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Summaries of Papers in this Issue

pH Cells for Over-all Temperature Compensation in the Measurement of the pH of Boiler Feedwater

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Keywords: *pH*; *temperature compensation*; *glass electrode*; *reference electrode*; *boiler feedwater*

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Analyst, 1982, **107**, 1297–1308.

Kinetic Determination of Nitrite in Waters by Using a Stopped-flow Analyser

Automatic kinetic methods for the determination of nitrite in waters with a stopped-flow analyser are described. The methods are based on the diazotisation of sulphanilamide, the product being coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye, which is measured at 540 nm. A single-point kinetic procedure uses a delay time of 10 s and one measurement of 0.7 s. A multi-point reaction rate method uses a delay time of 0.8 s and a measurement time of 1.5 s. The methods are fast, sensitive, accurate and precise, and without serious interference. The sample throughput for routine analysis can be up to 360 samples per hour in the range 0.025–2.00 p.p.m. of nitrite-nitrogen.

Keywords: *Nitrite determination*; *water analysis*; *kinetic method*; *stopped-flow analyser*; N-(1-*naphthyl*)*ethylenediamine dihydrochloride*

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Analyst, 1982, **107**, 1309–1315.

Simplified Procedure for the Determination of Chemical Oxygen Demand Using Silver Nitrate to Suppress Chloride Interference

A sealed-flask procedure for the determination of the chemical oxygen demand of wastewaters is described. Samples are digested at 150 °C in a glass-stoppered flask using springs to retain the stopper. The use of sealed-flask conditions offers two advantages over reflux conditions: simplified apparatus and experimental procedure and improved suppression of chloride interference. The proposed procedure is similar to the standard procedure in accuracy and reproducibility of results.

Keywords: *Chemical oxygen demand*; *sealed flask*; *wastewaters*

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Analyst, 1982, **107**, 1316–1319.

Extraction of Iron(III) from Aqueous Solution with Mixtures of Aliquat 336 and Ferron in Chloroform

Ferron is efficiently extracted by Aliquat 336 in chloroform and the ion pair ($R_4N^+HL^-$) forms dark green complexes with iron(III), in the organic phase. On the basis of solvent extraction data for iron, as well as absorbance measurements, it is assumed that two iron complexes, one with ferron and one with the alkylammonium cation, can be present in the organic phase [$FeL_5^{3-}(R_4N^+)_5$ and FeL_2-R_4N^+]. The latter complex can be formed at high iron concentration in aqueous solution, where part of the ferron is also stripped from the organic phase and forms coloured complexes with iron in the aqueous phase. The absorbance of solutions of Aliquat 336 and ferron in chloroform is very low, in the visible region. The absorbance of iron complexes with ferron and Aliquat 336 is higher than the absorbance of iron complexes with ferron in aqueous solutions and three absorbance maxima were found: 370 nm ($\epsilon = 7.7 \times 10^3$ l mol^{-1} cm^{-1}), 465 nm ($\epsilon = 6.86 \times 10^3$ l mol^{-1} cm^{-1}) and 610 nm ($\epsilon = 5.95 \times 10^3$ l mol^{-1} cm^{-1}). The absorbances at 465 and 610 nm can be utilised in the extraction - spectrophotometric determination of iron. Beer's law is obeyed in the concentration range 0.1–10 mg l^{-1} of iron in aqueous solution. The method is selective for the determination of iron and only copper interferes when present in amounts that exceed the iron concentration. The proposed method was used to determine iron in two samples of natural waters, and the results of 0.48 and 5.7 mg l^{-1} are in good agreement with results obtained by atomic-absorption spectrometry and a spectrophotometric method using thiocyanate.

Keywords: *Alkylammonium salt extractants*; *Aliquat* 336 *extraction*; *ferron extraction*; *iron determination in water*; *spectrophotometry*

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Analyst, 1982, **107**, 1320–1329.

Water Analysis by Inductively Coupled Plasma Atomic-emission Spectrometry after a Rapid Pre-concentration

A method is described in which large batches of 10-ml water samples are pre-concentrated by evaporation and rapidly analysed for 16 elements at average river concentrations, by simultaneous inductively coupled plasma spectrometry. The effects of background interference, and its on-peak correction, on realistic detection limits of 30 elements were studied on solutions with high levels of calcium and magnesium and were found to place minor constraints on the determination of some elements. Recoveries of 32 elements during pre-concentration were examined and 24 were found to be quantitative. The applicability of this method to the analysis of fresh water is considered in comparison with average river water concentrations and the EEC 1980 Council Directive.

Keywords: *Water analysis*; *pre-concentration*; *background interference*; *inductively coupled plasma*; *atomic-emission spectrometry*

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Analyst, 1982, **107**, 1330–1334.

Rapid Flow Analysis with Inductively Coupled Plasma Atomic-emission Spectroscopy Using a Micro-injection Technique

An introduction system for liquid micro-samples in inductively coupled plasma atomic-emission spectroscopy is described that allows the injection of 5–500-μl volumes into a rapidly flowing carrier reagent stream leading to the nebuliser. The effect on analyte signal was studied as a function of flow-rate, injection volume and sample concentration. It is shown that the carrier flow-rate determines the response time, sensitivity, precision and sample carry-over in the nebuliser. By the use of relatively rapid flow-rates of up to 7.5 ml min^{-1}, fast injection of 10-μl samples is achieved at an injection rate of 240 h^{-1} with a relative standard deviation of 1.5% for a single-element analogue readout. Digital readout is used for multi-element determinations with similar or better precision. Detection limits of the order of 0.1 mg l^{-1} are obtained for 10-μl injections, limited by the volume injected, with a proportionate decrease in detection limit for increasing volumes.

Keywords: *Rapid injection*; *inductively coupled plasma*; *serum electrolyte*

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Analyst, 1982, **107**, 1335–1342.

Indirect Determination of Iodine by Cold Vapour Atomic-absorption Spectrophotometry Utilising the Interfering Effect of Iodine Against Mercury. Part I. General Study and Application to the Determination of Iodine in Seaweed

A simple and rapid method is described for the indirect determination of iodine as an interferent against mercury in cold vapour atomic-absorption spectrophotometry. The interference effect is due to the formation of mercury(II) iodide complexes, which in highly acidic solutions cause a decrease in absorbance for mercury(II) proportional to the amount of iodine present. Within certain concentration limits a straight-line calibration graph is obtained, making the determination of small amounts of iodine possible. The detection limit is 2.5 $\pm$ 0.7 μg of iodine if it is allowed to interfere (as iodide or iodate) against 100 ng of mercury(II) in 3.0 M nitric acid solution. Interferences for the method are, in general, the same as in the cold vapour atomic-absorption determination of mercury. Chloride and moderate amounts of bromide do not interfere. The possible interference of certain metals, especially the noble metals, is discussed. The proposed method has been used with satisfactory results in the determination of iodine in a seaweed sample (*Ascophyllum nodosum*) that had been analysed earlier by neutron activation analysis.

Keywords: *Indirect iodine determination*; *cold vapour atomic-absorption spectrophotometry*; *interference of iodine against mercury*

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Analyst, 1982, **107**, 1343–1349.

Effect of the Type of Organometallic Iron and Copper Compounds on the Determination of Both Metals in Petroleum Samples by Flame Atomic-absorption Spectroscopy

The effect of the type of organometallic iron or copper compounds on the value of the absorbance signals of these metals in an air - acetylene flame and for iron in a dinitrogen oxide - acetylene flame is reported. The determination of iron and copper in xylene solutions of the asphaltene and resinous petroleum fractions was carried out using various organometallic compounds of both metals as standards and by both the calibration graph and standard additions techniques. There are serious limitations to the direct flame atomic-absorption spectrometric methods, owing to transport interferences. In addition, with iron, there are matrix interferences caused by the structure of the petroleum samples being analysed and also owing to the various forms of iron present in these samples. It is recommended that determination of these elements in heavy petroleum fractions should be carried out by dry mineralisation and flame atomic-absorption spectrometric analysis of aqueous solutions.

Keywords: *Iron determination*; *copper determination*; *atomic-absorption spectroscopy*; *petroleum*; *organometallic compounds*

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pH Cells for Over-all Temperature Compensation in the Measurement of the pH of Boiler Feedwater

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Strict control of the water chemistry in modern high-pressure boilers often requires that the measured pH of boiler feedwater is referred to a standard temperature of 25 °C. At present most of the pH meters installed in power stations cannot meet this requirement unless the sample temperature is controlled to 25 °C, because their temperature compensation circuits cannot correct for temperature-induced changes in the pH of alkaline feedwater. These changes can be considerable because the temperature coefficient of feedwater is of the order of -0.033 pH °C^{-1}.

Experimental glass electrodes have been developed which, when used in conjunction with a specified reference electrode, can provide over-all temperature compensation in ammonia-dosed feedwater over the range 15–35 °C. The chemistry of these electrodes has been arranged such that the cell e.m.f. only responds to changes in pH brought about by changes in alkalinity. At constant ammonia concentrations equivalent to pH values in the range 9–9.3, the temperature-induced variations in the cell e.m.f. over the temperature range 15–35 °C were equivalent to less than 0.05 pH for a pH electrode containing *N*-glycylglycine in its internal reference solution and using silver - silver chloride/0.1 mol l^{-1} potassium chloride or calomel/3 mol l^{-1} potassium chloride reference electrodes.

Keywords: *pH*; *temperature compensation*; *glass electrode*; *reference electrode*; *boiler feedwater*

The CEGB specification for the measurement of the pH of ammonia-dosed feedwater in once-through boilers requires that the pH is referred to a standard temperature of 25 °C. This has been considered necessary because under conditions of varying sample temperatures the measurement of pH by instruments which display the correct pH at the temperature of the sample will record changes in pH that are due solely to the effects of temperature on the chemical equilibria in the sample. These changes can be considerable because the temperature coefficient in boiler feedwater is of the order of -0.033 pH °C^{-1}. Without additional information, plant operators can be in doubt as to whether a difference in pH arises from a change in alkalinity or a change in sample temperature.

Most pH meters installed in plant display the correct pH at the temperature of the sample. What is required is a meter that will display the pH at the standard temperature of 25 °C and for our purposes such a pH measuring system would be described as having over-all temperature compensation.

This paper describes experiments in which the temperature-dependent properties of glass pH electrodes and silver - silver chloride and calomel reference electrodes were investigated with the aim of producing a pH cell that was self-compensating for the measurement of pH to a reference temperature of 25 °C in ammonia-dosed feedwater over the range 15–35 °C. The e.m.f., and hence the indicated pH, of such a cell would be independent of temperature under conditions of constant ammonia dosage. As such it would have distinct advantages over existing methods of providing over-all temperature compensation that depend on a measurement of temperature and the application of a linear correction factor to a temperature-dependent e.m.f. obtained from the pH cell.

Over-all Temperature Compensation

The e.m.f. of a pH cell (E_{cell}) is conventionally written as the difference between the potentials of the glass (E_{glass}) and external reference (E_{ref}) electrodes:

$$E_{cell} = E_{glass} - E_{ref} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Equation (1) can be expanded to include terms for the e.m.f. of the internal reference electrode (E_{int}), which is inside the glass electrode, and the pH of the solution in which it is immersed (pH_{int}), together with the pH of the external solution (pH_{ext}) and the potential of the liquid junction (E_J):

$$E_{cell} = (E_{int} + k\mathrm{pH}_{int})_{glass} - k\mathrm{pH}_{ext} - E_{ref} + E_J \quad .. \quad .. \quad (2)$$

where k is the Nernst coefficient, equal to $2.303RT/F$. When the temperature of the pH cell changes, each of the terms in equation (2) alters and the temperature dependence of the pH cell may be expressed by differentiating equation (2) with respect to temperature:

$$\frac{dE_{cell}}{dT} = \left(\frac{dE_{int}}{dT} + \frac{k\,d\mathrm{pH}_{int}}{dT} + \mathrm{pH}_{int}\cdot\frac{dk}{dT}\right)_{glass} - \frac{k\,d\mathrm{pH}_{ext}}{dT} - \mathrm{pH}_{ext}\cdot\frac{dk}{dT} - \frac{dE_{ref}}{dT} + \frac{dE_J}{dT} \quad (3)$$

No attempt was made to compensate for the temperature dependence of the liquid junction as it is considered to be very small.[1] On examination of equation (3), it can be seen that, excluding the term dE_J/dT, there are three pairs of terms (dE/dT, $k\,d\mathrm{pH}/dT$ and $\mathrm{pH}\cdot dk/dT$) each pair of which is identical except for sign and subscript.

The most direct approach to self-compensation was first attempted by equating the terms in each of these pairs. For the measurements in feedwater, this requires that the pH of the filling solution inside the glass electrode is typical of that of feedwater and also has a similar temperature coefficient ($d\mathrm{pH}/dT$). The value of the latter depends to some extent on the temperature dependence of the dissociation constant of the base, but in dilute solutions the variation of the dissociation constant of water with temperature dominates the relationship. Three bases were used. Ammonia was an obvious choice because it is the base most commonly added to control the pH of feedwater. Two other bases, tris(hydroxymethyl)-aminomethane (Tris) and *N*-glycylglycine, were selected because calculations indicated that $d\mathrm{pH}/dT$ in their solutions is very close to that expected in feedwater (-0.033 pH °C^{-1}). In addition, these bases are non-volatile and as such could have some advantage over ammonia, which might be gradually lost from the internal filling solution of the glass electrode.

The two terms in equation (3) of the general form dE/dT involve the temperature dependence of the reference electrodes and as the silver - silver chloride electrode is used almost exclusively as the internal reference electrode in glass electrodes, it was the first choice as the external reference electrode. Experiments were carried out using silver - silver chloride electrodes in contact with 3 mol l^{-1} potassium chloride as internal and external reference electrodes in the same cell. Subsequently, attempts to equate the terms dE/dT were discontinued and external electrodes differing in either concentration of electrolyte or type were used in an attempt to achieve the desired compensation, *i.e.*, of $dE_{cell}/dT = 0$. The most successful results were obtained with calomel (3 mol l^{-1} potassium chloride) and silver - silver chloride (0.1 mol l^{-1} potassium chloride) electrodes.

Experimental

Apparatus and Procedure

Dilute ammonia solutions were prepared using the apparatus shown in Fig. 1. A stream of de-ionised water was dosed with ammonia by diffusion of the gas through a length of silicone-rubber tubing from concentrated ammonia solution. Under conditions of constant temperature, flow-rate of de-ionised water and diffusion of ammonia, ammonia solutions of constant conductivities, comparable to those observed in feedwater, were produced. The temperature of these simulated feedwaters could be adjusted to known values by passing them through a temperature-controlled heat exchanger before measuring the changes in e.m.f. they produced in a number of pH electrodes contained in a flow cell. The apparatus was housed in an air-conditioned room whose temperature was controlled at 25 $\pm$ 1 °C.

In these investigations the pH values of the ammonia solutions were calculated from accurate measurements of their temperatures and conductivities. In order to facilitate simultaneous readings of temperature, conductance and the e.m.f. of the pH cells, signals from sensors for each of these parameters were fed to the central data acquisition and processing system (CDAPS) at CERL. These measurements were logged on a PCD–DCH70/72 cassette recorder and could be simultaneously displayed on a VDU. The output impedance of the conductance bridges and the impedance of the pH cells were too high to be fed direct to the measuring system and it was necessary to interpose an amplifier between these signal sources and CDAPS.

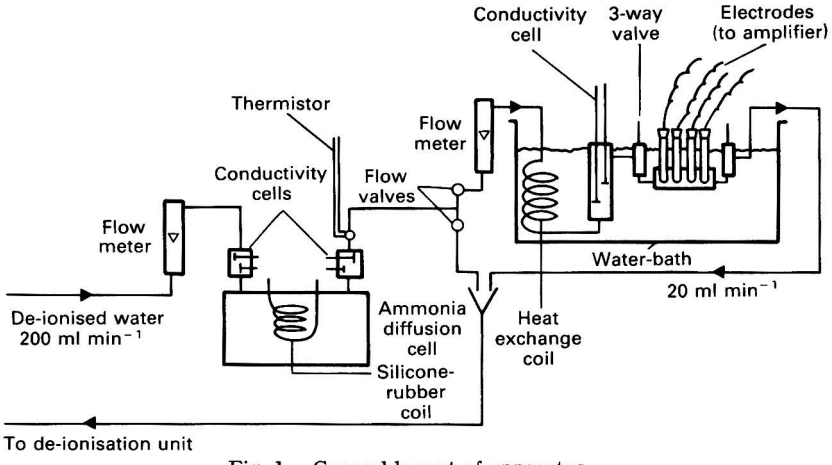

Fig. 1. General layout of apparatus.

Measurement of Temperature

The temperature of the stream of dilute ammonia solution was monitored at two points: directly after the ammonia diffusion cell and inside the conductivity cell that precedes the pH cell (see Fig. 1). Both of these measurements were used in the calculation of pH from the temperature - conductivity relationship in dilute ammonia solutions. In both of these positions, the temperature was sensed by a 100-kΩ precision thermistor (Type YSI 44011, Swift-Sasco, Crawley). In the first position the thermistor was mounted in a small glass flow cell through which the total output of about 200 ml min^{-1} from the diffusion cell flowed. The second thermistor was incorporated inside the conductivity cell by the manufacturer, where it measured the temperature of that portion of the total flow that was directed through the pH cell. The thermistors were powered from a 9.1-V d.c. power supply and the voltage across a 100-Ω resistor, placed in series with the thermistor, was measured by CDAPS. At 25 °C this was of the order of 9 mV. The thermistors were calibrated in flowing solution over the temperature range 15–35 °C against certificated mercury-in-glass thermometers. The measured voltages were fitted to an equation of the general form $mV = ae^{bT}$, where a and b are constants. The equations so obtained had correlation coefficients greater than 0.99 and temperatures could be measured with an accuracy of better than 0.1 °C.

Measurement of Conductivity

The conductivity of the solutions could be measured at three places (see Fig. 1): directly before and after the ammonia diffusion cell and immediately before the pH cell. All of the conductivity cells were made from stainless steel and were of the flow-through type. Those on either side of the ammonia diffusion cell were Type EFD 001 (cell constant *ca.* 0.01 cm^{-1}) and that in the water-bath Type ESC/005/200E/100K (cell constant *ca.* 0.05 cm^{-1}), both types being manufactured by Electronic Instruments Limited (EIL). Accurate values of the cell constants of these cells were obtained by calibrating them against a standard conductivity cell in a flowing solution whose specific conductivity was comparable to that of feedwater. The conductances of the solutions were measured using Wayne Kerr Auto-

balance Universal Bridges, Model B642 (Wilmot Breeden Electronics Limited, Bognor Regis) with an accuracy of the order of 0.1%.

