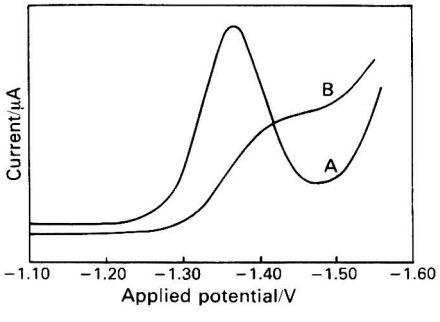

Fig. 1. Differential-pulse and d.c. polarographic waves for trimethoprim (5×10^{-4} M) in 1 M acetic acid - Walpole's acetate (pH 4.0). A, Differential-pulse wave, current 200 μA; and B, d.c. wave, current 20 μA.

tablets involved the use of 1 M acetic acid. This circumvented all interferences except that caused by the presence of povidone, a substance that was included in the formulations of one manufacturer. As expected, the povidone behaved like a surface-active agent and suppressed the height of the differential-pulse polarographic wave. Experimentation with known, synthetic povidone-containing samples revealed that the addition of a fixed, small amount of 1% gelatin solution overshadowed the influence of the povidone and decreased the peak height by a constant amount. Consequently, products containing povidone were assayed by the alternative direct comparison method in which the reference contained an equivalent amount of gelatin solution. The results of the analyses of all of the products appear in Table I and good agreement with the manufacturers' data is noted in each instance. Owing to the formulation, it was not possible to assay one suspension by the proposed procedure.

In their report, Ellaithy and Volke[12] stated that over the pH range 3–4, the number of electrons consumed per molecule was always 4. In this laboratory, controlled-potential coulometry revealed that the reduction process involved two electrons at pH 3.6.

TABLE I

ASSAY OF TRIMETHOPRIM PRODUCTS BY DIFFERENTIAL-PULSE POLAROGRAPHY

			Trimethoprim content found, %	
Manufacturer	Form	Label claim/mg	Manufacturer's result	Differential-pulse polarography*
A	Tablet	100	97.4	96.1 ± 0.4
	Tablet	80	98.8	99.7 ± 0.3
	Tablet	160	99.9	99.8 ± 0.4
	Tablet	20	98.3	99.3 ± 0.5
B	Tablet	80	102.0	99.9 ± 0.4
	Tablet	160	100.3	101.6 ± 0.5
	Suspension	80 mg ml^{-1}	97.7	99.7 ± 0.5

* Each value is the average of five determinations.

Results at a Platinum Electrode

The anodic oxidation of trimethoprim at a platinum electrode leads to two irreversible waves (Fig. 2), both of which are apparently one-electron oxidations by examination of the peak heights and shapes. Both peaks may be used for analytical measurements, as can be seen in Fig. 3. These results at first seem to be in direct conflict with those of Ellaithy and Volke,[12] who tried to observe oxidation waves in essentially the same system at a rotating platinum microelectrode. It should be noted, however, that the electrode becomes fouled after one forward sweep owing to the formation of a surface film that passivates the electrode towards further oxidation on subsequent scans. The results obtained with either vitreous carbon or gold working electrodes indicate that the reactions are the same as those obtained with platinum. Consequently, any of the three may be used at anodic potentials for the determination of trimethoprim. A single forward scan, however, with a clean working electrode, always exhibits two well defined anodic waves, both of which are analytical.

The first oxidation product (cation radical), sulphamethoxazole (Fig. 4), reacts with the substrate through a reaction that has a relaxation time that is observable on the same slow time scale that was used for recording the cyclic voltammograms in these experiments. It is apparent from the curves in Fig. 5 that the current of the second reduction is much less sensitive to scan rate than a simple (scan rate)$^{\frac{1}{2}}$ relationship, and that the current is also depressed by more than a linear relationship with concentration of substrate. Both of these factors are expected by an electrochemical - chemical (EC) mechanism where the product of the first electron-transfer reaction is involved. The first wave may be used for analytical measurements, as illustrated in Fig. 5.

If mixtures of trimethoprim and sulphamethoxazole are investigated anodically by cyclic voltammetry, it is seen that a new peak appears in the voltammogram at 0.78 V (Fig. 6).

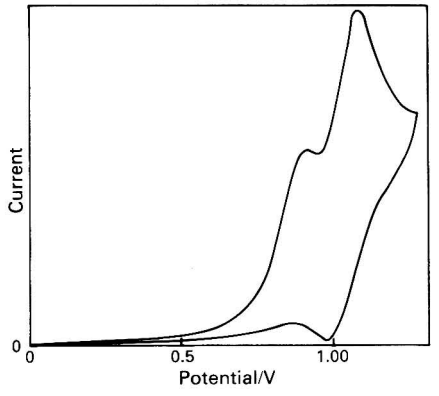

Fig. 2. Cyclic voltammogram of 1.0 mM trimethoprim; v = 150 mV s^{-1}.

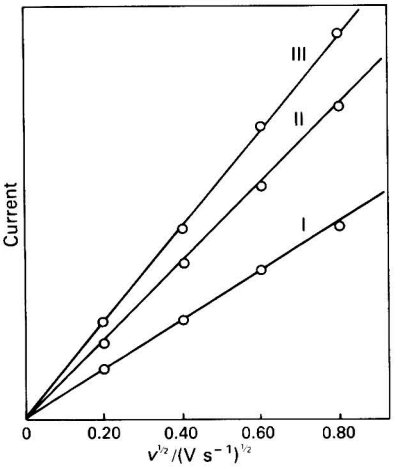

Fig. 3. Peak current dependence on (scan rate)$^{\frac{1}{2}}$ I, 0.5 mM Trimethoprim, 1st anodic peak; II, 5.0 mM, trimethoprim, 2nd anodic peak; and III, 5.0 mM trimethoprim, 1st anodic peak.

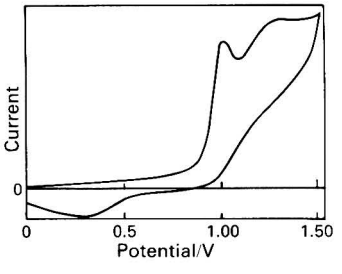

Fig. 4. Cyclic voltammogram of 1.0 mM sulphamethoxazole; v = 160 mV s^{-1}.

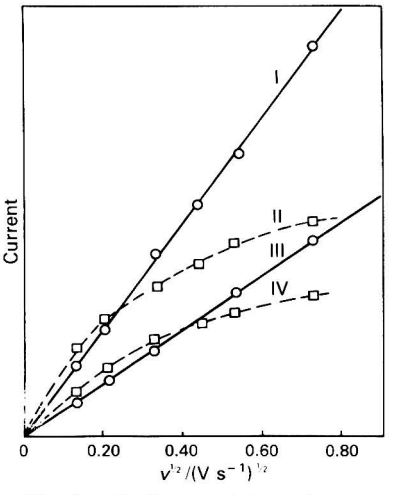

Fig. 5. Peak current dependence on (scan rate)$^{\frac{1}{2}}$. I, 10.0 mM sulphamethoxazole, 1st anodic peak; II, 10.0 mM sulphamethoxazole, 2nd anodic peak; III, 5.0 mM sulphamethoxazole, 1st anodic peak; IV, 5.0 mM sulphamethoxazole, 2nd anodic peak.

For a given trimethoprim concentration the peak reaches a maximum value when the molar concentration ratio of sulphamethoxazole to trimethoprim is 3:1. The peak moves more cathodically by about 0.06 V per decade increase in sulphamethoxazole concentration. The wave is thus probably due to the oxidation of sulphamethoxazole anion which remains after protonation of trimethoprim:

$$HSMZ \rightleftharpoons SMZ^- + H^+$$

$$H^+ + TMP \rightleftharpoons HTMP^+$$

where HSMZ = sulphamethoxazole and TMP = trimethoprim.

The appearance of the new peak at the lower potential is accompanied by the disappearance of the first anodic wave of trimethoprim. These acid - base interactions render the analysis of trimethoprim by anodic techniques difficult, and for such mixtures the differential-pulse polarographic method is preferable. As mentioned, however, analysis by anodic oxidation at a platinum electrode is reliable if either substance is present alone.

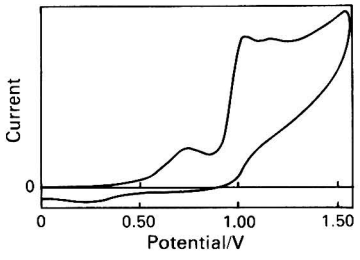

Fig. 6. Cyclic voltammogram of 0.43 mM trimethoprim and 1.18 mM sulphamethoxazole; $v = 100\,mV\,s^{-1}$.

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Differential Electrolytic Potentiometry with Periodic Polarisation

Part XXV.* Direct and Mark-space Biased Periodic Current Polarisation in Acid - Base Titrimetry in Acetic Anhydride - Acetic Acid

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The applications of direct current and mark-space bias square wave differential electrolytic potentiometric techniques to acid - base titrations in an acetic anhydride - acetic acid mixed solvent have been examined. Antimony metal - metal oxide electrodes were used as indicating systems. Titration curve shapes are similar to those obtained from aqueous acid - base titrations, but the required current density and percentage bias are much higher. Several compounds that do not exhibit basic properties in water were titrated successfully with perchloric acid. The advantage of using an aprotic solvent in conjunction with acetic anhydride is demonstrated.

Keywords: Differential electrolytic potentiometry; antimony electrodes; acetic anhydride solvent; non-aqueous acid - base titrimetry

The technique of direct current differential electrolytic potentiometry (d.c. DEP) has been applied to all types of ion-combination and oxidation - reduction reactions in aqueous media. Various types of electrodes have been employed. Antimony electrodes have been used mainly in acid - base reactions,[1–4] while silver - silver halide electrodes are appropriate for precipitation reactions.[5–7] Platinum electrodes have been applied in oxidation - reduction reactions[8–10] and gold amalgam electrodes in complexation reactions.[11,12] Periodic current differential electrolytic potentiometry (p.c. DEP) has also been applied to different types of titrimetric reactions.[13,14] Bishop and Webber[13] were the first to realise that the best periodic signal titration curves can be obtained when the applied periodic wave is perfectly symmetrical. Any bias or distortion in this wave produces a deterioration of the periodic titration curve. The use of one type of bias, *viz.*, a mark-space or time bias on a periodic wave, destroys the periodic differential curve (E_Δ) but produces a d.c. component, which may itself be used to polarise the electrodes. Hence, the technique takes the name mark-space bias DEP (m.s.b. DEP).

In order to investigate the parameters related to mark-space bias DEP, Hartshorn[14] designed an instrument that is capable of producing a signal of 0–99% bias. The optimum range of percentage bias has been found to be from 5 to 50. It has also been found that the peak height of the differential curve is proportional to the logarithm of the percentage bias.[14] The fast speed by which the electrodes attain their equilibrium potentials when m.s.b DEP is applied has been attributed to two reasons[14]: firstly, that the total amount of the current applied is larger than in d.c. DEP; and secondly, that the continual reversing of the signal prevents build up of films such as oxide layers on platinum electrodes and so keeps the electrodes active.

The method of DEP has been applied exclusively in aqueous media[1–10,13] and very little work has been carried out in non-aqueous media.[14,15] This paper presents the application of d.c. DEP and m.s.b DEP in a medium that is completely free from water. Acid - base titrations in an acetic anhydride - acetic acid mixed solvent have been considered; d.c. DEP, m.s.b DEP and zero-current potentiometry were performed simultaneously in order to compare their performance.

Experimental

Apparatus

Two sources were used throughout this work to polarise the electrodes: the d.c. source and the mark-space bias source, and their construction has been described elsewhere.[14,16]

* For Part XXIV of this series, see *Analyst*, 1978, **103**, 162.

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Preparation of antimony - antimony oxide electrodes

Spectrographically pure (Johnson-Matthey) antimony was melted in a crucible in a fume cupboard. A piece of precision bore Pyrex glass tubing (diameter 4 mm), which had been pre-heated in an electric oven was clamped vertically above the surface of the molten metal and then lowered so that one end was just immersed in the antimony. A pipette-filling bulb was attached to the other end and was used to draw molten antimony up the tube to a height of about 5 cm. On cooling, the glass tubing was carefully broken to release the body of the electrode, which was then placed in a holder made from PTFE.[17] The electrodes were then activated by exposing a fresh surface using a diamond saw.[3,18] Their response was checked by a method described earlier[3,18] and they were stored in distilled water. Prior to their use the electrodes were dried with tissue paper and immersed in acetic anhydride for 6 h.

Titration cell

The method normally used for the construction of the titration cell[18] was adopted with slight modification. The new cell was designed to be air tight and to hold volumes of up to 100 ml. An external gas inlet was made in order to pass oxygen through the solution.[14] The lid was machined from 1-cm thick PTFE in order to give a firm fit and to act as a stout support for the electrodes. Various holes were made in this lid[18] to accommodate the electrodes, the burette and the non-aqueous salt bridge.

Non-aqueous double bridge

A non-aqueous double bridge was used to avoid direct contact between the aqueous salt bridge of the saturated calomel electrode (S.C.E.) and the non-aqueous solution in the titration cell. This double bridge consists of two glass tubes: one tube is of U-shape and the other of Y-shape (see Fig. 1). The diameter of the Y-shaped tube is small to enable one side to be inserted into the U-tube. The ends of the two equal length sides of the Y-tube were

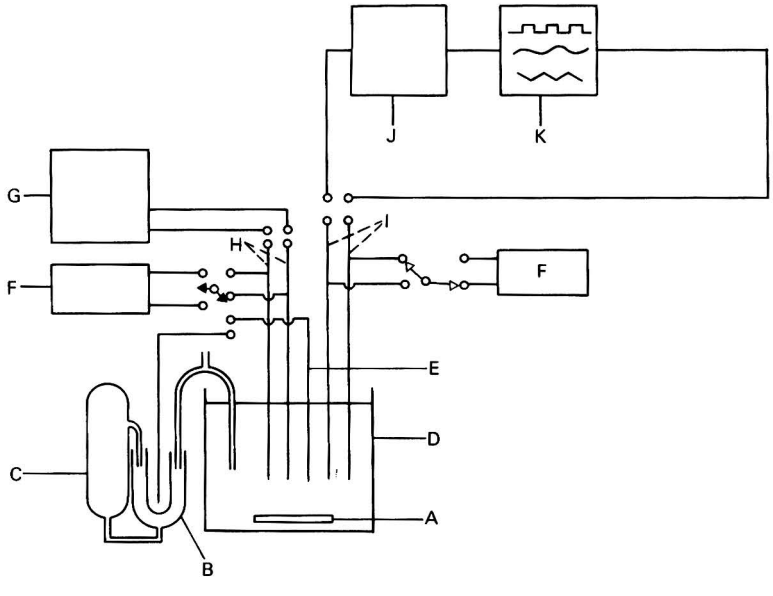

Fig. 1. General titration assembly: A, magnetic stirrer; B, non-aqueous double bridge; C, saturated calomel electrode; D, titration cell; E, zero-current electrode; F, pH meter; G, d.c. DEP unit; H, d.c. DEP electrodes; I, mark-space bias DEP electrodes; J, biasing unit; and K, waveform generator.

sealed with ceramic plugs of low leakage rate. The third side of this tube was employed as an inlet to charge the tube with the same non-aqueous solution of electrolyte as is used in the titration cell. One of the two parallel sides of the Y-tube was dipped into the solution contained in the titration cell, while the other side was immersed into one side of the U-tube. The salt bridge of the calomel electrode was dipped into the other side of the U-tube.

Time biased square wave

The square wave was produced by a Feedback Ltd. TWG 300 Test Waveform Generator and monitored.[18] The resulting symmetrical wave was then used as a signal source for the biasing unit.[14] The values of the unequal periods of the biased wave were measured by the Venner clock TSA 3314 and the percentage bias was calculated as shown in Fig. 2.

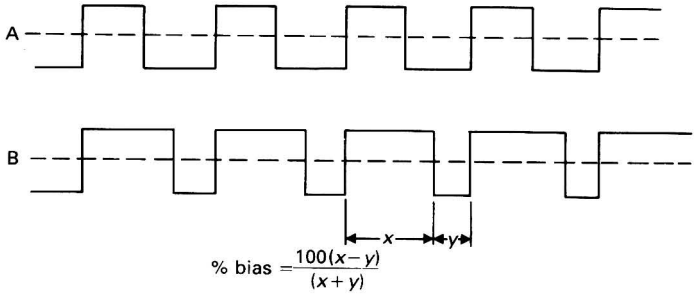

Fig. 2. (A) Symmetrical square wave; and (B) time-biased square wave. x, Duration of the positive half-cycle; and y, duration of the negative half-cycle.

Glassware

The calibrated glassware was of N.P.L. "A" grade. It was cleaned with a mixture of 25 ml of a saturated solution of AnalaR chromium(VI) oxide and 2.5 l of AnalaR sulphuric acid. After thorough washings to remove all trace amounts of adsorbed chromium species, the glassware was left to dry. Prior to its use it was rinsed several times with acetic anhydride.

Reagents

All of the chemicals used in this work were of analytical-reagent grade unless otherwise stated.

Perchloric acid solution, 0.05 M. Perchloric acid (Aristar) (72% *m/m*) was diluted with a mixture of acetic acid (AnalaR) and acetic anhydride (AnalaR) in a volume ratio of 95 + 5. The perchloric acid solution was standardised against a 0.01 M solution of potassium hydrogen phthalate in acetic anhydride. The end-point was located by both DEP and zero-current potentiometry.

Potassium hydrogen phthalate solution, 0.01 M. This solution was prepared by direct weighing of the solid and then dissolving it in 1.0 ml of distilled water. The solution was then diluted with acetic anhydride and left for 2 d before use.

Test solutions. Solutions of the following compounds were prepared by dissolving the required amount of each in acetic anhydride to give 0.01 M solutions: pyridine, 2,2′-bipyridyl, *NN*-dimethylaniline, tributylamine, quinoline, codeine, dimethyl sulphoxide, dimethylformamide, caffeine, 2,4,6-(2-pyridyl)-1,3,5-triazine (TPTZ) and sodium acetate.

Lithium chloride and tetramethylammonium bromide solutions. Both are insoluble in acetic anhydride, but 0.01 M solutions of each were prepared by dissolving the required mass in 1 ml of distilled water then adding acetic anhydride. These solutions were left standing for 2 d during which time the water reacted with acetic anhydride to give acetic acid.

Lithium perchlorate solution, 0.05 M. This solution in acetic anhydride was made by direct weighing of anhydrous lithium perchlorate, which serves as a supporting electrolyte.

General Procedure

A schematic layout is shown in Fig. 1 with the electrodes for m.s.b. DEP, d.c. DEP and

zero-current potentiometry indicated. To avoid contamination from water, the non-aqueous double bridge, with the Y-tube charged with the solution of lithium perchlorate in acetic anhydride, was used to connect the saturated calomel reference electrode to the cell. The titrant (the perchloric acid solution) was contained in a microburette. The cell was charged with 40 ml of the solution of lithium perchlorate in acetic anhydride, 10.00 ml of the titrand solution were added and the mixture titrated with the perchloric acid solution. For the purpose of comparison, d.c. DEP, m.s.b. DEP and zero-current potentiometric measurements were all made after the addition of each increment. A current density of 4×10^{-6} A cm^{-2} was used for d.c. DEP, and a mark-space bias of 28%, which gives m.s.b. peaks of about the same height as the d.c. peaks, was used for m.s.b. DEP. The frequency is without influence[13,14]; the best range is 4–110 Hz. The solution was stirred magnetically, and oxygen was passed to sustain the oxide film on the antimony electrodes.[3,4] The anode - S.C.E., cathode - S.C.E., zero-current - S.C.E. and anode - cathode (E_Δ potential) potentials of d.c. DEP were switched in sequence to and measured by means of a Corning EEL, Model 12, pH meter, and E_Δ for the m.s.b. DEP was measured on a second meter. Readings were noted when the potential drift became less than 1 mV min^{-1}.

Results and Discussion

Acetic anhydride has been found to be suitable for the titration of a variety of weak bases that are not easily titrated in other solvents.[19–21] This has been attributed to the absence in acetic anhydride of even trace amounts of water, which is basic and hence reduces the acidity of the solvated proton.[21] If perchloric acid is used as a titrant in the presence of acetic anhydride, strong acidic species will be formed.[22–25] These species will extend the acidic end of the potential range and consequently sharp titration curves will be obtained. It has been reported that these species include $H_2OOCH_3^+.ClO_4^-$ and the acetylium ion CH_3CO^+, which are stronger acids than H_3O^+.[26,27]

The enhanced polarisation current density demanded in aqueous titrimetry of weak acids or weak bases has been noted,[1,28] and has been ascribed to the need to overcome the poising of the solution when the equivalence point pH deviated from 7. The evident separation horizontally of anode and cathode from the zero-current graphs in the figures demonstrates that the current density for satisfactory differentiation in acetic anhydride is even greater, and consequently the differential peaks are somewhat less sharp. The change in solvation[29] also contributes to the higher current requirement, which is less for m.s.b. periodic than for d.c. polarisation.

Titration Curves

The usual pattern for the curves obtained by DEP, when acid - base titrations in aqueous media are involved, is as follows (Fig. 3): provided that the optimum current density is applied during the titrations, initially there is a small potential difference between the anode and cathode, and after the end-point has been reached, the potential difference again becomes small. However, in the vicinity of the end-point, the anode potential begins to diverge from the cathode and the zero-current potentials. At the end-point the difference in potential between the anode and cathode reaches its maximum, hence the peak is obtained at this point. Thereafter, the anode potential remains steady as the cathode potential increases to join that of the anode and the zero-current electrode.

The titration curves associated with this work are shown in Figs. 3–10. The normal behaviour[9] of antimony electrodes in acetic anhydride is confirmed by the titration curves of tetramethylammonium bromide in Fig. 3. It is obvious that the inflections are sharp and the differential curves of d.c. DEP (see Fig. 3, a_1) and mark-space bias DEP (Fig. 3, a_2) are smooth and symmetrical. This pattern characterises the fast electrode reactions and has been termed as type I.[9] The titration curves of lithium chloride, potassium hydrogen phthalate and sodium acetate also belong to this type as indicated by their differential curves presented in Fig. 4. These three compounds together with tetramethylammonium bromide have been reported to be strong anionic bases in acetic anhydride.[21] The general increase in their strength has been attributed to the low dielectric constant of acetic anhydride, which favours the formation of uncharged species. The smoothness and the symmetry of the differential curves (Fig. 4) are indicative of the stronger basic character of these anionic compounds in acetic anhydride.

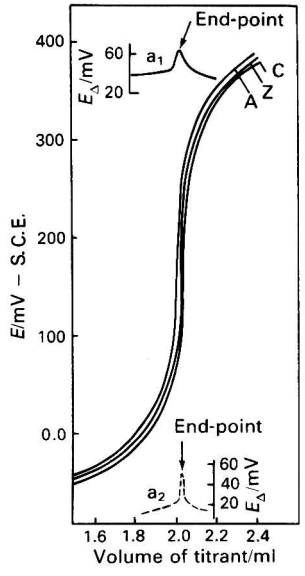

Fig. 3. Titration of 10 ml of 0.01 M tetramethylammonium bromide with 0.05 M $HClO_4$. A, Anode—saturated calomel electrode (S.C.E.) potential; Z, zero-current (indicator electrode) — S.C.E. potential; and C, cathode — S.C.E. potential. a_1, D.c. DEP differential curve, E_Δ ($E_\Delta = E_A - E_C$); a_2, mark-space bias DEP differential curve.

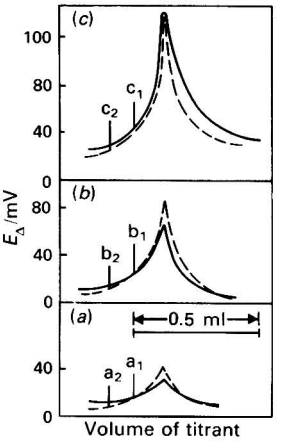

Fig. 4. Differential curves resulting from the titration of 10 ml of 0.01 M of (*a*) lithium chloride, (*b*) potassium hydrogen phthalate and (*c*) sodium acetate with 0.05 M $HClO_4$. a_1, a_2, D.c. DEP and m.s.b. DEP differential curves of lithium chloride; b_1, b_2, d.c. DEP and m.s.b. DEP differential curves of potassium hydrogen phthalate; and c_1, c_2, d.c. DEP and m.s.b. DEP differential curves of sodium acetate.

Neutral molecules in acetic anhydride are known to be generally of lower basicity than anionic species.[21] However, most of the neutral compounds in this work demonstrated considerable basic character, which resulted in successful titrations. The titration curves of quinoline are shown in Fig. 5. The shapes of these curves indicate that antimony electrodes behave in a more or less similar manner in both aqueous[3] and non-aqueous media. The crossing of zero-current (Z) and anode (A) curves in Fig. 5 is due to the change in slope of the zero-current electrode,[30] and the two curves being close enough together to show the effect.

Fig. 6 presents the differential curves obtained for tributylamine, codeine and *NN*-dimethylaniline. It can be seen that these curves are almost symmetrical, except for a slight asymmetry shown by the d.c. DEP differential curve of *NN*-dimethylaniline (Fig. 6, c_2). This asymmetry, which is attributed to the high overpotential of the anode, becomes more significant in the differential curves of pyridine and 2,2′-bipyridyl in Fig. 7. The observed increase in the overpotential of the anode might be due to the thickening of the oxide film at the anode surface. There is less marked asymmetry in the mark-space bias differential curves than in d.c. DEP curves. The reason for this can be attributed to the continual reversing of the signal, which prevents build-up of films on the surfaces of the electrodes and this keeps the electrodes more active.

Fig. 8 illustrates the titration curves of dimethyl sulphoxide. The anode in this instance behaves as if it were less active than with the other titrands. Repeating the titration with freshly activated electrodes gave the same type of titration curves. Owing to the anode behaviour, the differential curves have changed from the first type (completely symmetrical) to the second type (Z-shaped), which has been previously termed "falling Z."[9] The latter type of curves characterise slow electrode reactions. The weak basic character of dimethyl sulphoxide in acetic anhydride is probably the main reason for this abnormality.

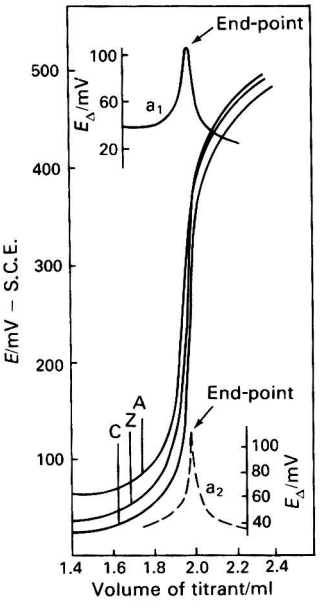

Fig. 5. Titration of 10 ml of 0.01 M quinoline with 0.05 M $HClO_4$. For meaning of symbols, see Fig. 3.

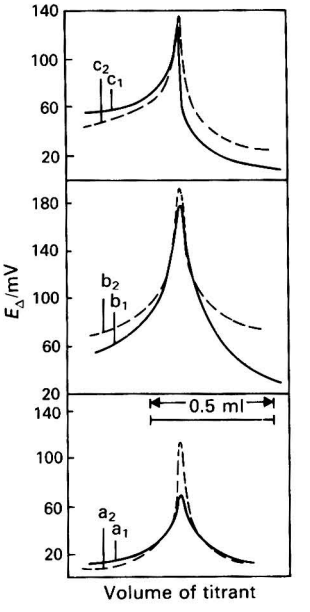

Fig. 6. Titration of 10 ml of 0.01 M base with 0.05 M $HClO_4$. Differential curves of codeine (a_1, a_2), tributylamine (b_1, b_2) and *NN*-dimethylaniline (c_1, c_2).

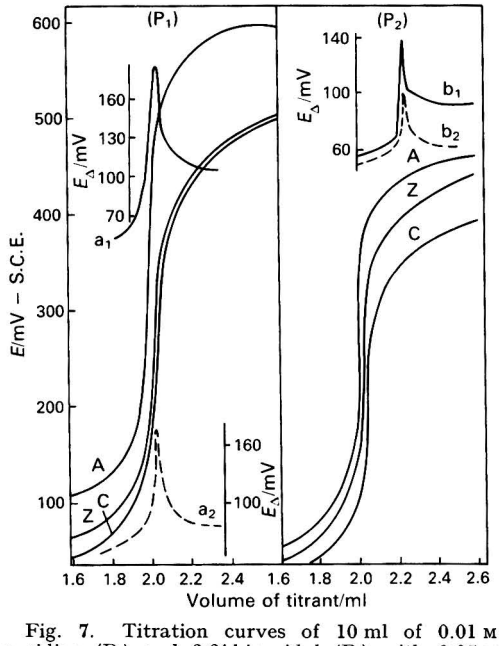

Fig. 7. Titration curves of 10 ml of 0.01 M pyridine (P_1) and 2,2′-bipyridyl (P_2) with 0.05 M $HClO_4$. For meaning of symbols, see Fig. 3.

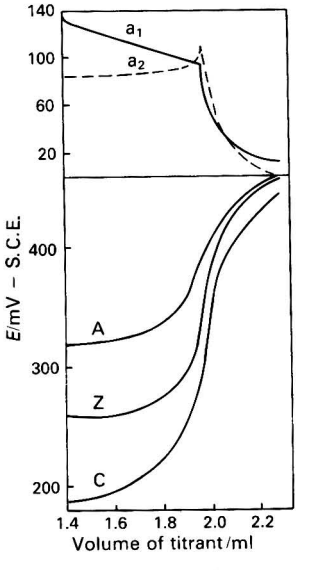

Fig. 8. Titration of 10 ml of 0.01 M dimethyl sulphoxide with 0.05 M $HClO_4$. For meaning of symbols, see Fig. 3.

Titrations of caffeine and 2,4,6-(2-pyridyl)-1,3,5-triazine (TPTZ) have been found to be difficult to carry out in acetic anhydride. As seen in Fig. 9, the curves are poor with small inflections but the end-points can be easily located from the corresponding differential curves. It was noted during these titrations that the potential spans of the inflections were compressed. It is possible that this is due to the narrow potential range of acetic anhydride and to its levelling effect on these weak bases. On carrying out these titrations in a mixture of 1 + 1 *V*/*V* acetic anhydride and toluene, a great improvement is observed. This is shown in Fig. 10 where the titration curves of TPTZ (Fig. 9, P_2) are normal with sharp inflections. The titration curves of caffeine (Fig. 9, P_1), although they exhibit sharp inflections, also show that the differences between the zero-current potential and the anode or cathode potentials are larger than usual prior to the end-point. This behaviour is in turn reflected in the shape of the differential curves. The behaviour of antimony electrodes may be due to the amphoteric nature of caffeine and to adsorption on the surfaces of the electrodes.

The use of toluene, which is an aprotic solvent, is responsible for the observed improvements in the titrations of TPTZ and caffeine. Aprotic solvents possess wide potential ranges and, owing to their generally low dielectric constants, they have the tendency to suppress the solubility of the product species. Hence, the buffer zones of the titration curves will decrease and sharp inflections will result even if weak acids or bases are involved.

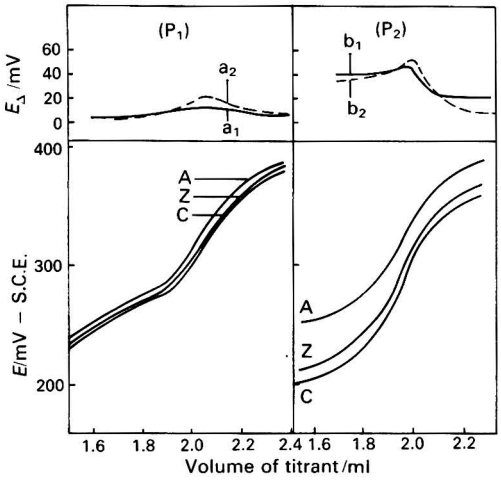

Fig. 9. Titration curves of 10 ml of 0.01 M caffeine (P_1) and TPTZ (P_2) with 0.05 M $HClO_4$. For meaning of symbols see Fig. 3.

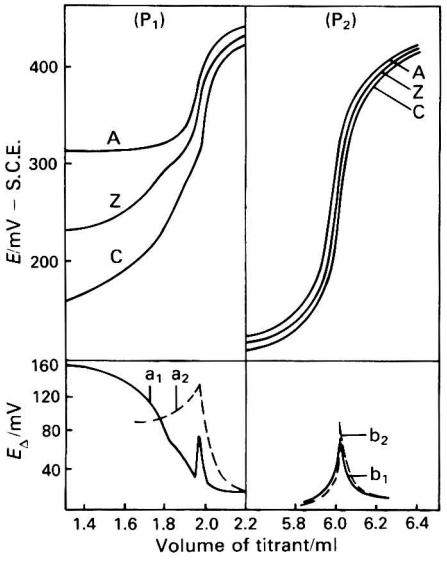

Fig. 10. Titration of 10 ml of 0.01 M caffeine (P_1) and TPTZ (P_2) in a 1 + 1 *V*/*V* mixture of toluene and acetic anhydride. For meaning of symbols, see Fig. 3.

Response Time

The response time required for the zero-current electrode to reach its equilibrium was found to be long. In the vicinity of the end-point a slow drift was usually observed. More than 15 min were required to reach an acceptable drift rate; with d.c. DEP 10 min were usually enough to reach the equilibrium potentials. In m.s.b. DEP the electrodes respond faster than in d.c. DEP. In most of the titrations the equilibrium potentials were reached within 2 min or less. This indicates the great advantage of m.s.b. DEP over both d.c. DEP and zero-current potentiometry. It is most likely that the shorter response time of m.s.b. electrodes is directly related to the continual reversing of the signal, which restrains the build-up of oxide layer on the surfaces of the electrodes. Accordingly, the electrodes will be always active.

Analytical Validity

In all titrations the m.s.b. peak, the d.c. peak and the zero-current inflection were in complete accord; the error by both DEP methods is therefore nil.

The analytical validity of these methods was further investigated. Six identical samples of potassium hydrogen phthalate solution were titrated under similar conditions. A standard deviation of 0.014 ml was obtained, which proved to be independent of the equivalence volume of from 1 to 10 ml. This value is generated by manipulation and is within the experimental range of high precision titrimetry.

Conclusions

It seems pertinent to conclude that the DEP technique is as applicable to acid - base titrimetric reactions in non-aqueous media as it is in aqueous media. Mark-space bias DEP minimises the time required to perform the titration because the continual reversing of the signal keeps the electrodes more active. For the first time, the behaviour of polarised antimony electrodes in acetic anhydride has been investigated. In addition, the feasibility of the aprotic solvents in improving the titrations of weak bases has been examined.

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NOTE—References 1, 2, 3, 4, 5, 6, 9, 10, 13 and 18 are to Parts VI, VII, XIV, XV, V, VIII, II, III, XXIII and XXI of this series, respectively.

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Flow Injection Voltammetric Determination of Nitrite by Reduction at a Glassy Carbon Electrode in Acidic Bromide or Chloride Media

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Nitrite can be determined by reduction at a glassy carbon electrode held at +0.3 V *versus* a saturated calomel electrode by flow injection voltammetry at concentrations of greater than 1×10^{-6} M by direct injection of sample solution (25 μl) into an eluent 3.2 M in hydrochloric acid and 20% *m/V* in potassium bromide. Alternatively, the nitrite may be pre-reacted with acidic bromide before injection and determined at concentrations above 1×10^{-7} M. The use of an acidic chloride medium is less satisfactory.

Keywords: *Flow injection analysis*; *nitrite determination*; *voltammetry*

In developing an automated amperometric micro-titration technique for the determination of low concentrations of pharmaceutically important sulphonamides by diazotisation with nitrite, Coenegracht *et al.*[1] investigated the use of the biamperometric end-point detection method that is commonly used for determinations at higher levels of sulphonamide.[2] Extrapolation of current *versus* volume graphs is necessary at low concentrations in order to obtain satisfactory precision, but unfortunately the biamperometric graphs after the end-point were curved. They then studied the system monoamperometrically using a rotating platinum working electrode and showed that for a 5×10^{-6} M solution of nitrite that is 1 M in hydrochloric acid and 20% *m/V* in potassium bromide there is no potential range over which zero current is obtained. Instead, a composite oxidation - reduction wave is obtained with zero current at +0.6 V *versus* a saturated calomel electrode (S.C.E.). The anodic part of this wave is due to the oxidation of bromide and the cathodic part is due to the reduction of nitrosyl bromide formed by reaction of nitrite and bromide. At these high levels of bromide the current on the anodic side is not limited, but a well formed cathodic voltammetric wave is given by nitrosyl bromide. The procedure adopted by Coenegracht *et al.*[1] involved titrating the sulphonamide in 1 M hydrochloric acid solution containing 25% *m/V* potassium bromide with standard 0.001–0.05 M nitrite solution whilst monitoring the reduction current of nitrite, or rather nitrosyl bromide, at a rotating platinum electrode held at +0.35 V *versus* an S.C.E.

Coenegracht *et al.*[1] also showed that in a 5×10^{-6} M solution of nitrite that is 1 M in hydrochloric acid and 20% *m/V* in sodium chloride zero current was obtained over the potential range +0.7 to +0.9 V. Oxidation of chloride occurred above +0.9 V and reduction of nitrite below +0.7 V, but the reduction wave due to nitrosyl chloride was not as well formed as that due to nitrosyl bromide.

Although the end-point detection method of Coenegracht *et al.* was not adapted by them to the direct determination of nitrite it clearly can be used for this purpose. In this paper the solution conditions have been optimised for the determination of nitrite using voltammetric flow injection analysis at a glassy carbon electrode.

Experimental

Flow of eluent was produced with an Ismatec Mini-S peristaltic pump, and injections (25 μl) were made with a Rheodyne injection valve (5020). The injection valve was connected to a Metrohm detector cell (EA 1096), fitted with a glassy carbon electrode (EA 286), by means of suitable lengths of 0.58 mm bore tubing. The detector cell was used as described previously,[3–5] partially immersed in 0.01 M sulphuric acid with the counter and reference electrodes removed. Electrical contact from a platinum counter electrode and a potentiometric calomel reference electrode to the sulphuric acid was made by means of salt bridges. It was found to be unnecessary to de-gas the eluent. The potential of the glassy carbon electrode was held at +0.30 V *versus* an S.C.E., except as indicated otherwise, using a

PAR-174 polarographic analyser (Princeton Applied Research). Current signals were monitored on a Tarkan 600 Y - t recorder. Coefficients of variation (3–10 measurements) were generally less than 1%.

Preliminary Studies

Coenegracht *et al.*[1] obtained satisfactory voltammograms at a rotating platinum electrode for 5×10^{-6} M nitrite in 1 M hydrochloric acid and 20% m/V potassium bromide solution, and in 1 M hydrochloric acid and 20% m/V sodium chloride solution, and these solution conditions were adopted here for preliminary investigation. Initial studies were made at the 2×10^{-4} M level of nitrite.

The chloride system was studied first. Injections of nitrite solution, with added sodium chloride, into hydrochloric acid and of a pre-reacted nitrite - sodium chloride - hydrochloric acid solution into acidic chloride eluent were made. For the injection of a neutral nitrite solution containing chloride into hydrochloric acid of different concentrations a double peak indicating incomplete reaction was observed at sodium chloride concentrations of less than 15%, but only a single peak was observed at 15%. The peak height increased with increasing acidity from 0.01 to 2 M hydrochloric acid. At a concentration of hydrochloric acid of 5 M, sodium chloride precipitated and a double peak was observed above 3.8 M. Thus, it appeared that 2 M hydrochloric acid with 18% m/V sodium chloride was the optimum solution condition. The length of the delay coil was optimised at 4 m during this development: a double peak was observed with a 2-m length.

The study of injecting nitrite pre-reacted with acidic chloride indicated that complete reaction was reached rapidly, no difference in peak height being observed between solutions kept for up to 40 min before injection and those injected immediately after mixing. Comparison of peak heights with a 4-m delay coil for neutral nitrite and pre-reacted nitrite injection indicated that the yield in the former was 74% of that in the latter.

Whereas solutions in which nitrite reacted with acidic chloride remained colourless, those in which nitrite reacted with acidic bromide became increasingly darker yellow with time. Furthermore, the injection of these pre-reacted solutions gave a voltammetric signal which was some ten times larger than that obtained with the chloride system and which increased continuously and markedly for pre-reaction times of up to 40 min. The injection of neutral nitrite containing potassium bromide into acid, however, gave a voltammetric signal of the same order of magnitude as for the chloride system. For the pre-reacted system increased acidity gave more rapid formation of colour and signal. At 20% m/V potassium bromide concentration and 3.2 M hydrochloric acid the voltammetric peak obtained was 18.5 μA after 5 min and 23 μA after 20 min. The optimised eluent for direct injection of neutral nitrite was found to be 2 M hydrochloric acid with 20% m/V potassium bromide.

Attention was then directed to obtaining an indication of the detection limit of the bromide and chloride systems. Measurable peaks were obtained for 1×10^{-5} M nitrite solutions for direct injection of nitrite into the chloride system and for a 1×10^{-6} M solution for injection of pre-reacted acidic chloride solution. For the bromide system 1×10^{-6} M of nitrite was detectable by direct injection. A much lower limit was expected from the pre-reacted system in view of the large increase in yield of nitrosyl bromide on standing the pre-reacted 1×10^{-4} M nitrite solution for 40 min, but in practice the signal, surprisingly, disappeared rather abruptly at nitrite concentrations of 1×10^{-6} M and less. This seemed to indicate that, although rapid formation of nitrosyl bromide occurs at high acidity, low concentrations of nitrosyl bromide are unstable in this medium. The use of deoxygenated eluent and sample solutions did not prevent the loss of the signal.

Further study indicated that an approximately 10-fold dilution of the acidic bromide reagent made possible the determination of 1×10^{-7} M bromide in sample solutions using the pre-reaction method.

Determination of Nitrite in Acidic Bromide or Chloride Media

Reagents

Standard sodium nitrite solution, approximately 1×10^{-2} M. Dissolve approximately 0.172 g of analytical-reagent grade sodium nitrite, accurately weighed, in water and dilute

to 250 ml in a calibrated flask. This solution is 1×10^{-2} M in nitrite. Prepare more dilute standard solutions from this solution.

Hydrochloric acid, 0.3 M. Dilute 13.8 ml of concentrated hydrochloric acid to 500 ml with water.

Acidic bromide solution, 20% m/V in potassium bromide and 3.2 M *in hydrochloric acid.* Dissolve 100 g of potassium bromide in 350 ml of water, add 138 ml of concentrated hydrochloric acid, cool the solution, dilute to 500 ml and mix.

Acidic chloride solution, 18% m/V in sodium chloride and 2 M *in hydrochloric acid.* Dissolve 90 g of sodium chloride in 400 ml of water, add 86 ml of concentrated hydrochloric acid, cool the solution, dilute to 500 ml and mix.

Sodium chloride solution, 30% m/V. Dissolve 150 g of sodium chloride in water and dilute to 500 ml with water.

Procedures

Direct injection into acidic bromide eluent. Inject 25 μl of nitrite sample or standard solution (1×10^{-6}–1×10^{-4} M) into the acidic bromide solution and note the reduction current signal. Use a 4-m delay coil.

Injection of nitrite pre-reacted with acidic bromide solution. Transfer by pipette an aliquot of less than 45 ml of nitrite sample or standard solution (1×10^{-7}–1×10^{-4} M) into a 50-ml calibrated flask. Add 4 ml of acidic bromide solution, mix the solution and allow it to stand for 20 min. Dilute to 50 ml, mix and inject 25 μl into 0.3 M hydrochloric acid solution. Note the reduction current signal. Use a 3-m delay coil.

Direct injection into acidic chloride eluent. Inject 25 μl of nitrite sample or standard solution (1×10^{-5}–5×10^{-4} M) into acidic chloride solution and note the reduction current signal. Use a 3-m delay coil.

Injection of nitrite pre-reacted with acidic chloride solution. Place 30 ml of 30% *m/V* sodium chloride solution and 8.6 ml of concentrated hydrochloric acid (by calibrated pipette) in a 50.0-ml calibrated flask. Add, by pipette, an aliquot of less than 10 ml of nitrite sample or standard solution (1×10^{-5}–5×10^{-4} M), dilute to 50 ml with water and mix. Inject 25 μl into 0.3–1 M hydrochloric acid and note the reduction current signal. Use a 3-m delay coil.

Results

Results in Table I show the effect on the signal obtained of the amount of acidic bromide reagent added to various levels of nitrite before injection into 0.3 M hydrochloric acid. The instability of the reductand at lower concentrations and in higher acidity solutions is seen clearly. The use of 4 ml of reagent was adopted as this gave a rectilinear signal over three orders of magnitude from 2×10^{-7} to 2×10^{-4} M nitrite. This is illustrated by the signals shown in Fig. 1. The length of the delay coil of between 1 and 4 m has a relatively small effect on peak height although the peaks become broader. The peak heights at different delay-coil lengths are given in Table II, which also includes the flow-rates obtained. These flow-rates apply to all the results given in this paper.

TABLE I

PRE-REACTED ACIDIC BROMIDE SYSTEM: EFFECT OF VOLUME OF ACIDIC BROMIDE REAGENT ADDED TO GIVE 50 ml OF PRE-REACTED SOLUTION ON THE REDUCTION CURRENT SIGNAL AT VARIOUS LEVELS OF NITRITE CONCENTRATION

Delay-coil length = 3 m; results are the reduction current signal in microamps.

Volume of acidic bromide reagent added/ml	Equivalent concentration of nitrite in pre-reacted solution/M			
	2×10^{-4}	2×10^{-5}	2×10^{-6}	2×10^{-7}
2	2.50	0.30	0.03	0.009
4	12.0	1.18	0.118	0.011
6	13.0	1.40	0.070	0
8	12.4	1.50	0	0

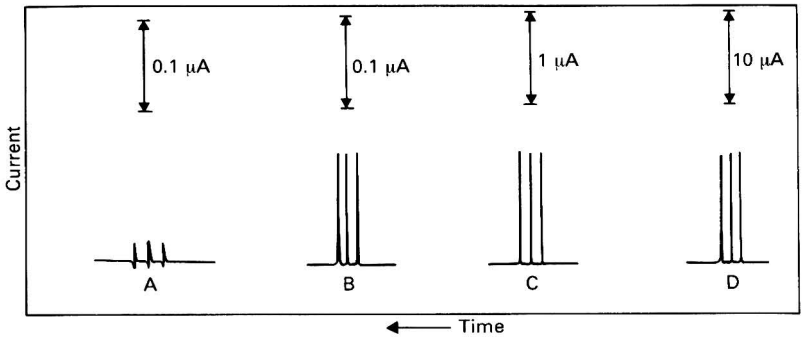

Fig. 1. Injection of nitrite pre-reacted with acidic bromide solution using recommended procedure. Equivalent nitrite concentration in pre-reacted solution injected: A, 2×10^{-7}; B, 2×10^{-6}; C, 2×10^{-5}; and D, 2×10^{-4} M.

Table II

Pre-reacted acidic bromide system: effect of delay-coil length on signals obtained and on the flow-rate using the recommended procedure

Equivalent nitrite concentration = 2×10^{-5} M.

Parameter	Delay-coil length/m					
	0.5	1.0	2.0	3.0	4.0	5.0
Peak current/μA	1.70*	1.40	1.36	1.26	1.04	0.85
Flow-rate/ml min^{-1}	9.6	8.0	5.2	4.2	3.3	2.9

* Double peak.

The effect of the acid concentration of eluent containing 20% m/V potassium bromide, and that of the potassium bromide concentration of the eluent 3.2 M in hydrochloric acid, on the signal obtained on the direct injection of nitrite are shown in Tables III and IV. The effect of the delay-coil length on the signal for direct injection into the optimised eluent is shown in Table V. A 4-m coil is required to remove the double peak but otherwise there is little change in peak height between 0.5 and 4 m. Signals obtained for direct injection of 2×10^{-6}–2×10^{-4} M nitrite solutions are shown in Fig. 2.

Signals obtained for the injection of nitrite pre-reacted in acidic chloride solution are shown in Fig. 3. The effect of the acid concentration of eluent containing 18% m/V sodium chloride, and that of the sodium chloride concentration of the eluent 2 M in hydrochloric acid, on the signal obtained on direct injection of nitrite are shown in Tables VI and VII. The effect of delay-coil length on the signal for the direct injection into the optimised eluent is shown in Table VIII.

Table III

Direct injection into acidic bromide: effect of acid concentration of eluent on signal obtained

Potassium bromide concentration = 20% m/V and nitrite concentration = 2×10^{-4} M.

Hydrochloric acid concentration/M	1	2	3	3.2	3.4
Peak current/μA	1.90*	3.90*	5.30	6.90	6.60

* Double peaks.

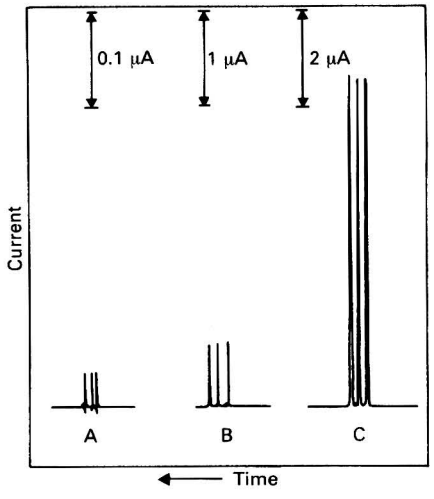

Fig. 2. Direct injection into acidic bromide solution using recommended procedure. Equivalent nitrite concentration in injection solution: A, 2×10^{-6}; B, 2×10^{-5}; and C, 2×10^{-4} M.

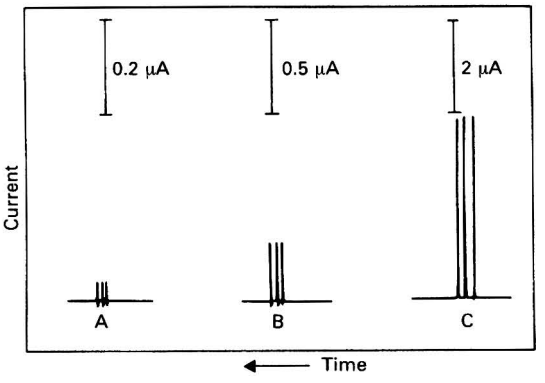

Fig. 3. Injection of nitrite pre-reacted with acidic chloride solution. Equivalent nitrite concentration in pre-reacted solution injected: A, 2×10^{-6}; B, 2×10^{-5}; and C, 2×10^{-4} M.

TABLE IV

DIRECT INJECTION INTO ACIDIC BROMIDE: EFFECT OF POTASSIUM BROMIDE CONCENTRATION ON SIGNAL OBTAINED

Hydrochloric acid concentration = 3.2 M and nitrite concentration = 2×10^{-5} M.

Potassium bromide concentration, % *m*/*V*	5.0	10.0	15.0	20.0	25.0
Peak current/μA	0.30	0.41	0.49	0.69	Ppt*

* Ppt = precipitate.

TABLE V

DIRECT INJECTION INTO ACIDIC BROMIDE: EFFECT OF DELAY-COIL LENGTH ON SIGNAL OBTAINED USING RECOMMENDED ELUENT

Nitrite concentration = 2×10^{-4} M.

Delay-coil length/m	0.5	1.0	2.0	3.0	4.0	5.0
Peak current/μA	6.50*	6.40*	6.40*	6.50*	6.90	5.60

* Double peak.

TABLE VI

DIRECT INJECTION INTO ACIDIC CHLORIDE: EFFECT OF ACID CONCENTRATION OF ELUENT ON SIGNAL OBTAINED

Sodium chloride concentration = 18% *m*/*V* and nitrite concentration = 2×10^{-4} M.

Hydrochloric acid concentration/M	0.5	1.0	1.5	2.0
Peak current/μA	0.20	0.26	0.50	1.60

TABLE VII

DIRECT INJECTION INTO ACIDIC CHLORIDE: EFFECT OF SODIUM CHLORIDE CONCENTRATION OF ELUENT ON SIGNAL OBTAINED

Hydrochloric acid concentration = 2 M and nitrite concentration = 2×10^{-4} M.

Sodium chloride concentration, % *m*/*V* ..	3.0	6.0	9.0	12.0	15.0	18.0	20.0
Peak current/μA	0.38	0.50	0.70	0.85	1.12	1.30	Ppt*

* Ppt = precipitate.

TABLE VIII

DIRECT INJECTION INTO ACIDIC CHLORIDE: EFFECT OF DELAY-COIL LENGTH ON SIGNAL OBTAINED USING RECOMMENDED ELUENT

Nitrite concentration = 2×10^{-4} M.

Delay-coil length/m	0.5	1.0	2.0	3.0	4.0
Peak current/μA	3.50*	3.00*	2.50*	1.50	0.70

* Double peak.

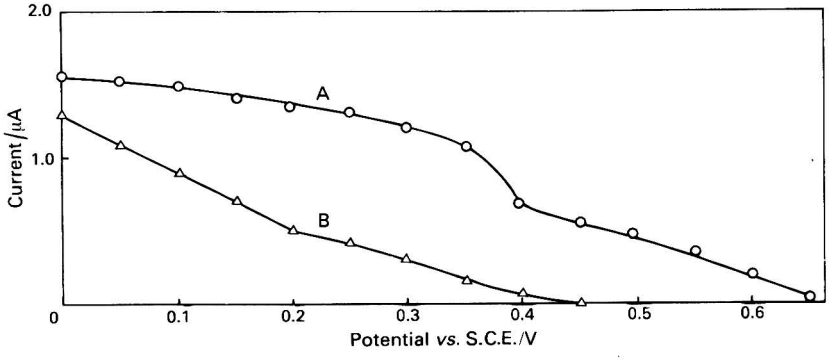

Fig. 4. Effect of the potential of the glassy carbon electrode on the signal obtained for injection of nitrite pre-reacted with (A) acidic bromide and (B) acidic chloride into 0.3 M hydrochloric acid. Equivalent nitrite concentration: 2×10^{-5} M.

The effect of the potential of the glassy carbon electrode on the signal obtained for nitrite injected after pre-reaction with acidic bromide or chloride into 0.3 M hydrochloric acid solution is shown in Fig. 4.

Discussion

Nitrite down to a level of 1×10^{-6} M can be determined very conveniently by flow injection voltammetry at a glassy carbon electrode by direct injection into an acidic bromide solution. By injecting nitrite pre-reacted with acidic bromide into 0.3 M hydrochloric acid solution the determination can be extended to the 1×10^{-7} M level. A high potassium bromide concentration (20% *m*/*V*) is used in the eluent for the direct injection procedure, and therefore the use of the pre-reacted system that uses a lower concentration of bromide would allow a considerable saving of reagent. Clearly, pre-reaction could be carried out in a smaller volume than that used in the recommended procedure (50 ml).

As well as giving a lower detection limit, the acidic bromide medium has the additional advantage that adsorption is not a problem with this system. The glassy carbon electrode can be used extensively with this system without the need to clean or polish it. A glassy carbon electrode used with the acidic chloride system requires more frequent cleaning, *e.g.*, after approximately 5 and 10 injections at the 1×10^{-4} and 1×10^{-5} M levels, respectively.

In the reaction of nitrite with bromide and chloride in acidic media an equilibrium appears to be set up, and clearly in the procedures recommended here conversion of nitrite into nitrosyl bromide or nitrosyl chloride is incomplete. More extensive formation of nitrosyl bromide was shown to be obtained at the 1×10^{-4} M level, for example, by increasing the acidity and bromide concentration even higher.

This study is now being extended to examine the possibility of determining amines by flow injection voltammetry indirectly by the loss of the nitrite signal on diazotisation.

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Determination of Chemical Oxygen Demand of Wastewaters Without the Use of Mercury Salts

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The use of silver nitrate solution to suppress chloride interference in the chemical oxygen demand test is described. Chlorides in wastewater are precipitated as silver chloride and in this form are only slightly oxidised. The proposed procedure obviates the use of toxic mercury(II) sulphate and is similar to the standard procedure in accuracy and reproducibility of results over a wide range of chloride concentrations.

Keywords: Chemical oxygen demand without mercury; wastewaters

The chemical oxygen demand (COD) test is widely used to determine the organic content of wastewater. The earliest form of the COD test was described by Adeney and Dawson[1] but modern procedures are based on the work of Muers,[2] who introduced the use of silver sulphate to catalyse the oxidation of volatile carboxylic acids. Moore *et al.*[3,4] applied the COD test to general wastewater analysis.

All of the authors cited above discussed the interference arising from the oxidation of chloride by dichromate.

$$Cr_2O_7^{2-} + 6Cl^- + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O \quad .. \quad .. \quad (1)$$

Moore *et al.*[3] suggested that the theoretical COD, attributable to chloride oxidation, could be deducted from the observed COD. Cameron and Moore[5] demonstrated that this theoretical deduction gave inaccurate results for standard solutions of organic compounds spiked with chloride. The deduction procedure was also criticised by Dobbs and Williams,[6] who pointed out that its use frequently overestimated the contribution of chloride oxidation to effluent COD and gave rise to negative results in certain instances. They introduced the use of mercury(II) sulphate as a complexing agent to decrease the concentration of free chloride ions and thus reduce the availability of chloride for reaction with dichromate. Dobbs and Williams somewhat overestimated the efficiency of mercury(II) in preventing chloride oxidation and claimed complete suppression of chloride interference when using a ratio of 4 mg of mercury(II) sulphate to 1 mg of chloride. Cripps and Jenkins[7] demonstrated that even at a ratio of 10 to 1, approximately 7% of the chloride was oxidised. Modern standard methods[8,9] are based on the work of Cripps and Jenkins.