The conductances of the dilute ammonia solutions were monitored and changes in conductivity were registered (by CDAPS) as changes in the potentiometric recorder output from the Wayne Kerr bridge. Each bridge was calibrated on the range applicable to the conductivity of feedwater (about 2–8 μS cm^{-1}) using a standard resistance box; thus the voltage measured by the data logging system could be converted to a conductance by a linear equation of the form $\mu S = a(mV) + c$, where a and c are constants. Using this procedure, conductances could be measured with an error of less than 0.5%.

Measurement of the E.m.f. of pH Cells

It was decided to control the temperature of the pH cell at as near to 15, 20, 25, 30 and 35 °C as possible, as most of the constants used in calculating the pH from measurements of conductivity had been accurately determined at these temperatures. The simplest way to obtain temperature control to better than ± 0.1 °C was by the use of a high-quality proportional heater such as a Tecam Model TU-14 in an insulated water-bath. Some difficulty was experienced in measuring the e.m.f. due to the presence of earth loops, as the dilute ammonia stream was connected to the earth terminal of the mains electrical supply through the stainless-steel heat exchanger and metal conductivity cell immersed in the water-bath. These earth loops were eliminated by using a differential potentiometric measurement with the water-bath (or more correctly the water-bath potential) as the common point.

A Keithley, Model 604, differential amplifier made the high-impedance e.m.fs from the pH cells acceptable to the CDAPS measuring system. The stability of this system was such that the standard deviation of the e.m.f. measured in buffer solutions over a period of 10 min was of the order of 0.03 mV. In simulated feedwater the precision was considerably reduced, but even then it was usually less than 0.5 mV when measured over a similar time period.

During a logging sequence, the e.m.fs of all of the electrodes (glass and reference) were measured with respect to one reference electrode. Subsequently, the e.m.f. of any glass - reference electrode combination could be obtained by difference.

Experimental Glass Electrodes

Refillable glass electrodes were first tried but these proved unsuitable because the inadequacies of the electrical screening gave high signal noise and poor stability. The alternative approach, which was completely satisfactory, was to obtain from EIL a supply of unfilled glass electrodes of the same type of glass as that used in their current range, and to fill these with the appropriate experimental internal reference solutions. These solutions contained 3 mol l^{-1} potassium chloride saturated with silver chloride, together with a base present at a concentration in the range 10^{-3}–10^{-2} mol l^{-1}. The pH of these solutions was adjusted, where necessary, to 9.0 $\pm$ 0.1 by the addition of potassium hydroxide solution.

External Reference Electrodes

The basis of designing a self-compensating pH cell was that the pH and external reference electrodes would be subjected to the same temperature changes. This requires that both of these electrodes are immersed in the sample solution, unlike some industrial pH cells where a remote junction is favoured.

A Corning Type 476029 silver - silver chloride reference electrode, which had a side-arm for connection to a remote reservoir, was emptied of its 4 mol l^{-1} potassium chloride solution and refilled with 3 mol l^{-1} potassium chloride solution saturated with silver chloride. This electrode was allowed to equilibrate with its new filling solution for about 10 d until its potential became constant. A second silver - silver chloride electrode of similar construction was used but in this instance the original electrolyte was replaced with 0.1 mol l^{-1} potassium chloride solution. The electrode was again allowed to attain a steady potential before being used.

An EIL RJ23 calomel electrode, also modified to take a remote reservoir, was the third reference electrode used. This was filled with 3 mol l^{-1} potassium chloride solution.

All of the electrodes were used in conjunction with remote reservoirs providing about 500 mm head of electrolyte solution and in every instance the liquid junctions were formed at ceramic frits.

pH Flow Cell

The pH flow cell was made from Perspex and was designed for use with eight electrodes (see Fig. 2). The cross-section of the cell was rectangular, 50 mm wide by 40 mm high, and the overall length was 122 mm. The electrodes were mounted vertically in the cell and held in position in tapered holes by silicone-rubber bungs, cast to fit the taper of the holes. A support lid was held in position on top of the flow cell by eight 4BA studs (see Fig. 2). This rectangular piece of 10-mm Perspex sheet was necessary to prevent any movement of the stems of the electrode that could lead to ingress of water from the water-bath. The inlet and outlet of the cell were $\frac{1}{4}$-in Drallim bulkhead couplings (Drallim Tube Couplings Limited, Bexhill-on-Sea), which were connected by 50-mm lengths of $\frac{1}{4}$-in PTFE tubing to Chemcon three-way PTFE valves (Production Techniques Limited, Fleet, Hampshire). These valves were necessary to isolate that part of the circuit containing the dilute ammonia solution from the flow cell during standardisation with buffer solutions.

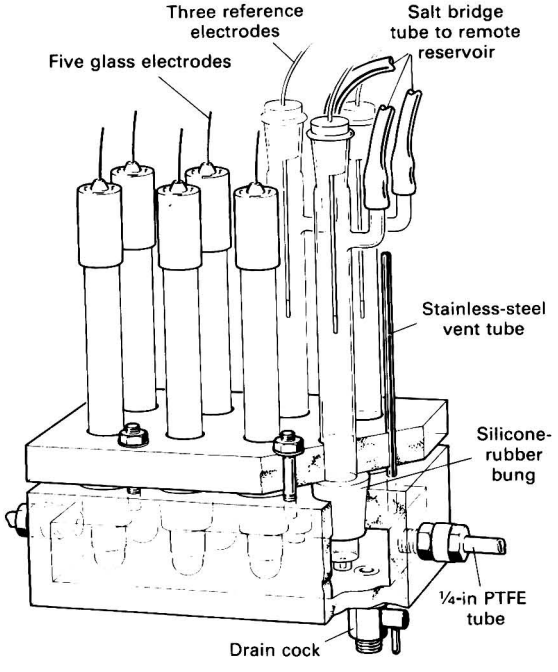

Fig. 2. Perspex flow cell.

Operating Procedure

The ammonia diffusion cell was designed to produce solutions of constant conductivity at an accurately known temperature of about 25 °C at a flow-rate of about 200 ml min^{-1}. A portion of the total flow, usually 15–20 ml min^{-1}, was passed through the heat exchange coil, conductivity cell and pH flow cell, all contained in the temperature-controlled water-bath.

The apparatus was run overnight to reach constant conditions of conductivity and temperature. During this period the temperature of the water bath was set at 15.0 °C, the lowest temperature of the range investigated. In the morning, readings were logged at 1-min intervals for a period of 30 min. The temperature in the water-bath was then re-set to 20.0 °C and after approximately 90 min data were again logged for 30 min. This procedure was repeated at 5 °C intervals up to 35 °C.

Only small differences (0.1–0.2 mV) were observed between the e.m.fs in flowing and static buffer solutions. Because of the operational simplicity of the latter, the pH electrodes

were standardised in the flow cell using static buffer solutions. During this procedure the flow cell was isolated from the circuit containing dilute ammonia solution by the use of the Chemcon three-way valves. The same temperature sequence described above was followed for the buffer solutions. At each temperature the responses of the electrodes were monitored and logged.

Calculation of pH from the Conductivity of Dilute Ammonia Solutions

At the ionic strengths considered here ($I < 5 \times 10^{-5}$), insignificant errors arise from the use of limiting equivalent conductances and the assumption that the activity coefficients are unity. In the conditions described for the diffusion cell, only three ionic species are considered to be present and, therefore, the specific conductance, κ (μS cm^{-1}), can be written as

$$\kappa = ([NH_4^+]\lambda^\circ_{NH_4} + [H^+]\lambda^\circ_{H} + [OH^-]\lambda^\circ_{OH})10^3 \quad .. \quad .. \quad (4)$$

where the terms in square brackets are the concentrations (mol l^{-1}) and the λ° terms are their corresponding limiting equivalent conductances (cm^2 S equiv.$^{-1}$). From consideration of the equations for electroneutrality and the dissociation constant of water, K_W, equation (4) can be rearranged and written as

$$[H^+]^2(\lambda^\circ_{H} - \lambda^\circ_{NH_4})10^3 - [H^+]\kappa + K_W(\lambda^\circ_{NH_4} + \lambda^\circ_{OH})10^3 = 0 \quad .. \quad .. \quad (5)$$

Equation (5) can be solved for [H$^+$] (the smaller positive root). At the temperatures of the experiments, values of the limiting equivalent conductances were obtained directly or interpolated from those given by Robinson and Stokes.[2] Values for K_W at the same temperatures were calculated from the following polynomial:

$$-\log K_W = \frac{4471.33}{T} - 6.0846 + 0.017054\,T$$

where T is the absolute temperature.

Results

Initial Stability of Experimental Electrodes

At intervals over a few weeks before starting the experiments in simulated feedwater, the e.m.fs of three experimental electrodes *versus* a Radiometer K100 saturated calomel electrode were measured in standard phosphate and borax buffers. Between measurements, the electrodes were stored at 25 °C in a small volume of distilled water. The variations in e.m.f. in phosphate buffer for the *N*-glycylglycine-, ammonia- and Tris-filled electrodes are shown in Fig. 3. Changes in e.m.f. could be due to a number of factors: variations in the boundary potentials of the sensing glass, changes in the standard potential of the internal reference electrode or changes in the pH of the internal filling solution. None of these can be measured directly. The e.m.f. of an unused EIL E$_0$7 glass electrode was monitored in a similar manner and, as this electrode was the same as the experimental electrodes except for the filling solution and had been assembled for at least 12 weeks before use, changes in its e.m.f. were taken as a measure of the changes which occur following hydration of the external surface of the glass. Initially its glass sensing surface was dry and on contact with aqueous solution small changes in the electrode potential were observed, much smaller than the changes observed with the experimental electrodes over the same period of time. Therefore, it was probable that the changes that occurred with the experimental electrodes were due to changes in the standard potentials of the internal reference electrodes or changes in the pHs of the internal filling solutions. However, after a period of about 3 weeks (see Fig. 3), the rate of change of potential of all of the experimental electrodes was only about -0.5 mV d^{-1}. This was acceptable for the temperature compensation experiments as measurements at any one level of ammonia were completed within 24 h.

Slope Factor of the Experimental Glass Electrodes

During the sequence of tests with pure ammonia solutions the slope factors, $k = 2.303RT/F$, of the glass electrodes were determined about once per week at 15, 20, 25 and 35 °C by

measuring the e.m.fs in NBS standard borax and phosphate (1 + 1) buffer solutions. These measurements were made in static solutions in the flow cell and the average reading calculated from ten measurements at 1-min intervals. The values of k obtained for all of the experimental electrodes were always greater than 99% of the theoretical values at 25, 30 and 35 °C. At the two lowest test temperatures (20 and 15 °C), values slightly less than 99% were found, particularly at 15 °C where a value as low as 98.4% was calculated for the Tris-filled electrode on one occasion.

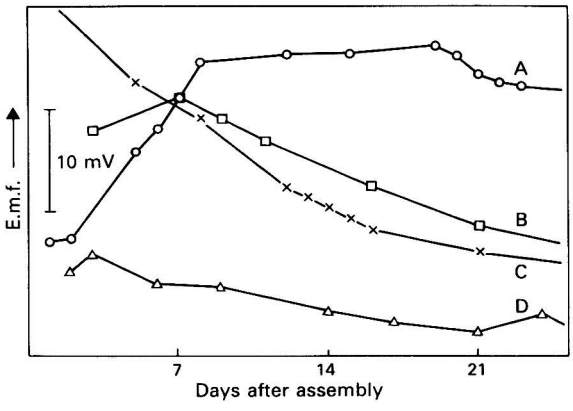

Fig. 3. Variation of e.m.f. in phosphate buffer of experimental pH electrodes *versus* S.C.E. A, Tris-filled; B, *N*-glycylglycine-filled; C, ammonia filled; and D, EIL $E_0 7$.

Stability of Silver - Silver Chloride Electrodes in Alkaline Solutions

The internal silver - silver chloride electrodes in conventional glass pH electrodes are in contact with neutral or acidic solutions containing chloride ions and under these conditions the internal reference electrode potential is stable. In the experimental glass electrodes the internal filling solutions were alkaline and although the stability was satisfactory for measurements over a 24-h period additional information was required on the variation of their e.m.f.s over a longer period. As it was not possible to carry out unambiguous tests on these internal reference electrodes because they were part of the glass electrodes, separate experiments were made using chloridised silver wire electrodes.

Pairs of silver - silver chloride electrodes were tested for long-term stability in solutions of 3 mol l^{-1} potassium chloride containing either ammonia or *N*-glycylglycine (*ca.* 10^{-3}–10^{-2} mol l^{-1}) adjusted to pH 9. The performance of these electrodes was compared with that of two similar electrodes in neutral 3 mol l^{-1} potassium chloride solution. All of these solutions were saturated with silver chloride. Pairs of electrodes and their respective solutions were sealed in 50-ml Pyrex glass flasks and their e.m.f.s measured at intervals over a period of 3 weeks by introduction of a separate reference electrode. Their variations in e.m.f. over this period are shown in Fig. 4 and it can be seen that no significant differences were detected between the variations in e.m.f. in the neutral and basic potassium chloride solutions. Further, comparison of the variations in e.m.f. for the two alkaline solutions with those of the experimental glass electrodes in Fig. 3 suggests that the internal filling solutions and their associated silver - silver chloride electrodes were not the major cause of the initial irregular behaviour.

Temperature Dependence of pH Cells in Flowing Solutions of Ammonia

The variations of the e.m.f. of pH cells consisting of the experimental glass electrodes *versus* a stated reference electrode in flowing ammonia solution were measured over the temperature range 15–35 °C. During any one run the ammonia level was kept constant at a concentration

giving a pH in the range 8.6–9.3. Duplicate experiments were carried out at each ammonia level, although owing to the nature of the apparatus it was not always possible exactly to reproduce the ammonia levels.

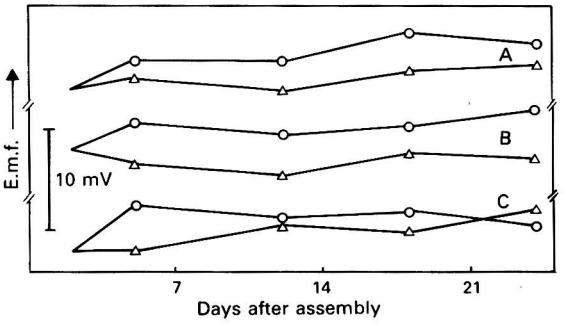

Fig. 4. Variation of e.m.f. of pairs (○, △) of silver - silver chloride electrodes in 3 mol l^{-1} potassium chloride solution and bases: A, no base; B, ammonia (pH 9); and C, Tris (pH 9). Results for each pair in solution were normalised with respect to the mean of the first readings of e.m.f.

Temperature Dependence of pH Cells with a Silver - Silver Chloride/3 mol l^{-1} Potassium Chloride External Reference Electrode

The temperature dependences of pH cells with ammonia-, Tris- and *N*-glycylglycine-filled experimental glass electrodes were measured in solutions containing from 0.09 mg l^{-1} (pH 8.59) to 0.47 mg l^{-1} (pH 9.17) of ammonia. This combination of glass and reference electrodes did not produce the temperature independence expected from the symmetry of the cell. The general trend in results for any one electrode combination was the same at all ammonia levels. The pH cells had temperature coefficients of 0.5–0.6 mV $°C^{-1}$. Examples of the typical behaviour are given in Figs. 5 and 6. It was not possible to say whether the positive coefficient arose because the temperature coefficient of the glass electrode was too high or that of the external reference too low. In order to resolve this uncertainty, tests

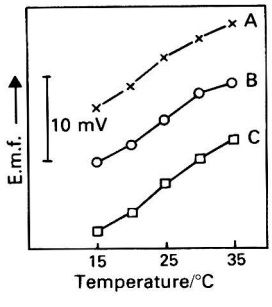

Fig. 5. Temperature dependence of experimental pH electrodes *versus* silver - silver chloride/3 mol l^{-1} potassium chloride external reference electrode in ammonia solution; pH = 8.59 at 25 °C. A, Ammonia-filled; B, Tris-filled; and C, *N*-glycylglycine-filled pH electrode.

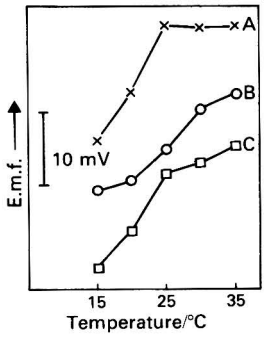

Fig. 6. Temperature dependence of experimental pH electrodes *versus* silver - silver chloride/3 mol l^{-1} potassium chloride external reference electrode in ammonia solution; pH = 9.17 at 25 °C. A, Ammonia-filled; B, Tris-filled; and C, *N*-glycylglycine-filled pH electrode.

were made with a calomel/3 mol l^{-1} potassium chloride reference electrode whose temperature coefficient (*ca.* 0.5 mV $°C^{-1}$) is higher than that of the silver - silver chloride electrode.[1]

Temperature Dependence of Cells with a Calomel/3 mol l^{-1} Potassium Chloride External Reference Electrode

In general, the cell temperature coefficients were much smaller than those obtained with the external silver - silver chloride/3 mol l^{-1} potassium chloride reference electrode, confirming that the coefficients of the glass electrodes were higher than those calculated from equation (3). The results shown in Fig. 7 indicate that over-all compensation was essentially achieved, with a variation in e.m.f. equivalent to only 0.05 pH over the span of 20 °C.

Temperature Dependence of Cells with a Silver - Silver Chloride/0.1 mol l^{-1} Potassium Chloride External Reference Electrode

Although a pH cell containing a calomel/3 mol l^{-1} potassium chloride external reference electrode gave essentially the desired over-all temperature compensation, a silver - silver chloride reference electrode is preferred in circumstances where changes in temperature may occur, as its response to, and recovery from, temperature excursions is much more rapid.[3] In order to decrease the cell coefficient from the value found with the silver - silver chloride/3 mol l^{-1} potassium chloride external reference electrode, it is necessary to reduce the concentration of the potassium chloride solution to about 0.1 mol l^{-1} in the external reference electrode.[1]

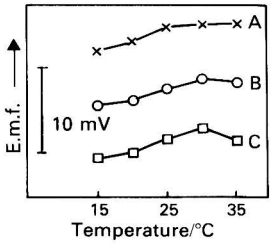

Fig. 7. Temperature dependence of experimental pH electrodes *versus* calomel/3 mol l^{-1} potassium chloride external reference electrode in ammonia solution; pH = 9.17 at 25 °C. A, Ammonia-filled; B, Tris-filled; and C, *N*-glycylglycine-filled pH electrode.