Surprisingly little importance appears to have been attached to the environmental significance of the COD test, in which 0.2–0.4 g of mercury salts are used, depending on the procedure adopted. As mercury salts are highly toxic,[10] it would be desirable to find an alternative means of suppressing chloride interference. Cripps and Jenkins[7] reported a significant reduction in chloride oxidation when using a ten-fold increase in silver sulphate concentration. They did not develop the procedure because of high cost and poor precision. Zietz[11] has described the use of silver nitrate in the COD analysis of pure organic compounds in saline solutions. His procedure was carried out in a 500-ml sealed flask, in an oven at 140 °C. Chlorides were precipitated with an equivalent concentration of silver nitrate solution before adding dichromate and sulphuric acid - silver sulphate solutions. The silver ions in the latter reagent served to suppress dissociation of silver chloride and hence also reduced chloride oxidation. It is likely that low chloride oxidation should also be attributed to the use of an 0.05 N dichromate solution for certain samples. It has been observed[12] that although the sealed tube and standard reflux COD methods show different responses to chloride oxidation, the difference is only 7% at a level of 1000 mg l^{-1} of chloride. It was therefore deduced that the mechanism controlling chloride oxidation might also serve to minimise chloride interference in a standard reflux procedure for COD. Such a procedure would be attractive because of the comparatively low toxicity of silver compounds in biological systems[13] and the ease of recovery of silver from waste solutions.[9]

Experiments have been carried out which showed that a more efficient use of silver salts was obtained by omitting silver sulphate from the sulphuric acid catalyst solution. All silver ions were then added as silver nitrate solution. The concentration of silver nitrate has been selected to give results comparable with those of the UK standard procedure,[9] for samples containing up to 1000 mg l^{-1} of chloride or having a chloride to COD ratio of 3 or less.

Experimental

Apparatus

The Tecator DS 20 block digester and water-cooled reflux condenser system were used. Clean new apparatus before use by refluxing with the sulphuric acid - dichromate mixture for 4 h. Cap the open tops of the condensers with 50-ml tall-form beakers. After cleaning, dismantle the apparatus and rinse with de-ionised water.

Reagents

Use analytical-reagent grade reagents throughout. Use distilled or de-ionised water with a suitably low COD for all reagents and blanks.

Sulphuric acid. AnalaR grade (d_{20} 1.84).

Silver nitrate solution, 25% m/V. Dissolve 25 $\pm$ 0.5 g of silver nitrate in water and dilute to volume in a 100-ml calibrated flask. Store this solution in amber-glass bottles and use each batch within 2 weeks.

Potassium dichromate solution, 0.125 N. Dissolve 6.129 g of potassium dichromate (dried at 140 °C for 1 h) in water and dilute to volume in a 1-l calibrated flask. Renew this solution after 4 weeks.

Potassium dichromate solution, 0.0625 N. Dissolve 3.065 g of potassium dichromate (dried at 140 °C for 1 h) in water and dilute to volume in a 1-l calibrated flask. Renew this solution after 4 weeks.

Ferroin indicator solution, 0.025 M. 1,10-Phenanthroline - iron(II) sulphate complex solution, obtained from BDH Chemicals Ltd.

Ammonium iron(II) sulphate solution, 0.025 N. Dissolve 9.80 $\pm$ 0.01 g of ammonium iron(II) sulphate in about 100 ml of water. Carefully add 20 $\pm$ 0.5 ml of AnalaR sulphuric acid (d_{20} 1.84), cool and dilute to volume in a 1-l calibrated flask. Standardise this solution before starting each batch of analyses, using the procedure described below.

Anti-bumping granules. Obtained from BDH Chemicals Ltd. Reflux with acidic dichromate for 2 h and wash with water before use.

Procedure

Standardisation of 0.025 N *ammonium iron(II) sulphate solution*

Dispense potassium dichromate (5 $\pm$ 0.05 ml, 0.125 N) into a 250-ml Erlenmeyer flask and dilute to approximately 50 ml with water. Carefully add sulphuric acid (15 $\pm$ 0.25 ml, d_{20} 1.84), mix and cool to ambient temperature. Add ferroin indicator solution (2 drops) and titrate with the ammonium iron(II) sulphate solution. The end-point colour change is from pale blue to red. Calculate the normality (N) of the ammonium iron(II) sulphate solution from

$$N = \frac{0.625}{V}$$

where V = ammonium iron(II) sulphate titre.

Samples containing up to 1000 mg l^{-1} *of chloride or having a chloride to COD ratio of* 3:1 *or less**

Pipette 10 $\pm$ 0.1 ml of sample having a COD of less than 450 mg l^{-1} (or an appropriate volume of stronger sample diluted to 10 ml) into a digestion tube. Add a few anti-bumping granules and 1.0 $\pm$ 0.1 ml of 25% *m/V* silver nitrate solution. Mix and allow to stand for between 5 and 15 min. Add 5 $\pm$ 0.05 ml of 0.125 N potassium dichromate solution.* Mix

* For samples with a COD of less than 200 mg l^{-1}, use 5 $\pm$ 0.05 ml of 0.0625 N dichromate solution.

and carefully add 15 ± 0.25 ml of sulphuric acid (d_{20} 1.84). Place the rack of digestion tubes into the heating block, which has been previously heated to 190 °C,* and position the condenser assembly. Reflux for 2 h (±5 min). Allow to cool for not less than 10 min and then remove the condenser assembly.† Carefully remove the rack of digestion tubes and stand on a heat-proof surface, in the fume cupboard, until emission of white fumes has ceased. Immerse the tubes in cold water until the contents are cold and then add 25 ± 1 ml of water and a magnetic stirrer bar. Titrate the residual dichromate as described above, stirring the contents of the digestion tube on a magnetic stirrer. Carry out duplicate blanks using 10 ± 0.1 ml of water in place of the sample.‡

Caution—Oxides of sulphur and other toxic gases are emitted during addition of sulphuric acid and during refluxing. Use the block digester and add sulphuric acid in a fume cupboard. Examine digestion tubes carefully before use and reject any showing cracks.

Calculation

$$\text{COD (mg l}^{-1}) = \frac{8000\,N}{S_v}\,(V_B - V_S)$$

where V_B = blank titre, V_S = sample titre and S_v = sample volume.

Performance Characteristics of Proposed Procedure

Precision

The precision of the procedure described above was determined for real samples and standard potassium hydrogen phthalate solutions. Within-batch precision was determined from repeat analyses of samples. Total precision of potassium hydrogen phthalate COD analysis was determined from analyses of pairs of samples on four different days by different analysts. Because of the instability of real samples, the total precision error in sewage COD analysis was determined from the differences between duplicate analyses of similar strength sewage samples. These were analysed on five different days by different analysts. A similar procedure is described in the A.O.A.C. statistical manual[14] which relies on the assumption that the precision does not change over the range of concentrations studied. Any error introduced by this assumption applies to both procedures and the data presented are certainly of comparative, if not absolute value. The performance of the method has been compared with the current UK standard method.[9] Results of precision tests are presented in Table I.

Bias

Wastewater analyses

Duplicate samples of sewage and wastewaters were analysed by the proposed and standard procedures.[9] The results are presented in Table II.

Spiking with sodium chloride

AnalaR sodium chloride (ashed for 1 h at 500 °C) was used to prepare all chloride solutions. Samples spiked with sodium chloride were analysed by the proposed and standard procedures. A mercury(II) sulphate to chloride ratio of 40 to 1 was maintained in all analyses carried out by the standard procedure on samples containing more than 500 mg l^{-1} of chloride. Mercury(II) sulphate solution (1 ml; 20% *m*/*V* in 10% *V*/*V* sulphuric acid) was used for samples containing 500 mg l^{-1} of chloride or less. The results of these analyses are presented in Fig. 1 and Table III.

* This temperature setting should ensure gentle refluxing of the test solution.

† Accurate results have been obtained without rinsing the condenser with water before removing.

‡ Duplicate blanks should not differ by more than 0.3 ml and the average blank should not differ by more than 1 ml from the volume of ammonium iron(II) sulphate solution used in standardisation. It is important to note that the blank for 0.0625 N dichromate solution is not exactly half the blank for the 0.125 N solution and separate blanks must be determined for each solution.

TABLE I

PRECISION OF PROPOSED PROCEDURE

Sample	Chloride content/ mg l^{-1}	Degrees of freedom	Mean result for COD/mg l^{-1}		Relative standard deviation, %	
			Standard method	Proposed method	Standard method	Proposed method
Within batch precision—						
Potassium hydrogen phthalate standard, COD = 250 mg l^{-1}	—	5	249	245	1.5	0.6
Settled sewage	500	5	302	306	1.5	1.7
Settled sewage	2000	4	424	440*	1.2	0.9
Sewage effluent	500	7	72	72	4.6	2.1
Total precision—						
Potassium hydrogen phthalate standard, COD = 250 mg l^{-1}	—	7	248	245	1.4	0.9
Settled sewages	*ca.* 90	8	248	258	1.8	1.1

* Analysed by proposed procedure using 1 ml of 50% *m*/*V* silver nitrate solution.

TABLE II

WASTEWATER COD ANALYSES BY PROPOSED AND STANDARD PROCEDURES

Sample	Chloride content/ mg l^{-1}	COD/mg l^{-1}		Mean bias of proposed procedure, %
		Standard procedure	Proposed procedure	
Settled sewage	<100	215, 216	229, 229	+6
	<100	308, 298	305, 307	+1
	<100	326, 342	320, 328	−3
	<100	408, 420	400, 400	−4
Sewage effluents	<100	31, 33	34, 34	+6
	<100	66, 68	70, 72	+6
	<100	66, 70	70, 68	−1
	<100	124, 89	104, 112	+1
	<100	96, 92	95, 97	+2
Trade effluents—				
Laundry	103	949, 966	896, 929	−5
Abattoir	279	15500, 15600	15500, 15600	0
Organic chemicals	751	3828, 3808	4008, 4050	+5
Abattoir	81	792, 796	828, 828	+4
Farm	1620	11400, 11600	11900, 11900	+3
Food	2590	5000, 5000	5090, 5070	+2
Food	2590	4550, 4570	4590, 4620	+2

TABLE III

ANALYSIS OF SAMPLES SPIKED WITH SODIUM CHLORIDE

Sample	COD by standard procedure (no added chloride)/mg l^{-1}	Chloride added/ mg l^{-1}	COD after addition of chloride/mg l^{-1}	
			Standard procedure	Proposed procedure*
De-ionised water	<10	2000	34, 35	18, 34
Settled sewage	268, 266	4080	280, 284	300, 306
Sewage effluent	60, 62	4060	90, 104	94, 112

* Analysed by proposed procedure, using 1 ml of 50% *m*/*V* silver nitrate solution.

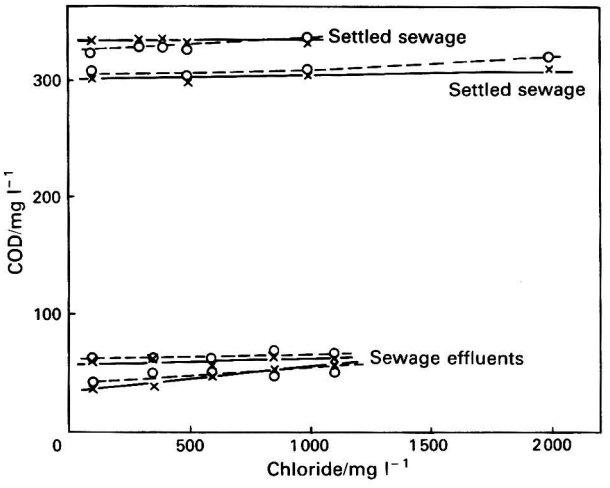

Fig. 1. COD analyses of sewage samples spiked with sodium chloride. Each point represents the average of two determinations. ○, Proposed procedure (1 ml of 25% *m/V* silver nitrate solution); and ×, standard procedure.

Discussion

Development of Procedure

The performance of the proposed procedure in the analysis of saline samples has been defined in terms of a chloride to COD ratio. Various authors[6,15] have noted that both the chloride and organic contents of samples influence the extent of chloride oxidation. It is insufficient to qualify the performance with a statement such as "suitable for samples containing up to 500 mg l^{-1} chloride." This statement assumes equivalent interference in samples containing, for example, 500 mg l^{-1} of chloride with zero COD and 500 mg l^{-1} of chloride with 500 mg l^{-1} of COD. This is clearly incorrect.[9]

The order of addition of silver nitrate solution and the time delay before adding other reagents were found to be very important. There was no significant suppression of chloride interference unless silver nitrate solution was added first. The suppression level was reduced if the dichromate solution and sulphuric acid were added immediately after the silver nitrate solution, although a delay of greater than 5 min did not further enhance suppression of chloride interference. In the proposed procedure, all silver ions are added as silver nitrate. A 1-ml aliquot of 25% *m/V* solution contains 50% more silver than is present in the 15-ml aliquot of silver sulphate - sulphuric acid solution that is used in the standard procedure. The increased expenditure on silver salts is partly off-set by eliminating the use of between 0.2 and 0.4 g of mercury(II) sulphate. The use of 0.0625 N dichromate solution is proposed for samples with an expected COD of less than 200 mg l^{-1}. During development of the procedure, attempts were made to use 0.125 N dichromate solution in the analysis of samples with less than 200 mg l^{-1} COD. The proposed procedure produced results 10–20% higher than the standard procedure when such samples contained 500–1000 mg l^{-1} chloride. A number of experiments were made using a lower strength silver nitrate solution (15% *m/V*). The results were generally 10–15% higher than those produced by the standard procedure.

Precision

It may be deduced from Table I that the proposed procedure is similar to the standard procedure in its reproducibility. Application of the *F*-test indicates that the within-batch sewage effluent and standard solution data differ significantly at the 95% confidence level. The results indicate that, in both instances, the proposed procedure was more precise. The variations in duplicate blank determinations, performed on eight different days by different

analysts, were analysed. The proposed and standard procedures give average blank 0.025 N ammonium iron(II) sulphate solution losses of 0.48 and 0.53 ml, respectively. The respective standard deviations in blank determination were 0.11 and 0.07 ml.

Bias

Wastewater analyses

Table II can only be judged in the light of a specification for an acceptable degree of bias in COD analyses. COD is a non-specific determinand and the result is defined by the analytical procedure. It is therefore considered unrealistic to expect identical results when different reagents are used. Application of the *t*-test shows that the observed differences are not significant at the 95% confidence level. It may be considered, however, that the relatively poor precision in sewage effluent analysis impairs the sensitivity of the *t*-test.

High chloride samples

Inspection of Fig. 1 confirms that, for low COD samples, the proposed procedure produces results that are about 5 mg l^{-1} higher than those obtained by the standard procedure. It is significant that this slight bias is sensibly independent of chloride concentration in the range 100–1100 mg l^{-1}. Table III presents the results of a limited study of the application of silver nitrate to the analysis of highly saline samples. This work stemmed from the encouraging results obtained in the analysis of aqueous chloride solutions, which indicated that, given sufficient silver nitrate, suppression of chloride oxidation is similar to that obtained using mercury(II) sulphate. It should be noted that precision data for samples containing 2000 mg l^{-1} of chloride has been presented in Table I, with the proposed procedure giving a result only 4% higher than that obtained using the standard procedure. Inspection of Table III indicates that there are problems in obtaining agreement at high chloride concentrations. This problem is compounded by the fact that the standard procedure itself does not give accurate results at high chloride to COD ratios, even when 40 mg of mercury(II) sulphate to 1 mg of chloride are added. This observation has already been made by other authors[15] and in Table III it can be seen that the COD of sewage effluent, spiked with 4000 mg l^{-1} of chloride, is 60% higher than the result for the unspiked sample. It is possibly relevant to question the value of a procedure which employs 1.6 g of a mercury salt in producing an inaccurate result. It is certainly significant that both Cripps and Jenkins[7] and Burns and Marshall[16] concluded that an empirical correction was necessary for COD analysis of saline samples.

Conclusions

A procedure has been described which allows the determination of COD without using mercury salts to suppress chloride oxidation. Good agreement with the standard procedure is obtained for samples containing up to 1000 mg l^{-1} of chloride or with a chloride to COD ratio of 3 to 1 or less. Data has also been presented to show that satisfactory agreement may also be possible for chloride to COD ratios of up to 5 to 1. Above this ratio the standard and proposed procedures produce results that are significantly affected by chloride.

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Determination of Organic Pharmaceuticals with *N*-Bromosuccinimide

Part III.* Some Pyrazolone Derivatives by Direct Titration

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Seven members of the pyrazolone group have been studied, namely, 3-methyl-1-phenyl-3-pyrazolin-5-one (MPP), phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), 4-aminophenazone (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), aminophenazone (amidopyrine; 2,3-dimethyl-4-dimethylamino-1-phenyl-3-pyrazolin-5-one), dipyrone [sodium (2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)methylaminomethanesulphonate], morazone [2,3-dimethyl-4-(3-methyl-2-phenylmorpholinomethyl)-1-phenyl-3-pyrazolin-5-one] and propyphenazone (4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one). The direct titration of the pyrazolone group with standard *N*-bromosuccinimide was only successful for MPP and phenazone in glacial acetic acid using methyl orange as the indicator. The concentration of the acetic acid must be not less than 9 N at the end of the titration. MPP can also be titrated successfully in 1 N hydrochloric acid, the end-point being detected either by methyl orange or potentiometrically. The molar ratio of the reaction was found to be always 1:1 (phenazone : *N*-bromosuccinimide) and 1:2 (MPP : *N*-bromosuccinimide).

A reaction mechanism is suggested involving the formation of a 4-bromo derivative during the titration of either MPP or phenazone with *N*-bromosuccinimide. A second molecule of *N*-bromosuccinimide is consumed in brominating the secondary amino group in the case of MPP.

Keywords: *Titrimetry*; *bromination*; *pyrazolone derivative determination*; *N-bromosuccinimide*

Several methods have been reported for the determination of pyrazolone derivatives. These include acidimetric,[1–3] oxidimetric,[4–6] complexometric,[7,8] gravimetric,[9,10] coulometric,[11] polarographic[12] and spectrophotometric[13,14] methods.

N-Bromosuccinimide has been proved to be a valuable reagent for the determination of many organic compounds.[15] Its usefulness is mainly due to its high selectivity together with a high yield of the products.[15] Recently, we have reported the successful determination of pyrazolidinedione derivatives by direct titration with *N*-bromosuccinimide.[16,17] No mention was found in the literature available concerning the use of *N*-bromosuccinimide for the determination of the chemically related pyrazolone derivatives. The object of this work was to investigate the extent to which *N*-bromosuccinimide can be used as a titrant for the determination of these compounds.

Experimental

Materials

The 3-methyl-1-phenyl-3-pyrazolin-5-one (MPP), 4-aminophenazone (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) (Aldrich, Milwaukee, WI), and propyphenazone (4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) (Hoffman-La Roche, Basle, Switzerland) used were of pure pharmaceutical grades; their identity and purity were checked by ultraviolet spectrophotometry of ethanolic solutions. The phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) (Lancet Pharmaceuticals Ltd, Mildenhall, Suffolk) was of pure pharmaceutical

* For details of Part I of this series, see reference list, p. 1059. For Part II, on the analysis of some Egyptian pharmaceutical products, see *Egypt. J. Pharm. Sci.*, 1982, **22**, in the press.

grade; its purity was checked by ultraviolet spectrophotometry of a solution in 0.1 N sulphuric acid. The aminophenazone (amidopyrine; 2,3-dimethyl-4-dimethylamino-1-phenyl-3-pyrazolin-5-one) (Geigy Pharmaceuticals, Basle, Switzerland) used was of pure pharmaceutical grade; its purity was checked by titration of a solution in benzene with standard perchloric acid solution. The dipyrone [sodium (2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)methylaminomethanesulphonate] (Hoechst, Frankfurt, Germany) used was of pure pharmaceutical grade and its purity was checked by titration with standard iodine solution. The morazone [2,3-dimethyl-4-(3-methyl-2-phenylmorpholinomethyl)-1-phenyl-3-pyrazolin-5-one] (Ravensberg Chemische, Ravensberg, Germany) used was of pure pharmaceutical grade, obtained in a crystalline state; its melting-point was checked.[18] The purity of these materials was also checked by thin-layer chromatography. Table I shows the purity of the samples used.

TABLE I

PURITY OF SAMPLES

Material	Purity, %	Method of assay	Reference	TLC* No. of spots	TLC* Colour	TLC* $R_F \times 100$
MPP	100.28	UV† at 280 nm	19	One	Blue	78.52
Phenazone	98.94	UV† at 280 nm	13	One	Brown	63.70
Propyphenazone	101.40	UV† at 248 nm	13	One	Blue	81.48
Aminophenazone	98.97	Non-aqueous titration	20	One	Blue	72.59
Dipyrone	97.33	Iodimetry	21	One	Blue	59.26
Morazone	—	—	—	One	Blue-green	61.48
4-Aminophenazone	—	—	—	One	Blue	61.00

* Thin-layer chromatography: silica gel G; chloroform - methanol (9 + 1); detection with hexacyanoferrate(III) - iron(III) chloride reagent.
† Ultraviolet spectrophotometry.

Procedures

Titration in acetic acid medium

Dissolve 50 mg of the compound in 10 ml of glacial acetic acid. Add an equal volume of glacial acetic acid followed by 2–3 drops of methyl orange and titrate with a 0.02 M standard solution of *N*-bromosuccinimide until the red colour of the indicator changes to colourless or pale yellow. When the volume of the titrant reaches the same volume as the sample taken, add an additional volume of glacial acetic acid for each volume of the standard *N*-bromosuccinimide solution consumed so as to keep the concentration of the acid at 9 N or above at the end-point. Carry out a blank titration.

Titration in 1 N *hydrochloric acid medium*

Dissolve 5 mg of the compound in 5 ml of 1 N hydrochloric acid. Add 10 ml of 1 N hydrochloric acid, followed by 2–3 drops of methyl orange and titrate with 0.01 M standard *N*-bromosuccinimide solution until the red colour of the indicator changes to colourless. Carry out a blank titration.

The amount of pyrazolone derivative is calculated using the following equivalency factors:
1 ml of 0.01 M *N*-bromosuccinimide = 0.867 mg of MPP;
1 ml of 0.02 M *N*-bromosuccinimide = 1.734 mg of MPP;
1 ml of 0.02 M *N*-bromosuccinimide = 3.764 mg of phenazone.

Determination by official method

Phenazone was also determined by the Egyptian Pharmacopoeia official method[22]; the results were then compared with those of the suggested *N*-bromosuccinimide methods.

Results

The direct titration of the pyrazolone group with standard *N*-bromosuccinimide solution was successful only for phenazone and MPP in glacial acetic acid using methyl orange as the indicator (Table II). It is clear from Table II that the proposed method could be used for

the determination of 4–100 mg of phenazone and MPP at room temperature using 0.005–0.05 M solutions of *N*-bromosuccinimide. The concentration of acetic acid should not be allowed to fall below 9 N at the end of the titration. Concentrations of acetic acid of less than 9 N at the end-point cause the change in colour of the indicator to be less sharp, leading to difficulties in end-point detection. Moreover, titration of MPP in more dilute acetic acid solutions is always accompanied by precipitation of the bromo derivative.

TABLE II

DETERMINATION OF PHENAZONE AND MPP IN GLACIAL ACETIC ACID USING *N*-BROMOSUCCINIMIDE

Phenazone			MPP		
Taken/mg	Found/mg	Recovery, %	Taken/mg	Found/mg	Recovery, %
4.70	4.52	96.20	4.35	4.21	96.78
9.40	9.30	98.94	8.70	8.44	97.01
14.10	13.97	99.10	13.05	12.80	98.08
18.80	18.66	99.26	17.40	17.05	97.99
23.50	23.44	99.74	21.75	21.14	97.20
25.00	25.30	101.20	25.00	24.56	98.24
50.00	50.12	100.24	50.00	48.73	97.46
100.00	100.60	100.60	100.00	97.45	97.45
Mean		91.41			97.53
Standard deviation		1.51			0.53
Coefficient of variation		1.52			0.05

Attempts to titrate solutions of phenazone in 1 N hydrochloric acid with *N*-bromosuccinimide were unsuccessful; the colour change of the indicator was not sharp enough to allow an accurate detection of the end-point. However, solutions of MPP in 1 N hydrochloric acid were titrated successfully with *N*-bromosuccinimide (Table III). It is clear from Table III that the proposed method could be used for the determination of 1–9 mg (about 5–50 μmol) of MPP using 0.005–0.01 M solutions of *N*-bromosuccinimide. Although separation of the bromo derivative occurred during the titration of MPP with *N*-bromosuccinimide solution in hydrochloric acid medium, a very sharp and stoicheiometric end-point was observed both visually (using methyl orange) or potentiometrically. Fig. 1 shows the potentiometric first derivative titration curve using a platinum - silver chloride electrode system. A sharp jump in the voltage was noticed at the expected end-point.

Several attempts were made to render the direct titration feasible for other pyrazolones. These included the use of different solvents and temperatures (10–70 °C), the addition of catalysts, *e.g.*, sodium bromide and sodium acetate and also the utilisation of different detection procedures, *i.e.*, various colour indicators, a potentiometric technique, and dead-stop end-point detection using two identical platinum electrodes at 0.2 mV. However, all of these methods gave inconsistent results and in all instances the reaction was non-stoicheiometric. Moreover, no sharp change at the end-point could be detected visually or potentiometrically and for the dead-stop technique no current could be detected.

TABLE III

DETERMINATION OF MPP IN 1 N HYDROCHLORIC ACID USING *N*-BROMOSUCCINIMIDE

Taken/mg	Found/mg	Recovery, %
0.87	0.86	98.85
1.74	1.73	99.43
2.61	2.60	99.62
3.48	3.38	97.13
4.35	4.20	96.55
5.22	5.10	97.70
6.09	5.91	97.04
8.70	8.42	96.78
Mean		97.89
Standard deviation		1.23
Coefficient of variation		1.2

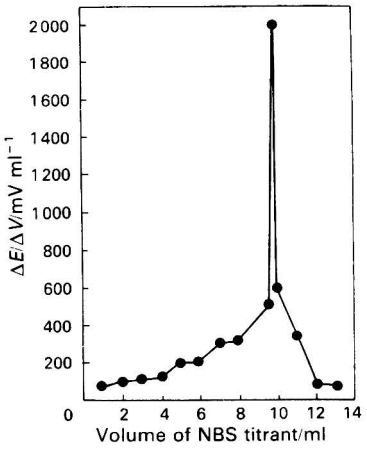

Fig. 1. Typical first-derivative potentiometric titration curve for MPP in 0.1 N hydrochloric acid.

Phenazone was also analysed according to the official method reported in the Egyptian Pharmacopoeia.[22] The results obtained were compared statistically with those of the proposed method using Student's *t*-test.[23] The value calculated for *t* confirms the suitability of the proposed method for the determination of phenazone with acceptable accuracy and precision, which are comparable to those obtained using the established official method (Table IV).

TABLE IV

DETERMINATION OF PHENAZONE USING *N*-BROMOSUCCINIMIDE AND THE EGYPTIAN PHARMACOPOEIA OFFICIAL METHOD[22]

N-Bromosuccinimide method			Official method		
	Found			Found	
Taken/mg	mg	%	Taken/mg	mg	%
50	49.91	99.82	100	100.22	100.22
50	50.32	100.64	100	99.00	99.00
50	49.91	99.82	100	98.00	98.00
Mean		100.09 ± 0.48		99.07 ± 1.11	
No. of determinations		3		3	
Variance		0.230		1.232	
t		1.474 (2.776)*			

* The value in parentheses is the reported[23] value for *t* at $p = 0.025$ (4 degrees of freedom).