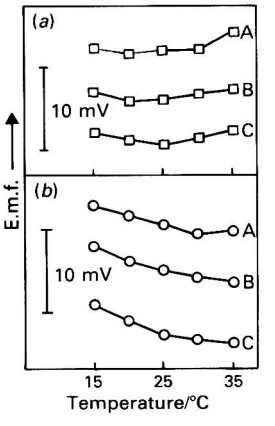

Fig. 8. Temperature dependence of experimental pH electrodes *versus* silver - silver chloride/0.1 mol l^{-1} potassium chloride external reference electrode in ammonia solutions: A, pH 9.0; B, pH 9.16; and C, pH 9.26 at 25 °C. (*a*) *N*-Glycylglycine-filled pH electrode; and (b) Tris-filled pH electrode.

The temperature dependences of pH cells with an external reference electrode containing this electrolyte solution were measured at ammonia levels of 0.28 mg l^{-1} (pH 9.0), 0.46 mg l^{-1} (pH 9.16) and 0.63 mg l^{-1} (pH 9.26). Results of e.m.f. *versus* temperature for pH cells containing the Tris- and *N*-glycylglycine-filled experimental electrodes are shown in Fig. 8 and the variations in pH with temperature are given in Tables I–III. It can be seen that the Tris-filled electrode cell had slightly the higher temperature dependence but in the worst instance this was only equivalent to a variation of 0.08 pH over the range of 20 °C. The cell with the *N*-glycylglycine-filled electrode had a variation in pH usually less than ± 0.03.

TABLE I

VARIATION IN INDICATED pH WITH TEMPERATURE IN CELLS HAVING AN EXTERNAL SILVER - SILVER CHLORIDE/0.1 mol l^{-1} POTASSIUM CHLORIDE REFERENCE ELECTRODE (pH = 9.00 AT 25 °C)

		Tris-filled experimental electrode		*N*-Glycylglycine-filled experimental electrode	
T/°C	pH calculated from μS cm^{-1}	E.m.f./mV	Indicated pH*	E.m.f./mV	Indicated pH*
15	9.35	−92.5	8.96	−127.9	9.00
20	9.17	−93.8	8.98	−129.5	9.02
25	9.00	−94.8	9.00	−128.1	9.00
30	8.84	−96.0	9.02	−128.0	9.00
35	8.70	−95.5	9.01	−125.9	8.96

* Values of pH at *T* °C are normalised with respect to the conductivity value at 25 °C.

Discussion

From the theoretical expression for the temperature dependence of the pH cell [equation (3)], the best over-all temperature compensation was expected from a combination of the experimental glass electrodes and a silver - silver chloride reference electrode each containing the same concentration of potassium chloride in the reference electrolyte. Under these conditions, according to equation (3) the terms associated with the inside of the glass electrode should exactly balance those of the sample and the external reference electrode, and thus the cell should have a temperature coefficient of zero. However, with electrodes that contained 3 mol l^{-1} potassium chloride a coefficient of about 0.6 mV $°C^{-1}$ was observed. A closer examination of the individual terms of equation (3) was made to ascertain where this difference could have arisen.

TABLE II

VARIATION IN INDICATED pH WITH TEMPERATURE IN CELLS HAVING AN EXTERNAL SILVER - SILVER CHLORIDE/0.1 mol l^{-1} POTASSIUM CHLORIDE REFERENCE ELECTRODE (pH = 9.16 AT 25 °C)

		Tris-filled experimental electrode		*N*-Glycylglycine-filled experimental electrode	
T/°C	pH calculated from μS cm^{-1}	E.m.f./mV	Indicated pH*	E.m.f./mV	Indicated pH*
15	9.51	−103.4	9.11	−139.0	9.14
20	9.32	−105.2	9.14	−140.1	9.16
25	9.16	−106.3	9.16	−140.0	9.16
30	8.99	−107.1	9.17	−139.4	9.15
35	8.84	−107.7	9.18	−138.8	9.14

* Values of pH at *T* °C are normalised with respect to the conductivity value at 25 °C.

One possible source was a difference in the temperature coefficients of the silver - silver chloride electrode in the alkaline solution inside the glass electrode and in the neutral solution of the external reference electrode. Experiments showed that the coefficients differ by only 0.1 mV $°C^{-1}$ and therefore this difference was not solely responsible for the cell coefficient described above. Another possibility was a difference between the solution coefficient (dpH/dT) inside the glass electrode (where the electrolyte is mainly 3 mol l^{-1} potassium chloride), and in the dilute ammonia solution. Measurements of pH in 3 mol l^{-1} potassium chloride solution adjusted to pH 9 with ammonia were made with a standard pH cell calibrated with buffer solutions at 25 and 35 °C. The value of −0.033 pH $°C^{-1}$ found for dpH/dT corresponded to that found in dilute ammonia solution containing no added potassium chloride. Another possible explanation of the unexpected cell coefficient was that there was a reaction between the internal filling solution of the glass electrode and the glass, which produced a change in the pH of the filling solution. If the known values of the coefficients

are substituted in equation (3) and the observed value of 0.6 mV °C^{-1} used for dE_{cell}/dT, then the equation can be solved for pH_{Int}. From these calculations the internal filling solution would be required to change from pH 9 to 12. This is in contradication to the observed trend in change of potential of the experimental electrode (see Fig. 3) where two electrodes demonstrated a change equivalent to a slow decrease in internal pH while the other had an initial change equivalent to a small increase (+0.3 pH) but was followed by a decrease in pH. On the basis of these results it is concluded that equation (3), derived from a simple equation for the e.m.f. of a glass electrode pH cell, does not fully describe the temperature dependence of the cell. Although these cells had significant self-compensation characteristics (the coefficient being 0.01 pH °C^{-1} compared with −0.033 pH °C^{-1} for ammonia solutions) they were still too temperature dependent for satisfactory application in power station feedwater.

TABLE III

VARIATION IN INDICATED pH WITH TEMPERATURE IN CELLS HAVING AN EXTERNAL SILVER - SILVER CHLORIDE/0.1 mol l^{-1} POTASSIUM CHLORIDE REFERENCE ELECTRODE (pH = 9.26 AT 25 °C)

		Tris-filled experimental electrode		*N*-Glycylglycine-filled experimental electrode	
T/°C	pH calculated from μS cm^{-1}	E.m.f./mV	Indicated pH*	E.m.f./mV	Indicated pH*
15	9.61	−110.4	9.20	−145.8	9.22
20	9.43	−112.4	9.23	−146.7	9.25
25	9.26	−114.0	9.26	−147.4	9.26
30	9.11	−114.7	9.27	−146.7	9.25
35	8.96	−115.1	9.28	−145.6	9.23

* Values of pH at *T* °C are normalised with respect to the conductivity value at 25 °C.

Given the positive over-all temperature coefficient observed for cells expected to be self-compensating, an improvement in the over-all compensation was anticipated using the same experimental electrodes but with an external reference electrode whose coefficient was *ca.* 0.5 mV °C^{-1} greater than the 0.2 mV °C^{-1} of the silver - silver chloride/3 mol l^{-1} potassium chloride electrode at 25 °C. The calomel/3 mol l^{-1} potassium chloride electrode has a coefficient of 0.5–0.6 mV °C^{-1} at 25 °C and therefore the over-all coefficient of a cell incorporating such a calomel electrode was expected to be nearer zero. At an ammonia concentration equivalent to pH 9.17, the experimentally determined coefficients using the calomel external reference electrode were between 0.1 and 0.15 mV °C^{-1}, which is equivalent to a pH change over the 20 °C range of ⩽0.05. The main disadvantage of the calomel electrode is that it responds less rapidly and reversibly to changes in temperature than the silver - silver chloride electrode.[3] For this reason, an alternative form of the silver - silver chloride electrode was investigated. In order to increase the temperature coefficient of a silver - silver chloride external reference electrode to give an over-all cell coefficient of zero with the same experimental glass electrodes, the concentration of potassium chloride in the filling solution of the external reference electrode must be reduced to 0.1 mol l^{-1}. With this solution in the external reference electrode, the over-all cell coefficient in ammonia solutions of pH 9.0–9.26 was <0.1 mV °C^{-1} for the *N*-glycylglycine-filled pH electrode and −0.2 mV °C^{-1} (0.003 pH °C^{-1}) for the Tris-filled pH electrode (Fig. 8). Even in the latter instance the compensation would be acceptable for pH measurements in boiler feedwater.

Self-compensating cells incorporating commercially available glass electrodes are possible in principle, but as such electrodes usually have internal filling solutions of pH 7 an external reference electrode with a high temperature coefficient would be needed. With silver - silver chloride reference electrodes, the filling solution would need to be so dilute to achieve this high temperature coefficient that a stable liquid junction would be difficult to obtain. The calomel electrode would give higher temperature coefficients than the silver - silver chloride electrode at the same potassium chloride concentration and would enable reasonable potassium chloride concentrations to be used if the glass electrode had a filling solution of

pH 7. For example, with EIL Type 1070 glass electrodes, which have an internal pH of 7.0 ± 0.3, a calomel electrode containing potassium chloride at a concentration of 0.1–0.5 mol l^{-1} would be required. As noted previously, these reference electrodes would be less reliable in circumstances where the sample temperature was fluctuating.

The type of pH meter required for a self-compensating electrode system is much simpler than those normally used to measure pH in conditions where the sample temperature is changing. The major simplification is the absence of any feedback circuit from a temperature sensor in the pH cell since the temperature compensation is brought about chemical changes in the electrodes themselves. A meter suitable for use with these electrodes need only have the facility for manual alteration of the slope factor together with a control which offsets the e.m.f. to the value corresponding to the standardisation pH. A limitation of this measuring system might arise if conventional buffer solutions are used for standardisation because the electrode system treats all solutions as feedwater and so applies an incorrect compensation for dpH/dT. This effect can be avoided by buffering at the reference temperature (25 °C). For the most accurate control of pH, the standardisation is best made by means of dilute ammonia solutions of known temperature and conductivity,[4] and if this technique is used for standardisation the limitation would disappear.

In most instances, the pH meters currently installed in power stations are unsuitable for use with self-compensating electrodes without some alteration to their temperature compensation circuits. In particular the isopotential control must be set at zero and the resistance thermometer removed and replaced by a fixed resistor whose value is equal to that of the thermometer at 25 °C.

Conclusions

Experimental glass pH electrodes were constructed with filling solutions formulated to compensate for the temperature-induced changes in both the e.m.f. of the external reference electrode and the pH of alkaline feedwater. When both the internal silver - silver chloride electrode of the glass electrode and the external silver - silver chloride reference electrode contained 3 mol l^{-1} potassium chloride solution in addition to a weak base the cell coefficient in simulated feedwater was about 0.01 pH $°C^{-1}$. This was not only greater than expected from theory but was not considered to be adequate for accurate measurements in feedwater.

Over-all compensation suitable for an accuracy of ±0.05 pH over a 20 °C range in temperature was obtained by selecting an external reference electrode whose temperature coefficient exactly compensated for the combined effects of temperature on the experimental pH electrodes and feedwater. Suitable reference electrodes were shown to be a calomel/3 mol l^{-1} potassium chloride electrode or a silver - silver chloride/0.1 mol l^{-1} potassium chloride electrode. For application in conditions where the temperature can vary rapidly, the latter reference electrode is preferred as its response is more rapid and reversible.

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Kinetic Determination of Nitrite in Waters by Using a Stopped-flow Analyser

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Automatic kinetic methods for the determination of nitrite in waters with a stopped-flow analyser are described. The methods are based on the diazotisation of sulphanilamide, the product being coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye, which is measured at 540 nm. A single-point kinetic procedure uses a delay time of 10 s and one measurement of 0.7 s. A multi-point reaction rate method uses a delay time of 0.8 s and a measurement time of 1.5 s. The methods are fast, sensitive, accurate and precise, and without serious interference. The sample throughput for routine analysis can be up to 360 samples per hour in the range 0.025–2.00 p.p.m. of nitrite-nitrogen.

Keywords: *Nitrite determination*; *water analysis*; *kinetic method*; *stopped-flow analyser*; N-(1-*naphthyl*)*ethylenediamine dihydrochloride*

Nitrite, an intermediate stage in the nitrogen cycle, occurs in water as a result of the biological decomposition of proteinaceous materials. When correlated with the concentration of other nitrogen forms, trace amounts of nitrite can indicate organic pollution. The role of nitrite ion as an important precursor in the formation of nitrosamines, many of which have been shown to be potent carcinogens, has been studied.[1,2] Therefore, sensitive and rapid methods for the routine determination of nitrite are desirable.

Many spectrophotometric methods[3–6] have been reported for the determination of small amounts of nitrite, most of them being based on the Griess reaction, *i.e.*, the reaction of nitrite with a primary aromatic amine to form a diazonium salt, which is then coupled with another aromatic compound to form an azo dye whose absorbance is measured. Many of these methods have been adapted to automatic air-segmented continuous-flow systems,[7–10] *e.g.*, the Technicon AutoAnalyzer system, and flow injection analysers[11–13] for the determination of nitrite and also nitrate after its reduction by various reductor columns.

The stopped-flow technique for the rapid mixing of chemical reagents has had widespread use in basic studies on the rates of rapid chemical reactions and more recently it has been shown that a modified automated stopped-flow system is a very valuable tool for analytical purposes.[14] High precisions are possible because of the very precise delivery of sample and reagent and rapid, quantitative mixing of the solutions. Reaction rate methods used with the stopped-flow system[15] demonstrate the potential advantages over other automatic analysers.

Recently, we have described the construction and analytical applications of a compact automated microprocessor-based stopped-flow analyser.[16] Results are shown here using this system for an automated fast kinetic determination of nitrite in waters using the Shinn[17] reaction, *i.e.*, the formation of a reddish purple azo dye produced by coupling diazotised sulphanilamide (SAA) with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDD). This reagent was chosen because of its high sensitivity and relative freedom from interferences.

The determination of nitrite must be carried out promptly on fresh samples to prevent bacterial conversion of nitrite into nitrate or ammonia. For short-term preservations for 1–2 days, the sample should be frozen at −20 °C or 40 mg of mercury(II) chloride per litre of sample should be added, with storage at 4 °C.

Experimental

Apparatus

The automated, microprocessor-based stopped-flow analyser (SFA) was developed in our laboratory.[16] The entire system is automated using a Rockwell AIM 65 microcomputer for

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control of all operations, data acquisition and reduction, display and printout of results. The sampling - mixing module is used to sample 150-μl volumes of reagent and standards or samples on the turntable with a reproducibility better than 0.1%, to mix them efficiently and transfer them into the observation cell. The photometric system is an easily constructed module with a 1-cm flow cell, automatic shutter, light source, photomultiplier and interference filters. An interference filter with a 10-nm band pass at 540 nm was used in the photometer.

An investigative program was used to evaluate the optimum parameters for the analytical procedure and a routine reaction rate program for the calibration graph and the analysis of the samples.

All measurements were carried out in an air-conditioned laboratory maintained at a nominal temperature of about 25 °C.

Reagents

All chemicals used were of analytical-reagent grade and de-ionised water was used in all experiments.

Nitrite standard solution, 1000 p.p.m. of NO_2^--N. A 4.926-g amount of sodium nitrite (Baker Analysed, 99.7%), oven-dried at 100–105 °C for 2 h, was dissolved in de-aerated, de-ionised water and diluted to 1 l. A pellet of sodium hydroxide and 1 ml of spectroscopic-grade chloroform were added in order to prevent liberation of nitrous acid and bacterial growth. It was kept in a refrigerator and replaced every 2 weeks. Working standard solutions in the range 0.025–2 p.p.m. of NO_2^--N were prepared daily by appropriate dilution.

Reagent solution. The colour reagent was prepared by dissolving 10.0 g of SAA, 0.50 g of NEDD and 30 ml of 85% orthophosphoric acid in water and diluting to 1 l. This solution was stored in an amber-glass bottle in a refrigerator.

Interfering ion solutions. These were prepared so as to contain 1000 p.p.m. of each ion to be tested.

Samples

Waste-water samples from East-Central Illinois, USA, collected and preserved as recommended,[3] were analysed. Before analysis, only samples containing suspended solids were filtered and highly coloured organic matter was removed by adding an aluminium suspension (2 ml per 100 ml), stirring and filtering.

Procedure

The turntable is loaded with the blank (de-ionised water), three to five standards in the range 0.025–2 p.p.m. of NO_2^--N and the samples in 5-ml disposable polystyrene micro-beakers. One channel of the SFA is used to take aliquots of the reagent solution and the other the standards and the samples. The appropriate BASIC and machine language programs are loaded from the cassette recorder into the computer's memory. Then, in execution of the program, the blank is injected into the flow cell, an integration time of 0.7 s is selected and the dark current and 100% transmittance are measured automatically. The program then prompts the operator to select the time parameters. A delay time of 10 s and a measurement time of 0.7 s (one integration) are used for the single-point kinetic procedure, and a delay time of 0.8 s and a measurement time of 1.5 s for the multi-point kinetic procedure. The number of standards and samples to be measured, the number of measurements to be averaged and the number of flushes are then requested by the computer. The program then sequences through each standard, flushing the system between each standard (four flushes are needed) and prompting the operator for its concentration. After the standards have been measured, the microcomputer calculates the linear least-squares regression line and prints its slope, intercept, correlation coefficient and the standard error of the estimate. Samples are automatically measured, after which the concentration of the nitrite in the sample is calculated and printed.

A dedicated program can also be used with all the information, time parameters and concentration of standards contained in the software. Once the operator has input the number of samples, the analysis will be completed automatically.

Results and Discussion

Optimisation of the Procedure

The behaviour of the reaction using various concentrations of the colour reagent constituents was investigated, using an investigative program that prints out 50 absorbance values during a pre-selected time period. The reaction curve was plotted on the paper printer and the reaction rate was calculated at various parts of the curve together with linearity factors. From these data the optimum reagent concentration, the delay time and measurement time were evaluated.

The effect of the nitrosated species (SAA) concentration on the reaction is shown in Fig. 1. Both the reaction rate and the final absorbance values increase with increasing SAA concentration. An SAA concentration of 1% was chosen as the optimum.