Discussion

Seven members of the pyrazolone group were studied, namely, MPP, phenazone, 4-aminophenazone, aminophenazone, dipyrone, morazone and propyphenazone, the chemical structures of which are shown in Fig. 2. Only phenazone and MPP can be directly titrated with *N*-bromosuccinimide. The main difference between these two compounds and the other pyrazolones studied is the absence of any substituents at C-4 of the pyrazolone nucleus (Fig. 2). The hydrogen atom at the 4-position of the pyrazolone nucleus is easily replaced

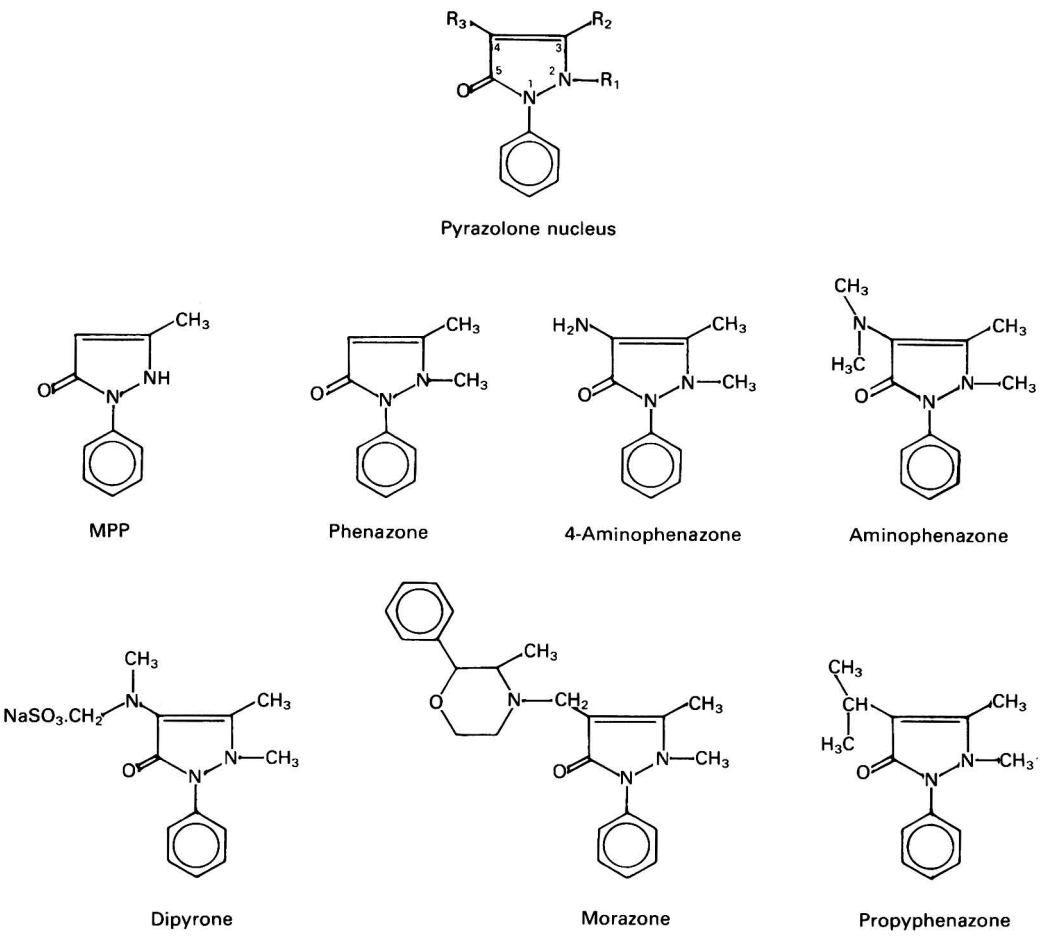

Fig. 2. Structures of the pyrazolone derivatives.

by stronger electrophilic agents.[24] Accordingly it is assumed that the reaction product could be a 4-bromo derivative. In support of this theory it has been shown[24] that the 4-bromo derivative of phenazone can be prepared by heating equimolecular amounts of phenazone and *N*-bromosuccinimide in carbon tetrachloride. Moreover, we have found that pyrazolidinedione derivatives were brominated readily and quantitatively by *N*-bromosuccinimide in acetic acid giving 4-bromo derivatives.[16]

The molar ratio of the reaction was found to be always 1:1 (phenazone:*N*-bromosuccinimide) and 1:2 (MPP:*N*-bromosuccinimide), *i.e.*, one molar equivalent was consumed by phenazone while two molar equivalents were consumed by MPP. The only difference between the two compounds is the presence of a secondary amino group in the 2-position of MPP instead of the tertiary one present in phenazone (Fig. 2). Accordingly, it is reasonable to assume that bromination of the amino group of MPP takes place, consuming a second molecule of *N*-bromosuccinimide and producing a 2,4-dibromo derivative. Previous reports have indicated that *N*-bromoamines are obtained by the reaction of *N*-bromosuccinimide or *N*-bromophthalimide with amines.[25] The mechanism by which *N*-bromosuccinimide brominates the different amines is not fully understood. However, it has been reported

that *N*-bromosuccinimide in water may liberate molecular bromine,[26] and hence bromine atoms at low concentration, which preferentially give *N*-bromo rather than *C*-bromo derivatives.[27] It has also been reported that *N*-halo compounds are easily converted into halogen and amines whereas *C*-halogenation is non-reversible.[28] Accordingly, the bromine atom on the nitrogen at the 2-position of MPP is labile and will liberate iodine from potassium iodide under the conditions of the back-titration.[29]

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NOTE—References **16**, **17** and **29** are to Parts I, II and IV of this series, respectively.

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A Study of the Formation and Stability of the Iron(III) - Thiocyanate Complex in Acidic Media*

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The formation of the iron(III) - thiocyanate complex in sulphuric and perchloric acids has been studied spectrophotometrically. Under no circumstances has any evidence been found that more than one thiocyanate ion enters the complex, contrary to widely held beliefs that up to six ions may be involved. Hydroxyl, sulphate and hydrogen sulphate ions and water molecules complete the co-ordination sphere. Decomposition products have been isolated and identified. Three ranges of acid concentration are identified, each region characterised by the manner of variation of the molar absorptivity and formation constant with change in acid concentration, and an interpretation is offered. Decomposition of both reagent and complex render thiocyanate unsatisfactory as a reagent for the precise determination of iron.

Keywords: Iron(III) - thiocyanate complex; iron determination; spectrophotometry; reaction mechanisms

The blood red complex of iron(III) with thiocyanate continues to receive attention,[1–12] and has recently been studied by fast reaction techniques.[13–16] The formation of the complex appears to be very fast, and it is suggested that in aqueous media there are two reaction paths, one independent,

$$[Fe(H_2O)_6]^{3+} + (NCS)^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Fe(NCS)(H_2O)_5]^{2+} + H_2O \quad .. \quad .. \quad (1)$$

and the other dependent on hydrogen ion concentration,

$$[Fe(H_2O)_6]^{3+} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [Fe(OH)(H_2O)_5]^{2+} + H^+ \quad .. \quad .. \quad .. \quad (2)$$

$$[Fe(OH)(H_2O)_5]^{2+} + (NCS)^- \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} [Fe(OH)(NCS)(H_2O)_4]^+ + H_2O \quad .. \quad (3)$$

At higher thiocyanate concentrations it is supposed that sequential fast ligand exchange progresses until the hexaisothiocyanatoferrate(III) species is attained.[17–22] That the thiocyanate is nitrogen bonded[23–25] is supported by the analogy with the monoazide - iron(III) complex. The intensity of the colour has not previously been investigated, although some qualitative observations have been perpetuated on misapprehensions.[18] In moderately acidic solutions, decrease in absorbance is ascribed loosely to the direct reduction of iron(III) by thiocyanate. Thiocyanate is oxidisable by reagents such as cerium(IV) and hydrogen peroxide,[26–30] and the latter has been suggested as an additive in the colorimetric process,[18] but there is no mention of identification of reaction products.

Experimental

Reagents

Water. Quartz-processed dust- and grease-free high-purity water[31] was used throughout.

Acids. Aristar perchloric (72%) and sulphuric acids (98%) were diluted to appropriate stock strengths and assayed. The stock solutions were diluted precisely to an appropriate

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concentration of between 0.1 and 0.5 M, which were then used to titrate weighed portions of primary standard dried sodium carbonate to an end-point using methyl orange, a colour matched with blanks of the corresponding concentration of sodium perchlorate or sulphate to which 0.01 ml of the relevant acid had been added. Precisely dispensed amounts of the standardised stock solutions were used in the preparation of the iron and thiocyanate solutions to provide determinate acid concentrations.

Iron(III) solutions, 3×10^{-3} M. Anhydrous iron(III) perchlorate (Alpha), dried at 40 °C over magnesium perchlorate, or assayed AnalaR ammonium iron(III) sulphate dodecahydrate were used. The required amount was weighed by difference and dissolved in water containing the required amount of the appropriate stock acid solution and made up to volume.

Thiocyanate solution, 3×10^{-3} M. The solution was prepared, as described for iron(III), from AnalaR ammonium thiocyanate immediately before use.

Apparatus

Spectrophotometers. Cary 16 and Unicam SP 1800, with an AR 25 recorder, spectrophotometers with matched sets of W210/uu/10.00 mm Thermal Syndicate cells were used for the visible region. A Perkin-Elmer 398 spectrophotometer was used with 100-mm gas cells with sodium chloride windows for infrared measurements.

Reaction product collection. An assembly was blown from three 250-ml vessels in vacuum line fashion for total exclusion of the atmosphere and for argon purging so that reactants could be out-gassed, mixed and allowed to react, and the reaction products collected, dried if necessary, and transferred into the infrared gas sample cells.

Results and Discussion

At ambient temperature, absorption spectra of diverse concentrations of iron(III) in a wide range of excesses of thiocyanate in sulphuric or perchloric acid concentrations varying from 0.01 to 4.0 M were run against a reagent blank in the range 350–750 nm. A single, broad, flat band was always obtained, with $\lambda_{max.}$ invariably at 474 nm in perchloric acid and at 478 nm in sulphuric acid. These values, confirmed on the calibrated Cary 16, were then used as the working wavelengths. This small difference in octahedral splitting energies arises from the higher ligand field in sulphuric acid and is otherwise independent of conditions. The molar absorptivity is more than twice as great in perchloric acid as in sulphuric acid of the same concentration. There is a considerable measure of charge transfer in the absorption, probably via electron tunnelling through nitrogen and carbon to the terminal sulphur in the thiocyanate, and this appears to be hindered by sulphate or hydrogen sulphate in the co-ordination sphere.

The stoicheiometry was extensively probed by the method of continuous variations (Job's method[32,33]) over a wide range of conditions and under varying acid concentrations. An example for each acid is shown in Fig. 1(*a*) and (*b*), from among the large number of graphs generated. Rapid manipulation was required to achieve absorbance readings before the onset of decomposition, which occurred within about 1 min. In no instance did the ratio of iron(III) to thiocyanate deviate from 1.00:1.00. Attempts were made to establish the existence of higher ratios by the molar ratio method, but, despite interference from decomposition, no evidence was secured. Nor did the slope ratio method, even at reagent ratios of 5000:1, offer any sign of departure from the 1:1 stoicheiometry. The maximum of 1:6 so widely reported[17–22] would not appear to be attainable at an acid concentration adequate to prevent hydrolytic precipitation of iron(III), nor at higher acid concentrations. This, incidentally, confirms the results of some 600 undergraduates who have studied the reaction in their final year course. The most probable situation in acid of up to about 0.5 M is that the "solvated" iron(III) ion contains one hydroxyl ion, the remaining five sites being occupied by water molecules and perhaps one perchlorate ion in perchlorate media, and certainly at least one sulphate or hydrogen sulphate ion in sulphate media. The thiocyanate will enter by fast ligand exchange with a water molecule *trans* to the hydroxyl ion, which exerts the *trans* effect.[19,34]

As is evident from the results in Tables I and II, in both acids the change in colour intensity occurs in three phases, labelled A, B and C in Fig. 2, in three ranges of acid concentration. Molar absorptivities are higher in perchloric acid, while formation constants are higher in

sulphuric acid. It is not surprising that conflicting reports appear in the literature. In any phase in either acid, as one parameter increases, the other decreases, the effect being nearly inverse as the log(molar absorptivity × formation constant) in the tables indicates. In perchloric acid, the mean log value of the product is 3.249 and the standard deviation is

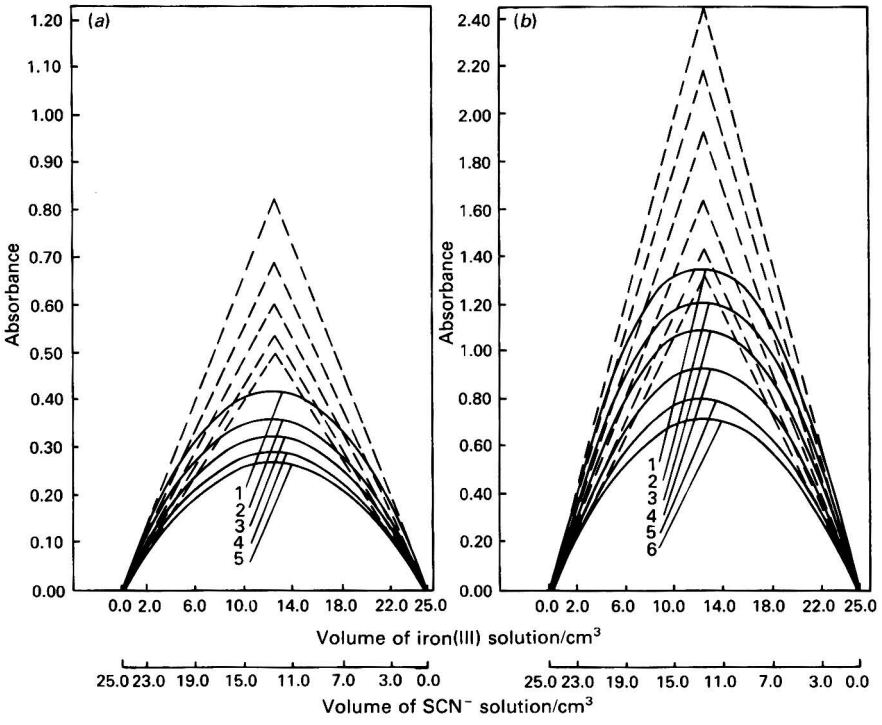

Fig. 1. Job plots for 3 × 10^{-3} M solutions of iron(III) and thiocyanate. (*a*) In sulphuric acid: (1) 0.0364 M; (2) 0.0727 M; (3) 0.1455 M; (4) 0.2909 M; and (5) 0.5819 M. (*b*) In perchloric acid: (1) 0.0244 M; (2) 0.0487 M; (3) 0.0975 M; (4) 0.1949 M; (5) 0.390 M; and (6) 0.780 M.

TABLE I

MOLAR ABSORPTIVITY (ϵ) AT $\lambda_{max.}$ OF 478 nm AND FORMATION CONSTANTS ($K_{form.}$) OF $[Fe(NCS)L_5]$ IN SULPHURIC ACID

Phase	Sulphuric acid concentration/M	ϵ/l mol^{-1} cm^{-1}	$K_{form.}$/l mol^{-1}	Log(ϵ × $K_{form.}$)
A	0.0364	552.1	2.505	3.141
	0.0727	437.5	3.249	3.153
	0.1455	375.0	3.879	3.163
	0.2909	343.75	4.445	3.184
	0.5819	312.5	4.981	3.192
B	0.5819	312.5	4.981	3.192
	1.1766	364.6	4.917	3.254
	1.8385	385.4	4.365	3.226
	2.5739	458.3	3.244	3.172
	3.3093	541.7	2.444	3.123
C	4.4124	489.6	3.707	3.259
	5.1478	416.7	5.161	3.333
	5.5155	343.8	5.929	3.309
	6.330	0	0	—

TABLE II

MOLAR ABSORPTIVITY (ϵ) AT $\lambda_{max.}$ OF 474nm AND FORMATION CONSTANT ($K_{form.}$) OF $[Fe(SCN)L_5]$ IN PERCHLORIC ACID

Phase	Perchloric acid concentration/M	ϵ/l mol^{-1} cm^{-1}	$K_{form.}$/l mol^{-1}	Log($\epsilon \times K_{form.}$)
A	0.0244	1667	1.106	3.265
	0.0487	1458	1.284	3.274
	0.0975	1292	1.527	3.295
	0.1949	1125	1.791	3.235
	0.3900	950.0	1.963	3.271
	0.7800	854.2	2.010	3.235
B	1.9494	958.3	1.850	3.249
	2.4368	1167	1.498	3.249
	2.9242	1208	1.461	3.242
	3.4115	1250	1.339	3.224
	3.8989	1375	1.114	3.185
	4.3862	1583	0.750	3.325
C	4.8740	1038	1.791	3.269
	5.3614	979	1.827	3.253
	5.8488	792	1.862	3.169
	6.0925	583.3	1.998	3.067
	6.3362	437.5	2.941	3.110
	7.000	0	0	—

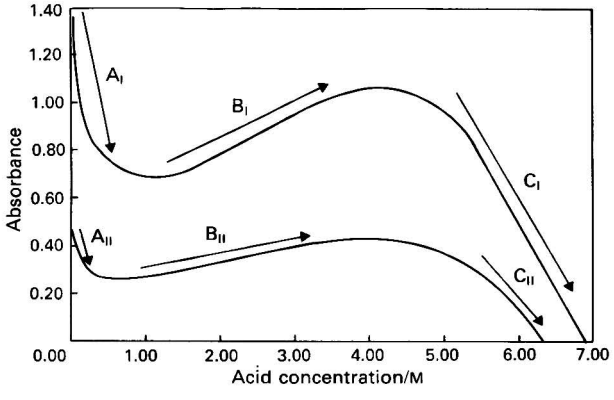

Fig. 2. Change in absorbance of solutions 1.5×10^{-3} M in iron(III) and thiocyanate in (I) perchloric acid and (II) sulphuric acid of varied concentration.

0.039, while in sulphuric acid the mean is 3.187 with a standard deviation of 0.047: the means are not significantly different. There is no trend in perchloric acid, but in sulphuric acid the product increases slowly in phase A and decreases in phase B.

In phase A, for both acids molar absorptivity decreases and formation constant increases with increase in acid concentration. The complex is unstable, acid weakening the *trans* effect, and the terminal sulphur separating from the thiocyanate and precipitating. The nitrogen-linked cyano group is released, picks up a proton and is evolved as hydrogen cyanide gas, which was collected and identified by the twin peak at 2050 cm^{-1}.

Contrarily in phase B, the molar absorptivity increases and the formation constant decreases as the acid concentration is increased. Here the hydroxyl ion content of the complex is diminished by protonation to water molecules, so removing the propitious *trans* effect, and in accordance with the hydrogen ion concentration dependence postulated by Below *et al.*[13] At the same time decomposition is aggravated, sulphur being precipitated and hydrogen cyanide appearing in the gaseous products.

In phase C, molar absorptivity decreases and formation constant increases with increasing acid concentration. The colour finally disappears when the concentration of sulphuric acid reaches 6.3 M or if perchloric acid reaches 7.0 M. Although decomposition of the complex doubtless continues, this is overshadowed by decomposition of thiocyanate, probably as thiocyanic acid, to hydrogen sulphide and hydrogen cyanide. The hydrogen sulphide reacts with iron(III) producing iron(II) and sulphur, which precipitates. The hydrogen sulphide was identified by reaction with lead acetate and with nitrosopentacyanoferrate(II). Thiocyanate in the absence of iron(III) in either acid at a concentration greater than 4 M decomposes rapidly, yielding hydrogen sulphide and hydrogen cyanide, which were identified. A diligent search was made throughout for any trace amounts of thiocyanogen or cyanogen among the reaction products, but none was found.

It may be concluded that at acid concentrations adequate to prevent hydrolytic precipitation of iron(III), the complex with thiocyanate takes up no more than one thiocyanate ion, that the pattern of behaviour of the molar absorptivity and the formation constant with change of acid concentration is regular but unusual, and that, because of decomposition of complex or reagent, thiocyanate is unsuitable for the precise determination of iron(III) and inadequate for reaction rate measurements involving iron(III).

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Spectrophotometric Determination of Cobalt in Hydrofining Catalysts, Alloys and Salts with Biacetylmonoxime 2-Pyridylhydrazone

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Biacetylmonoxime 2-pyridylhydrazone (BMPH) has been examined to evaluate its usefulness as a selective spectrophotometric reagent for cobalt. The yellow coloration is formed at an initial pH of 5–6 in a medium containing 20% of dimethylformamide and measured at 430 nm at a final concentration of 0.5 N perchloric acid. The molar absorptivity is 3700 l mol^{-1} cm^{-1}. The method has been applied to the determination of cobalt in synthetic mixtures, hydrofining catalysts, alloys and chemicals with good results. The BMPH chromogen shows a wide range of application.

Keywords: *Biacetylmonoxime 2-pyridylhydrazone reagent*; *cobalt determination*; *spectrophotometry*

Numerous pyridylhydrazone compounds and related types have been synthesised and investigated for the purpose of exploiting their ability to chelate trace metals.[1] During studies on pyridylhydrazones derived from α-diketones in this laboratory,[2] it was found that biacetylmonoxime 2-pyridylhydrazone (BMPH) behaves as a selective reagent for the spectrophotometric determination of cobalt. In aqueous solution (pH 8), BMPH reacts with cobalt(II) to form an orange complex, which, on addition of acid, undergoes a hipsochromic shift, changing from orange to yellow. It was observed that the quantitative determination of cobalt can be achieved by measuring the absorbance of the yellow coloration at 430 nm in solutions of final acidity about 0.5 N in perchloric acid in the presence of a large number of ions. The detailed development and testing of a method involving the use of BMPH is described below. The method has been applied to the determination of cobalt in synthetic mixtures, aluminium - molybdenum - nickel - cobalt catalysts, alloys and salts, and compares favourably with other methods used for the same purpose.

Experimental

Reagents

Biacetylmonoxime 2-pyridylhydrazone (BMPH) was used as a 0.2% *m/V* solution in dimethylformamide.[3]

A standard solution of cobalt(II) (3.0009 mg ml^{-1}) was prepared from cobalt nitrate hexahydrate and was standardised by EDTA titration.[4] Working solutions were prepared by appropriate dilution.

All chemicals used were of analytical-reagent grade unless otherwise stated, and distilled water was used throughout.

Apparatus

Two spectrophotometers were used: a Perkin-Elmer, Model Coleman 55, (digital) instrument was used for measuring absorbances at a fixed wavelength; and a Pye Unicam SP 800 recording spectrophotometer for absorbance scanning. Matched glass cells of 1.00 cm optical path length were used. An Orion, Model 501, pH meter with a combined saturated calomel - glass electrode was used for pH measurements. All experiments and measurements were carried out at ambient temperature.

Recommended Procedure

Into a 25-ml calibrated flask transfer a volume of solution (up to 18 ml) containing 25–

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375 μg of cobalt, adjust the pH to 5–6 and add 2.5 ml of 0.2% *m/V* BMPH solution in dimethylformamide and 2.5 ml of dimethylformamide. Allow the solution to stand in the flask at room temperature for a few minutes and dilute with perchloric acid and water to give a final concentration of about 0.5 N in perchloric acid (0.8 ml of 60% perchloric acid per 25 ml for pure cobalt solutions). Measure the absorbance at 430 nm against distilled water. Use a suitable calibration graph or empirical equation to convert absorbance into concentration.

Preparation of Sample Solutions

The methods published by Bahamonde *et al.*[5] (sample 2) and García Montelongo *et al.*[6] (samples 1 and 3) were followed for the dissolution of hydrofining catalysts. The solutions of nickel stone, Raney nickel and aluminium alloys were the same as those used by Rosales and Cano-Pavón,[7] diluted 10-fold before use. The required amount of pure reagent salts were weighed and dissolved in distilled water (adding hydrochloric acid until dissolution was complete where necessary). These solutions were diluted to the required volume with water.

Results and Discussion

Absorption Spectra and pH Effect

When a few drops of an ethanolic or dimethylformamide solution of BMPH are added to a cobalt(II) solution, an orange complex ($\lambda_{max.}$ at 480 nm) is formed instantaneously at basic pH. The orange colour of the complex changes with decrease in pH to yellow ($\lambda_{max.}$ at 390 nm), the system being reversible. The spectra of a series of solutions varying in final pH, but for which the colour had been initially developed at the pH resulting from mixing pure cobalt(II) solutions and BMPH reagent, were recorded and are shown in Fig. 1(*a*) and 1(*b*). A clear isosbestic point is apparent at 430 nm.

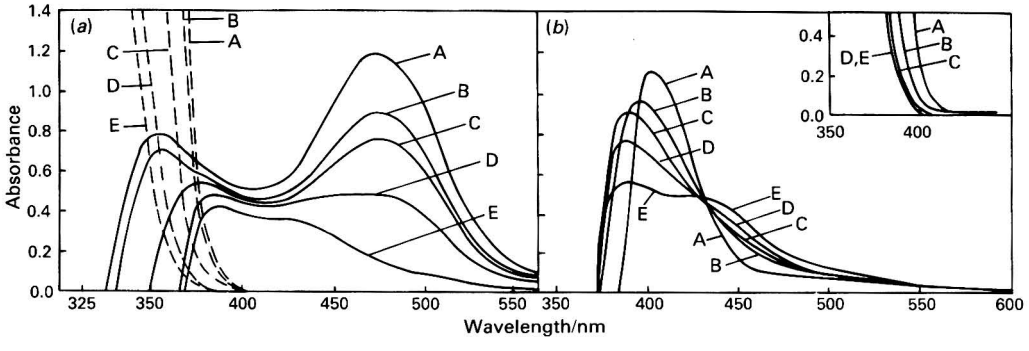

Fig. 1. Absorption spectra of the cobalt - BMPH system in 20% aqueous dimethylformamide. (*a*) A, pH 8.14–9.73; B, pH 6.50 ($H_2PO_4^-$ - HPO_4^{2-} buffer); C, pH 6.17 (CH_3COO^- - CH_3COOH buffer); D, pH 4.98 (CH_3COO^- - CH_3COOH buffer); and E, pH 3.20 (phthalate - HNO_3 buffer). C_{Co} = 6.75 p.p.m. C_R = 2.5 ml of BMPH at 0.2% in dimethylformamide per 25 ml. Broken lines: reagent blanks. (*b*) A, in 40% $HClO_4$; B, in 20% $HClO_4$; C, in 10% $HClO_4$; D, at pH 0.8; and E, at pH 2.5. Inset, blank values. C_{Co} = 8.1 p.p.m. C_R = 2.5 ml of BMPH at 0.2% in dimethylformamide per 25 ml.

Choice of Optimum Wavelength and pH

To determine the most suitable wavelength, the absorbance *versus* pH graph for the cobalt complexes were determined at several wavelengths (Fig. 2). No change in absorbance was observed over the pH range 8–12 (orange complex). A range of constant absorbance was also observed at acidities between 2 and $10^{-3.2}$ M (pH 3.2) at 430 nm (yellow complex). Formation of the cobalt complex at as low a pH as possible increases the selectivity for cobalt and also avoids a tendency for metal ions to hydrolyse from solution. It was found that the minimum pH for the development of maximum absorbance of the yellow coloration is about 4 (apparent pH) [Fig. 2(*a*)]. However, for greatest accuracy subsequent studies

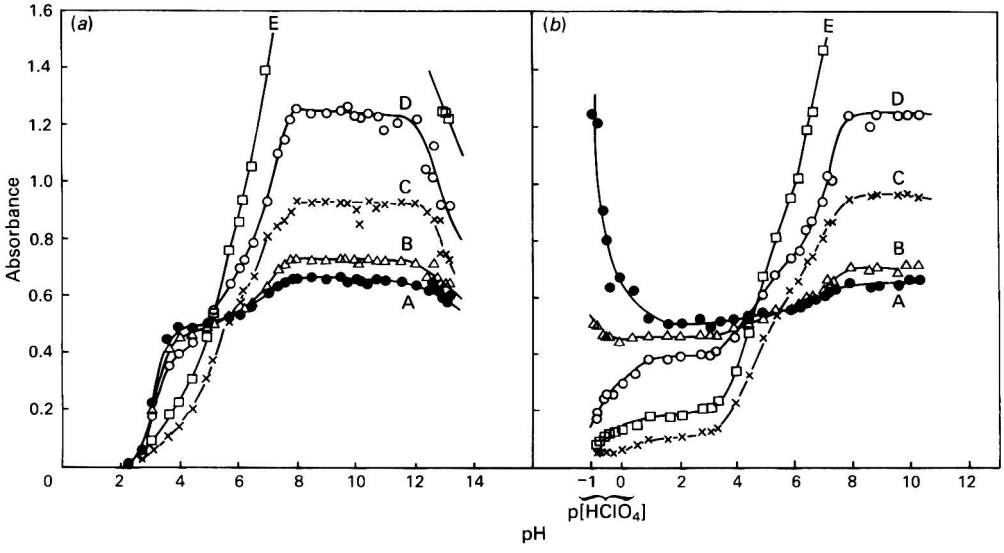

Fig. 2. Absorbance *versus* pH graphs for the cobalt - BMPH system. Order of addition of reagents: (*a*) cobalt, perchloric acid and BMPH; and (*b*) cobalt plus BMPH (pH 10) and perchloric acid. Absorbance at (A) 410 nm, (B) 430 nm, (C) 450 nm, (D) 480 nm and (E) 510 nm. C_{Co} = 6.75 p.p.m. C_R = 2.5 ml of BMPH at 0.2% in dimethylformamide per 25 ml.

were carried out at an initial pH of 5.5–6.0, but the absorbance was measured in solutions of final acidity of about 0.5 N in perchloric acid. The wavelength of maximum absorption of the yellow coloration was not suitable for analytical use because of its blank absorption and the pH dependence on the absorbance. In addition, the interference of the excess of reagent was easily circumvented by measurement at 430 nm, where absorption of the reagent is negligible.

Choice of Solvent

The absorption characteristics of the cobalt - BMPH system at pH 1 at 430 nm in various solvents miscible with water were similar (Fig. 3). The absorbance of the coloured complex

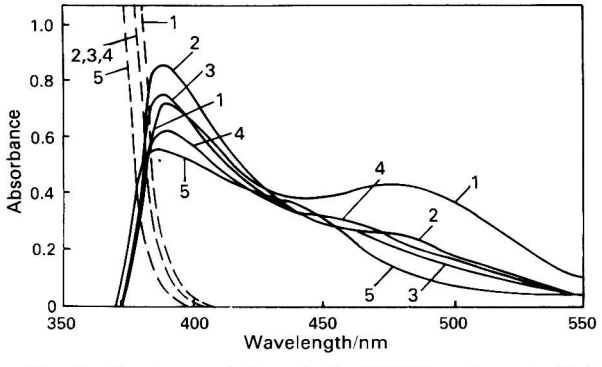

Fig. 3. Spectrum of the cobalt - BMPH system at pH 1 in various solvents miscible with water. Solvents: 1, dimethylformamide; 2, dioxan (16% of dimethylformamide); 3, methanol (8% of dimethylformamide); 4, ethanol (8% of dimethylformamide); and 5, water (20% of dimethylformamide). Broken lines: reagent blanks. C_{Co} = 6.75 p.p.m. C_R = 2.5 ml of BMPH at 0.2% in dimethylformamide per 25 ml.

measured at $\lambda_{max.}$ decreased slowly with time, the percentage decrease per day being 16.5, 21.2, 15.1 and 18.6 for dimethylformamide, ethanol, methanol and dioxan, respectively. The ratio of ethanol or dimethylformamide to water in the final solution (20–50% *V*/*V*) was unimportant. A medium containing a 20% *V*/*V* of dimethylformamide was selected for the recommended procedure. The quality of the dimethylformamide (Merck, Carlo Erba, UCB and Panreac) had no effect on the absorbance measurements.