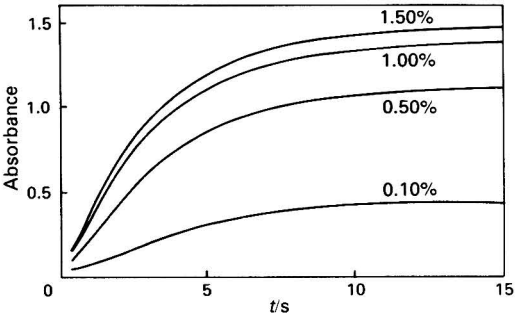

Fig. 1. Effect of sulphanilamide concentration on the reaction. NO_2^--N, 1 p.p.m.; NEDD, 0.05%; and H_3PO_4, 10% *V*/*V*.

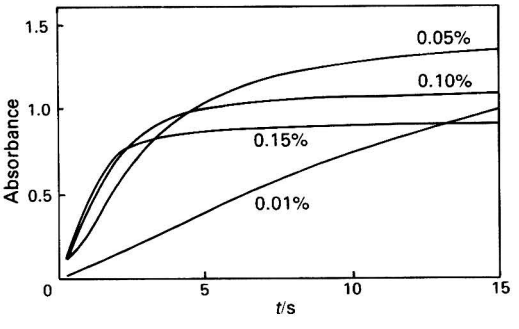

Fig. 2. Effect of *N*-(1-naphthyl)ethylenediamine dihydrochloride concentration on the reaction. NO_2^--N, 1 p.p.m.; sulphanilamide, 1%; and H_3PO_4, 10% *V*/*V*.

The effect of NEDD concentration was also investigated and the reaction curves for 15 s are shown in Fig. 2. As illustrated, the initial reaction rate increases with increase in NEDD concentration but the final absorbance value decreases. Because of the simultaneous addition of nitrosated species (SAA) and the coupling reagent (NEDD) there is a competition for nitrite ion. At low concentrations of NEDD the conversion of diazonium ion into the coloured pigment is slow and incomplete. Conversely, at high concentrations appreciable amounts of nitrite react with NEDD and pigment formation is reduced. The resultant effect is that there is a point of maximum pigment production with varying NEDD concentration.[18] Under our experimental conditions a 0.05% concentration was found to be the optimum. This allows a 30-fold excess of SAA.

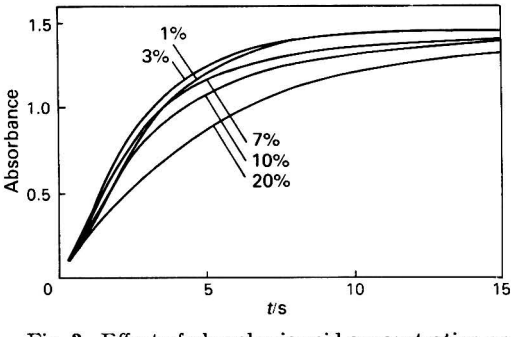

Fig. 3. Effect of phosphoric acid concentration on the reaction. NO_2^--N, 1 p.p.m.; sulphanilamide, 1%; and NEDD, 0.05%.

The effect of orthophosphoric acid concentration was also investigated in the range 1–20%. As shown in Fig. 3, there is only a small dependence of the reaction rate on the acid concentration. Small increases in the reaction rate and the final absorbance value are initially observed as the acid concentration increases but at higher concentration they both decrease. The optimum concentration was 3%, which provides a pH of 1.43 in the mixed solution. This effect is a balance between too little nitrous acid (and then of the nitrosating species, N_2O_3) at higher pH values, and protonation of the NED (which causes the decrease in the reaction rate) and/or acid decomposition of the pigment at lower pH values.[18]

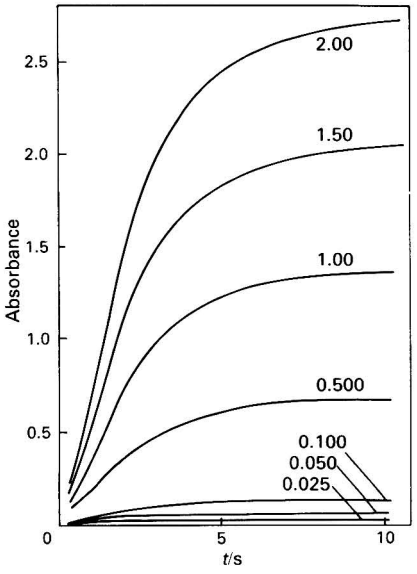

Fig. 4. Reaction curves for various nitrite concentrations used for the calibration graphs. Sulphanilamide, 1%; NEDD, 0.05%; and H_3PO_4, 3% *V*/*V*.

Using the optimum concentrations of reagents found, the reaction curves shown in Fig. 4 were obtained for various nitrite concentrations. The reaction is almost completed after 10 s so both multi-point reaction rate and single-point kinetic procedures can be used. For the multi-point reaction rate procedure a delay time of 0.8 s and a measurement time of 1.5 s gave the best results. The calibration graph obtained using this procedure was linear

TABLE I

NITRITE CALIBRATION GRAPHS

Single-point kinetic method*			Multi-point reaction rate method†		
NO_2^--N, p.p.m.	Absorbance	RSD, ‡%	NO_2^--N, p.p.m.	Rate/mA s^{-1}	RSD, ‡%
0.025	0.035	4.0	0.100	26.8	3.4
0.050	0.071	3.2	0.500	184.6	1.0
0.100	0.138	0.5	1.00	376.6	0.2
0.500	0.708	0.2	1.50	563.9	0.2
1.00	1.413	0.2	2.00	747.8	0.1
1.50	2.112	0.1			
Slope = 1.410			Slope = 379.3		
Intercept = 0.0002			Intercept = −6.9		
Correlation coefficient (r) = 0.999995			Correlation coefficient (r) = 0.99990		

* Delay time = 10 s, measurement time = 0.7 s.
† Delay time = 0.8 s, measurement time = 1.5 s.
‡ Relative standard deviation (n = 5).

from 0.100 to 2.00 p.p.m. of NO_2^--N. For the single-point procedure a delay time of 10 s and one measurement of 0.7 s gave a calibration graph that was linear from 0.025 to 1.50 p.p.m. of NO_2^--N. Using a delay time of 5 s a calibration graph linear up to 2.00 p.p.m. can be obtained. Table I shows typical results for calibration graphs obtained using both procedures.

Accuracy and Precision of the Method

The accuracy of the proposed method was examined by measuring the nitrite concentration of waste water samples before and after a standard addition. Recovery was calculated after addition of 100 μl of 10 p.p.m. NO_2^--N standard to 10 ml of each assayed sample. Because of the low concentrations of the samples the more sensitive single-point procedure was used. The results obtained (Table II) show an average recovery of 98.6%. The precisions of both procedures are given in Table I. Similar results were obtained for the analysis of samples.

TABLE II

RECOVERY DATA FOR THE DETERMINATION OF NITRITE IN WASTE WATERS (SINGLE-POINT PROCEDURE)

	Nitrite content, p.p.b.* NO_2^-–N			
		After standard addition		
Sample No.	Before standard addition	Expected	Determined	Recovery, %
1	156	253	252	99
2	38	137	138	101
3	129	227	225	99
4	232	329	325	99
5	185	282	274	97
6	37	136	133	98
7	32	131	128	98
8	175	272	272	100
9	55	153	150	98
10	25	124	120	97
			Average:	98.6

* Parts per 10^9.

Interference Study

The effect of various potential interferents was investigated. The results of these experiments are shown in Table III. Major interference is caused by reducing or oxidising ions. The low results caused by copper(II) ion because of its catalysis of the decomposition of the diazonium salt in methods with long reaction times[3] are avoided with the proposed method. The serious sulphite interference that appears during the analysis of nitrite in beet sugar factory juices[19] with the Shinn method is also almost eliminated in this method. The sulphide interference can be eliminated by adding excess of cadmium ions and filtering. Addition of 200 p.p.m. of Cd^{2+} to a waste water sample containing 1 p.p.m. of NO_2^--N and 50 p.p.m. of sulphide eliminated the error.

Sample Throughput

Four flushes are used to change from one solution to another (flush volume 150 μl for sample and reagent and instrument cycle time about 1.5 s) and, assuming one measurement per sample, 1 s for the turntable position increment and 0.5 s for the computer calculation and printing time, the analysis rates for the aforementioned methods are 200 samples per hour for the single-point procedure (270 samples per hour if a 5-s delay time is used for the range 0.1–2.0 p.p.m.) and 360 samples per hour for the multi-point reaction-rate procedure.

Conclusion

The proposed kinetic determinations of nitrite are sensitive, accurate and precise. The useful analytical range can be extended by using a shorter delay time. The ions commonly

TABLE III

EFFECT OF DIVERSE IONS

Nitrite concentration: 1 p.p.m.

Ion investigated	Ratio of ion to nitrite (*m*/*m*)	Error,* %	
		Single-point procedure	Multi-point procedure
Acetate	200	n	n
Bromide	200	−23.5	−27.3
Bromide	20	−4.9	−4.7
Bromide	2	n	n
Carbonate	200	n	n
Chloride	200	n	n
Cyanide	200	n	n
Dichromate	200	+32.3	+5.3
Dichromate	20	+4.7	n
Dichromate	2	n	n
Fluoride	200	n	n
Iodate	200	n	n
Iodide	200	−41.7	−46.7
Iodide	20	−10.9	−9.1
Iodide	2	n	n
Nitrate	200	n	n
Oxalate	200	n	n
Perchlorate	200	n	n
Phenol	200	n	n
Sulphate	200	n	n
Sulphide	200	−78.9	−78.4
Sulphide	20	−16.3	−5.9
Sulphide	2	−2.2	n
Sulphite	200	n	+3.4
Aluminium	200	n	n
Ammonium (NH_4Cl)	200	−3.1	−4.2
Cadmium	200	n	n
Cobalt ($CoCl_3$)	200	n	−5.0
Copper	200	n	n
Iron(II)	200	n	n
Iron(III)	200	−30.3	−33.1
Iron(III)	20	−4.0	−7.0
Iron(III)	2	n	n
Lead	200	n	n
Magnesium ($MgCl_2$)	200	n	−6.9
Mercury(II)	200	+2.4	+3.2
Tin(II)	200	−98.8	−99.7
Tin(II)	20	−12.5	−13.4
Tin(II)	2	n	n

* n = negligible (less than 2%).

present in waters do not interfere and the interferences from copper(II) and sulphite ions are eliminated. Only a small amount of sample is needed, the sample throughput is high (up to 360 samples per hour) and the analyst effort is small. Compared with the standard Technicon AutoAnalyzer method it has the same sensitivity, a superior rate of analysis, better precision and accuracy and less interference from copper(II) ions. This relatively simple automated stopped-flow analyser can be used for a wide variety of constituents in environmental samples.

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Simplified Procedure for the Determination of Chemical Oxygen Demand Using Silver Nitrate to Suppress Chloride Interference

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A sealed-flask procedure for the determination of the chemical oxygen demand of wastewaters is described. Samples are digested at 150 °C in a glass-stoppered flask using springs to retain the stopper. The use of sealed-flask conditions offers two advantages over reflux conditions: simplified apparatus and experimental procedure and improved suppression of chloride interference. The proposed procedure is similar to the standard procedure in accuracy and reproducibility of results.

Keywords: Chemical oxygen demand; sealed flask; wastewaters

The determination of chemical oxygen demand (COD) in wastewaters, using silver nitrate to suppress chloride interference, has been recently described.[1] The procedure employed the standard reflux conditions commonly used in Water Authority laboratories. The sealed-tube COD procedure[2] offers several advantages over the reflux procedure, notably a saving in bench space and cost of equipment. In spite of these advantages and general comparability with reflux procedures[2,3] in results, the sealed-tube procedure has not been adopted as a standard method. It is understood that the Department of the Environment's Standing Committee of Analysts decided not to endorse a sealed-tube COD procedure because of safety considerations. Thermal degradation of the tube caps and liners is also found to occur. This creates difficulties in unscrewing caps and occasionally causes contamination of samples, particularly if an oven is used to heat the tubes.

The apparatus described below overcomes the problems cited above and offers several advantages over the silver nitrate reflux procedure. It is not necessary to use a weaker dichromate solution for COD levels of 200 mg l^{-1} or less. The suppression of chloride interference is improved and lower concentrations of silver nitrate are used.

Experimental

Apparatus

Flasks

Thick-walled Pyrex conical flasks, of 100-ml capacity, with ground-glass stoppers (24/20 socket) were used. Hooks on the stoppers and flask shoulders allow the stoppers to be retained by a pair of wire springs. Clean new apparatus before use by shaking the stoppered flasks containing a sulphuric acid - dichromate mixture and then heating in the oven at 150 °C for 2 h. After heating, rinse the flasks and stoppers with low COD water. Dry the flasks and stoppers before use.

Oven

A fan convection oven, capable of heating the flasks at 150 ± 2 °C, was used. Use a mercury-in-glass thermometer to check any variation in temperature within the working space.

Reagents

Use analytical-reagent grade reagents, except where stated otherwise. 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 ± 0.5 g of silver nitrate in water and dilute to the mark in a 100-ml calibrated flask. Store this solution in amber glassware and use each batch within 2 weeks.

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

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

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

Procedure

Caution—Oxides of sulphur and other toxic gases are emitted during addition of sulphuric acid. Add sulphuric acid in a fume cupboard. Examine flasks carefully before use and reject any showing cracks.

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

Dispense potassium dichromate (5 ± 0.05 ml of 0.125 N) into a conical flask and dilute to approximately 50 ml with water. Carefully add sulphuric acid (15 ± 0.25 ml), mix and cool to ambient temperature. Add Ferroin indicator (2 drops) and titrate with the ammonium iron(II) sulphate solution. The end-point colour change is 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 4000 *mg* l^{-1} *of chloride, irrespective of COD level*

Do not allow sample or reagents to wet the neck of the flask during addition or mixing (NOTE 1).

Pipette 10 ± 0.1 ml of sample having a COD of less than 400 mg l^{-1} (or an appropriate volume of stronger sample diluted to 10 ml) into a flask. Add silver nitrate (1.0 ± 0.1 ml of 25% m/V solution) (NOTE 2). Mix and allow to stand for between 5 and 15 min. Add 5 ± 0.05 ml of 0.125 N potassium dichromate solution, then mix and carefully add 15 ± 0.25 ml of sulphuric acid. Mix carefully, stopper the flasks (immediately securing the stoppers with springs) and place in the oven which has previously been heated to 150 ± 2 °C. Leave for 2 h (±5 min). Remove the flasks from the oven and allow to cool to room temperature (NOTE 3). Remove the stoppers, add 25 ± 1 ml of water and titrate the residual dichromate as described above. Carry out duplicate blanks using 10 ± 0.1 ml of water in place of the sample (NOTE 4).

Calculation

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

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

NOTES—

1. Crystallisation of dissolved salts has been found to cause the stoppers to stick.
2. Use silver nitrate (1.0 ± 0.1 ml of 15% m/V solution) for samples having an expected chloride to COD ratio of 2 or less.
3. Do not titrate the solution while still warm. In warm solution, iron(II) is oxidised by oxides of nitrogen to iron(III), causing reversion of the end-point (see under Discussion).
4. 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 used in standardisation.

Performance Characteristics

The precision of the proposed procedure and bias with respect to the standard procedure[4] were estimated by the method described earlier.[1] Within-batch relative standard deviation in sewage analysis ranged from 2.2% at levels of 60 mg l^{-1} of COD to 0.8% at 380 mg l^{-1} of COD (4 degrees of freedom). Total relative standard deviation in analysis of a 300 mg l^{-1}

COD potassium hydrogen phthalate solution was 1.0% (8 degrees of freedom). These results did not differ significantly ($p = 0.05$) from data obtained using the standard procedure or the silver nitrate reflux COD procedure.[1]

TABLE I

ANALYSES OF WASTEWATERS BY STANDARD AND PROPOSED PROCEDURES (1 ml OF 25% m/V SILVER NITRATE SOLUTION)

Sample	Chloride/mg l^{-1}	COD*/mg l^{-1} Standard procedure†	COD*/mg l^{-1} Proposed procedure	Mean bias of proposed procedure, %
Settled sewage	<100	398	382	−4.0
	<100	329	313	−4.9
	<100	490	484	−1.2
	2900	361	366	+1.4
Sewage effluent	<100	68	63	−7.4
	<100	38	39	+2.6
	2800	143	145	+1.4
Settled sewage‡	4000	339	327	−3.5
Sewage effluent‡	4000	91	92	+1.1
	1000	74	78	+5.4

* Results are the means of two determinations.

† Determinations were made using mercury(II) sulphate (1 ml of 20% m/V solution in 10% V/V sulphuric acid) at chloride levels of 500 mg l^{-1} or less. At higher chloride levels a mass of mercury(II) sulphate 40-times greater than the mass of chloride in the aliquot was used.

‡ Samples spiked with chloride.

Analyses of saline sewage and chloride-spiked sewage are presented in Table I. Wastewaters having a chloride to COD ratio of 2 or less were analysed by the proposed procedure but using silver nitrate (1 ml of 15% m/V solution). These results are given in Table II. Standard solutions of potassium hydrogen phthalate, spiked with chloride, were also analysed. These results are compared with those given for the standard procedure in Table III.

TABLE II

ANALYSES OF LOW CHLORIDE WASTEWATERS BY STANDARD AND PROPOSED PROCEDURES (1 ml OF 15% m/V SILVER NITRATE SOLUTION)

Sample	Chloride/mg l^{-1}	COD*/mg l^{-1} Standard procedure†	COD*/mg l^{-1} Proposed procedure	Mean bias of proposed procedure, %
Sewage effluent	70	75	78	+4.0
	90	68	63	−7.4
	100	54	56	+4.0
Settled sewage	112	300	307	+2.3
Potassium hydrogen phthalate standard (200 mg l^{-1} COD)	0	202	199	−1.5
Trade effluents—				
Preserves and pickles	71	6790	6460	−4.9
Abattoir	94	305	302	−1.0
Laundry	139	591	587	−0.7
Laundrette	52	363	374	+3.0
Frozen foods	48	768	735	−4.3
Dairy	79	287	283	−1.4

* Results are the means of two determinations.

† Determinations were made using mercury(II) sulphate (1 ml of 20% m/V solution in 10% V/V sulphuric acid) at chloride levels of 500 mg l^{-1} or less. At higher chloride levels a mass of mercury(II) sulphate 40-times greater than the mass of chloride in the aliquot was used.