Stability

No change in the absorbance of the yellow coloration was observed during several days at 430 nm, but an increase in absorbance was observed at higher wavelengths. However, the yellow coloration remained stable for several weeks at least under strongly acidic conditions (2 M in perchloric acid).

Influence of the Amount of Reagent

A constant absorbance was obtained with a 1:3 ratio of reagent to cobalt. Large amounts of reagent (5 ml of BMPH at 1.0% *m*/*V* in dimethylformamide per 25 ml was the maximum amount tested) had no additional effect on the absorbance.

Effect of Mixing of Reagents

Complete reaction occurred immediately upon mixing the reagents.

Stoicheiometry

The method of continuous variation was applied to a series of solutions in which the total concentration of reactants (cobalt + BMPH) was kept constant at 3.04×10^{-4} M, but the molar fractions were varied. The maxima occurred at a 0.32 molar fraction of cobalt, indicating that the composition of the complex is 1:2. The molar ratio method was first applied to solutions of final concentration 1.09×10^{-4} M cobalt and varying BMPH concentration. Extrapolation of the lower and upper portions of the absorption graph indicated a molarity ratio of 1:2.1 (cobalt to BMPH). The molar ratio method was also applied to a series of solutions containing BMPH at fixed concentrations of 2.19×10^{-4} and 1.09×10^{-4} M, the concentration of cobalt being varied. A plot of absorbance *versus* moles of cobalt per mole of BMPH showed breaks at absorbances of 0.45 and 0.475, respectively, again confirming a reaction stoicheiometry of 1:2 (cobalt to BMPH). Measurements were made at 410, 430 and 440 nm in all instances. These findings were expected on the basis of the presence of three basic nitrogen atoms in the BMPH molecule. The formation constant of the complex is too high to be evaluated by conventional methods. When EDTA (16000 p.p.m.) was added after cobalt complexation (cobalt to BMPH ratios 1:20), no interference was observed.

We were not able to find a scheme that would explain the changes in the spectral characteristics of the cobalt - BMPH system. However, this does not affect the use of BMPH reagent for the spectrophotometric determination of cobalt. Apparently, the cobalt exists in the tervalent state in the complex, and a characteristic protonation of the cobalt complex with simultaneous redistribution of electrons occurs in an acidic medium. It is known that high stability in strongly acidic solutions is a characteristic of a spin-paired d^6 cobalt complex.[8]

Spectrophotometric Determination of Cobalt with BMPH

The relationship between the concentration and the colour intensity obeyed Beer's law in the range 0.8–15 p.p.m. of cobalt, the graph obtained being a straight line that passed through the origin. The molar absorptivity is 3700 l mol^{-1} cm^{-1}. The sensitivity of reaction as defined by Sandell is 0.016 μg cm^{-2}. The optimum concentration range as evaluated by a Ringbom plot is 3.24–15 p.p.m. The precision, expressed as relative standard deviation, was 3.15, 1.06, 0.40 and 0.76% for 0.81, 1.89, 3.24 and 5.40 p.p.m., respectively (15 samples). A negligible contribution of between-batch variation to the precision of the determination was found. For between-day variation, triplicate determinations were made on each of seven consecutive days. All absorbances were within the range 0.195–0.211 (3.24 p.p.m. of

cobalt). However, in both the interference and the application studies, triplicate pure cobalt solutions were prepared prior to measurements being made. As a check on the ability of the method to withstand variations in the amounts of reagents added to samples [BMPH reagent, dimethylformamide, perchloric acid (added before and after dilution with distilled water)], a factorial experiment was carried out as described by Dougan and Wilson.[9] The results obtained showed that negligible errors were caused by small variations in the reagents added, as expected. Measurements made at 430 ± 1.5 nm gave negligible differences in the absorbance values.

Effect of Foreign Ions

For the determination of 3.24 p.p.m. of cobalt by this method, foreign ions (added before the BMPH reagent) can be tolerated at the levels given in Table I. The criterion for an interference was an absorbance value varying by more than ±2% from the expected value of cobalt alone. Results of test tolerances with masking agents are given in Table II. Several mixtures were also analysed for cobalt (Table III). Good results were obtained in masking

TABLE I

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

Cobalt concentration 3.24 p.p.m.

Tolerance, p.p.m.	Foreign ion or species	Tolerance, p.p.m.	Foreign ion or species
10000	Zn(II), Cd(II), alkali and alkaline earth metals	60000	PO_4^{3-}
3200	Al(III), NH_4^+	40000	Thiourea
320	Mo(VI), W(VI), Bi(III), Tl(I), Ti(IV), Zr(IV)	32000	Trichloroacetic acid
64	V(V)*	20000	$P_2O_7^{4-}$, Cl^-, CH_3COO^-
32	Hg(II), Mn(II), V(V), UO_2^{2+}	6000	F^-, NO_3^-
16	Ni(II), Cr(III)	4000	Ascorbic acid
12	Cu(II)	3200	Br^-, tartrate, citrate, SO_3^{2-}, $B_4O_7^{2-}$
9	Fe(III)	960	AsO_4^{3-}, AsO_2^-
8	Fe(II)	320	SCN^-
1	Au(III)	160	$S_2O_3^{2-}$
0.5	Ag(I), Pd(II), EDTA	32	I^-
		6	NO_2^-, $C_2O_4^{2-}$

* Measured after 1 h.

TABLE II

ELIMINATION OF INTERFERENCES BY ADDITION OF MASKING AGENTS

Cobalt concentration 3.24 p.p.m.

Foreign ion	Amount tolerated, p.p.m. Without masking agent	With masking agent	Masking agent
Fe(III)	9	1600	Phosphate, 60000 p.p.m.
Cu(II)	12	32	Thiourea, 2.5 ml of 40% in DMF*
Ni(II)	16	320	Thiourea, 2.5 ml of 40% in DMF*
Ni(II)	16	32	Bicine,† 4000 p.p.m.
Hg(II)	32	64	Chloride, 3200 p.p.m.
Ag(I)	0.5	9.6	$S_2O_3^{2-}$, 160 p.p.m.
Ag(I)	0.5	320	Thiourea, 2.5 ml of 40% in DMF*
Au(III)	1	3.2	$S_2O_3^{2-}$, 160 p.p.m.
Mn(II)	32	3200	Thiourea, 2.5 ml of 40% in DMF*
Pb(II)	320	3200	Tartrate, 3200 p.p.m.
Sn(II)	320	3200	Tartrate, 3200 p.p.m.
Sb(III)	320	3200	Tartrate, 3200 p.p.m.
$C_2O_4^{2-}$	6	3200	Ca^{2+}, 3200 p.p.m.
NO_2^-	6	3200	Urea, 12000 p.p.m.

* DMF = dimethylformamide.
† *NN*-Bis(2-hydroxyethyl)glycine.

TABLE III

DETERMINATION OF COBALT IN THE PRESENCE OF SYNTHETIC MIXTURES OF FOREIGN IONS

Mixtures of foreign ions, p.p.m.	Masking agent, p.p.m.	Co added, p.p.m.	Co found, p.p.m.
150 Fe(III) + 50 Mo(VI) + 50 W(VI)	PO_4^{3-} (48000)	1.89	1.92
		3.24	3.15
5 Cr(III) + 5 V(V) + 10 Mn(II)	—	1.89	1.90
		3.24	3.11
150 Fe(III) + 32 Mn(II) + 12 Cu(II) + 10 Ni(II)	PO_4^{3-} (48000) and 0.5 ml of thiourea at 40% in DMF*	1.89	1.93
		3.24	3.01
300 Mn (II) + 50 Ni(II) + 1500 Fe(III)	PO_4^{3-} (48000) and 1 ml of thiourea at 40% in DMF*	1.89	2.18
		3.24	2.28
300 Al(III) + 60 Mo(VI) + 6 Ni(II)	—	1.89	1.85
		3.24	3.25
150 Fe(III) + 40 Mn(II) + 20 Cr(III) + 3 V(V) + 2 Mo(VI) + 32 W(VI) + 10 Cu(II)	PO_4^{3-} (48000) and 2.5 ml of thiourea at 40% in DMF*	1.89	1.95
		3.24	2.96
160 Fe(III) + 32 Mn(II) + 32 Cu(II) + 10 Ni(II)	PO_4^{3-} (6000) and 2.5 ml of thiourea at 40% in DMF*	1.89	1.87
		3.24	3.20

* DMF = dimethylformamide.

copper(II) and nickel with thiourea and iron(III) with phosphate. Mixtures of phosphate (first added as sodium hydrogen orthophosphate) and thiourea were effective in masking the interferences due to several mixtures of foreign ions. Any precipitate obtained was dissolved after addition of perchloric acid. In some instances complete dissolution was not achieved and precipitates were removed by filtration from the solutions.

Applications

The recommended procedure for the determination of cobalt was applied in a variety of situations to evaluate its effectiveness. The results obtained in the determination of cobalt in hydrofining catalysts are shown in Table IV. A series of recovery experiments were carried out by adding standard pure cobalt solution to aliquots of dissolved sample alloys such as Aluminium alloy 20b, Aluminium bronze 32a, Raney nickel and nickel stone, and to samples of various salts. The results obtained are given in Tables V and VI, and were typical of those expected for good spectrophotometric procedures.

TABLE IV

DETERMINATION OF COBALT IN HYDROFINING CATALYSTS

Molybdenum - cobalt - nickel - aluminium catalysts.

Catalyst	Volume taken/ml	Co content,* %
Sample 1 (0.2029 g per 100 ml) . .	5	1.62
	4	1.58
	3	1.60
Sample 2 (0.2101 g per 100 ml) . .	15 + 4.59 p.p.m. of Co	0.045
Sample 3 (0.2216 g per 100 ml) . .	15	—
	10	—

* Measured against sample solution.

Comparison with Other Chromogens for Cobalt

Although the molar absorptivity of the BMPH procedure compares unfavourably with the values obtained with other pyridylhydrazones reported previously,[1,2] BMPH reagent is cheaper and it is very easily synthesised. The attraction of the BMPH procedure is its good reproducibility and selectivity, which can be greatly enhanced by use of appropriate masking agents. Bipyridylglyoxal dithiosemicarbazone,[5] sodium diethyldithiocarbamate,[10]

TABLE V

RECOVERY OF COBALT ADDED TO RANEY NICKEL, NICKEL STONE AND ALUMINIUM ALLOYS

Sample	Concentration of solution/ g l^{-1}	Volume of sample solution taken/ ml	Co added,* %	Thiourea/ g	BMPH reagent (0.2%)/ ml	Co found,† %
Raney Ni	0.9665	2.5	1.995	0.5	5	1.835 ± 0.063
Ni Stone	1.045	1	4.521	1	7.5	4.376 ± 0.040
Aluminium alloy 20b ..	5.0031	3	0.315	0.6	5	0.331 ± 0.019
Aluminium bronze 32a ..	1.066	0.5	8.865	1	7.5	7.679 ± 0.095

* 1.89 p.p.m. of Co in the final solution in all instances.
† Triplicate determinations.

3-hydroxypicolinaldehyde azine[11] and 3-(2′-thiazolylazo)-2,6-diaminotoluene[6] have been proposed as chromogenic reagents for the spectrophotometric determination of cobalt in hydrofining catalysts. All of these methods, however, have some disadvantages, such as interferences, high reagent costs, the need to extract the coloration or a long time for constant absorbance, and therefore their application in routine analysis is not completely satisfactory. A number of methods based on the use of 2-nitroso-5-dimethylaminophenol,[12] 1-nitrosonaphth-2-ol[13] and 1-(2-pyridylazo)naphth-2-ol[14] and the effect of cobalt on the rate of reaction between catechol and hydrogen peroxide,[15] have been proposed for the spectrophotometric determination of cobalt in salts. The very good precision of direct spectrophotometric measurements achieved with BMPH permits the determination of cobalt in salts in the 10^{-4}% *m/m* range.

TABLE VI

DETERMINATION OF COBALT IN VARIOUS SALTS

Cobalt added: 1.89 p.p.m.

Salt	Amount added/ g l^{-1}	Absorbance values with cobalt added*	Absorbance value without cobalt added*	Cobalt concentration, % *m/m* × 10^4
$Zn(NO_3)_2.4H_2O$	38	0.129, 0.127, 0.129	0.008	3.0 ± 0.4
$Cd(NO_3)_2.xH_2O$	34	0.123, 0.126, 0.122		
$3CdSO_4.xH_2O$	43.5	0.123, 0.122, 0.122		
$Pb(CH_3COO)_2.xH_2O$ ..	47	0.131, 0.129, 0.128	0.005	3.0 ± 0.2
$SnCl_2$	15	0.108, 0.122, 0.113		
KNO_3	40	0.122, 0.124, 0.115		
NaCl	49	0.124, 0.122, 0.120		
$SbCl_3$	46	0.112, 0.117, 0.117		
Na_2HPO_4	290	0.131, 0.131, 0.133	0.009	0.58 ± 0.04
CH_3COONH_4	138	0.135, 0.135, 0.139	0.011	1.7 ± 0.2

* Measured against sample solution because of the absorbance of these salts at the concentrations used (less than 0.010 absorbance unit in all instances). Absorbance values for 1.89 p.p.m. of Co: 0.120, 0.122 and 0.119.

Conclusion

The BMPH method for cobalt is rapid, precise, selective and easy to use. The procedure as described can be performed with chemicals and equipment common in most laboratories. Nevertheless, some limitations of the method are evident, *e.g.*, interferences due to large amounts of copper and chromium and low sensitivity. While not advocating that the method should replace the usual procedures for the determination of cobalt, the features of the BMPH method demonstrated here show that it should not be precluded as an analytical technique.

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Micellar Enhanced Fluorimetric Determination of 1-*NN*-Dimethylaminonaphthalene-5-sulphonyl Chloride and *o*-Phthalaldehyde - 2-Mercaptoethanol Derivatives of Amino Acids

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The effects of various surfactant micellar systems upon the spectrofluorimetric method for the determination of amino acids by Roth's method and the dansyl chloride procedure have been assessed. Specifically, the fluorescence intensity of dansyl glycine was found to be remarkably enhanced when in the presence of cationic hexadecyltrimethylammonium chloride or dodecyltrimethylammonium chloride and zwitterionic *N*-dodecyl-*NN*-dimethylammonium-3-propane-1-sulphonic acid micellar systems. Likewise, the lysine derivative of *o*-phthalaldehyde - 2-mercaptoethanol exhibited intensified fluorescence when in the presence of non-ionic Brij-35 or Triton X-100 and anionic sodium dodecylsulphate micelles. Depending upon the type of surfactant micellar system employed, fluorescence enhancements of from 8 to 20 were observed in comparison with that in water alone. Owing to this micellar phenomenon, the sensitivity of the fluorimetric methods for the determination of these two species was correspondingly increased from 8- to 20-fold over the conventional procedures.

The spectral parameters, quantum yields, lower detection limits and analytical figures of merit for these two substances in the micellar systems and water alone are compared. Possible reasons for this observed micellar induced enhanced fluorescence are given. A brief prospective on the general applicability of using micelles to enhance other fluorimetric methods is also given.

Keywords: *Micellar enhanced fluorescence*; *Roth's method*; *dansyl chloride procedure*; *lysine*; *dansyl glycine*

Of the many fluorimetric reagents available for the determination of amino acids, the use of 1-*NN*-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride)[1,2] and Roth's reagents, *o*-phthalaldehyde (OPTA) - 2-mercaptoethanol (MERC),[3,4] appears to be the most popular. These reagents react with most amino acids (as well as many amines) to form the fluorescent derivatives, 1-*NN*-dimethylaminonaphthalene-5-sulphonamides and 1-alkylthio-2-alkylisoindoles, respectively.[1–4] The fluorescence quantum yields of these amino acid derivatives are reportedly very sensitive to the environment about their excited states.[5–7] In fact, as the dielectric constant of the solvent composition is decreased, fluorescence enhancements of from 2 to 10 were observed for dansyl amino acids.[5,6] The intensity of the OPTA - MERC derivatives is likewise increased by factors of from 1.7 to 17 when in the presence of 32–85% aqueous dimethyl sulphoxide (DMSO) solvent mixtures.[6,7] Although impressive, this solvent-induced enhanced fluorescence does not usually evolve into a more sensitive procedure for these amino acids because the increased fluorescence obtained is more than offset by the dilution effect (owing to the addition of the co-solvent to the normally aqueous sample of the amino acid to be determined).

In this preliminary work, we report the use of aqueous micellar systems as an alternative to the use of mixed solvent systems in these fluorimetric amino acid assays. Upon reaching a certain minimum concentration (termed the critical micelle concentration, CMC), amphiphilic surfactant molecules tend to associate dynamically in aqueous solution to form

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molecular aggregates termed micelles.[8–10] The local micro-environment encountered by a solute associated with a micellar system can be drastically different from that which it experiences in a bulk homogeneous solvent system. Consequently, the solute's fluorescence parameters can be affected. For many solutes in micelles, significant increases in fluorescence lifetimes and quantum yields have been observed.[8–11] These effects have been rationalised in terms of favourable alteration of such "solvent" properties as micro-polarity, micro-fluidity (viscosity) and dielectric constant about a solute associated with a micelle in addition to the micellar effects upon the various photophysical rate processes.[8–11] The net result is that the micelle provides a protective environment for the excited singlet state.

Despite the implications of these published results, only sparse analytical advantage has been taken of micellar enhanced fluorescence. Most of the work carried out utilising micelles in this respect has concerned the determination of metal ions via formation of fluorescent metal - dye complexes.[12–18] We have previously reported micellar improved fluorimetric methods for the determination of cyanide ion,[19,20] pyrene[21,22] and other polynuclear aromatic hydrocarbons.[22] A review of the literature revealed that no study had been made of micellar effects upon the fluorescence determination of dansyl amino acids, which is surprising as several have been used as probes of micellar structure and dynamics.[23–25] Those results show that the fluorescence intensity can be greatly increased in the presence of micelles. Additionally, several papers have casually mentioned that surfactant systems can increase the fluorescence of some OPTA - MERC amino acid derivatives[7,26–28]; however, no thorough comparative study of the micellar effects has been made.

We have found that the fluorescence intensity of dansyl glycine and the OPTA - MERC derivative of lysine is enhanced by 8.5–20.4 times when in the presence of surfactant micelles compared with that in water. As the concentration required to form micelles is low (*i.e.*, 1×10^{-5}–1×10^{-2} M), only a small amount of the surfactant needs to be added to the original sample (as either a concentrated solution or the pure material). Hence, there is no appreciable loss of sensitivity due to dilution. In addition to the remarkably enhanced fluorescence and improved sensitivity, the use of aqueous surfactant micelles offers advantages in terms of being less expensive, less toxic and less volatile when compared with the usual mixed organic - aqueous solvent systems employed.

Experimental

Materials and Apparatus

All salts (ACS grade or better) used to prepare the buffers, *o*-phthalaldehyde (analytical-reagent grade), DMSO (spectroanalysed) and water (HPLC grade) were obtained from Fisher Scientific Company (Raleigh, NC, USA). Dansyl glycine and β-cyclodextrin (β-CD) were procured from Sigma Chemical Company (St. Louis, MO, USA). L-(+)-Lysine dihydrochloride, 2-mercaptoethanol (98+ %), dodecyltrimethylammonium chloride (DTAC) and hexadecyltrimethylammonium chloride (CTAC) were purchased from Eastman Kodak Company (Rochester, NY, USA). 1-(1,1-Dimethyl-3,3-dimethylbutane)-4-polyethyleneoxybenzene [Triton X-100 (TX-100)] and sodium dodecylsulphate (NaLS), both electrophoresis purity reagents, were obtained from Bio-Rad Laboratories (Rockville Centre, NY, USA). *N*-Dodecyl-*NN*-dimethylammonium-3-propane-l-sulphonic acid [sulphobetaine 12 (SB 12)] (99+ %) was a Serva Biochemical Company (Garden City Park, NY, USA) product. All reagents were used as received. In most instances, distilled, de-ionised water was used to prepare all solutions.

The fluorescence spectra were recorded and measurements made on an Aminco-Bowman spectrophotofluorimeter (American Instruments) equipped with a xenon arc source, 1P21 PM tube and a J10-222A PM micro-photometer using conventional 1.00 × 1.00 × 4.50 cm quartz cells with the exit, entrance and detector slits all set at 2.0 mm unless otherwise noted. Absorption measurements and spectra were obtained using a Varian Cary, Model 219, spectrophotometer.

Procedures for the Measurement of Dansyl Glycine

Solvent effect studies

Concentrated stock solutions of 1-*NN*-dimethylaminonaphthalene-5-sulphonylglycine (dansyl glycine) were freshly prepared by dissolving a weighed amount of the pure solid in ethanol and diluting with distilled water (final composition was 20% *V*/*V* ethanol). The

standard fluorescence - concentration calibration graphs for dansyl glycine in the different surfactant and solvent systems were then prepared by serial dilution of the dansyl glycine stock solutions with distilled water and appropriate amounts of a concentrated solution of the surfactant or cyclodextrin system being studied. The total ethanol concentration was never above 0.3% *V/V*. After thorough mixing, the fluorescence intensity was measured at the optimum fluorescence excitation and emission wavelengths.

Recommended procedure for the determination of dansyl glycine

To a measured aliquot of the sample of dansyl glycine solution is added sufficient hexadecyltrimethylammonium chloride (CTAC) to ensure the formation of micelles (*i.e.*, [CTAC] > CMC $\approx 1.3 \times 10^{-3}$ M). After mixing, the fluorescence intensity was measured ($\lambda_{ex.} = 350$ nm, $\lambda_{em.} = 510$ nm). A blank containing only the surfactant solution should be run concurrently. The concentration of dansyl glycine can be determined in the 1.0×10^{-9}–3.2×10^{-5} M concentration range by comparison with known standards or from calibration graphs prepared under the same experimental conditions.

Determination of Lysine as the OPTA - MERC Derivative

The OPTA - MERC - lysine derivative was prepared using a modification of Roth's procedure.[3,4] A 2.00-ml aliquot of the aqueous lysine sample was buffered to a pH of 10.10 using disodium tetraborate. To this solution were added 30 μl of solutions of 10 mg ml^{-1} OPTA in ethanol, 15 μl of 10 μl ml^{-1} MERC in ethanol and 100 μl of an appropriate concentrated stock solution of the surfactant. This solution was mixed vigorously by shaking. After 5 min reaction time, the fluorescence intensity was measured ($\lambda_{em.} = 430$–440 nm, depending on the surfactant employed; $\lambda_{ex.} = 344$ nm). A blank solution containing the same composition of OPTA, MERC and solvent system was run concurrently. Standard calibration graphs in the different media were prepared in a similar fashion by varying the lysine concentration by serial dilution of a concentrated stock solution. Lysine can thus be determined in the 1×10^{-8}–4×10^{-5} M concentration range.

Quantum Yield Determinations

The relative fluorescence quantum yields for dansyl glycine and the OPTA - MERC - lysine derivative in the different solvent systems were determined by comparison with a standard quinine sulphate solution (1.00 μg ml^{-1} in 0.05 M sulphuric acid) in the usual manner.[29]

Results and Discussion

Description and Properties of Surfactant Micelles

Upon reaching their CMC, amphiphilic molecules dynamically associate in aqueous solution to form aggregates termed micelles. Each micelle is composed of a certain number of monomer surfactant molecules referred to as its aggregation number, *N*. The shape of these micellar ensembles is roughly spherical with the hydrophilic head groups directed towards and in contact with the aqueous solution, thus forming a "polar" surface, while the hydrophobic alkyl tails are directed away from the water, forming a central non-polar core.[8,30] The charge of the micellar surface is determined by the nature of the hydrophilic headgroup that is attached to the hydrophobic backbone of the surfactant molecule. In this work, cationic CTAC and DTAC, anionic NaLS, non-ionic TX-100 and Brij-35 and zwitterionic SB 12 were the surfactants employed.

Table I summarises the micellar parameters (CMC and *N*) for each of these surfactants along with their "solvent" properties.[30–37] For comparison purposes, the properties of some common bulk solvents are also included. As can be seen, compared with water, the microscopic polarity, as expressed by the empirical $E_{T(30)}$ scale,[31] and dielectric constant are reduced while the viscosity is greatly increased in these surfactant micellar systems. Judging from the published information on solvent dependence of the fluorescence for dansyl or OPTA - MERC derivatives of amino acids,[1–7] the microenvironment provided by these micelles should be ideal for the observation of enhanced fluorescence.

TABLE I

SUMMARY OF MICELLAR PARAMETERS AND SOLVENT PROPERTIES

Micellar (or solvent) system	CMC × 10^3/ M*	N*	$E_{(T30)}$†	Effective dielectric constant	Microscopic viscosity/ cP
Hexadecyltrimethylammonium chloride (CTAC)	1.3	78‡	53.2§	28–49§¶	18–31§
Dodecyltrimethylammonium chloride (DTAC)	20.3	—	54.2§	30–40§	—
Sodium dodecylsulphate (NaLS)	8.1	62	57.5§	40±8,¶‖ 51–55§	9–12,‡ 19–31‖
N-Dodecyl-*NN*-dimethylammonium-3-propane-1-sulphonic acid (SB 12)	1.2–4.6**	—	—	—	—
1-(1,1-Dimethyl-3,3-dimethylbutane)-4-polyethyleneoxy(9.5)benzene (TX-100)	0.24–0.42	143	53.0§	28±8§‖	28–35‖
Polyoxyethylene(23)dodecanol (Brij-35)	0.06–0.09	40	52.8§	27–29§	—
Water	—	—	63.1††	78.54‡‡	0.8904‡‡
Methanol	—	—	55.5††	32.63‡‡	0.547‡‡
Ethanol	—	—	51.9††	24.3‡‡	1.20‡‡
Acetonitrile	—	—	46.0††	37.5‡‡	0.345‡‡
Dimethyl sulphoxide	—	—	45.0††	46.68	1.96
NN-Dimethylformamide	—	—	43.8††	36.7‡‡	0.80

* Taken from references 8 and 30 unless otherwise noted.
† Empirical microscopic solvent polarity parameter, see reference 31 for more details.
§ Taken from reference 32.
¶ Taken from reference 33.
‡ Taken from references 34–36.
‖ Taken from reference 37.
** Taken from reference 39.
†† Taken from reference 31.
‡‡ Taken from reference 38.

Characterisation of Dansyl Glycine and the OPTA - MERC - Lysine Derivative in Different Surfactant Systems

The absorption and fluorescence spectral behaviours of DG and OPTA - MERC - lysine in the surfactant systems compared with that in water alone are very similar. In water, dansyl glycine and OPTA - MERC - lysine exhibit absorption bands centred at 330 and 340 nm, respectively, while in the presence of surfactant micelles, these bands bathochromatically shift to 338 and 343 nm, respectively. Within the limits of experimental error, the intensity of these absorption bands appears to remain essentially constant (Table II).

As the surfactant concentration is increased, the fluorescence emission peaks of both dansyl glycine and OPTA - MERC - lysine progressively shift towards shorter wavelengths. Concomitantly, the fluorescence intensity of these peaks significantly increases. Depending upon the surfactant micelle employed, fluorescence enhancements ranging from 8 to 20 are

TABLE II

SUMMARY OF MOLAR ABSORPTIVITY AND QUANTUM YIELD DATA FOR DANSYL GLYCINE AND OPTA - MERC DERIVATIVE OF LYSINE IN DIFFERENT MEDIA

	Dansyl glycine		OPTA - MERC - lysine	
Solvent system	$\epsilon \times 10^3$ l mol^{-1} cm^{-1} (at 350 nm)	ϕ	$\epsilon \times 10^3$ l mol^{-1} cm^{-1} (at 344 nm)	ϕ
Water	3.88	0.01 (0.065)*	5.05	0.04 (0.034)†
CTAC (2 g l^{-1})	4.32	0.19	—	—
DTAC (15 g l^{-1})	—	0.10	—	—
β-CD (10 mM)	4.32	0.11	—	—
SB 12 (5 g l^{-1})	3.88	0.13	—	—
NaLS (2.5 g l^{-1})	—	—	5.25	0.15
Brij-35 (2.5 g l^{-1})	—	—	5.60	0.27
TX-100 (1.0 g l^{-1})	—	—	5.60	0.35

* Taken from reference 5.
† Taken from reference 7.

observed. These parameters ($\lambda_{em.}$ and fluorescence intensity) tend to reach a limiting value at surfactant concentrations just above the CMC for each surfactant. Under these conditions, the fluorescence emission of dansyl glycine shifted from 550 nm in water to 512 $\pm$ 2 nm when in the presence of micellised CTAC, DTAC or SB 12, while that of OPTA - MERC - lysine shifted from 455 nm (in water) to 435 $\pm$ 3 nm (in micellar TX-100, Brij-35, NaLS or CTAC).

Interestingly, the positions of the absorption and fluorescence emission bands of dansyl glycine and OPTA - MERC - lysine in aqueous surfactant micelles are very similar to those observed for these species when in dipolar aprotic solvents such as DMSO or acetonitrile. This implies that the micro-environment provided by these micellar systems mimics that of lower dielectric constant organic solvents as far as these two solutes are concerned. This should not be too surprising in view of the data presented in Table I concerning the "solvent" properties of these respective micelles. (Chen has suggested that the observed solvent effects upon $\lambda_{em.}$ of dansyl glycine may be rationalised in a qualitative fashion in terms of Lippert's theory.[5] However, our results indicate that these solvent effects can be better understood and rationalised in terms of the multiple dependence on the solvochromatic parameters π^*, α and β, which have been developed by Kamlet *et al.*,[40] Kolling[41] and Taft *et al.*[42]).

The fluorescence intensity - surfactant concentration dependence of both dansyl glycine and OPTA - MERC - lysine indicates that the presence of micelles is required for observation of the maximum values of enhanced fluorescence. The addition of tetramethylammonium chloride (TMAC) or tetrabutylammonium chloride (TBAC) did not change either the absorption or fluorescence parameters of dansyl glycine or OPTA - MERC - lysine compared with that in water. Both TMAC and TBAC have the same ionogenic group as do CTAC and DTAC, except that they are not capable of forming micelles. Clearly, this indicates that the presence of micelles, rather than just formation of hydrophobic ion pairs, is required for the enhanced fluorescence observed in this study. Consequently, it is necessary to ensure that the surfactant concentrations in the sample solutions are slightly above their CMC values in the recommended procedures. Our results suggest that some of the discrepancies in the published work concerning the use of Brij-35 in Roth's procedure[26–28] are most probably the consequence of an insufficient concentration of the surfactant. For all of the quantitative data obtained for dansyl glycine and OPTA - MERC - lysine in this study, the surfactant concentrations were adjusted to the approximately 2–4 times that of their respective CMC values (Table I).

Possible Reasons for the Enhanced Fluorescence in Micelles

In the absence of any other changes in the experimental conditions, the observed enhancements in the micellar systems must stem from an increase of either the molar absorptivity (ϵ) at the exciting wavelength and/or quantum yield (ϕ) of the species compared with that in bulk solvents alone. As ϵ for both dansyl glycine and OPTA - MERC - lysine in the various media is essentially constant, the observed enhancements are primarily due to an increase in ϕ (refer to Table II). Clearly, the rate constants for deactivation of the excited stated by the radiationless processes are significantly reduced when these species are in the presence of micelles.

In the dansyl amino acid derivatives (such as dansyl glycine), the amino side-chain is thought to play a significant role in radiationless transitions from the excited state,[5] while for the OPTA - MERC - lysine derivative, interaction between the two isoindole groups of the disubstituted lysine reportedly quenches the fluorescence.[7] Additionally, water is capable of interacting with indole's excited state to form an exciplex, which competes with the desired radiative pathway for deactivation of the excited state.[6] These non-radiative photophysical processes are obviously greatly retarded when these amino acid derivatives are in a micellar environment. Our spectral studies show that both dansyl glycine and OPTA - MERC - lysine are effectively compartmentalised when they partition to and bind the micellar system. These "bound" solutes are probably much more restricted (less mobile) in such an environment compared with the situation in a bulk solvent. Additionally, the micro-polarity is altered (Table I). Owing to a combination of these factors, the radiationless and/or quenching interactions mentioned for dansyl glycine and OPTA - MERC - lysine are diminished. This makes possible the observed dramatic increase in the quantum yield

(or fluorescence intensity) of these species. Similar arguments have been used to explain the increased fluorescence exhibited by other substances in micelles.[8–19]

Analytical Parameters and Figures of Merit

The standard calibration graphs of fluorescence intensity *versus* concentration for OPTA - MERC - lysine and dansyl glycine in the different media are shown in Figs. 1 and 2, respectively. As is evident, the intensity of fluorescence for each species is much greater in the micellar systems. For instance, the fluorescence intensity of dansyl glycine in micellar

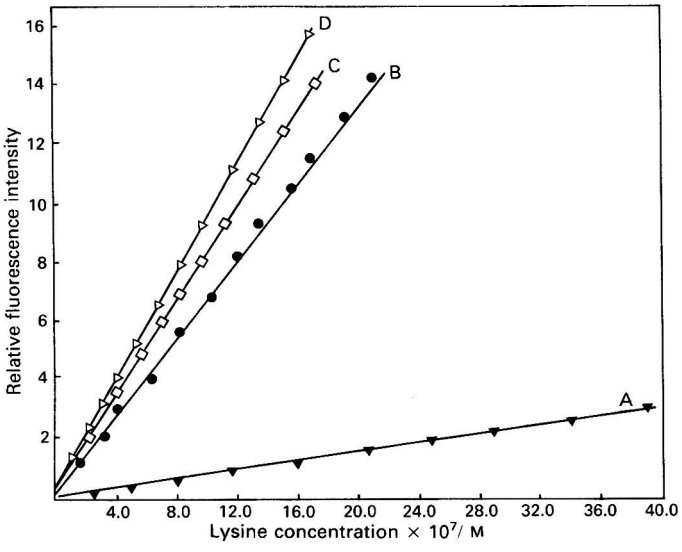

Fig. 1. Typical calibration graphs of OPTA - MERC - lysine fluorescence intensity ($\lambda_{ex.}$ = 344 nm, $\lambda_{em.}$ = 444 nm) *versus* lysine concentration in: A, water, B, 17.4 mM NaLS; C, 2.1 mM Brij-35; and D, 1.6 mM TX-100.

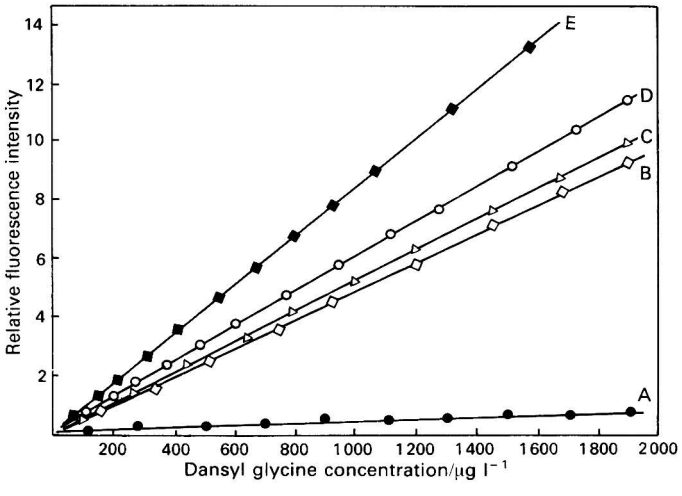

Fig. 2. Analytical calibration graphs of fluorescence intensity ($\lambda_{ex.}$ = 350 nm, $\lambda_{em.}$ = 510 nm) *versus* concentration of dansyl glycine in: A, water; B, 24.0 mM DTAC; C, 10 mM β-CD; D, 14.9 mM SB 12; and E, 6.3 mM CTAC.

DTAC, SB 12 and CTAC is 11.8, 14.6, and 20.4 times greater, respectively, compared with that in water. Similar enhancements were observed for other dansyl amino acids in micelles.[43] It should also be noted that the fluorescence intensity of dansyl glycine in CTAC is approximately twice that observed in β-cyclodextrin (β-CD), which has been employed to increase the sensitivity of the dansyl amino acid assay.[45] Likewise, in the presence of micellar NaLS, Brij-35 and TX-100, the OPTA - MERC - lysine derivative's fluorescence is enhanced by factors of 8.5, 10.3 and 11.4, respectively. Enhancements of 1.2–4.4 (simple amino acids) and 8–70 (peptides, amides or amines) were noted for other OPTA - MERC derivatives of amino compounds.[43,44] These enhancements were as great as or better than those obtainable in pure or mixed aqueous dipolar aprotic solvent systems.

In all instances, the observed fluorescence enhancements directly lead to lower detection limits. The limit of detection for dansyl glycine in CTAC is 1.00×10^{-9} M, and the linear dynamic range spans four decades. By comparison, the limit of detection for dansyl glycine in water is only 2.5×10^{-8} M, under the same instrumental conditions. Likewise, the limit of detection for the determination of lysine by Roth's method is significantly diminished (from approximately 1×10^{-7} M in water to approximately 1×10^{-8} M in micellar TX-100). Thus, addition of the appropriate surfactant to an aqueous solution of the amino acid to be determined increases dramatically the sensitivity of these assays. As only very small volumes of a concentrated surfactant stock solution need be added to the original sample, there is no loss of sensitivity due to dilution. The net effect is that the fluorimetric determination of amino acids as their dansyl or OPTA - MERC derivatives in the presence of surfactant micellar solutions rivals that of any other analytical methods currently available.

In addition to the lowered detection limits, the accuracy and precision of the procedures in micelles are as good as or better than those in water. The slope of all log (fluorescence intensity) *versus* log (concentration) calibration graphs was 1.00 ± 0.06. The correlation coefficients of these graphs for OPTA - MERC - lysine were 0.9988, 0.9980, 0.9984 and 0.9904 in TX-100, Brij-35, NaLS and water, respectively. For the calibration graphs of dansyl glycine in all systems, the correlation coefficients were 0.9980 ± 0.0015. In CTAC, for two series of seven measurements each, on 0.50 and 3.00 μg l^{-1} dansyl glycine solutions, relative errors of 2.7 and 1.9% with relative standard deviations of 3.1 and 2.4% were obtained, respectively. For the determination of OPTA - MERC - lysine at 2.00×10^{-7} M in TX-100, a relative error of 2.4% and relative standard deviation of 2.8% were obtained (based upon three series of five measurements each). Thus, at concentrations near the limit of detection, the precision and accuracy of the amino acid assay are much better in the micellar systems.

Conclusion

A fluorimetric procedure for the determination of dansyl glycine and the *o*-phthalaldehyde - 2-mercaptoethanol derivative of lysine has been described that is much more sensitive than existing procedures. The approach is simple, convenient, rapid and accurate. The technique is based upon the use of micellar systems and their unique properties that lead to drastically enhanced fluorescence. Such use of micelles obviates the current need for organic co-solvent systems (along with their associated problems) in these procedures. The enhancement of fluorescence and concomitant reduction of the detection limits by use of micelles should prove to be a very useful technique in chemical analysis. The general approach as outlined in this work for dansyl glycine and OPTA - MERC - lysine should be applicable to many analytical systems, especially in view of the fact that many other types of compounds have been shown to exhibit increased fluorescence when in the presence of micelles.[8–25,37] In fact, our own work indicates that more sensitive procedures can be developed for the fluorimetric determination of such substances as cyanine and merocyanine dyes,[43,44] polynuclear aromatic hydrocarbons,[21,22] quinones,[43] cyanide ion[19,20] and different vitamins[43] via use of micellar enhanced fluorescence. Recently Armstrong *et al.*[46] reported the first known example of micellar enhanced fluorescence and stabilised room temperature liquid phosphorescence detection in high-performance liquid chromatography. Many other such applications will no doubt soon be reported. Further work in this area will lead to improved and extremely sensitive fluorimetric methods.

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Infrared Determination of Petroleum Oil

Part II.* Extraction from Water and Removal of Interferences

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A brief review is given of the methodologies used in the infrared determination of petroleum oil. Some performance characteristics are presented for a method that measures hydrocarbons after a Florisil column clean-up. Extraction efficiencies were determined for dispersions of gas oil in water for the solvents carbon tetrachloride and Freon 113. The removal of interferences was also studied.

Keywords: Petroleum oil determination; infrared spectroscopy; solvent extraction; hydrocarbons; interferences

Methods for the determination of oil in water by extraction into carbon tetrachloride and measurement of the infrared absorbance in the region 3400 cm^{-1} (2.94 μm)–2600 cm^{-1} (3.85 μm) have been described by a number of workers since 1951.[1–8] The approaches to the calibration used in these methods have recently been discussed.[9]

Some methods have removed interfering substances, such as natural oils, by the use of a Florisil column,[7] Florisil with shaking,[3] stirring,[5] or molecular sieve 5A.[8] Other methods[1,2,4,6] make no attempt to separate these substances before measuring the infrared absorbance of the extract. The less toxic solvent Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) has been used by two groups as an alternative to carbon tetrachloride in the extraction.[4,6] All workers add sodium chloride or potassium bromide and all but one[8] acidify to improve the extraction efficiency.

The method employed in this laboratory uses a carbon tetrachloride extraction, after the sample has been acidified and sodium chloride has been added to give a concentration approximately the same as that of sea water, and a Florisil column clean-up of the extract. The aim of this study was to investigate some performance characteristics of the method incorporating the new calibration procedure,[9] to compare this method with that using Freon 113 extraction and to investigate the removal of interfering substances by the Florisil column.

Experimental

Apparatus

Double-beam infrared spectrophotometer. Pye Unicam SP 1200. This was fitted with an IR 50 detector.

Cells. These were 10- and 2-mm matched infrared-grade silica cuvettes.

Bottles. These were 1-l glass bottles with PTFE-lined screw-caps.

PTFE screw-cap - B24 adaptor. Jencon's Scientific Ltd.

Solvent removal device. See Fig. 1.

Chromatographic column. A short column, approximately 150 × 10 mm i.d. (Quickfit CR 12/10).

Syringe. Hamilton 10-μl syringe.

Electrically driven rollers, 60 rev min^{-1}. Biddulph and Co. (M/c) Ltd., Manchester.

Reagents and Materials

Carbon tetrachloride. Analytical-reagent grade.

1,1,2-*Trichloro*-1,2,2-*trifluoroethane* (*Freon* 113). BDH Chemicals Ltd., Poole, Dorset.

Florisil, 60–80 mesh. This was prepared to a fixed activity by the following procedure. The Florisil was heated at 400 °C in a silica basin for 2 h and allowed to cool to 200 °C in the furnace. The basin was removed into a desiccator and allowed to cool to room temperature. A weighed amount of this Florisil was placed in a wide-mouthed glass bottle, 6% by mass of water was added and the bottle was capped, shaken and rolled on electrically driven rollers for 1 h.

* For details of Part I of this series, see reference list, p. 1085.

Hexadecane.
2,6,10,14-*Tetramethylpentadecane* (*Pristane*). Koch-Light Laboratories Ltd.
Toluene. Analytical-reagent grade.
Sodium chloride. Commercial grade.
Sodium sulphate, anhydrous granular. Analytical-reagent grade.
Hydrochloric acid, concentrated. Analytical-reagent grade.
Oils. These were from a reference collection held at this laboratory.

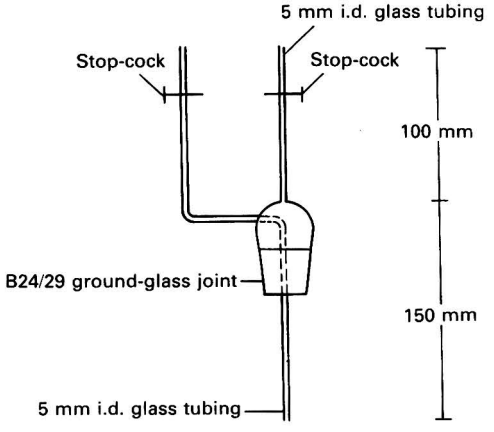

Fig. 1. Solvent removal device.

Procedure

Dispersions of a gas oil in water were prepared by injecting known volumes with a micro syringe into approximately 900 cm³ of tap water in 1-l glass bottles. The bottles were then sealed with PTFE-lined screw-caps and then shaken vigorously by hand for 2 min. Standard solutions of the gas oil in carbon tetrachloride and Freon 113 were prepared by injecting 5 μl of the gas oil into 25 cm³ of solvent in a calibrated flask.

Extraction of the dispersed oil into carbon tetrachloride or Freon 113 was carried out in the following manner. A 40-g amount of sodium chloride, 2 cm³ of concentrated hydrochloric acid and 25 cm³ of solvent were added to the bottle. The bottle was sealed, shaken vigorously by hand for 1 min and then rolled for a further 30 min on electrically driven rollers.

The solvent layer was removed, by means of the solvent removal device shown in Fig. 1, into a small beaker, after having first decanted most of the aqueous layer. The extract was dried by adding 4 g of anhydrous sodium sulphate.

A column of Florisil was prepared by wet packing the chromatographic column to a depth of approximately 3 cm and then with a further 1 cm of anhydrous sodium sulphate (bed volume 2.5 cm³). The column was washed through with 5 cm³ of carbon tetrachloride.

The extract was then passed through the column and the first 5 cm³ were discarded. Further extract was eluted to fill the cuvette and the infrared spectrum of the eluate was recorded between 2500 and 3400 cm^{-1} using suitable cuvettes with the appropriate solvent in the reference beam. The absorbances were measured at 2930, 2960 and 3030 cm^{-1}.

Blank determinations were carried out for each solvent by the above procedure using tap water.

The infrared spectra of the standard solutions were recorded, after passage through the Florisil column, in a similar manner using 10-mm cuvettes (Fig. 2).

Solutions of interfering substances were prepared by weighing and dissolving in a known volume of carbon tetrachloride. The infrared spectra of the solutions were recorded both before and after passage through the Florisil column.

Calibration of the instrument for both carbon tetrachloride and Freon 113 solvents was

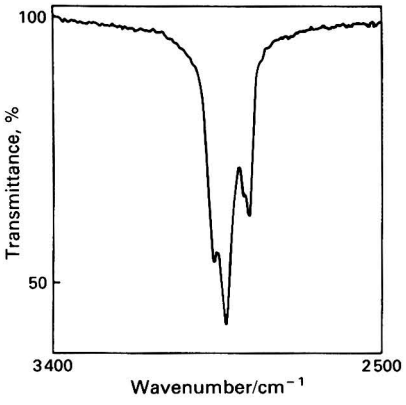

Fig. 2. Infrared spectrum of gas oil standard in carbon tetrachloride.

carried out as described previously[9] for carbon tetrachloride using solutions of hexadecane, pristane and toluene. The calibration was checked using solutions of a gas oil, white spirit, 3-star petrol and 20/50W lubricating oil.

Results and Discussion

Table I shows the calculated concentrations and precisions for the extracts in both solvents for the different volumes of gas oil dispersed in water and also the calculated concentrations for the standards in both solvents. Dispersions of the oil and the standards are based on volume, as injecting the oil into water and shaking was found to be the only practical method of preparing dispersions. A 5-μl volume of gas oil injected into 900 cm^3 of water gives a concentration of 4.4 mg l^{-1}.

The measured concentrations in standards and extracts are calculated by the equation

$$C = \left[xA_{2\,930} + yA_{2\,960} + z\left(A_{3\,030} - \frac{A_{2\,930}}{F}\right)\right]\frac{10}{L} \quad .. \quad .. \quad (1)$$

where C mg l^{-1} is the concentration of oil in the extract or standard; $A_{2\,930}$, $A_{2\,960}$ and $A_{3\,030}$ are the absorbances corresponding to the C–H stretch absorbances of CH_2 groups, CH_3 groups and CH (aromatic) groups, respectively; x, y and z are calibration factors[9] and are found by the solution of three simultaneous equations of the form of equation (1) for individual solutions in the appropriate solvent of known concentration of the compounds

TABLE I

EXTRACTION RECOVERIES OF GAS OIL USING CARBON TETRACHLORIDE AND FREON 113

Each based on 7 replicates; concentrations shown are in the extracts. The recoveries shown are based on the measured concentration of the standard solution and thus eliminate calibration bias.

	Carbon tetrachloride			Freon 113		
Volume of gas oil injected/μl	Measured concentration/ mg l^{-1}	Standard deviation/ mg l^{-1}	Recovery, %	Measured concentration/ mg l^{-1}	Standard deviation/ mg l^{-1}	Recovery, %
5*	150	2.36	—	169.6	6.48	—
1.25	40.6	3.40	108.3	34.8	4.92	82.1
5	144.0	3.96	96.0	146.1	6.72	86.1
25	703.8	22.0	93.7	744.3	25.0	87.8

* Standard.

hexadecane, pristane and toluene; F is a correction factor to compensate for the contribution to the absorbance at 3030 cm^{-1} by the peaks at 2930 cm^{-1} and 2960 cm^{-1}. In practice a suitable value for F may be found from the absorbance values found for the hexadecane solution by letting $F = A_{2930}/A_{3030}$, as hexadecane has no aromatic absorbance. This makes the term $A_{3030} - A_{2930}/F$ in equation (1) equal zero for hexadecane and an assumption is made that this term can also be ignored in the equation for pristane when solving for x, y and z. L mm is the cuvette path length.

Table I also shows the calculated recoveries for the extracted samples. These recoveries are based on the calculated concentration for a standard prepared directly in the appropriate solvent:

$$R = \frac{C_e V_s}{C_s V_d} \times 100 \qquad (2)$$

where R is the percentage recovery or extraction efficiency, C_e and C_s mg l^{-1} are the calculated concentrations for the extract and standard, respectively, and V_s and V_d μl are the volumes of gas oil used in preparing the standard and dispersion, respectively.

The extraction recoveries calculated by equation (2) are corrected for calibration bias[9] of the oil used by virtue of the nature of the calculation. The calibration bias for a particular oil may be found by measuring the appropriate absorbances for a solution of the oil of known concentration (prepared by weighing) in the solvent of interest. The measured concentration can then be calculated from equation (1) and compared with the true concentration.

If the calculated concentration of oil in water was used and compared with the true concentration of oil in water, the efficiency calculated would include the calibration bias for the oil, as well as the actual extraction efficiency, and it is to avoid this confusion that concentrations are compared between the extracts and a standard in the solvent.

The extracts of the tap-water blanks have no measurable infrared absorbance in the region of interest.

The concentration of oil in an unknown sample would be calculated by an expanded form of equation (1):

$$C_w = \left[xA_{2930} + yA_{2960} + z\left(A_{3030} - \frac{A_{2930}}{F}\right)\right] \frac{10D}{LW} \qquad (3)$$

where C_w mg kg^{-1} is the concentration of oil in water, D is any dilution factor applied to the extract before measurement, L mm is the cuvette path length and W kg is the mass of sample extracted.

Table II shows some possible interferences that are completely removed by the Florisil column. The concentration column shows the actual concentration of each compound in carbon tetrachloride. The apparent concentrations shown are those calculated using equation (1) if the appropriate absorbances are measured before the solution is passed through the Florisil column. The capacity of the column was found to be about 25 mg of tallow.

Halogenated hydrocarbons are known to cause interferences as they are not removed by the Florisil column.[10] However, it is only rarely that these compounds will occur in such concentrations as to cause a problem in the types of sample being analysed.

Table II

Interferences removed by Florisil column

All the interferences shown were completely removed by the Florisil column.

Substance	Concentration/mg l^{-1}	Apparent concentration before Florisil/mg l^{-1}
Soya bean oil	151	169
Herring oil	166	185
Tallow	258	236
Phenol	196	100
Anachidic alcohol	187	100

Table III shows the calibration factors and the results of the calibration checks with four commercial oils for both solvents. These factors and the "bias recoveries" are found as discussed above. The calibration check results show the calibration to give a similar bias for the two solvents.

Although the extraction efficiency of Freon 113 is slightly lower than that of carbon tetrachloride (Table I), when large numbers of routine samples are involved it would be acceptable to use Freon 113 provided that the extraction efficiency had been determined for the particular laboratory and an appropriate correction made.

As we have found in our work that oil pollution of inland waters is due largely to petroleum products, the aim of our method is to give a quantitative measure of hydrocarbons of petroleum origin over a wide boiling range. The method described above has been used in this laboratory, with carbon tetrachloride as solvent, for a number of years. Here we present some performance characteristics of this method at levels of interest in waters and industrial effluents. The levels used here correspond to the range 1–20 mg l^{-1} in water. The method can be used, with modifications, to measure lower concentrations using cuvettes of longer path length and different sample to solvent ratios. However, Freon 113 is not suitable for measuring low concentrations as the transmittance of this solvent in the infrared region used is low and carbon tetrachloride is more satisfactory.

TABLE III

CALIBRATION FACTORS AND CHECKS FOR CARBON TETRACHLORIDE AND FREON 113

Calibration factors for equation (2)—

	Carbon tetrachloride	Freon 113
x	177.5	132.8
y	299.4	183.4
z	1960	1255
F	34.6	31.2

Calibration checks—

	Recovery, %*	
Oil	Carbon tetrachloride	Freon 113
White spirit	108.1	110.5
Petrol, 3 star	102.2	98.0
Gas oil	95.3	101.0
Lubricating oil, 20/50W	87.7	89.7

* Reference 9.

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NOTE—Reference 9 is to Part I of this series.

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SHORT PAPERS

Direct Titrimetric Determination of the Antihypertensive Drugs Methyldopa and Propranolol in Pharmaceutical Preparations

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Keywords: *Methyldopa determination*; *propranolol determination*; *titrimetry*; N-*bromosuccinimide*

Methyldopa and propranolol are valuable selective antihypertensive drugs.[1] A number of methods have been reported for the study of pharmacological actions and the assay of antihypertensive drugs.[2–6]

N-Bromosuccinimide (NBS) has been used as a quantitative brominating and oxidising agent for several organic compounds and drugs.[7–9] In this work, we have carried out the semi-micro determination of methyldopa and propranolol with NBS in various pharmaceutical preparations.

Experimental

Reagents

Pure methyldopa and propranolol and pharmaceutical samples (25 mg) were dissolved in 10 ml of concentrated sulphuric acid (AnalaR, sp. gr. 1.84) by gentle warming on a water-bath for 2 min and the solutions were diluted to 50 ml with distilled water in calibrated flasks.

N-*Bromosuccinimide* (*NBS*), 2×10^{-2} M *solution*. A 0.3560-g amount of NBS was dissolved in the minimum amount of warm distilled water and the solution was diluted to 100 ml with cold distilled water and standardised iodimetrically.[10]

Procedure

An aliquot containing 1–5 mg of the sample is placed in a 100-ml iodine flask followed by the addition of 5 ml of distilled water and two drops of 0.04% methyl red indicator. The reaction mixture was shaken well and titrated with standardised 2×10^{-2} M NBS solution with continuous stirring. The addition of the reagent was continued until the pink colour of the indicator had disappeared. Excess of NBS added, if any, was calculated by back-titrating the reagent iodimetrically. A blank experiment was also run under identical conditions.

The recovery of the sample was calculated from the difference in the volume of NBS consumed in the actual and blank experiments by using the following expressions:

Mass of methyldopa (M_r 211) sample (mg) = 1.055 × volume of NBS consumed (ml)

Mass of propranolol (M_r 259) sample (mg) = 1.036 × volume of NBS consumed (ml)

The validity of the quantitative oxidation of methyldopa and propranolol was proved by performing recovery experiments. A small amount of the pure drug was added to the suitably prepared dosage form and the total amount of the drug was assayed. By subtracting the amount found for the preparation without the addition of pure drug, the amount recovered was calculated and expressed as a percentage of the total amount added (Table I).

Results and Discussion

Results obtained with the recommended procedure are quantitative, reproducible and stoicheiometric with a precision of $\pm 1.0\%$. The effects of the amount of sulphuric acid (AnalaR, sp. gr. 1.84) and the reaction temperature were studied. It was found that the recommended amount of sulphuric acid was appropriate (it helps in removing any carbohydrates present). Heating of the reaction mixture tends to give inaccurate results and creates difficulties in detecting a sharp end-point, because of the instability of NBS at higher temperatures. NBS reagent should be prepared freshly and kept in a cold, dark location. As methyl red indicator reacts with the reagent, only a small amount (2 drops) should be used. The solvent used for the reaction (distilled water) should be free from organic impurities. The method is applicable in the presence of excipients without interferences. It has been verified experimentally that carbohydrates such as glucose, lactose, sucrose and starch do not interfere under the reaction conditions used. The method is also applicable to large sample sizes without any decrease in accuracy.

It was found that methyldopa and propranolol consume 4 and 5 equivalents of NBS reagent, respectively. Considering the available literature[9–14] and the stoicheiometry, it may be postulated that the compounds undergo decarboxylation and bromination at suitable positions.

Table I

Recovery of methyldopa and propranolol in pharmaceutical preparations

For methyldopa, the amount of drug in each dosage form was 250 mg and the amount of pure drug added was 25 mg. For propranolol, the amount of drug in each dosage form was 10 mg and the amount of pure drug added was 1 mg. Each result is the average of five determinations.

Drug	Dosage form (tablet)	Amount assayed for the dosage form/mg (*A*)	Amount assayed for the dosage form + pure drug added/ mg (*B*)	Amount found for pure drug/ mg ($B - A$)	Recovery, %
Methyldopa	Aldomet, MSD	256.94	281.78	24.84	99.36
	Cardopa, Dolphin	245.00	269.98	24.98	99.92
	Dopagyt, Themis Chemicals	248.33	273.20	24.87	99.48
	Meldopa, Dey's	251.25	276.40	25.15	100.60
	Emdopa, IDPL	252.08	277.00	24.92	99.68
	Lopa, Gufic	250.82	275.84	25.02	100.08
Propranolol	Carditab, Martel Hammer	10.17	11.18	1.01	101.00
	Ciplar, Cipla	9.94	10.93	0.99	99.00
	Inderal, ACCI	9.93	10.92	0.99	99.00

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Simple Determination of Glutathione by Two-phase Titration

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Keywords: Glutathione determination; two-phase titration; tetrabromophenolphthalein ethyl ester

Thiols such as glutathione and cysteine are widely distributed in living tissues and are involved in important biological functions. The extensive use of the thiol compounds in both medical and industrial applications has stimulated the development of numerous techniques for their instrumental determination in a variety of different systems.[1–3] The reaction of silver(I) with glutathione affords a mercaptide. However, a high concentration of silver(I) cannot be used in practice for the determination of a small amount of glutathione. The principal reason for the limited use of such reactions is the lack of suitable indicators for titration with a dilute titrant.