TABLE III

ANALYSES OF CHLORIDE-SPIKED POTASSIUM HYDROGEN PHTHALATE SOLUTIONS BY STANDARD AND PROPOSED PROCEDURES (1 ml OF 25% *m*/*V* SILVER NITRATE SOLUTION)

Expected COD/mg l^{-1}	Observed COD at given chloride level*†/mg l^{-1} Cl				
	0	500	1000	1500	2000
0	—	15 (10)	19 (23)	21 (41)	32 (59)
100	102	112 (103)	115 (105)	121 (109)	128 (121)
200	199	203 (206)	209 (214)	221 (215)	217 (228)
300	295	300 (308)	297 (308)	311 (311)	316 (318)
400	391	401 (404)	403 (404)	405 (412)	407 (419)

* Results are the means of two determinations.
† Values in parentheses are means of results obtained using the standard procedure.[4]

Discussion

As noted above, it is important to cool the diluted digest to room temperature before titration. Above 30 °C, the indicator reverts from red to blue within a few minutes. This is attributed to oxidation of iron(II) by nitrogen oxides, formed by a brown-ring type reaction of the nitrate ion. It is assumed that these oxides are expelled under reflux conditions. The reaction does not affect the end-point, as it does not occur in the presence of chromate.[5] It is possible, however, that the reversion might cause an inexperienced analyst, titrating a hot solution, to overshoot the end-point.

Table I shows that the sealed-flask COD procedure extends the range of comparability with the standard procedure up to 4000 mg l^{-1} of chloride. None of the observed differences are significant ($p = 0.05$). Table II demonstrates the possibility of using a lower concentration of silver nitrate solution when the chloride to COD ratio is 2 or less. The observed differences are again not significant ($p = 0.05$). Table III confirms the general comparability of the proposed and standard procedures over a wide range of chloride concentrations. The proposed procedure may be considered more economical and versatile than reflux procedures using mercury(II) sulphate. Current costs for silver salts in the proposed procedure at 100 and 4000 mg l^{-1} of chloride are 10 p and 17 p per test, respectively. Equivalent costs for silver and mercury salts in a reflux procedure are 12 p and 28 p, respectively. The versatility of the proposed procedure arises from the use of a single reagent to suppress chloride interferences. When using mercury(II) sulphate it is necessary to add this salt in a 40-fold excess over the mass of chloride ion in the sample aliquot. Thus the mass of mercury(II) sulphate required depends on the chloride concentration in the sample and the volume taken for analysis.

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Extraction of Iron(III) from Aqueous Solution with Mixtures of Aliquat 336 and Ferron in Chloroform

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Ferron is efficiently extracted by Aliquat 336 in chloroform and the ion pair ($R_4N^+HL^-$) forms dark green complexes with iron(III), in the organic phase. On the basis of solvent extraction data for iron, as well as absorbance measurements, it is assumed that two iron complexes, one with ferron and one with the alkylammonium cation, can be present in the organic phase [$FeL_5^{3-}(R_4N^+)_5$ and $FeL_2{-}R_4N^+$]. The latter complex can be formed at high iron concentration in aqueous solution, where part of the ferron is also stripped from the organic phase and forms coloured complexes with iron in the aqueous phase. The absorbance of solutions of Aliquat 336 and ferron in chloroform is very low, in the visible region. The absorbance of iron complexes with ferron and Aliquat 336 is higher than the absorbance of iron complexes with ferron in aqueous solutions and three absorbance maxima were found: 370 nm ($\epsilon = 7.7 \times 10^3$ l mol^{-1} cm^{-1}), 465 nm ($\epsilon = 6.86 \times 10^3$ l mol^{-1} cm^{-1}) and 610 nm ($\epsilon = 5.95 \times 10^3$ l mol^{-1} cm^{-1}). The absorbances at 465 and 610 nm can be utilised in the extraction - spectrophotometric determination of iron. Beer's law is obeyed in the concentration range 0.1–10 mg l^{-1} of iron in aqueous solution. The method is selective for the determination of iron and only copper interferes when present in amounts that exceed the iron concentration. The proposed method was used to determine iron in two samples of natural waters, and the results of 0.48 and 5.7 mg l^{-1} are in good agreement with results obtained by atomic-absorption spectrometry and a spectrophotometric method using thiocyanate.

Keywords: *Alkylammonium salt extractants*; *Aliquat* 336 *extraction*; *ferron extraction*; *iron determination in water*; *spectrophotometry*

The extraction of anionic complexes of metals with sulphonated chelating reagents by solutions of amines with high relative molecular mass or of quaternary alkylammonium salts has been utilised in the extraction - spectrophotometric determination of metals, because extraction often increases the sensitivity and selectivity of conventional methods used for the determination of metals with such reagents in aqueous solutions.[1,2] Ziegler *et al.*[3,4] improved the colorimetric method for determining iron(III), based on the extraction of the coloured complex of the metal with ferron (7-iodoquinolin-8-ol-5-sulphonic acid) by isoamyl alcohol, by adding tributylamine acetate to the aqueous solution. Although this method is more selective, in comparison with conventional spectrophotometric methods for the determination of iron with ferron in aqueous solution, the relatively high extraction of ferron by higher alcohols[5] and the solubility of tributylamine salts in water make it difficult to determine the extraction mechanism of iron and to investigate the complex formation in the organic phase. Our earlier experiments on chromatographic methods (paper extraction chromatography and moist-paper techniques)[6] as well as static extraction[7,8] indicated that ferron and other sulphonated metal chelating reagents can be effectively extracted with solutions of ternary amines, their salts or quaternary alkylammonium salts in organic diluents and that Aliquat 336 (methyltricaprylylammonium chloride, where caprylyl is an alkyl C_8–C_{10}) appeared to be the most effective extracting agent. Therefore, the extraction of ferron by Aliquat 336 and the extraction of iron(III) by the ion pair produced by Aliquat 336 and ferron was investigated in order to utilise the extraction in the extraction - spectrophotometric determination of iron(III).

Experimental

All experiments were performed at ambient temperature (23 $\pm$ 2 °C).

Apparatus

A Zeiss VSU 2P (Jena, GDR) spectrophotometer with 1-cm silica cells was used for the absorbance measurements. The pH measurements were made with a Mera-Elwro N 517 (Wroclaw, Poland) direct reading pH meter with a glass - calomel electrode assembly. A Pye Unicam SP 192 single-beam atomic-absorption spectrometer was used for iron determination in the aqueous phase after extraction.

Reagents

Aliquat 336 (General Mills Chemicals, Inc., Kankakee, IL, USA) containing 93.3% *m/m* quaternary alkylammonium chloride was purified, to ensure that no iron was present, by shaking a 0.01 M solution in chloroform with equal volumes of doubly distilled water five times and then filtering the organic phase through a cellulose filter. All other reagents were of analytical-reagent grade.

Procedure

Extraction of ferron was performed by shaking equal volumes (5 or 10 ml) of aqueous ferron solution with a chloroform solution of Aliquat 336 of the same concentration for 10 min in a cylindrical separating funnel. The organic phase was then filtered through a cellulose filter to remove any remaining aqueous solution and the aqueous phase was centrifuged. Ferron was determined in the aqueous phase spectrophotometrically at 286 nm (or at 435 nm, for higher reagent concentrations), using appropriate calibration graphs.

Extractions of iron(III) by Aliquat 336 plus ferron solutions of equimolar concentrations in chloroform were performed in a similar manner. Aqueous iron(III) solutions were prepared from Titrisol-grade standard iron(III) chloride (E. Merck, Darmstadt, FRG) solution adjusted to an appropriate pH value by adding dilute hydrochloric acid, acetate buffer or ammonia solutions. Iron(III) was determined by atomic-absorption spectrometry in the aqueous phase after extraction, and using calibration graphs. Spectrophotometric measurements were performed usually at 465 or 610 nm against a blank solution of Aliquat 336 plus ferron in chloroform.

Results and Discussion

Earlier preliminary experiments indicated that Aliquat 336 is a stronger extracting agent for ferron than trioctylamine or trioctylammonium chloride; therefore, this quaternary alkylammonium salt was used as a liquid anion exchanger in all the extraction experiments

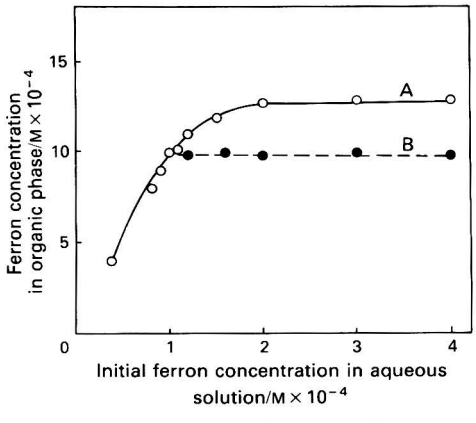
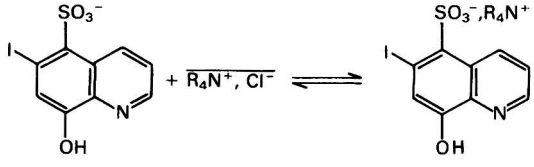


Fig. 1. Concentration of ferron in the organic phase *vs.* initial ferron concentration in aqueous solution. (A) Extractant 10^{-3} M Aliquat 336 in chloroform. (B) Ferron extraction by Aliquat 336 after subtraction of the results of ferron extraction by chloroform.

performed in this work. Chloroform was chosen as a diluent owing to the better phase separation obtained than with benzene solutions of Aliquat 336 when the tendency to form an emulsion was observed. Pure chloroform partially extracts ferron, with extraction coefficients of 0.1–0.12.[8]

The extraction results for ferron presented in Fig. 1 suggest that only the sulphonic group of the reagent is bound in an ion pair with the alkylammonium cation (taking into account the partial extraction of an excess of ferron by chloroform) and thus ferron should maintain the complexing ability for metal ions. It was found that the extraction process is very rapid, a 2-min shaking time was sufficient to reach equilibrium, and the organic phase after extraction was colourless, in contrast with the intense yellow colour of aqueous ferron solutions. Ferron is almost quantitatively extracted by Aliquat 336 and extraction coefficients of higher than 400 were obtained when a low excess (10%) of liquid anion exchanger, relative to ferron, was used for the extraction. As was found previously,[8] chloride ions pass almost quantitatively into the aqueous phase after extraction of ferron by Aliquat 336, and the extraction of the reagent can be described by an anion exchange reaction:



(1)

It is thus presumed that the ion pair composed of alkylammonium cation and ferron should form complexes with iron(III) in the organic phase.

It was found that the iron extraction process with Aliquat 336 and ferron in chloroform is not as rapid (10 min shaking time was necessary to reach equilibrium) as the extraction of ferron by Aliquat 336 (Fig. 2). The organic phase after extraction of iron is dark green and the colour intensity does not change even after 7-d storage of the organic phase in a closed vessel. It is well known that ferron forms complexes with iron in aqueous solutions very rapidly and for the maximum stable intensity of colour, a large excess of reagent is required.[9] Owing to the strong extraction of ferron by Aliquat 336, the possibility of iron complexation in the aqueous phase after contact with the organic solution of the reagent seems to be of low probability. Thus, the low rate of iron extraction can be explained by complex formation at the interface (additionally, the stepwise complexation of iron cannot be excluded) and then the complex is transported into the organic phase; each of these processes can limit the rate of the extraction. However, this supposition needs additional investigations concerning the extraction kinetics.

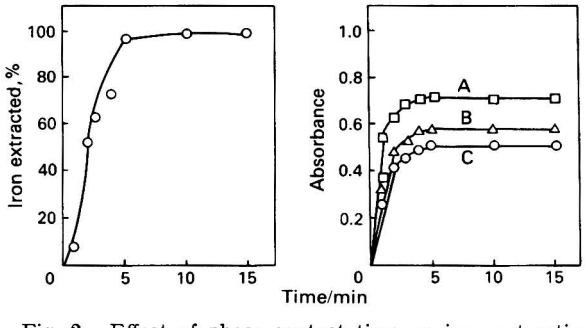


Fig. 2. Effect of phase contact time on iron extraction and on absorbance of the organic phase. Concentration of iron(III) in initial aqueous solution (pH 2.5), 5 mg l^{-1}. Extractant, 10^{-3} M Aliquat 336 + ferron in chloroform. Absorbance was measured at (A) 375 nm, (B) 465 nm and (C) 610 nm.

The results for iron(III) extraction by 10^{-3} M Aliquat 336 and ferron solutions in chloroform are presented in Fig. 3 in the form of an extraction isotherm. Although the graph becomes significantly less steep at iron concentrations in the organic phase equal to approximately 3.3×10^{-4} M, an excess of iron is extracted relative to the amount corresponding to the complex $FeL_3^{3-}(R_4N^+)_3$ at higher initial metal concentrations in the aqueous solution.

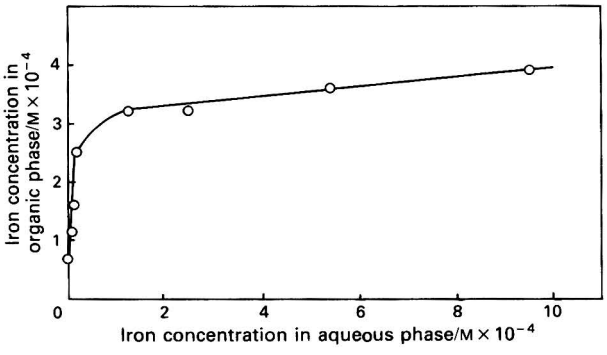


Fig. 3. Extraction isotherm for iron(III). Extractant, 10^{-3} M Aliquat 336 + ferron in chloroform; pH of aqueous phase, 2.4–2.6.

Because it is known that ferron in aqueous solutions with iron(III) forms the complexes FeL, FeL_2 and FeL_3[10] and that only anionic complexes can be extracted by liquid anion exchangers, the extraction of iron(III) by an ion pair composed of ferron and alkylammonium cation can be described by the two following reactions:

(*i*) The complex anion FeL_3^{3-} is predominant in the organic phase if an excess of extractant is used for iron extraction:

$$Fe^{3+} + \overline{3R_4N^+HL^-} \rightleftharpoons \overline{FeL_3^{3-}(R_4N^+)_3} + 3H^+ \quad .. \quad .. \quad (2)$$

(*ii*) Besides the complex FeL_3^{3-}, the anionic complex FeL_2^- can be present in the organic phase if an excess of iron is present in the aqueous solution, which also contains chloride ions:

$$Fe^{3+} + Cl^- + \overline{2R_4N^+HL^-} \rightleftharpoons \overline{FeL_2^-R_4N^+} + \overline{R_4N^+Cl^-} + 2H^+ \quad .. \quad (3)$$

It should be noted that after extraction the aqueous phase was bright green (the characteristic colour for iron complexes with ferron in aqueous solutions) when a large excess of iron relative to the extractant was present in the aqueous solution, which suggests the possibility of the following additional reactions:

$$Fe^{3+} + Cl^- + \overline{R_4N^+HL^-} \rightleftharpoons \overline{R_4N^+Cl^-} + FeL^+ + H^+ \quad .. \quad .. \quad (4)$$

$$Fe^{3+} + 2Cl^- + \overline{2R_4N^+HL^-} \rightleftharpoons \overline{2R_4N^+Cl^-} + FeL_2^- + 2H^+ \quad .. \quad .. \quad (5)$$

$$Fe^{3+} + 3Cl^- + \overline{3R_4N^+HL^-} \rightleftharpoons \overline{3R_4N^+Cl^-} + FeL_3^{3-} + 3H^+ \quad .. \quad (6)$$

The possibility of such reactions seems to be indicated by the reaction of the ion pair composed of alkylammonium cation and strongly hydrophyllic tiron (1,2-dihydroxybenzene-3,5-disulphonic acid disodium salt) with aqueous iron(III) solution. In this instance the blue complex of iron with tiron was formed only in the aqueous phase.[11]

Iron(III) is almost quantitatively extracted when a sufficient excess of extractant is used at pH values higher than 1.6 (see Fig. 4) and the slope of the straight line of the relationship between the logarithm of extraction coefficient and pH indicates that in this instance the complex $Fe(R_4N^+L^-)_3$ is predominant in the organic phase.

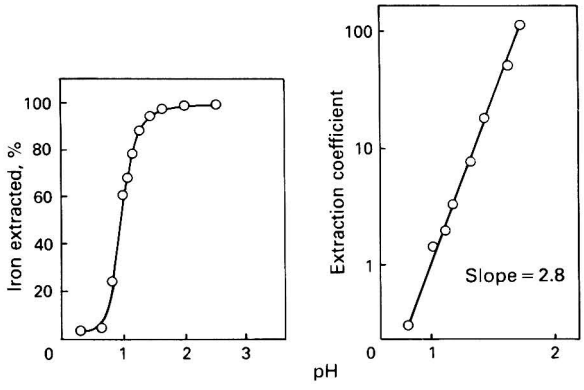


Fig. 4. Percentage of iron extracted and extraction coefficients for iron as a function of pH of the aqueous phase. Initial iron(III) concentration in aqueous solution, 5 mg l^{-1}. Extractant, 10^{-3} M Aliquat 336 + ferron in chloroform.

Absorption Spectra

Absorption spectra for aqueous ferron solutions as well as for organic solutions containing equimolar concentrations of ferron and Aliquat 336 in chloroform are shown in Fig. 5. The absorption maximum at 440 nm (characteristic of aqueous ferron solutions) disappears after extraction of the reagent by Aliquat 336 in chloroform and an absorbance of lower than 0.002 was found at wavelengths higher than 440 nm for freshly prepared 4×10^{-3} M Aliquat 336 plus ferron solutions in chloroform. It should be noted that the absorbance of organic ferron solutions in the wavelength range 400–600 nm was not changed after storage for 48 h.

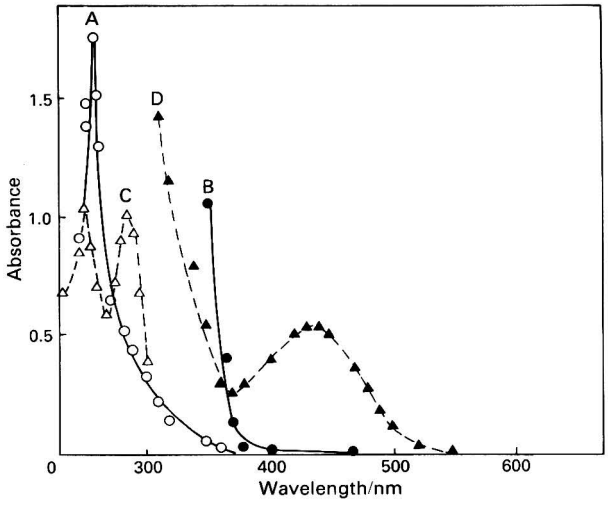


Fig. 5. Absorption spectra of aqueous ferron solutions (concentrations: C, 4×10^{-5} M; and D, 4×10^{-4} M) and Aliquat 336 + ferron solutions in chloroform (ferron concentrations: A, 4×10^{-5} M; and B, 4×10^{-3} M). Blanks: water or Aliquat 336 in chloroform (4×10^{-5} or 4×10^{-3} M).

Absorption spectra for complexes of iron with ferron in aqueous solutions and for organic phase containing iron complexes with ferron and Aliquat 336 are shown in Fig. 6; the results were obtained at comparable experimental conditions (iron concentration 5 mg l^{-1}, pH of initial aqueous iron solutions 2.6, ferron concentration in aqueous solution or in organic phase 4×10^{-3} M and Aliquat concentration 4×10^{-3} M). The lines for iron complexes with ferron in water (A) and in organic extracts (B and C) are parallel in the wavelength

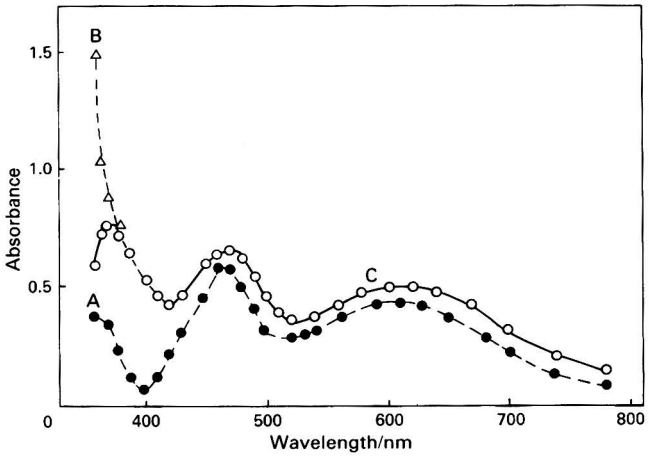


Fig. 6. Absorption spectra of iron (5 mg l^{-1}) complexes with ferron. A, Aqueous solution (ferron concentration 4×10^{-3} M) measured against aqueous reagent blank: B, organic phase after extraction of iron complex with ferron with 4×10^{-3} M Aliquat 336 in chloroform, measured against Aliquat 336 in chloroform; and C, organic phase after extraction of iron with 4×10^{-3} M Aliquat 336 + ferron in chloroform measured against reagent blank. pH of aqueous iron solutions, 2.6.

range 450–780 nm and show maxima at 465 and 610 nm; however, the absorption coefficients for iron corresponding to these absorption maxima are higher for the iron complexes with ferron plus Aliquat 336. The strong absorbance at 465 nm, which is not suitable for iron determination using ferron in aqueous solutions, can be utilised for the extraction - spectrophotometric determination of iron, owing to the very weak absorbance of ferron in a chloroform solution of Aliquat 336. The absorption coefficients for iron extracted by Aliquat 336 plus ferron in chloroform, corresponding to the three absorption maxima (370, 465 and 610 nm) decrease with the wavelength. However, at 370 nm a relatively strong absorbance for the extractant (Aliquat 336 plus ferron) was observed, see Fig. 5. One experiment was also performed for the iron complex with ferron extracted from aqueous solution containing an excess of the ferron with 4×10^{-3} M Aliquat 336 in chloroform; the strong absorbance between 360 and 370 nm (line B in Fig. 6) indicates that besides the complex anion FeL_3^{3-} the free anion HL^- is also extracted by the quaternary alkylammonium salt.

Effect of pH on Absorbance

The graphs of absorbance *versus* pH shown in Fig. 7 indicate that the pH values for the development of maximum absorbance are between 1.6 and 2.9 and that the absorbance strongly decreases at pH values higher than 3, contrary to the absorbance *versus* pH graphs reported by Ziegler *et al.*[4] for iron complexes with ferron and tributylamine salt extracted with isoamyl alcohol (they found the constant, maximum absorbance to occur between pH 2.5 and 5).

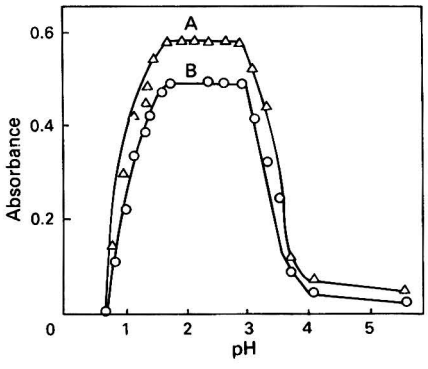


Fig. 7. Influence of pH on absorbance of iron (5 mg l^{-1}) complex with Aliquat 336 + ferron measured at (A) 465 nm and (B) 610 nm. Extractant, 10^{-3} M Aliquat 336 + ferron in chloroform.

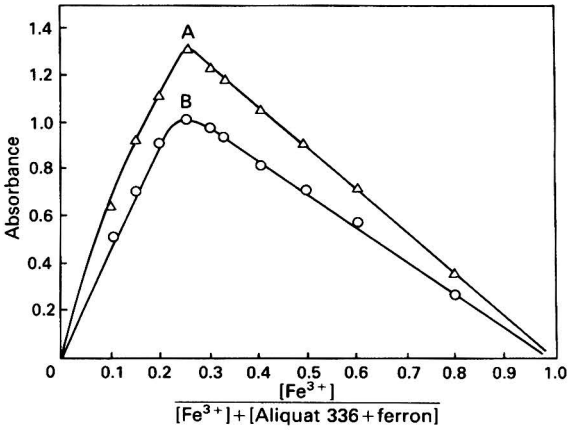


Fig. 8. Stoicheiometry of iron complex with Aliquat 336 + ferron in the organic phase (continuous variation method). Measurements at (A) 465 nm and (B) 610 nm.

Composition of Complexes in the Organic Phase

The results obtained with the method of continuous variation (Fig. 8) suggest the formation of the complex $FeL_3^{3-}(R_4N^+)_3$ in the organic phase, in agreement with extraction data for iron (see Figs. 3 and 4) and with reaction 2. However, the shape of the graph for the extraction - spectrophotometric titration (Fig. 9) suggests the formation of two iron complexes with Aliquat 336 plus ferron, and to obtain a constant and maximum value of absorbance a large excess of extractant (about 15 times greater than the iron concentration in the initial aqueous solution) is required. It is therefore supposed that the extraction process can be described by reactions of stepwise formation of iron complexes with ferron and Aliquat 336 in the organic phase:

$$Fe^{3+} + \overline{2R_4N^+HL^-} + Cl^- \rightleftharpoons \overline{FeL_2^-R_4N^+} + \overline{R_4N^+Cl^-} + 2H^+ \quad .. \quad (7)$$

$$\overline{FeL_2^-R_4N^+} + \overline{R_4N^+HL^-} + \overline{R_4N^+Cl^-} \rightleftharpoons \overline{FeL_3^{3-}(R_4N^+)_3} + H^+ + Cl^- \quad .. \quad (8)$$

The green colour of the aqueous phase [whose intensity increases with the iron concentration in the initial aqueous solution (Fig. 10)], with an excess of iron relative to the extractant

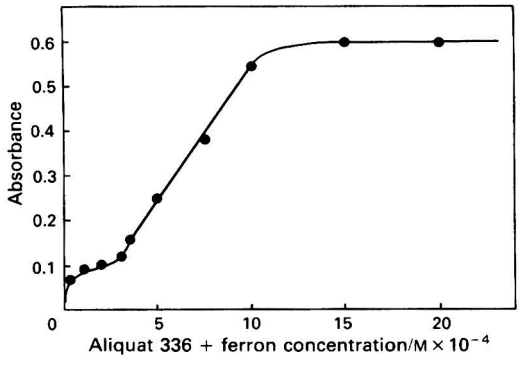


Fig. 9. Absorbance of the organic phase measured at 610 nm *vs.* Aliquat 336 + ferron concentration. Iron concentration in initial aqueous solution (pH 2.4), 10^{-4} M.

concentration, was also observed in these series of experiments, which confirms the possibility of the additional reactions (4), (5) and (6). Therefore, in further experiments the concentrated (4×10^{-3} M) solutions of Aliquat 336 plus ferron in chloroform were used for iron extraction in order to ensure constant and maximum absorbance values.

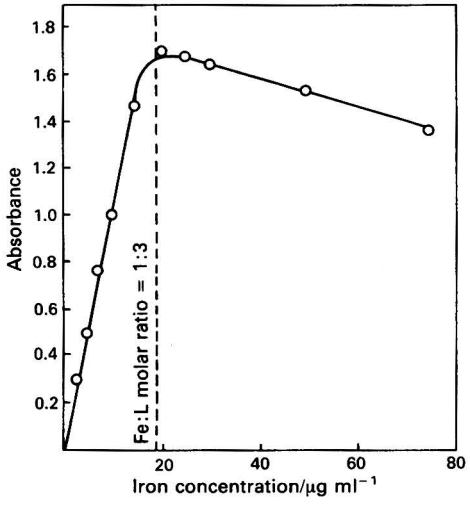


Fig. 10. Absorbance of the organic phase measured at 610 nm as a function of iron concentration in aqueous solution (pH 2.4). Extractant, 10^{-3} M Aliquat 336 + ferron in chloroform.

Interferences

The effect of some common cations and anions on the absorbance of the organic phase is given in Table I.

The tolerance limits given in Table I could probably be increased, especially for metal ions forming complexes with ferron, if more concentrated solutions of Aliquat 336 plus ferron were used for iron extraction. Negative errors in the determination of iron occurred in the presence of amounts of magnesium, sulphate, phosphate and citrate ions larger than the limiting

TABLE I

EFFECT OF SOME CATIONS AND ANIONS ON THE DETERMINATION OF IRON

Iron concentration, 5 ng l^{-1}; pH, 2.3–2.8; extractant, 4×10^{-3} M Aliquat 336 plus ferron in chloroform.

Foreign ion	Form added	Tolerance limit*/ mg l^{-1}	Foreign ion	Form added	Tolerance limit*/ mg l^{-1}
Al^{3+}	$Al(NO_3)_3$	1000	Zn^{2+}	$ZnCl_2$	50
Ca^{2+}	$CaCl_2$	2000	Cl^-	NaCl	5000
Cd^{2+}	$CdCl_2$	50	F^-	NH_4F	190
Co^{2+}	$CoCl_2$	2000	HPO_4^{2-}	Na_2HPO_4	10
Cu^{2+}	$CuSO_4$	5	NO_3^-	$NaNO_3$	5000
K^+	KNO_3	2000	SO_4^{2-}	Na_2SO_4	80
Mg^{2+}	$MgCl_2$	200	CH_3COO^-	CH_3COOH	5000
Mn^{2+}	$MnCl_2$	1000	Citrate	Trisodium citrate	40
Na^+	NaCl	5000	Tartrate	Disodium tartrate	1500
Ni^{2+}	$NiCl_2$	2000			

* Corresponding to a 2% change in absorbance measured at 610 nm.

amounts indicated in Table I, whereas larger amounts of copper, cadmium and zinc enhanced absorbance. It is interesting that contrary to the method for the determination of iron with ferron in aqueous solution and contrary to the method recommended by Ziegler *et al.*,[4] large amounts of cobalt, nickel and aluminium do not interfere in this determination of iron even without the addition of masking agents and only copper interferes if present in amounts minimally exceeding the iron concentration.

Extraction - Spectrophotometric Determination of Iron in Natural Waters

The proposed method was used for the determination of iron in two samples of natural waters and the results were compared with those obtained with conventional methods, *i.e.*, atomic-absorption spectrometry and a thiocyanate spectrophotometric method (Table II).

TABLE II

DETERMINATION OF IRON IN WATER

The values are the means of five determinations on separate samples.

	Iron found, mg l^{-1}		
	Spectrophotometric methods		
Sample	Proposed method	Thiocyanate method	AAS
Drinking water (hardness at 20 °C)	0.484 ± 0.007*	0.45 ± 0.03	0.46 ± 0.11
Mineral water, "Zuber"†	5.67 ± 0.09	5.55 ± 0.18	5.65 ± 0.10

* Iron was extracted from a 50-ml sample into 10 ml of 4×10^{-3} M Aliquat 336 plus ferron in chloroform.

† Declared composition: K^+ 343, Na^+ 7240, Li^+ 12.8, Ca^{2+} 133, Mg^{2+} 307, Fe^{2+} 6.9, Cl^- 1180, Br^- 2.42, SO_4^{2-} 77, HCO_3^- 19700 and $H_2SiO_3^-$ 37.1 mg l^{-1}.

It should be noted that the precision of the proposed method for the determination of iron in drinking water (containing low amounts of iron) was markedly higher in comparison with the thiocyanate or the atomic-absorption spectrometric method owing to the distinctly higher values of absorbance obtained when extraction was performed at a phase volume ratio (V_{aq}:V_{org}) equal to 5:1.

Recommended Procedure

Prepare a 4×10^{-3} M solution of Aliquat 336 in chloroform and shake it five times with equal volumes of doubly distilled water in a separating funnel. Shake the purified organic solution with an equal volume of a 4×10^{-3} M aqueous solution of ferron and after separating the phases filter the lower organic phase through a quantitative cellulose filter. The solution of extractant so prepared is stable for at least 1 week.

Prepare a stock solution of iron(III) chloride containing 10 mg of iron in 1 l. Transfer 5–35 ml volumes of the stock solution into a series of 50-ml calibrated flasks and add 5 ml of acetate buffer solution (pH 2.2–2.8) to each. Make the solutions up to the mark with doubly distilled water. Check the pH with a pH meter.

Treat the solution to be analysed with a few drops of 3% V/V hydrogen peroxide solution [to oxidise Fe(II) to Fe(III)], warm to the boiling-point and cool to the ambient temperature. Adjust an appropriate volume to a pH value of between 1 and 4, transfer into a 50-ml calibrated flask, add 5 ml of acetate buffer solution and make the solution up to the mark with doubly distilled water. Check the pH with a pH meter.

Transfer equal volumes (5 or 10 ml) of aqueous iron(III) solution and extractant solution into a small separating funnel, shake for 10 min and filter the lower organic phase through a quantitative cellulose filter. Measure the absorbance at 465 nm or at 610 nm (the latter is preferable if the analysed sample contains copper, zinc or cadmium) against the reagent blank. Determine the iron concentration with the aid of a calibration graph.

If the iron concentration in the analysed solution is lower than 1 mg l^{-1}, a somewhat modified procedure is recommended. A 45-ml aliquot of solution [iron(II) should be previously oxidised with hydrogen peroxide or ammonium persulphate] is transferred into

a 100-ml separating funnel, 5 ml of acetate buffer solution and 5 or 10 ml of extractant solution are added and the extraction is performed in the manner described above. Determine the iron concentration with the aid of calibration graphs obtained by the extraction of iron from aqueous working standard iron solutions (in the concentration range 0.1–1 mg l^{-1} of iron) under analogous experimental conditions, *i.e.*, at volume ratios of aqueous phase to organic phase equal to 5:1 or 10:1.

Conclusions

The proposed extraction - spectrophotometric method seems to be useful for the determination of iron owing to its simplicity, higher sensitivity (the molar absorption coefficients calculated from 30 independent measurements and corresponding to the three absorption maxima at 370, 465 and 610 nm have the following values: 7.7×10^3, 6.86×10^3 and 5.59×10^3 l mol^{-1} cm^{-1}) in comparison with the method using ferron in aqueous solutions. It should be noted that the phases separate well after extraction and the absorbance of the free extractant (Aliquat 336 plus ferron in chloroform) is very low in the visible region. It was found that Beer's law is obeyed in the concentration range 0.1–10 mg l^{-1} of iron and the precision of the method is sufficiently high.

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Water Analysis by Inductively Coupled Plasma Atomic-emission Spectrometry after a Rapid Pre-concentration

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A method is described in which large batches of 10-ml water samples are pre-concentrated by evaporation and rapidly analysed for 16 elements at average river concentrations, by simultaneous inductively coupled plasma spectrometry. The effects of background interference, and its on-peak correction, on realistic detection limits of 30 elements were studied on solutions with high levels of calcium and magnesium and were found to place minor constraints on the determination of some elements. Recoveries of 32 elements during pre-concentration were examined and 24 were found to be quantitative. The applicability of this method to the analysis of fresh water is considered in comparison with average river water concentrations and the EEC 1980 Council Directive.

Keywords: *Water analysis*; *pre-concentration*; *background interference*; *inductively coupled plasma*; *atomic-emission spectrometry*

We present a rapid method for the pre-concentration of fresh waters by evaporation, for subsequent simultaneous multi-element analysis by inductively coupled plasma atomic-emission spectrometry (ICP). Ten-millilitre samples of water are evaporated in test-tubes in batches of up to 250 in an aluminium block bath held at 99 °C until about 1 ml remains. The remainder is analysed by ICP, variations in the final volume being compensated for by an internal standard of lanthanum added before the evaporation. The recovery of a wide range of elements at low levels is essentially quantitative. A batch of samples can be concentrated within a working day because of the small sample and the high working temperature. The method has the attractive feature that the sample has contact only with the one vessel from the time of collection to the final analysis.

The ICP can provide a direct and very rapid analysis of surficial fresh waters for the following ten elements: sodium, potassium, magnesium, calcium, strontium, barium, iron, boron, sulphur and silicon. Other elements, including many toxic heavy metals that are subject to legislative control, have average abundances that are too close to their ICP detection limits for satisfactory determination. Pre-concentration by a factor of ten or more brings a wider group of elements into a useful range, and methods which have been reported in connection with the ICP are solvent extraction,[1] ion exchange[2–5] and coprecipitation.[6,7] These methods, while possessing the virtue of selectivity against the major ionic constituents of waters and therefore minimising interference, are undoubtedly time consuming and exclude analyte species which are not in a labile ionic form [*e.g.*, manganese(II) and chromium(III)].

Pre-concentration by evaporation is generally regarded as an inferior method because of several factors, *viz.*, possible loss of volatile species, possible contamination through the long exposure of the sample to the atmosphere in an open vessel, the lengthy procedure and the non-selectivity. However, we have shown that the scope of the ICP in the analysis of fresh waters can be considerably expanded by a ten-fold pre-concentration by evaporation.