Wang and Panzardi[4] published a general survey and background of two-phase titration (ion-pair extraction titration) employing indicators for the analysis of synthetic detergents. Visual end-point detection can, however, be a problem in two-phase titration methods, because of colour reflection between the phases and differences in shades or hues of the colours in the two phases.[4] These problems occur because the colour change at the end-point occurs on transfer of the indicator dye from one phase to the other. The use of hydrophobic indicators[5,6] made it possible to detect the end-point in a single phase. In this paper, a hydrophobic dye, tetrabromophenolphthalein ethyl ester,[7] is suggested as an indicator for the visual titration of glutathione with silver(I) in the presence of 1,2-dichloroethane and 2,2′-bipyridyl as a chelating agent. The indicator colour changes occur in one phase without transfer of the indicator from one phase to the other. As a result, this type of indicator affords a basis for a sensitive two-phase titration for glutathione.

Experimental

Apparatus

A Toa, Model HM–5ES, pH meter and a 50-ml burette were used.

Reagents

Indicator, 0.03% *m/V*. Dissolve 30 mg of potassium tetrabromophenolphthalein ethyl ester in 100 ml of ethanol.

Titrant, 2×10^{-4} M. Prepare a 1×10^{-1} M solution of silver nitrate and standardise it according to the Mohr method; use it after accurate dilution to 2×10^{-4} M.

Glutathione solution. Dissolve 30–35 mg of glutathione in distilled water and dilute to 500 ml.

2,2′-Bipyridyl solution, 2×10^{-2} M. Dissolve 620 mg of 2,2′-bipyridyl in 200 ml of 0.05 M sulphuric acid.

Buffer solution, pH 6.7. Adjust the pH of a solution of disodium hydrogen orthophosphate (3×10^{-1} M) to 6.7 by the dropwise addition of 5 M sulphuric acid.

Procedure

Place 5 ml of glutathione solution, 2 ml of 2,2′-bipyridyl solution, 5 ml of phosphate buffer solution, 1 drop of the indicator and 1.5 ml of 1,2-dichloroethane in a 200-ml Erlenmeyer flask. Titrate the mixture against silver(I) solution with intermittent hand shaking

to ensure equilibrium between the two phases. A colour change from faint yellow to sky blue takes place in the organic phase at the equivalence point.

1 ml of silver(I) solution (2×10^{-4} M) $\equiv$ 61.48 μg of glutathione.

Results and Discussion

Titration Conditions

A blank titration is unnecessary because the organic phase turns blue on addition of one drop of titrant. The upper aqueous layer remains colourless throughout the titration, because the indicator is insoluble in it.

Various water-immiscible solvents were tested: benzene, butyl acetate, carbon tetrachloride, chlorobenzene, chloroform, 1,2-dichloroethane, isoamyl alcohol, hexane, nitrobenzene, nitroethane, 4-methylpentan-2-one and toluene. Of these, 1,2-dichloroethane gave the best end-point. 1,2-Dichloroethane forms a film on the surface of the aqueous layer and this was found to assist end-point detection. The colour change in the film is clearly detected. Initial volume fluctuations of the aqueous layer (8–20 ml) and the organic layer (1–2 ml) had no influence on the determination of the titration end-point.

The effects of the pH and the amount of the reagent were examined; the titre was constant between pH 5.5 and 7.5 and a sharp colour change at the end-point was observed with the addition of between 1 and 5 ml of the 2,2′-bipyridyl solution. The results of titrations of glutathione at several different concentrations, shown in Table I, indicate the high sensitivity of the proposed titrimetric method.

The following ions did not interfere at the 10^{-3} M level; K^+, Ca^{2+}, Mg^{2+}, NO_3^-, Cl^-, trichloroacetate, sulphosalicylate and cystine. Metal ions that react with thiols, such as mercury(II), cadmium(II) and zinc(II), caused negative errors at even the 10^{-6} M level. Positive interferences from iodide and cyanide were observed at levels in excess of maximum permissible concentration of 10^{-6} M.

TABLE I

EFFECT OF CONCENTRATION OF SILVER(I) AS A TITRANT

Titrant concentration/ M $\times 10^{-4}$	Glutathione concentration/ M $\times 10^{-4}$ (5 ml)	Mean titre*/ ml	Standard deviation/ ml
20.4	20.0	4.90	0.04
10.2	10.0	4.88	0.04
5.10	5.00	4.90	0.03
2.04	2.00	4.90	0.04
1.02	1.00	4.95	0.05
0.510	0.500	5.00	0.05

* Results for 10 sample solutions.

Application of the Titration

The method can be applied to the determination of cysteine and cystine in the presence of 1 ml of 2×10^{-1} M sodium sulphite solution.

The main thiol compound in mammalian tissues is glutathione.[2] Rat liver (0.5 g) was homogenised in the presence of 5 ml of 10% m/V trichloroacetic acid solution with ice cooling. After centrifuging, the supernatant liquid was neutralised with 1 N sodium hydroxide solution and titrated. The result was 3.78×10^{-6} mol g^{-1} glutathione by the proposed method, compared with 3.90×10^{-6} mol g^{-1} by an alternative spectrophotometric method[8,9] using nitrite and sulphanilamide. A recovery test using 5 ml of a 2×10^{-4} M glutathione solution yielded a result of 98%.

The method thus provides a simple and rapid determination of glutathione employing a visual indicator without the need for instrumentation.

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Micro-scale Thin-layer Chromatographic Method for the Screening of Urine Samples for Aspirin Metabolites

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Keywords: *Aspirin metabolites*; *urine*; *thin-layer chromatography*; *forensic investigations*; *clinical investigations*

Aspirin [acetylsalicylic acid; 2-(acetyloxy)benzoic acid] is a readily available drug that is frequently encountered in cases of self-poisoning; in 1975 aspirin was the fifth most frequent drug associated with suicide by poisoning in England and Wales.[1] Aspirin constituted 5.2% of drug-associated suicides in 1973, 5.4% in 1974 and 5.9% in 1975 (data calculated from Osselton *et al.*[1]); aspirin is therefore of importance as a self-poisoning agent.

In man approximately 60% of an orally administered dose of aspirin is excreted in the urine as *o*-hydroxyhippuric acid (salicyluric acid)[2]; smaller amounts of salicylic acid (2-hydroxybenzoic acid), gentisic acid (2,5-dihydroxybenzoic acid) and their glucuronic acid conjugates[3] and trace amounts of 2,3-dihydroxybenzoic acid[4] and 2,3,5-trihydroxybenzoic acid[5] are also excreted.

The rapid screening of urine samples for evidence of aspirin metabolites is a useful preliminary investigation to be undertaken by the clinical or forensic scientist before embarking on more sophisticated analysis techniques. In this paper a micro-scale thin-layer chromatographic method is presented that can be applied to the detection of non-glucuronic acid conjugated metabolites of aspirin, for rapid screening purposes, or applied to enzymically hydrolysed urine samples for total metabolite invesgitations.

Experimental

Urine Samples

Urine samples were obtained from three people approximately 3 h after they had received orally 300 mg of aspirin. Control urine samples were also collected shortly before the aspirin was given.

Extraction of Urine Samples

Non-glucuronide metabolites

The available urine sample was acidified with concentrated hydrochloric acid to pH 1.0 and extracted by agitation with four times its volume of diethyl ether. The ether extract was evaporated to dryness and the residue dissolved in acetone (1 ml).

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Total metabolites

The urine sample was diluted with an equal volume of acetate buffer (0.2 M; pH 5.0) and β-glucuronidase type-H1 (5 mg) (Sigma Chemical Co., Poole, Dorset) was added. The mixture was incubated for approximately 18 h at 37 °C, then the pH of the solution was adjusted to 1.0 with concentrated hydrochloric acid. The solution was then extracted as described above.

Thin-layer Chromatography

Approximately 10 μl of urine extract were applied to Camlab SilG thin-layer chromatographic plates (Macherey, Nagel & Co., Düren, West Germany) in discrete spots not exceeding 1.5 mm in diameter. The plate was developed in toluene - acetic acid - diethyl ether - methanol (120 + 18 + 60 + 1) over a distance of 5 cm in a vapour-saturated tank. When development was complete (approximately 10 min), the chromatogram was dried with a warm air blower and sprayed lightly with a 0.04% *m*/*V* solution of iron(III) chloride in 0.01 M hydrochloric acid.

Results and Discussion

The three major metabolites of aspirin, namely *o*-hydroxyhippuric acid, salicylic acid and gentisic acid, were present in urine samples from people given aspirin, both before and after treatment with β-glucuronidase. The individual metabolites were well resolved by the chromatographic system and gave characteristic colour reactions with the iron(III) chloride spray reagent (Table I). Control urine sample extracts demonstrated the absence of interfering materials, both with respect to co-chromatography and colour reaction with iron(III) chloride.

TABLE I

R_F VALUES IN TOLUENE - ACETIC ACID - DIETHYL ETHER - METHANOL (120 + 18 + 60 + 1) AND COLOUR REACTIONS WITH IRON(III) CHLORIDE SPRAY REAGENT OF THE THREE MAJOR METABOLITES OF ASPIRIN

Metabolite	R_F	Colour reaction with $FeCl_3$ spray reagent
Salicylic acid	0.77	Purple
Gentisic acid..	0.66	Blue
o-Hydroxyhippuric acid	0.48	Purple

The detection limit for *o*-hydroxyhippuric, salicylic and gentisic acids was 5 μg per spot. This corresponds to 2.6×10^{-8}, 3.6×10^{-8} and 3.2×10^{-8} mol, respectively.

The technique is well suited to application to routine forensic and clinical investigations, owing to its ease and rapidity of application. An important advantage attending the use of this technique in routine laboratory investigations is the exclusion of benzene from the chromatographic solvent system. Benzene is the major constituent of Cummings and King's[6] solvent mixture upon which the present system is based. The former solvent system is not recommended for routine applications owing to the cumulatively toxic nature of benzene.

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Determination of Monosodium Glutamate in Food Using High-performance Liquid Chromatography and Fluorescence Detection

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Keywords: Glutamic acid determination; fluorescence derivatisation; high-performance liquid chromatography; soups

As a flavour enhancer the use of monosodium glutamate is widespread, particularly in "fast foods." Normal concentrations range from 0.2 to 0.5 g per 100 ml in the liquid, whilst dried products contain from 1 to 10 g per 100 g. Excessive use of monosodium glutamate can give rise to symptoms that have been called the Chinese restaurant syndrome.

Various methods have been published that describe the determination of monosodium glutamate and include: a rapid paper-chromatographic method[1]; ion-exchange column separation followed by a Sorenson formalin titration[2]; and ion-exchange column separation followed by fluorescence determination using fluorescamine.[3]

In this paper we describe a high-performance liquid chromatographic method using pre-column dansylation and fluorescence detection. The procedure is similar to that used for the analysis of anabolic agents.[4] Dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl chloride or DNS-Cl) reacts with primary and secondary amino groups to form highly fluorescent derivatives.[5] The derivatives are readily separated using liquid chromatography whilst fluorescence detection gives excellent sensitivity. Sample preparation involves the dispersion of 3 g of the dried soup in 500 ml of water, followed by dansylation of 10 μl of the clear liquid after filtration.

Experimental

Equipment

Chromatograph. High-performance liquid chromatography was performed using a Perkin-Elmer, Model Series 3B, liquid chromatograph with a 6-μl Rheodyne injector and a high speed C_{18} (5 μm) column (Perkin-Elmer ODS–C_{18} bonded to silica), 12.5 cm $\times$ 4.6 mm. i.d.

Fluorescence detector. A Perkin-Elmer, Model LS–4, fluorescence spectrometer with a 3-μl flow cell was used.

Vials and heating module. Reacti-Vials (1 ml) and a Reacti-Therm heating module (Pierce Chemical Co. Rockford, IL) were used for derivatisation.

Reagents

All reagents were of AnalaR grade from BDH Chemicals, Ltd., Poole, Dorset, unless otherwise stated.

Glacial acetic acid.

Dansyl chloride solution. A solution of 1.5 mg ml^{-1} in dried acetone.

Methanol. HPLC grade.

Water. Distilled and filtered through a 0.45-μm millipore filter.

Buffer. A pH 10.5 solution of 4 g l^{-1} sodium hydrogen carbonate.

L-*Glutamic acid.* Sigma Chemical Co., St. Louis, MO. A 10 mg ml^{-1} standard solution in water.

Eluting solvents. Pump A, 1% *V*/*V* glacial acetic acid in methanol; and pump B, 1% *V*/*V* glacial acetic acid in 45% methanol + 55% water.

Dansylation Procedure

Aliquots of 10 μl of either the standard, sample or blank (water) were transferred into a Pierce 1-ml Reacti-Vial and 50 μl of the buffer solution were added; 100 μl of the dansyl chloride solution were then added and the solution mixed vigorously using a rotomixer.

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The vials were then placed in a Pierce Reacti-Therm heating module at 100 °C for 10 min in the dark. The vial contents changed from a pale yellow before reaction to colourless after reaction. The yield of dansylated product was not changed by longer reaction times. The vials were cooled with water and 300 μl of methanol were added to each to minimise any errors that might occur owing to loss of the reaction mixture through evaporation.

Separation and Detection Procedures

Elution was performed with a flow-rate of 3 ml min^{-1}. The solvent was held at 45% *V*/*V* methanol for 2.5 min after injection and then run up to 99.9% *V*/*V* methanol over 1 min and held for 2.5 min. The gradient was then reversed and the column allowed to equilibrate at 45% *V*/*V* methanol over 3 min before the next injection. The dansylated glutamic acid was detected with an excitation wavelength of 328 nm and an emission wavelength of 530 nm. The corrected excitation spectrum and uncorrected emission spectrum of the derivative was obtained by collecting the fraction containing the compound from the chromatograph and scanning the spectrum on a Perkin-Elmer, Model LS-5, luminescence spectrometer. Although the peak excitation is at 245 nm, Fig. 1(*a*), an excitation wavelength of 328 nm was used to minimise any interference from compounds that might absorb radiation in the ultraviolet region.

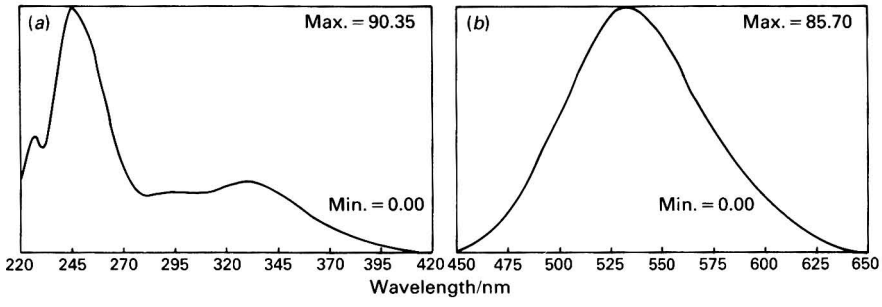

Fig. 1. Corrected (*a*) excitation and (*b*) emission spectra of dansyl-L-glutamic acid after blank subtraction. Slit width, 5 nm; scan speed, 120 nm min^{-1}.

Sample Preparation

A series of commercially available soups were analysed for monosodium glutamate. A 3-g sample of the dry mix was added to 400 ml of water in a beaker and the mixture stirred vigorously for 2–3 min. The contents were then transferred into a 500-ml calibrated flask and made up to volume with water. After mixing, the solution was filtered through a Whatman No. 1 filter-paper and the filtrate was used as the sample. Standard additions were made by adding known concentrations of L-glutamic acid to the filtrate.

Results and Discussion

Dansylation and Chromatography

Fig. 2(*a*) is the chromatogram from the dansylation of a beef soup and Fig. 2(*b*) is the chromatogram of a standard plus dansyl chloride. Fig. 2(*c*) is the chromatogram of a blank of the buffer solution plus dansyl chloride. The dansylated glutamic acid is eluted in 2.5 min at a flow-rate of 3 ml min^{-1} and is well resolved from any other dansylated products. Recoveries varied from 92 to 102%. Pure methanol containing 1% *V*/*V* glacial acetic acid was used to remove from the column dansylated products with retention times greater than that of glutamic acid. Although the pH of the eluent was kept acidic using acetic acid, slight tailing of the dansylated glutamic acid did occur owing to the highly polar nature of the product (two carboxylic acid groups per molecule, Fig. 3). The fluorescence maximum remained unchanged for at least 45 min after the addition of the methanol to the reaction mixture.

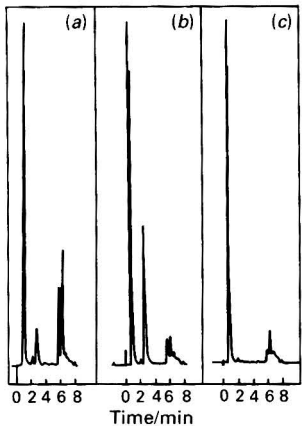

Fig. 2. (*a*) Chromatogram of the dansylation of beef and tomato soup filtrate; (*b*) chromatogram of the dansylation of L-glutamic acid; and (*c*) chromatogram of the dansylation of the blank buffer.

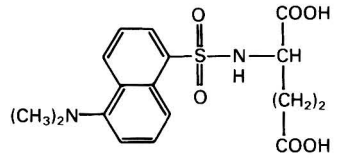

Fig. 3. Molecular structure of dansyl-L-glutamic acid.

Using the sample preparations and dansylation procedure indicated, 0.006 g per 100 g of monosodium glutamate could be detected in a diluted soup sample. Concentrations were calculated by constructing a calibration graph from a range of L-glutamic acid standard solutions (0–10 μg μl^{-1}) and converting into percentage of monosodium glutamate by multiplying the percentage of glutamic acid by 1.15. Concentrations in dried samples ranged from 3 to 10 g per 100 g with an average recovery of 97% and a standard deviation of 3.5% (Table I).

TABLE I

CONCENTRATION OF MONOSODIUM GLUTAMATE FOUND IN A VARIETY OF SOUPS

Product	Concentration/g per 100 g in dried product	Concentration/g per 100 ml in drinking form	Recovery, %
Oxtail soup	2.99	0.35	98
Chicken soup	5.40	0.30	92
Beef and tomato soup ..	4.99	0.52	97
Golden vegetable soup ..	3.79	0.42	102
Diet mushroom soup	9.87	0.58	99
Oxo	5.63	0.18	96

Conclusions

The high-performance liquid chromatographic method described here is both rapid and specific for the determination of monosodium glutamate in dried soup products and with suitable sample preparation could be extended to a range of other food products.

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Communication

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

Graphite-probe Atomisation for Carbon Furnace Atomic-absorption and Atomic-emission Spectrometry

Keywords: *Atomic-absorption spectrometry*; *atomic-emission spectrometry*; *graphite-probe sample introduction*; *electrothermal atomisation*; *interferences*

The advantages provided by sample vaporisation into a hot isothermal environment have been recognised from the earliest studies of electrothermal atomisation. Under these conditions, all analyte species experience the same high vapour temperature, irrespective of their volatilisation and atom appearance temperatures, and the dissociation of molecules produced from the sample material is enhanced. Thus atomisation efficiency is increased, particularly when the atomisation mechanism involves the dissociation of oxide or halide molecules of the analyte. Many vapour phase chemical interferences are also eliminated or reduced under these conditions, and thus both the sensitivity and practical performance achieved by atomic-absorption measurements are improved. The existence of the atoms of many elements at higher vapour temperatures also significantly enhances atom excitation and consequently the sensitivity of atomic-emission measurements.

Although some early designs of furnace atomisers[1–3] allowed vaporisation into a graphite cuvette at a high and constant temperature, commercial exploitation of these systems has not been undertaken. Most manufacturers have based their instruments on the furnace design introduced by Massmann,[4] in which the sample is deposited on to the wall of the carbon tube prior to heating. In this instance, it is impossible to independently control the temperature of the atomisation surface and the vapour phase in which analyte measurements are made. As a consequence, one of the major limitations of conventional electrothermal atomisation has been the occurrence of matrix or chemical interference effects,[5,6] particularly in the determination of volatile elements such as lead, cadmium and thallium analysed in halide matrices. Atoms of these elements are produced at comparatively low temperatures, at which volatilisation of matrix molecules also frequently occurs, and under conditions in which conversion of analyte atoms into halide molecules is thermodynamically favourable. The low vapour temperatures experienced by the atoms of volatile elements also leads to poor atomic-emission sensitivity in unmodified conventional atomisers.[7]

Many attempts have been made to increase the effective vapour temperature during the atomisation of volatile elements in conventional Massmann-type furnace atomisers. Most of these have involved the incorporation of a delay in the heating of the atomisation surface, while the rest of the furnace tube and the vapour phase are heated more rapidly. Reduction of the tube wall thickness at the ends[7] and the introduction of a sample cup at the centre[8,9] both have the desired effect of increasing atomic-emission sensitivity. However, such modifications to the tube design are time consuming to construct and less easily adapted in comparison with a third approach, platform atomisation, which has approximately the same effect on vapour temperatures. The platform concept, introduced by L'vov,[10] is becoming increasingly popular for both atomic-absorption[11,12] and atomic-emission measurements.[13,14] The sample is deposited on to the platform, which is heated by radiation from the tube wall. The delay in the heating of the platform ensures that the vapour temperatures experienced by analyte atoms are substantially higher than their atomisation temperature. The maximum difference that can sensibly be achieved between these temperatures is limited. An increase in platform mass increases the delay, but also decreases the heating rate of the platform and hence the rate of atomisation. Too large a platform also limits the maximum attainable temperature. Both factors can have detrimental effects on the sensitivity of atomic-absorption and -emission measurements, inhibit the removal of interference effects and indicate the limitations of the platform concept.

To obtain a combination of the highest possible vapour temperatures and rapid atomisation rates for all elements, it is clearly desirable to separate the heating of the vapour in the graphite tube from the volatilisation and atomisation of the analyte, as in the atomisers of L'vov[2] or Woodriff *et al.*[3] This can be achieved in a commercial Massmann-type furnace by the introduction of the sample on a probe which is inserted into a pre-heated constant temperature furnace. The use of a tungsten wire probe attached to a conventional autosampler and introduced through the injection hole of the graphite tube has been reported by L'vov and Pelieva[15] and Manning *et al.*[16] The major limitation of this type of probe is the small amount of sample that can be introduced by the wire. Slavin *et al.*[11] have also mentioned the use of a graphite probe but stated that their system "is not very convenient to use for real samples." In this paper we describe a graphite-probe device, which has been developed in our laboratory and that we believe is ideally suited for the analysis of real samples. The results of preliminary experiments in both atomic-absorption and atomic-emission spectrometry are given and the reduction of chemical interference effects is demonstrated.

Experimental

Atomic-absorption measurements were made with a Perkin-Elmer 306 spectrometer fitted with deuterium arc background correction and an HGA 72 heated graphite atomiser. A Spectrametrics echelle monochromator modified for wavelength modulation background correction[13] was used to measure atomic-emission signals generated in a Perkin-Elmer HGA 70 heated graphite atomiser.

For probe atomisation, a 9 mm diameter hole was cut in the front of the HGA 70/72 furnace housing to allow the introduction of a pyrolytic graphite probe into the tube through a 5 mm long by 2 mm wide slot cut in the wall at the centre of the tube. For most of the work, tubes made of pyrolytic graphite were used,[17] with dimensions similar to conventional HGA 70/72 tubes (*i.e.* 9 mm i.d., 53 mm long). The use of pyrolytic graphite tubes and probes gave a substantial increase in lifetime compared with standard graphite. For some atomic-emission studies, the length of the atomiser tube was reduced to 40 mm to increase the maximum attainable temperature.

Probe dimensions were typically 50 mm long by 3 mm wide and 400 μm thick, with a 4 $\times$ 3 mm sample head at one end capable of holding a 50-μl aliquot. The probe was held in a solid sampling tool clamped on to an optical bench mount. The probe was manually moved into and out of the furnace by movement of the mount along the optical bench placed at right-angles to the furnace workhead. A short graphite tube (9 mm o.d.) was used as an entrance tube, and was placed in the hole in the furnace housing at right-angles to the atomiser tube and close to but not touching it. This was used as a drying zone for evaporation of the sample droplet injected on to the probe, and also reduced the rate of loss of inert gas from inside the workhead during atomisation.

The procedure used to carry out the normal dry, ash and atomisation steps of the furnace programme with the graphite probe was as follows. A sample aliquot of 10, 20 or 50 μl was deposited on the probe-head with a micropipette and the probe moved into the drying zone while the atomiser tube was heated to 450 °C. Under these conditions, solvent evaporation was completed in a time comparable to conventional furnace operation. When drying is complete, the atomiser tube can be heated to a suitable temperature to allow ashing of the sample matrix if required. The probe was then removed from the furnace assembly and the atomiser tube heated to the required atomisation temperature. A period of 8–10 s was required to ensure constant tube temperature with the HGA 70/72 power supplies used. Accordingly, a total atomisation time of 12 s was selected and the probe was introduced directly into the hot atomiser tube after exactly 10 s. Atomisation temperatures were normally 2300–2500 °C and the inert gas flow was stopped a few seconds before probe introduction to maximise signal sensitivity.

Only the probe sample head enters the tube and is heated. The height of the probe was adjusted so that the probe head was close to but not touching the bottom surface of the atomiser tube. In this position, it does not interrupt the hollow cathode light beam in atomic-absorption measurements, and the probe image can be excluded from the entrance slit of the monochromator together with the tube wall image, thus minimising the background in atomic-emission measurements. The lack of contact between the atomiser tube and probe ensures that the probe is heated principally by radiative processes.

Optical pyrometer measurements indicated a probe heating rate of approximately 4000 °C s^{-1} when the atomiser temperature was set above 2300 °C. For the probe dimensions given above, the final equilibrium temperature of the probe is only 50–100 °C below that of the tube wall. The probe heating rate measured by this method is subject to error as the pyrometer does not distinguish

between radiation emitted by the probe and radiation from the tube wall scattered or reflected by the probe. However, the time responses of both atomic-absorption and atomic-emission signals indicate that the probe heating rate is extremely rapid. Peak widths at half height were found to be about 1–1.5 s and the peak maxima were normally observed within 1 s of the start of the signal. These preliminary measurements were made with a Servoscribe 541.20 chart recorder of 0.375-s full-scale deflection, and improved signal detection might be achieved with equipment with a faster response time.

Results and Discussion

Atomic-absorption detection limits and sensitivities (1% absorption) achieved with this system for five elements of varying volatilities are given in Table I. Detection limits are defined as twice the standard deviation of 5 or 6 measurements of an aqueous solution of the analyte giving an absorbance of between 0.05 and 0.10 units. Sensitivity values for the probe/HGA 72 system compare favourably with tube-wall atomisation in the HGA 500 atomiser where dilution of the atomic vapour is less. Detection limits are generally inferior to the corresponding sensitivity values, and this is related to the reproducibility obtained with the present manual probe movement system. Precisions, at concentrations of from 5 to 10 times the detection limit were in the 1–5% range, which is of a similar order to tube-wall atomisation with manual sample injection.

TABLE I

DETECTION LIMITS AND SENSITIVITY VALUES USING CARBON FURNACE ATOMIC-ABSORPTION SPECTROMETRY WITH PROBE ATOMISATION

Injections were all 20 μl.

Element	Wavelength/ nm	Detection limit (HGA 72/probe)/ $\mu g\ l^{-1}$	Sensitivity/$\mu g\ l^{-1}$ HGA 72 probe	Sensitivity/$\mu g\ l^{-1}$ HGA 500* tube wall
Aluminium	309.3	1.40	1.6	0.8
Gold	242.8	1.60	0.65	0.6
Cadmium†	228.8	0.20	0.05	0.03
Chromium	357.9	0.44	0.18	0.18
Lead	283.3	1.23	1.0	0.7

* Optimum choice of heating rate and tube material. Values from Perkin-Elmer manual.
† Electrodeless discharge lamp.

The most interesting and potentially useful aspect of probe atomisation is the ability to substantially reduce vapour phase interference effects. The results in Table II show that up to 2% m/V magnesium chloride and calcium chloride have no significant effect on the atomic-absorption signal of 100 $\mu g\ l^{-1}$ of lead, and suggest that the probe is more attractive than platform atomisation[18] for the analysis of trace elements in complex matrices.

TABLE II

INTERFERENCE OF MAGNESIUM CHLORIDE AND CALCIUM CHLORIDE ON THE CARBON FURNACE ATOMIC-ABSORPTION SPECTROMETRIC DETERMINATION OF LEAD

Interferent concentration, % m/V	Lead absorbance: Magnesium chloride*	Lead absorbance: Calcium chloride†
0	0.280	0.400
0.1	0.285	—
0.2	—	0.390
0.5	0.260	0.395
1.0	0.285	0.400
2.0	0.270	0.410

* Injections of 10 μl.
† Injections of 20 μl.

Atomic emission detection limits for elements of varying volatility and excitation potential are given in Table III. Detection limits are defined as the analyte concentration giving a signal equal to twice the peak to peak background noise. Probe atomisation gave increased emission intensities for all elements, compared with wall or platform atomisation using similar atomisation temperatures. The probe detection limits are compared in Table III with values obtained recently using platform

atomisation with the same spectrometer system.[13,14] The most significant improvements were achieved for the volatile elements, lead, cadmium, zinc and gold and these values are the lowest so far reported for these elements. For elements with most sensitive lines at longer wavelengths, for example, indium, chromium and manganese, improvements are less significant owing to the relatively greater increase in background emission intensity at higher wavelengths, which limits the improvement in signal to noise ratios at temperatures above about 2500 °C.