Experimental

Spectrometer

The ICP spectrometer used was an Applied Research Laboratories ARL 34000C with a 1-m vacuum spectrometer fitted with 36 lines, of wavelengths given in Table I. Data processing and instrument control were carried out by a dedicated PDP 11/04 computer. The ICP source was run at a forward power of 1250 W with a viewing height above the load coil

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of 14 mm. The nebuliser used was of the glass concentric type (Meinhard TR–30–3A) taking up 0.9 ml min^{-1} of sample with a flow of humidified argon at 1.0 l min^{-1}. The liquid flow-rate was restricted by the use of a flexible polyethylene capillary uptake tube 350 mm long and of 0.5 mm i.d. No peristaltic pump was used. The tip of the nebuliser was washed between samples with 0.5 ml of de-ionised water containing 1% *V*/*V* Photo-flo to prevent a build-up of solids at the nebuliser tip and to reduce nebuliser noise. The spray chamber was a double-pass Scott type and the torch was a Fassel type with argon flow-rates of 12 l min^{-1} of coolant and 0.4 l min^{-1} of plasma gas.

Heating Block

The aluminium heating block, made by Scienco-Western Ltd. (Cambridge), was controlled thermostatically to ± 0.5 °C. For the fastest evaporation rate, the holes in the block should fit the sample tubes closely and expose less than 10 mm of the top of the tube to minimise reflux action. The block, with 250 holes, fitted into a typical fume chamber. To minimise corrosion of the block and sample contamination, the top of the block was covered with fresh aluminium kitchen foil for each batch of samples.

Tubes

Graduated 15-ml centrifuge tubes with conical bottoms, made of borosilicate glass, were used. They were pre-cleaned by prolonged refluxing of nitric acid in the heating block, followed by rinsing with pure water.

Reagents

Hydrochloric acid. Spectosol, 36% *m*/*m*, diluted as appropriate with distilled, de-ionised water.

Lanthanum chloride solution. Spectrosol, 100 g l^{-1}, diluted to 10 mg l^{-1} with 1 M hydrochloric acid.

Calcium carbonate and magnesium carbonate. Specpure grade dissolved in hydrochloric acid.

Trace element solutions. Spectrosol, 1 g l^{-1}, standard solutions for atomic-absorption spectroscopy.

Tip wash additive. Photo-Flo 600 (Kodak, Catalogue No. 3256773).

Procedure

Water samples should be filtered and acid-stabilised soon after collection. Pipette 10.0 ml of each water sample or test solution into a centrifuge tube containing 1.00 ml of the lanthanum solution. Place the tubes in the hot block pre-heated to 99 °C and remove them when the volume has decreased to between 0.5 and 1.0 ml (about 8 h). Make the volume up to 1.0 ± 0.1 ml with pure water and mix. Analyse the resulting solution by ICP.

Calibration of the ICP

The trace elements were calibrated in units of micrograms per litre using a 1 M hydrochloric acid blank and a 10^4 μg l^{-1} multi-element standard. For reasons of chemical stability, elements in anionic form were segregated into a separate standard solution, as were the elements supplied as sulphates. Calcium and magnesium calibrations were prepared with six solutions each, from blank to 2500 mg l^{-1} for calcium and to 300 mg l^{-1} for magnesium, and fitted using the splined polynomial provided. The analytical procedure required a 20-s pre-flush followed by 3×5-s integration using a total volume of 0.6 ml of solution. Blank readings and sensitivities were checked after every ten solutions.

Calibration and correction calculations were carried out via a specially written program. On-peak interference data for the effect of magnesium and calcium on trace elements were recorded at the same time as the solutions were run for major element calibrations. Only those values of apparent analyte concentration that were above the respective detection limit were used in the calculation of the interference coefficients. Linear or polynomial regression was used as appropriate to relate interference to major element concentration. Results were corrected for interference effects before ratioing to the internal standard.

Results and Discussion

Detection Limits

In order to investigate the performance and likely sources of error in the proposed method, synthetic trial solutions resembling fresh waters were concentrated and analysed by the procedure outlined above. Realistic estimates of the effect on detection limits of increasing background corrections were obtained by concentrating and analysing ten replicate samples of de-ionised water and ten of the synthetic fresh waters containing high levels of calcium (200 mg l^{-1}) and magnesium (30 mg l^{-1}) only. The resulting detection limits are given in columns 4 and 5 of Table I.

The detection limits in column 3 are those estimated from ten contiguous sequential integrations of a single blank solution with no pre-concentration. Despite the more rigorous measure of detection limit for the pre-concentration method (*i.e.*, using replicate samples and not only replicate measurements), an approximately ten-fold improvement in detection limit was evident for most elements.

TABLE I

APPLICABILITY OF ICP TO WATER ANALYSIS

Column 3: detection limit (2σ) from 10 readings of blank solution by direct nebulisation.
Column 4: detection limit (2σ) from 10 replicate blank preparations by pre-concentration.
Column 5: detection limit (2σ) from 10 replicate samples with 200 mg l^{-1} of calcium and 30 mg l^{-1} of magnesium prepared by pre-concentration.
All values of detection limits are approximate and can vary by 100% by random fluctuations.
Column 6: the background interference from 200 mg l^{-1} of calcium and 30 mg l^{-1} of magnesium expressed in $\mu g\ l^{-1}$ of analyte; 0 signifies no measureable interference.
Column 7: * elements giving low recoveries on spikes at 50 and 500 $\mu g\ l^{-1}$; † elements giving low recoveries on spikes at 50 $\mu g\ l^{-1}$ only.
Column 8: average of median river concentrations from Wedepohl[8] and *Rose *et al.*[9] Question marks signify uncertain or unknown values.
Columns 9 and 10: EEC guide levels (GL) and maximum admissible concentrations (MAC) of 1980.[10]
Column 11: elements for which determination at average river levels is *suitable or †marginal.
Column 12: elements for which determination below EEC levels is *suitable or †marginal.

		Detection limits/$\mu g\ l^{-1}$					Concentrations/$\mu g\ l^{-1}$			Applicability	
			By pre-concentration					EEC			
Element	Wavelength/nm (order)	Direct	Soft	Hard	Interference/$\mu g\ l^{-1}$	Recovery	Average river water	GL	MAC	Average river water	EEC
1	2	3	4	5	6	7	8	9	10	11	12
Ag	328.1 × 2	2	0.3	0.5	1.3	*	0.3		10		
Al	308.2 × 2	50	15	6	0		400	50	200	*	*
As	193.8 × 2	30	2	1	0	†	2		50		†
Ba	455.4 × 1	4	0.4	0.3	0		10	100		*	*
Be	313.0 × 2	0.1	0.02	0.03	0.04	*	0.4?			†	
Bi	223.1 × 2	30	2	4	7.7		0.005*				
Ca	317.9 × 2	60	5	—	—		1.5×10^4	1×10^5		*	*
Co	228.6 × 3	5	0.6	0.5	0.87		0.2				
Cd	226.5 × 3	2	0.2	0.3	0.52		0.03*		5		*
Cr	267.7 × 2	3	0.2	0.8	3.0		1		50	†	*
Cu	324.8 × 2	2	0.2	0.3	2.0		7	100		*	*
Fe	259.9 × 2	40	8	5	0		100*	50	200	*	*
Hg	194.2 × 1	4	0.6	1.5	3.4		0.07		1		
K	766.4 × 1	100	9	9	0		2300	1×10^4	1.2×10^4	*	*
Li	670.8 × 1	1	0.1	0.1	0		3			*	
Mg	279.0 × 2	100	10	—	—		4100	3×10^4	5×10^4	*	*
Mn	257.6 × 2	10	1	2	0	†	7	20	50	*	*
Mo	281.6 × 2	5	0.6	4	31	†	1			†	
Na	589.0 × 1	50	30	20	0		6300	2×10^4	1.8×10^5	*	*
Ni	231.6 × 2	8	0.8	0.9	2.5		1.5*		50	*	*
P	178.3 × 2	20	3	2	8.6		20	175	2182	*	*
Pb	220.3 × 2	30	4	4	12.3		3		50		*
S	180.7 × 3	70	8	57	1500		3.7×10^3	8.3×10^3	8.3×10^4	*	*
Sb	206.8 × 2	80	5	6	8.3	*	1		10		
Se	196.1 × 2	80	8	12	45		0.2		10		
Sn	190.0 × 2	7	0.6	0.4	8.1		?				
Sr	407.8 × 1	2	0.2	0.1	1.6		50			*	
Te	214.3 × 2	30	2	5	5.8		?				
Ti	337.3 × 2	60	5	7	0	*	3				
V	311.1 × 2	2	0.2	0.1	2.8		0.9			*	
Zn	202.5 × 3	7	0.8	1.4	4.8		20	100		*	*
Zr	349.6 × 2	3	0.2	0.9	4.6	*	?				

The effect of high levels of calcium and magnesium on the practical detection limit can be seen by a comparison of columns 4 and 5. The detection limits in column 5 are calculated in a way that includes both inaccuracy and imprecision in the background correction. To the normal two standard deviations of noise over ten samples has been added the absolute value of the correction bias. For example, sulphur has a detection limit of 70 $\mu g\ l^{-1}$ by direct nebulisation, which is improved to 8 $\mu g\ l^{-1}$ by the pre-concentration method, in the absence of interfering elements. The interference, mainly from calcium, produced 1505 $\mu g\ l^{-1}$ of apparent sulphur with a standard deviation of 20 $\mu g\ l^{-1}$. The total calculated interference correction is 1488 $\mu g\ l^{-1}$, leaving an uncorrected residual of $+17\ \mu g\ l^{-1}$ of sulphur. The detection limit is therefore recorded as $(2 \times 20) + | +17 | = 57\ \mu g\ l^{-1}$. A deterioration of the detection limit due to this cause is evident in a number of elements, notably sulphur and molybdenum. Although the uncertainties in the detection limits make rigorous interpretation difficult, there appears generally to be an increase in the detection limit equal to approximately 10% of the total background interference.

The calcium and magnesium levels used in this study are approximately ten times higher than those in average river waters. The interference effects in river water analysis will therefore be proportionally reduced.

Precision

The precision measured using the ten replicate samples expressed as twice the coefficient of variation and averaged over 20 analytes was 8.0% at the 50 $\mu g\ l^{-1}$ level and 7.0% at the 500 $\mu g\ l^{-1}$ level. The presence of 200 $mg\ l^{-1}$ of calcium and 30 $mg\ l^{-1}$ of magnesium increased these figures to 8.8 and 7.8%, respectively, after interference correction.

Loss of Elements During Sample Pre-concentration

In order to study the recovery of 21 elements during sample pre-concentration, two levels of trace element spikes were added to ten-fold replicates of pure water. At the 500 $\mu g\ l^{-1}$ level only silver* (33% low) and antimony (17% low) showed recoveries that are significantly low at 95% confidence limits. At the 50 $\mu g\ l^{-1}$ level manganese (17% low), arsenic (16% low) and molybdenum (8% low) also showed values significantly below the spike added. Three other elements studied separately showed low results: titanium (up to 70% low), zirconium (up to 16% low) and beryllium up to (16% low). For these elements with high ionic potentials the low results probably represent loss of analyte by chemisorption or hydrolysis. For other elements low returns could be due to statistical inaccuracy and not losses (*e.g.*, manganese).

Recovery of the principal ionic constituents (Al^{3+}, Ca^{2+}, Fe^{3+}, K^+, Mg^{2+}, Na^+, PO_4^{3-} and SO_4^{2-}) was found to be quantitative in separate experiments with the pre-concentration of natural water samples. No significant loss of hydrochloric acid occurred during the evaporation.

Acidity Variation

The maximum possible variation in the acid concentration in the final solution was $\pm 10\%$, and the effect of this both on the blank response and on sensitivity levels was examined for all of the analytes. By monitoring the effects in the range 0.5–1.5 M the error in the 0.9–1.1 M region was established to be less than 1% relative error for all elements. Hence the variation in acid concentration cannot affect detection limits or sensitivity.

Applications of the Method

Excluding elements that are partially lost during pre-concentration and those present at too low a level, 16 elements can be determined by the proposed method in average river water, and are shown in column 11 of Table I. Of the other elements, molybdenum in soft water has a detection limit below the average river water concentration but interference (mainly from magnesium) and apparent small losses in preparation (8%) make the determination impracticable by this method. Chromium at average levels is similarly too close to the detection limits for reliable determination.

* The solubility of silver in 1 M hydrochloric acid is greater than 1000 $\mu g\ l^{-1}$, which is considerably higher than the level suggested by elementary solubility product calculations.

The pre-concentration method enables waters to be screened for 17 elements (column 12) for levels above the EEC 1980 Guide Levels (GL) and Maximum Admissible Concentrations (MAC).[10] Arsenic has a detection limit well below the EEC MAC and correction for losses might be possible, but other methods, such as that of Thompson *et al.*,[6] would be preferable for arsenic and also give values for antimony, bismuth, selenium and tellurium that also cannot be determined at the appropriate level by the proposed method.

Conclusions

The method described provides a rapid technique for measuring large numbers of small-bulk water samples for a wider range of major and trace elements than is possible by direct nebulisation into the ICP. Mercury (as $HgCl_2$) and selenium (as H_2SeO_3) were recovered quantitatively from spikes during pre-concentration. In natural waters, however, they may be present in part as volatile methylated compounds and therefore more prone to loss unless additional pre-treatment of the sample is employed.

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Rapid Flow Analysis with Inductively Coupled Plasma Atomic-emission Spectroscopy Using a Micro-injection Technique

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An introduction system for liquid micro-samples in inductively coupled plasma atomic-emission spectroscopy is described that allows the injection of 5–500-μl volumes into a rapidly flowing carrier reagent stream leading to the nebuliser. The effect on analyte signal was studied as a function of flow-rate, injection volume and sample concentration. It is shown that the carrier flow-rate determines the response time, sensitivity, precision and sample carry-over in the nebuliser. By the use of relatively rapid flow-rates of up to 7.5 ml min^{-1}, fast injection of 10-μl samples is achieved at an injection rate of 240 h^{-1} with a relative standard deviation of 1.5% for a single-element analogue readout. Digital readout is used for multi-element determinations with similar or better precision. Detection limits of the order of 0.1 mg l^{-1} are obtained for 10-μl injections, limited by the volume injected, with a proportionate decrease in detection limit for increasing volumes.

Keywords: *Rapid injection*; *inductively coupled plasma*; *serum electrolyte*

Aqueous or organic liquid nebulisation techniques in common use for inductively coupled plasma atomic-emission spectroscopy (ICPAES) require several millilitres of solution and uptake rates are of the order of 1 ml min^{-1}. When an adequate sample is available such usage rates present no special problems. However, in many environmental, clinical, forensic, toxicological, solid-state and other investigational areas, only micrograms to milligrams of the solid or microlitres of liquid are available. Techniques for the representative direct introduction of solid micro-samples into an ICP are being developed,[1] thereby avoiding more time-consuming sample processing. There are still several problems to be solved with the solid sample approach, including difficult representative sampling, matrix and volatilisation effects, pulse effects and plating out and memory effects in the transfer line to the plasma. However, solution processing that can be adapted to micro-samples has many advantages, including sub-sample homogeneity, possibilities of separation or concentration of the analyte, the use of internal standards or standard additions, the use of dilution techniques and various chemical modifications, including addition of matrix elements.

Developments for the introduction of liquid micro-samples for ICPAES commencing in 1969 have recently been reviewed.[2] Approaches include vaporisation of dried samples from graphite or metal filaments, introduction via micro-capillaries, cups or funnels, direct introduction into the plasma region after drying in a graphite cup and automatic flow methods.

Automatic flow methods have been developed using flow injection analysis (FIA)[3–6] and rapid flow analysis (RFA).[7] These approaches have the potential of very rapid multi-element analysis for microlitre samples. The FIA method described by Ito *et al.*[3] used volumes of between 0.5 and 50 μl and the determination of boron, copper and zinc in NBS orchard leaves was reported. Greenfield[4] investigated the effects of controlled dispersion and delineated concentration - time profiles using FIA. Calcium was determined in Portland cement and the relative standard deviation (RSD) was 3%. Our preliminary report on the application of RFA[5] has been extended and is now detailed. The novel aspect of our approach concerns the method of injecting samples into a peristaltic pump in order to damp out effects of pressure surges on the ICP pneumatic nebuliser system and simultaneous computer data processing.

In previous electrochemical studies using RFA,[7] electrode response times were shown to improve with increasing flow-rate. In this study with ICPAES, the transient signal produced after each sample injection is also shown to be dependent on flow-rate, either by

monitoring with a recorder or by computer (multi-element mode). This approach of rapid flow into the nebuliser is shown to be potentially useful for fast multi-element analysis using ICPAES.

Experimental

Reagents and Standards

All reagents were of analytical-reagent grade from BDH Chemicals Ltd. Metal stock and standard solutions were prepared from Specpure metal samples (Matthey Garrett Pty. Ltd.) according to reference 8, yielding multi-element solutions in dilute (6% *V/V*) aqua regia.

Instrumentation

The ICPAES instrumentation and operating conditions are listed in Table I.

TABLE I

ICP INSTRUMENTATION AND OPERATING CONDITIONS

Plasma power supply	Labtest, Model 2000, 0.4–2 kW, 27.12 MHz, crystal controlled
Nebuliser	Labtest GMK Nebuliser[9] (modified Babington type). Nominal sample flow-rate 0.2–2.0 ml min^{-1}
Spectrometer	Labtest V25 vacuum spectrometer, 1-m Paschen - Runge, reciprocal linear dispersion 0.46 nm mm^{-1}, 21 channels
Detector electronics	Simultaneous A/D conversion of all 21 phototube signals and data processing performed in Labtest CRT 100 A multi-processor minicomputer system. Any one channel monitored via Labtest auto ranging amplifier to chart recorder (Houston Omniscribe, Type EB 5117–X5R)
Plasma parameters	Coolant argon, 12.0 l min^{-1} Sample argon, 0.9 l min^{-1} Forward power, 1400 W Reflected power, <5 W

A conventional septum-injection valve (1 mm i.d.) from a normal liquid chromatograph was attached to the peristaltic pump, as shown in Figs. 1 and 2. The septum-injector was fitted to a Swagelock T-junction so that the sample could be injected into two separate reagents to give a single carrier stream, or the T-junction could be removed and the injector outlet attached directly to the pump to allow injection of the sample into a single carrier stream. SGE (Melbourne, Australia) micro-syringes (5–500 μl) were used throughout.