TABLE III

CARBON FURNACE ATOMIC-EMISSION DETECTION LIMITS USING PROBE ATOMISATION

Injections were all of 50 μl.

Element	Wavelength/nm	Detection limits/μg l^{-1}	
		Probe used in this work	Platform† atomisation
Indium	451.1	0.013	0.025
Chromium	425.4	0.025	0.023
Manganese	403.1	0.016	0.029
Lead	405.8	0.9	3.4
Cadmium	326.1	6	50
Zinc	307.6	47	N.D.*
Tin	284.0	6	N.D.*
Gold	267.6	6.2	25

* N.D. = not determined.
† Reference 13.

These preliminary results indicate the advantages of the probe method of sample introduction in electrothermal atomisation. Separate control of vapour-phase temperatures and atomisation conditions will be of great advantage in the analysis of real samples, a number of which are under active study in our laboratory. This system can accept relatively large sample volumes and is readily adaptable to current commercial Massmann-type furnaces. The probe mechanism could be automated relatively easily and thus should further improve detection limits of atomic-absorption and atomic-emission spectrometric measurements. It is also likely that probe atomisation will be of considerable assistance in the study of atom-formation processes, particularly those involving condensation/re-atomisation and in investigations of mechanisms of excitation and ionisation.

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Book Reviews

METHODS FOR THE EXAMINATION OF WATERS AND ASSOCIATED MATERIALS. By THE STANDING COMMITTEE OF ANALYSTS (TO REVIEW STANDARD METHODS FOR QUALITY CONTROL OF THE WATER CYCLE); DEPARTMENT OF THE ENVIRONMENT, NATIONAL WATER COUNCIL. HM Stationery Office.

Ultraviolet and Visible Solution Spectrophotometry and Colorimetry, 1980. *An Essay Review, by K. C. Thompson.* Pp. 38. 1981. Price £3.50. ISBN 0 11 751538 8.
Emission Spectrophotometric Multielement Methods of Analysis for Waters, Sediments and other Materials of Interest to the Water Industry, 1980. Pp. 82. 1982. Price £4.60. ISBN 0 11 750015 1.
Boron in Waters, Effluents, Sewage and Some Solids, 1980. Pp. 35. 1981. Price £3.20. ISBN 0 11 751583 3.
Phosphorus in Waters, Effluents and Sewages, 1980. Pp. 31. 1981. Price £2.80. ISBN 0 11 751582 5.
Silicon in Waters and Effluents, 1980. Pp. 27. 1981. Price £2.80. ISBN 0 11 751557 4.
Oxidised Nitrogen in Waters, 1981. Pp. 61. 1982. Price £4.50. ISBN 0 11 751593 0.

These booklets represent the latest products from the Standing Committee of Analysts, whose principal aim is the provision of a series of recommended analytical methods for use by the water industry, in succession to the Department of the Environment "green book," "Analysis of Raw, Potable and Waste Waters." A review of the most recent previously published booklets in this series appeared in *The Analyst*, 1982, **107**, 464. There is no doubt that the Committee are succeeding in producing an extremely valuable set of booklets which already give a wide coverage of relevant aspects of analytical chemistry. The methods booklets will be essential to all working water analysis laboratories, and others, such as the first two above, provide excellent reviews of topics of genuine interest in the water industry.

The description of each analytical procedure is extremely clear, and each stage is carefully amplified by notes that explain the principle behind the particular operation involved. This information, and the detailed specification of each procedure in terms of detection limit, precision, sensitivity, interferences and time for analysis, should ensure that the methods are not only used with confidence and in an informed manner, but also that high accuracy is achieved more readily. The Committee's efforts in precisely defining each procedure are almost unique in the field of standard methods, and their work should receive international as well as national recognition. Improved procedures will no doubt be introduced in the future, but the yardstick against which such methods must be evaluated is well characterised in these publications.

Of the above booklets, four are concerned with analytical procedures and each of these contains a number of alternative procedures for the relevant analyte(s). Thus the booklet dealing with boron contains four methods of which one is an automatic method and one is titrimetric. The phosphorus booklet details two procedures for different sample types but both are based on the phosphomolybdenum blue method. Two methods are given for silicon at different concentration ranges, and the booklet on nitrogen contains eight procedures, both automatic and manual, for total oxidised nitrogen, nitrate and nitrite. The first two booklets above are reviews, the first providing useful background information on a technique widely used in the water industry. The level is suitable for the average laboratory technician, and the coverage includes the principles, performance characteristics and routine maintenance of instruments, and most usefully, methods by which the performance of instruments can be routinely checked. This booklet can be recommended both outside and inside the water industry. Emission spectrometry has been of limited use in the water industry (obviously excepting the determination of sodium and potassium), but the recent commercial introduction of inductively coupled plasmas (ICP) is causing a rapid change in this, particularly in the USA, where the Environmental Protection Agency are now actively working on methods for routine use. The ICP is also particularly suited to water analysis, where spectral interferences are minimised by the nature of the matrix, elements such as boron, phosphorus and silicon, can be incorporated with the metals of common interest, and sensitivity is usually adequate. The booklet on emission spectrometric methods is therefore timely and will be helpful

to water laboratories considering the introduction of ICP hardware. Of the booklets published in this batch, however, this one is likely to have the shortest useful lifetime, as developments in analytical practice and technology are taking place most rapidly in this subject area.

J. M. OTTAWAY

LASER SPECTROSCOPY OF SOLIDS. Edited by W. M. YEN and P. M. SELZER. *Topics in Applied Physics, Volume* 49. Pp. xii + 310. Springer-Verlag. 1981. Price DM112; $53.40. ISBN 3 540 10638 3; 0 387 10638 3.

The subject of this book is perhaps better described as "The Electronic Spectra of Metal Ions in Solids," than by the more enticing "Laser Spectroscopy of Solids." The seven chapters by different authoritative authors explain the basic physics of the processes involved; these chapters contain extensive bibliographies, especially references to recent experimental work. Phonon absorption, phonon Raman scattering and similar processes are explained and widely invoked to explain relaxation features with due emphasis on the conservation of energy. Indeed much of the text forms a good postgraduate monograph on the static and dynamic features of these systems.

Although the general ideas are not especially recent, the advent of lasers, especially tunable dye lasers, has revolutionised the experimental side and has led to observations that are more informative and more easily interpreted than those using broad-band sources for exciting fluorescence. The advantage is that selective excitation to very specific excited states enables direct emission from these states to be examined from the prompt fluorescence, while non-radiative transfer can be monitored in detail by examining the change of fluorescence with time following pulsed excitation. In addition, not only can the laser be tuned to a particular absorption transition, but it can also be tuned throughout its line width. If the line is homogeneously broadened this width can be accurately measured. But more common is inhomogeneous broadening, which occurs when different individual ions are associated with slightly different environments and having slightly different spectra as a consequence. Such different environments may be the consequence of dislocations, strains, deliberate or accidental impurities, while in amorphous glasses the inhomogeneous width may be considerable because of the lack of any regularity. Thus for Eu^{3+} in a silicate glass at 4 K the $^5D_0 \rightarrow {}^7F_0$ homogeneous line width is less than 20 MHz (10^{-3} cm^{-1}), and the inhomogeneous width is 3 THz (100 cm^{-1}), a ratio of 10^{-5}:1. The excitation of a narrow sub-section of these sites often leads to a narrow fluorescence from the same sites, at least initially. One can thus see why the abbreviation, FLN, for fluorescence line narrowing appears throughout this volume; and also see the merit of monochromatic exciting light capable of being produced in short pulses.

This is very much a book written for physicists, and chemists should be alert to unfamiliar usages. "Optically active" has nothing to do with chirality but merely means electronically allowed and silicate glasses are described as containing Si^{4+} ions. Analytical chemists should find much of background interest and just possibly something of value for their own problems. However, typical is the one reference to analytical chemistry noticed by this reviewer (on p. 159) which concerns the problem of trace amounts of PO_4^{3-} ions to be found in $BaSO_4$ with europium doping. If, and it may be a big if, this is the problem on which the economic future of your enterprise depends you will be delighted to learn "that a Eu ion with a nearby PO_4 was clearly distinguishable from one in a distant, unperturbed site and thus could be used as a tool for quantitative analytical chemistry."

D. H. WHIFFEN

CHLORINATED DIOXINS AND RELATED COMPOUNDS. IMPACT ON THE ENVIRONMENT. PROCEEDINGS OF A WORKSHOP HELD AT THE INSTITUTO SUPERIORE DI SANITÀ, ROME, ITALY, 22–24 October 1980. Edited by O. HUTZINGER, R. W. FREI, E. MERIAN and F. POCCHIARI. *Pergamon Series on Environmental Science, Volume* 5. Pp. xii + 658. Pergamon Press. 1982. Price $75. ISBN 0 08 026256 2.

Dioxin has become a notorious and emotive word, a consequence of its use as an abbreviation for polychlorinated dibenzo-*p*-dioxins, which occurred as impurities in herbicides used in the Vietnam War causing much harm and were produced and dispersed in the much publicised disaster at the chemical plant in Seveso. This volume is an account of the proceedings of a workshop that began a sober assessment of the problems associated with dioxins and closely related

chlorinated dibenzofurans. There was a second meeting held on the same subject in Washington DC in October 1981.

The group of compounds provided a considerable challenge to the analyst because components do not occur individually and all components of mixtures need to be separated, identified and assayed, because extremely small doses of some produce very unpleasant reactions or even death in man and animals. Some are described and discussed. Consequently only a fraction of the volume is devoted to analytical chemistry and much appears to have been more fully described elsewhere so that few analysts are likely to purchase this volume. Nevertheless, the residue of the volume is of some interest to analysts in general because rarely is so much effort concentrated on such a narrow field and is so reliant on analytical technique. It provides examples of problems likely to be found with many natural and synthetic substances that are likely to find their way into the environment, especially the distribution, movement and persistence and metabolism of compounds that are only slowly degraded, and how much is important in the environment. This all supposes an adequate method of analysis and a means for identifying highly biologically active compounds that occur in complex mixtures of closely related substances. It presupposes a close collaboration between chemist, biologist and other specialists. Once the active chemical has been identified there remains the question of deciding whether it is likely to be hazardous, a question as difficult to answer as "how long is a piece of string?"

Finally this volume provides a good case for devoting effort to a study of the principles underlying the fate and behaviour of chemicals in the environment, free from the constraint of investigating the outcome of a disaster. The investigations of sources of dioxin formation, which include town-waste incineration, are a hopeful sign, but is this the most urgent problem? K. A. LORD

PHARMACEUTICAL ANALYSIS. MODERN METHODS. PART A. Edited by JAMES W. MUNSON. *Drugs and the Pharmaceutical Sciences, Volume* 11. Pp. xiv + 485. Marcel Dekker. 1981. Price SwFr154. ISBN 0 8247 1502 0.

This book is the first of a two-volume set devoted to a description of modern analytical techniques in current use within the area of pharmaceutical analysis. The first volume contains chapters on gas chromatography, pyrolysis gas chromatography, gas chromatography - mass spectrometry, luminescence spectroscopy, liquid scintillation counting and radioimmunoassay. A second volume will cover the remaining techniques, such as high-performance liquid and thin-layer chromatography, electrochemical methods, atomic spectroscopy and ultraviolet - visible spectrophotometry. The subject matter included in the present volume gives the appearance of having been chosen somewhat randomly. Approximately half of the book is devoted to gas chromatography; high-performance liquid chromatography however, is not included and thus the reader is unable to compare and contrast the two techniques. Indeed, this is perhaps the main criticism of the work. It would have been opportune to combine the chromatographic methods in one volume. In its present format, the reader must await publication of the second volume before he/she is able to ascertain the relative advantages of one chromatographic technique *versus* another.

However, having voiced this criticism it must be admitted that, in general, the texts are clear and concise, yet contain sufficient detail to enable the reader to gain an appreciation of the practical requirements needed for the successful application of a technique. Occasional lapses into the transatlantic use of the English language are irritating, but will be acceptable to all except the most pedantic of readers.

The description of radioimmunoassay and related techniques is worthy of particular mention as interest in this type of procedure continues to gain impetus. Whilst the raising of specific antibodies is beyond the scope of the average pharmaceutical analysis laboratory, there is no doubt that the quest for enhanced assay sensitivity, for example in the area of body-fluid analysis, will continue to proliferate the availability of the technique. In this respect, the monograph is a timely review. A welcome survey of the literature is included and, although not comprehensive, suitably illustrates the vast potential of the technique. A useful glossary enables the uninitiated to comprehend the attendant terminology.

According to the Preface, the book is intended to fill the gap between undergraduate texts and highly specific journal articles and this the volume admirably achieves. Without doubt the book will appeal most to those pharmaceutical analysts wishing to acquire a working knowledge of an unfamiliar technique and to students specialising in pharmaceutical analysis. W. F. HEYES

STATISTICS AND COMPUTER METHODS IN BASIC. By J. D. LEE and T. D. LEE. Pp. xii + 198. Van Nostrand Reinhold. 1982. Price £11.50 (hardback); £5.50 (softback). ISBN 0 442 30474 9 (hardback); 0 442 30475 7 (softback).

Mathematical developments have shown that many aspects of experimental work depend on the analysis (and general computation) of numerical data, and it is the task of statistics to understand and handle the latter correctly. Statistics is not only descriptive, dealing with methods of condensing large masses of data, but analytical as well, dealing with techniques which enable conclusions to be drawn from the data. The statistical (or better, perhaps, datametric) approach involves new ways of looking at experiments and new ways of thinking. In teaching statistics, it is imperative to avoid intimidating jargon and to sacrifice mathematical rigour in the interests of readability. This book does just that.

It is indeed a very commendable book, well produced and set out, and aimed at introducing the basic ideas of statistics to undergraduate students of science and engineering. It should also, incidentally, provide a useful read-through for the practising experimentalist.

The first half of the book covers a number of commonly encountered elementary statistical topics, including errors, averages and the spread of results, distributions, tests of dissimilarity (chi-squared), comparison of samples both large and small (*F*- and *t*-tests) and correlation coefficients. This is followed by four chapters dealing with some commonly encountered numerical techniques for the curve fitting of straight lines and polynomials, solving equations and calculating areas. Finally, in the last chapter, some techniques for sorting data are described. The techniques are introduced by means of detailed worked examples; exercises are included and one particularly welcome feature is that solutions indicating some of the intermediate steps as well as the answers are given. A computer program is provided with each method, written in an elementary subset of BASIC, so that they may be implemented with little difficulty on almost all computers. No attempt is made to teach programming.

In this well written book the authors do, I feel, succeed in their aim in providing a useful introduction to the subject for their intended readership.

The book certainly merits consideration by those concerned with work at this level.

A. B. CALDER

CHEMICAL CRIMINALISTICS. By ANDREAS MAEHLY and LARS STRÖMBERG. Pp. vii + 322. Springer-Verlag. 1981. Price DM162; $75.50. ISBN 3 540 10723 1; 0 387 10723 1.

There are at present two well established books on forensic science, H. J. Walls' "Forensic Science" and R. Saferstein's "Criminalistics," both of which have been written on the basis that the reader has little or no previous scientific knowledge. A more recent publication, "Forensic Science Handbook" by R. Saferstein, though more detailed, still starts from simple principles.

"Chemical Criminalistics" on the other hand makes no such concessions and is more suited to those with a good background in chemistry. The authors are, respectively, the director and divisional head in the Swedish National Laboratory of Forensic Science and as such the contents of the book very much reflect the activities of a laboratory of high reputation amongst forensic scientists.

There are three sections. Firstly, there is a brief one on forensic science history and a survey of the present day state of affairs. Thereafter follows the main part of the book under the heading "State of The Art." This consists of 13 chapters that are devoted to: narcotics and drugs; explosives; polymers; fibres; paints, varnishes and lacquers; glass; soil; firearms discharge residues; fire investigation; questioned documents; toxic substances in food; restoration of erased markings; and miscellaneous. The final section is headed "Auxiliary Activities" and comprises the forensic significance of physical evidence and its collection; reference collections; the forensic expert; sources of information on forensic science; and the organisation of a forensic science laboratory. The coverage is therefore relatively comprehensive in respect of the title, but there are one or two areas not included, *e.g.*, poisons and alcohol in samples from drunken drivers, which are usually dealt with by chemists in other forensic science laboratories.

It is interesting that the divisions of the main section are based on evidential types in contrast to other texts in this field which are technique oriented. In consequence, the chapters discuss the nature of the substrate and the main methods of examination used, amply reinforced with references and tables and illustrated by examples from actual cases. It is perhaps a little disappointing

that the authors did not come out strongly in favour of the methods which they preferred. A little controversy always adds zest to reading. Notwithstanding this though, there is much to recommend the book within the compass with which it sets itself.

However the price is a very great disadvantage, about £40 is a lot to pay for a specialist topic, no matter how well printed and what the quality of the paper is. In addition, the proof reading is very poor for a book of this quality. This reviewer noted over 70 typographical and similar errors without deliberately looking for them. Thus it is probable that this book may find a home in the reference library of laboratories but, however desirable it may be, it is beyond the pocket of individual scientists.

R. L. WILLIAMS

FLAVOUR '81. 3RD WEURMAN SYMPOSIUM. PROCEEDINGS OF THE INTERNATIONAL CONFERENCE, MUNICH, APRIL 28–30, 1981. Edited by PETER SCHREIER. Pp. xiv + 780. WALTER DE GRUYTER. 1981. Price DM198. ISBN 3 11 008441 4.

Since the inception of this conference in 1975, the number of contributions have increased from 20 to 57, which has naturally resulted in a corresponding increase in the size of the proceedings.

Contributions are divided under the headings of Sensory Methodology, Application of Sensory Methods, Instrumental Analysis, Formation of Flavour, Application and Technology and Molecular Aspects of Flavour. Like all conference reports, the publication cannot be regarded as a text-book, as the contents depend upon the contributions made by the authors. Its value, therefore, depends on whether it represents an up-to-date review of the state of the art.

In a paper entitled "The future of aroma research," by Rijkens and Boelans, in the proceedings of the 1975 symposium, it was stated that the important areas of future aroma research would lie in the study of the biogenesis of flavours, their formation by thermal processes and their interaction with each other. The authors further suggested that sensory research should concentrate on the mechanism of olfaction and taste.

In the 1981 proceedings, four contributions deal with the formation of flavours by thermal processes and nine with the biogenesis of flavours. The paper by Drawert and Berger deals especially with the production of flavours in plant tissue cultures. Only one contribution from a group of workers from Munich is concerned with the mechanism of taste. Anyone hoping that the proposals made at the 1975 symposium would be actively pursued and reported in the 1981 proceedings would probably be a little disappointed.

The two topical aspects of biotechnology and extraction of flavours with supercritical carbon dioxide have been either dealt with superficially or in the latter case, ignored.

However, the prospective reader should not think that the book lacks useful information. For those with a practical mind, several early chapters deal with mathematical methods for the expression of taste thresholds, together with some useful notes on errors that have clearly been made in the past. Sugisawa and Hirose describe an apparatus, complementary to the well known Likens and Nickerson apparatus, for the simultaneous distillation and adsorption of volatiles from essential oils. The following paper by Schaefer compares a number absorbents for their efficiency in concentrating volatiles from foodstuffs.

Tressl and co-workers at the University of Berlin characterise a number of novel flavour compounds arising from the reaction of maltose and glucose with proline or hydroxyproline. De Rijke and other workers describe the Shigematsu variation of the Maillard reaction between some sulphur amino acids and six sugars.

Drawert and Berger offer some valuable results, without experimental detail, in the important area of biotechnological production of flavours by plant tissue culture, which should stimulate further studies. E. W. Seitz, in a subsequent short contribution, introduces the concept of microbial formation of flavours. The possibility of selectively producing certain terpenes from specific strains of fungi is outlined by two authors from the University of Hamburg. Workers at the Givaudan Co. Ltd. review in some detail the formation of cheddar cheese flavour, and a corresponding group from the Brewing Research Foundation summarises the flavour constituents of malt.

The penultimate section contains a number of papers on the technological, industrial and legal aspects of flavours.

The final section contains two invaluable contributions on the molecular aspects of flavour, which should stimulate further ideas on the production of flavour compounds.

Many of the contributions are from continental authors whose mother-tongue is not English.

With this fact in mind, the standard is fairly high, but the publication is not without a series of rather quaint English terms, like "perforator" (p. 378) and "aliphatic rest" (p. 380). A number of misprints and spelling mistakes occur, "tymol" (p. 373) and "cristalisation" (p. 273). Poor reproduction of mass spectra on p. 271 make them almost unreadable.

At a price of DM198, the book will probably find a place on the shelves of libraries which serve a group of scientists dedicated to flavour research, but it is unlikely to reach a wide circulation.

M. J. SAXBY

TREATIES ON ANALYTICAL CHEMISTRY. PART 1. THEORY AND PRACTICE. Second Edition. VOLUME 5: SECTION F, SAMPLE PREPARATION; AND SECTION G, SEPARATION TECHNIQUES. Edited by PHILLIP J. ELVING, ELI GRUSHKA and I. M. KOLTHOFF. Pp. xxxii + 668. Wiley-Interscience. 1982. Price £48. ISBN 0 471 01837 6.

My main objection to an extensive encyclopaedia of this kind is that the various topics age at different rates and one can end with a collection of volumes that are, in part, obsolescent, whereas single monographs in a series can easily be replaced as they age. However, I had never visualised that a Second Edition could be started so soon after the First Edition was completed. Clearly, the First Edition must have been a commercial success, as it deserved to be, otherwise, in this hard world, a Second Edition would have had to wait a very long time before it could be launched.

The Editors, in their Preface to the Second Edition, state "like the First Edition, the Second Edition is not an extensive text book; it attempts to present a thorough introduction to the methods of Analytical Chemistry and to provide a background for detailed evaluation of each topic." This should be borne in mind when the chapters are consulted, for in places there is some superficiality.

The present volume contains the following chapters: "Decomposition and Dissolution of Samples; Inorganic," by Donald C. Bogen; "Decomposition and Dissolution of Samples; Organic," by E. C. Dunlop and C. R. Ginnard; "Principles of Chemical Separation," by J. Calvin Giddings; "Mechanical Methods," by James O. Osburn and Karl Kammermeyer; "Membrane Processes," by Sum-Tak Hwang and Karl Kammermeyer; "Preparation of Isotope Ultrafiltration Membranes"; "Biochemical and Biomedical Separations Using Liquid Membranes," by John W. Frankenfeld and Norman M. Ly; "Crystallisation and Precipitation," by William R. Wilcox and Joseph Estrin; "Adsorptive Double Separation Methods," by Robert B. Grieves; "Distillation," by Arthur and Elizabeth Rose; "Liquid-Liquid Extraction," by H. M. N. H. Irving; and "Counter Current Distribution," by H. O. Rothbart and R. A. Barford.

All of these various sections maintain the high standards set in the earlier edition, which is to be expected from authors of this calibre. Incredibly, the chapter on fusions does not contain tabulated information on the fusion-point of the various fluxes, and the US still has to discover that the oxygen-flask was used in the last century; indeed, in a few years time, it will enjoy its centenary.

When the limitations imposed by the Editors are considered, this is an outstanding volume which all analysts will find indispensable; by today's standards the price is very reasonable.

(the late) R. BELCHER

Spectrophotometric Determination of Cobalt in Hydrofining Catalysts, Alloys and Salts with Biacetylmonoxime 2-Pyridylhydrazone

Biacetylmonoxime 2-pyridylhydrazone (BMPH) has been examined to evaluate its usefulness as a selective spectrophotometric reagent for cobalt. The yellow coloration is formed at an initial pH of 5–6 in a medium containing 20% of dimethylformamide and measured at 430 nm at a final concentration of 0.5 N perchloric acid. The molar absorptivity is 3700 l mol^{-1} cm^{-1}. The method has been applied to the determination of cobalt in synthetic mixtures, hydrofining catalysts, alloys and chemicals with good results. The BMPH chromogen shows a wide range of application.

Keywords: *Biacetylmonoxime 2-pyridylhydrazone reagent*; *cobalt determination*; *spectrophotometry*

AGUSTIN G. ASUERO, DANIEL ROSALES and MANUEL M. RODRIGUEZ
Department of Bromatology, Toxicology and Applied Chemical Analysis, Faculty of Pharmacy, University of Seville, Seville-4, Spain.

Analyst, 1982, **107**, 1065–1072.

Micellar Enhanced Fluorimetric Determination of 1-*NN*-Dimethylaminonaphthalene-5-sulphonyl Chloride and *o*-Phthalaldehyde - 2-Mercaptoethanol Derivatives of Amino Acids

The effects of various surfactant micellar systems upon the spectrofluorimetric method for the determination of amino acids by Roth's method and the dansyl chloride procedure have been assessed. Specifically, the fluorescence intensity of dansyl glycine was found to be remarkably enhanced when in the presence of cationic hexadecyltrimethylammonium chloride or dodecyltrimethylammonium chloride and zwitterionic *N*-dodecyl-*NN*-dimethylammonium-3-propane-1-sulphonic acid micellar systems. Likewise, the lysine derivative of *o*-phthalaldehyde - 2-mercaptoethanol exhibited intensified fluorescence when in the presence of non-ionic Brij-35 or Triton X-100 and anionic sodium dodecylsulphate micelles. Depending upon the type of surfactant micellar system employed, fluorescence enhancements of from 8 to 20 were observed in comparison with that in water alone. Owing to this micellar phenomenon, the sensitivity of the fluorimetric methods for the determination of these two species was correspondingly increased from 8- to 20-fold over the conventional procedures.

The spectral parameters, quantum yields, lower detection limits and analytical figures of merit for these two substances in the micellar systems and water alone are compared. Possible reasons for this observed micellar induced enhanced fluorescence are given. A brief prospective on the general applicability of using micelles to enhance other fluorimetric methods is also given.

Keywords: *Micellar enhanced fluorescence*; *Roth's method*; *dansyl chloride procedure*; *lysine*; *dansyl glycine*

H. N. SINGH and WILLIE L. HINZE
Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, USA.

Analyst, 1982, **107**, 1073–1080.

Infrared Determination of Petroleum Oil. Part II. Extraction from Water and Removal of Interferences

A brief review is given of the methodologies used in the infrared determination of petroleum oil. Some performance characteristics are presented for a method that measures hydrocarbons after a Florisil column clean-up. Extraction efficiencies were determined for dispersions of gas oil in water for the solvents carbon tetrachloride and Freon 113. The removal of interferences was also studied.

Keywords: *Petroleum oil determination*; *infrared spectroscopy*; *solvent extraction*; *hydrocarbons*; *interferences*

W. A. McCRUM and P. J. WHITTLE

North West Water, Rivers Division Laboratory, Liverpool Road, Great Sankey, Warrington, Cheshire, WA5 3LP.

Analyst, 1982, **107**, 1081–1085.

Direct Titrimetric Determination of the Antihypertensive Drugs Methyldopa and Propranolol in Pharmaceutical Preparations

Short Paper

Keywords: *Methyldopa determination*; *propranolol determination*; *titrimetry*; N-*bromosuccinimide*

V. N. PATHAK

Department of Chemistry, University of Allahabad, Allahabad-211002, India.

Miss S. R. SHUKLA

Department of Pharmacology, M.L.N. Medical College, Allahabad-211002, India.

and I. C. SHUKLA

Department of Chemistry, University of Allahabad, Allahabad-211002, India.

Analyst, 1982, **107**, 1086–1087.

Simple Determination of Glutathione by Two-phase Titration

Short Paper

Keywords: *Glutathione determination*; *two-phase titration*; *tetrabromophenolphthalein ethyl ester*

MASAHIRO TSUBOUCHI

Laboratory of Chemistry, Kochi Medical School, Oko, Nankoku, Kochi 781–51, Japan.

and EIJI TAO

Laboratory of Chemistry, Kagoshima Women's College, Shibahara, Kagoshima, Japan.

Analyst, 1982, **107**, 1088–1090.

Micro-scale Thin-layer Chromatographic Method for the Screening of Urine Samples for Aspirin Metabolites

Short Paper

Keywords: *Aspirin metabolites*; *urine*; *thin-layer chromatography*; *forensic investigations*; *clinical investigations*

I. C. SHAW
31, Marlbrook Lane, Bromsgrove, Worcestershire.

Analyst, 1982, **107**, 1090–1091.

Determination of Monosodium Glutamate in Food Using High-performance Liquid Chromatography and Fluorescence Detection

Short Paper

Keywords: *Glutamic acid determination*; *fluorescence derivatisation*; *high-performance liquid chromatography*; *soups*

A. T. RHYS WILLIAMS and S. A. WINFIELD
Perkin-Elmer Limited, Post Office Lane, Beaconsfield, Buckinghamshire, HP9 1QA.

Analyst, 1982, **107**, 1092–1094.

Graphite-probe Atomisation for Carbon Furnace Atomic-absorption and Atomic-emission Spectrometry

Communication

Keywords: *Atomic-absorption spectrometry*; *atomic-emission spectrometry*; *graphite probe sample introduction*; *electrothermal atomisation*; *interferences*

S. K. GIRI, D. LITTLEJOHN and J. M. OTTAWAY
Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL.

Analyst, 1982, **107**, 1095–1098.

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