The computer was programmed to print integrated emission counts for each element at intervals of 3 s after sample injection for a total of ten discrete readings. A digital time response profile for each of the 21 elements could therefore be compiled. The computer was also programmed to calculate calibration plots, detection limits and precision for each element after sampling a range of concentrations (1–100 mg l^{-1}) and a blank. Calibration could be performed using peak height or area, with and without internal standardisation.

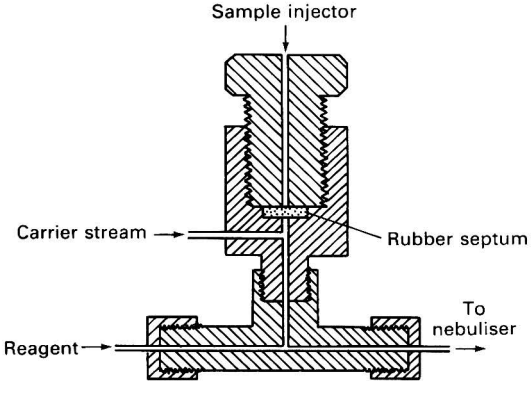

Fig. 1. Injector for rapid flow analysis - ICPAES.

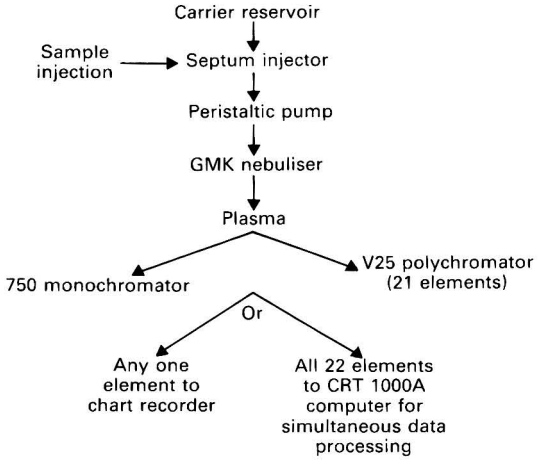

Fig. 2. Schematic diagram of rapid-flow analysis with ICPAES showing the sequencing of sample and carrier stream prior to the peristaltic pump.

The spectrometer was also fitted with an analogue emission signal readout, coupled to a chart recorder (Houston OmniScribe, Type EB 5117–X5R) for single-element analogue monitoring. For this study, zirconium was selected as a test metal for monitoring the response of the nebuliser plasma system to change in aspiration flow-rate. A zirconium solution (5 mg l^{-1}) was injected into the nebuliser with the fixed polychromator wavelength set at 343.8 nm, and the zirconium emission intensity was monitored continuously on the chart recorder or the digital printer was used to print out the integrated emission signal at 3-s intervals.

Flow Analysis Procedure

The pump used was a Gilson peristaltic pump with ten rollers, three of which were in constant contact with the pump tubing at any given time to reduce pulsing. Liquid samples of volumes ranging from 10 to 500 μl were injected into the carrier stream, which was pumped continuously into the nebuliser. The flow diagram is shown in Fig. 2. The pump tubing used in the peristaltic pump was of 1.02 mm i.d., but was replaced with tubing of 1.42 and 2.05 mm i.d. when higher flow-rates were required. The connecting tube to the pump was of 1.02 mm i.d. The flow-rate of the carrier stream was varied by use of the speed controller on the pump in conjunction with the above tubes. Flow-rates were varied by this method in the range 2.0–7.5 ml min^{-1}.

For a single metal determination in liquid samples, the samples were injected into the carrier stream and the resulting emission pulse was recorded on the chart recorder. The carrier stream used for all measurements was triply distilled water. Multi-element data, except for the recorder monitored element, was also printed out via the computer for the same sample slug injected into the nebuliser.

For these studies, we investigated the effect of varying the injection volume on the emission intensity for a single metal sample, and then studied the effect of flow-rate on the emission response signal. At the maximum sampling rate, the precision, carryover, sensitivity and detection limits were determined for a single element injected in a small sample volume. The same parameters were then studied for multi-element analysis of the same injected sample.

Results

Effect of Sample Injection Volume

Fig. 3 shows the steady-state emission signal from aspiration of zirconium (5 mg l^{-1}) continuously into the nebuliser at a fixed flow-rate of 2.0 ml min^{-1}. The effect of injecting

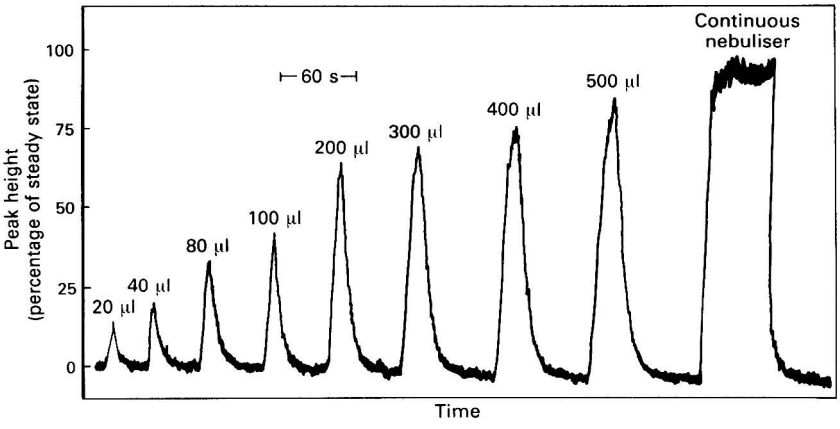

Fig. 3. Effect of sample injection volume on peak heights for 5 μg ml^{-1} of zirconium at a flow-rate of 2.0 ml min^{-1}.

small sample volumes into the carrier stream is also shown. The peak heights were found to approach the steady-state signal asymptotically as the injection volume was increased from 20 to 500 μl. Because of dilution and dispersion of the sample when injected into the stream, the peak height for small volumes (20 μl) was reduced by a factor of almost 10 in this system, giving reduced sensitivity. However, the use of micro-volumes is advantageous in many applications if the sensitivity and precision are sufficient, as the non-attainment of steady-state conditions means that washout is rapid, increasing the speed of analysis; compare Fig. 4(*a*) with Fig. 4(*b*).

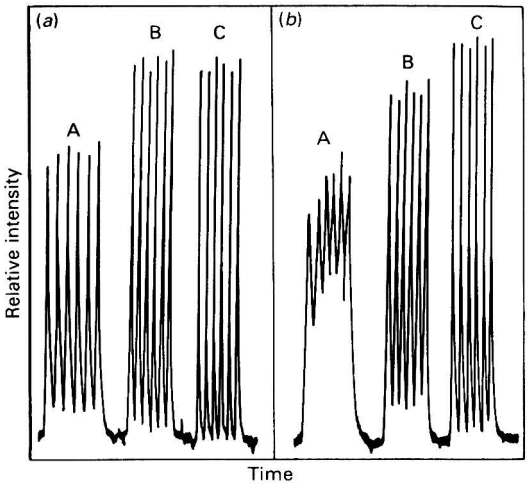

Fig. 4. Effect of flow-rate on peak-height precision at an injection rate of 240 h^{-1}.
(*a*) 10 μl of 100 μg ml^{-1} of zirconium injected at a sampling rate of 240 h^{-1}. A, 2.0 ml min^{-1} (RSD = 3.17%); B, 4.0 ml min^{-1} (RSD = 2.08%); and C, 7.5 ml min^{-1} (RSD = 1.45%). (*b*) 300 μl of 5 μg ml^{-1} of zirconium as in (*a*) A, RSD = 8.85%; B, RSD = 2.43%; and C, RSD = 1.40%.

Injection of a similar mass of analyte in 10- and 300-μl volumes indicates little change in precision (Fig. 4), except with a high injection volume and low flow-rate, where sample carryover becomes unacceptably high.

Effect of Flow-rate on Response Time

As in previous studies of electrode response times,[7] we found that the nebuliser response is considerably faster when the carrier flow-rate is increased. Fig. 5 shows emission response after injection of zirconium samples at flow-rates varying from 2.0 to 7.5 ml min^{-1}. Considerable dispersion and dilution of the injection slug occurred at 2.0 ml min^{-1}, and approximately 40 s were required for virtual (99%) washout of the sample from the nebuliser. The peak width was found to be inversely proportional to the flow-rate in the range studied, and also indicates improved sensitivity at the higher flow-rate due to less dispersion in the stream.

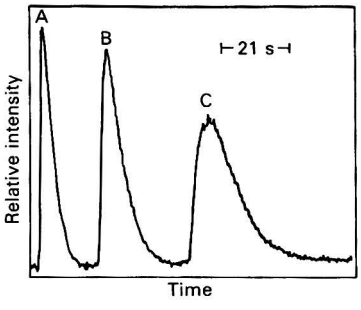

Fig. 5. Effect of flow-rate on response time. 300 μl of 5 μg ml^{-1} of zirconium injected at various flow-rates: A, 7.5; B, 4.0; and C, 2.0 ml min^{-1}.

These results show the advantage of using a high flow-rate with spectroscopic detectors, and indicate the possibility of faster sampling rates, because the rate of sampling is dependent only on the dispersion in the carrier stream and not on the detector characteristics.

Precision and Carryover

The precision of replicate peak-height measurements was also found to be dependent on the flow-rate of the carrier stream. Fig. 4(*a*) shows replicate peaks recorded for rapid injections of zirconium (1.0 μg) at different flow-rates with 10-μl injections made every 15 s. The slow flow-rate of 2.0 ml min^{-1} shows a poorer precision of 3.2% (RSD) compared with 1.5% at 7.5 ml min^{-1}. The results in Fig. 4 also indicate the greater peak overlap occurring at the slow flow-rate. This effect caused carryover between consecutive high and low samples of approximately 5% at slow flow-rates, whereas Fig. 4(*a*) shows complete return to the base line for sample emission at a flow-rate of 7.5 ml min^{-1}.

These results indicate that an over-all sample injection rate of at least 240 samples h^{-1} is permissible with rapid flow ICPAES analysis with sample injection volumes as low as 10 μl. Fig. 4(*b*) shows increasing carryover at higher injection volumes (300 μl).

Multi-element Determination

The ICPAES used in this study was programmed to give direct readout of 21 elements. The 13 elements listed in Table II, contained in one calibrating solution, were used as model elements. The other elements on the polychromator (arsenic, potassium, phosphorus, sulphur, selenium, tungsten and mercury) in other solution groupings were not investigated in detail when it became apparent that the behaviour of all elements was similar and thus predictable.

The spectrometer was programmed for rapid sample analysis to integrate the emission over ten successive 3-s periods, with a time delay after injection to synchronise a central 3-s period with the peak maximum (6-s delay for 2 ml min^{-1}; no delay for 4 and 7.5 ml min^{-1}).

TABLE II

MULTI-ELEMENT PRECISION (% RSD) *versus* CARRIER FLOW-RATE

Sample: 10 μl of 100 mg l^{-1} solution.

Element	RSD, %		
	2.0 ml min^{-1}	4.0 ml min^{-1}	7.5 ml min^{-1}
Al	7.89	4.44	3.39
Ba	3.42	1.99	2.53
Ca	3.47	2.41	4.77
Cr	5.99	2.16	2.28
Fe	5.30	2.38	1.74
Mg	3.90	2.01	2.23
Mo	2.89	1.40	3.44
Na	6.01	6.32	1.53
Si	9.88	8.35	15.95
Sn	3.25	3.13	3.55
Ti	5.28	0.99	2.01
V	5.88	2.52	1.61
Zr	6.97	2.42	2.15

The net peak height was obtained by subtracting the first (base-line) integration from the maximum (peak maximum) integration.

The peak height printed out by this method yielded precisions as low as 2–4% (RSD) (Table II), similar to the precision of the chart readout (Fig. 4).

Hence, with 10-μl samples injected at the rate of 240 samples h^{-1}, most of the 13 elements were determined with a precision of 1–3% (RSD).

Improved precision could be obtained by measuring peak area (*ca.* 2% RSD) or by use of internal standardisation (see Table III). The precision obtained (0.7–2% RSD) is significantly better than the 3–5% RSD reported for the micro-sampling technique of Knisely *et al.*[10] for single-element determinations.

TABLE III

EFFECT OF QUANTITATION METHOD ON PRECISION

Internal standard: 5 mg l^{-1} of barium. Flow-rate: 2.0 ml min^{-1}.

Quantitation method	RSD,* %
Peak-height calculation	4.39
Peak-area calculation	1.93
Internal standard (Zr peak height/Ba peak height)	0.70
Internal standard (Zr peak area/Ba peak area)	0.61

* Six replicates of 300 μl of 5 mg l^{-1} zirconium solution.

Detection Limits and Sensitivity

The drawback of this injection technique is the loss of sensitivity as a result of dispersion in the carrier stream, as shown in Fig. 3. We determined the detection limit for a 300-μl injection of sample solution containing 13 elements as twice the standard deviation of the background emission counts. The results shown in Table IV range from about 0.01 to 0.2 mg l^{-1}, depending on the particular element, for concentration detection limits. Although the concentration detection limits are much poorer than those reported by Knisely *et al.*[10] and Greenfield and Smith[11] for their micro-sampling technique, the absolute detection limits were better than 10 ng for many elements so that many of the samples suggested by Greenfield[4] and Greenfield and Smith[11] could be rapidly analysed by our rapid flow method.

The linear working range is not degraded to any great extent owing to dispersion of the sample in the stream, and the useful linear range is approximately four orders of magnitude. Improved detection limits and a wider working range could be obtained in this system by further reducing dispersion and dilution of the stream. This would require tubing of small internal diameter throughout and a more rapid pump motor. Injection after the pump

TABLE IV

DETECTION LIMIT FOR MULTI-ELEMENT DETERMINATIONS WITH MICROLITRE INJECTIONS

300-μl injection volumes with a carrier flow-rate of 4.0 ml min^{-1}.

Line/nm	Element	Detection limit (2 × S.D.) μg l^{-1}	ng	Continuous nebulisation/ μg l^{-1}
396.1	Al	80	24.0	23
233.5	Ba	16	4.8	4
317.9	Ca	23	6.9	7
267.7	Cr	24	7.2	3
259.9	Fe	(120)*	(36.0)*	3
279.5	Mg	3	0.9	21
202.0	Mo	25	7.5	10
588.9	Na	200	60.0	80
288.1	Si	700	210.0	200
189.9	Sn	36	10.8	30
334.9	Ti	13	3.9	3
309.3	V	36	10.8	17
343.8	Zr	17	5.1	5

* High value due to iron contamination from the syringe needle.

(Fig. 6) just before the nebuliser does afford less dispersion and very rapid sample throughput (600 samples h^{-1}), but at the cost of perturbing the plasma and even extinguishing it at higher injection volumes (100–300 μl). The use of a higher powered ICP as reported by Greenfield and Smith[11] should overcome the plasma instability problem.

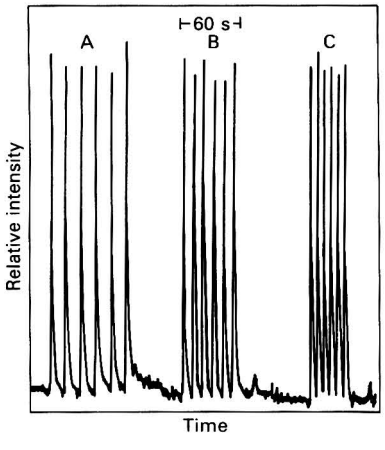

Fig. 6. Injection after pump into a carrier stream of 7.5 ml min^{-1}. 5 μl of 100 μg ml^{-1} of zirconium injected after pump. Injections per hour: A, 240 (RSD = 3.21%); B, 360 (RSD = 3.17%); and C, 600 (RSD = 2.56%).

Serum Electrolyte Analysis

Application of the technique to serum electrolyte analysis using small sample volumes (10 μl) permitted a high throughput (240 samples h^{-1}). Calibration against a 5% aqueous albumin matrix was necessary to overcome differences in nebuliser efficiency between the serum and the simple diluted acid standards.

TABLE V

SERUM ELECTROLYTE ANALYSIS

Injection	10 μl at 240 injections per hour
Standard	5% aqueous albumin matrix
Carrier flow-rate	4.0 ml min^{-1}

Element	Concentration in control serum*/mg l^{-1} RFA/ICP	RSD,‡ %	Assigned value range*
Na	3752 (4070)†	1.61	3522–3712
K	276 (156)	26.7	281–289
Ca	139 (120)	2.94	133–138
Mg	48.9 (44)	2.90	47–48
Fe	4.8 (—)	8.01	2.88

* Hyland Control Serum II, Lot No. 0368NOO3AA.
† Calibration against acid only (6% *V*/*V* aqua regia).
‡ From the mean of five determinations.

Results on a control serum (Table V) indicate good agreement with established values even when using the least precise peak-height quantitation procedure. The required accuracy in this application did not require the use of the more precise peak area or internal standardisation procedure. The poor precision obtained for potassium was caused by the low counts observed for the injected volume of 10 μl, for which the potassium concentration was close to the detection limit.

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Indirect Determination of Iodine by Cold Vapour Atomic-absorption Spectrophotometry Utilising the Interfering Effect of Iodine Against Mercury*

Part I. General Study and Application to the Determination of Iodine in Seaweed

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A simple and rapid method is described for the indirect determination of iodine as an interferent against mercury in cold vapour atomic-absorption spectrophotometry. The interference effect is due to the formation of mercury(II) iodide complexes, which in highly acidic solutions cause a decrease in absorbance for mercury(II) proportional to the amount of iodine present. Within certain concentration limits a straight-line calibration graph is obtained, making the determination of small amounts of iodine possible. The detection limit is 2.5 $\pm$ 0.7 μg of iodine if it is allowed to interfere (as iodide or iodate) against 100 ng of mercury(II) in 3.0 M nitric acid solution. Interferences for the method are, in general, the same as in the cold vapour atomic-absorption determination of mercury. Chloride and moderate amounts of bromide do not interfere. The possible interference of certain metals, especially the noble metals, is discussed. The proposed method has been used with satisfactory results in the determination of iodine in a seaweed sample (*Ascophyllum nodosum*) that had been analysed earlier by neutron activation analysis.

Keywords: *Indirect iodine determination*; *cold vapour atomic-absorption spectrophotometry*; *interference of iodine against mercury*

Iodine is a serious interferent in the determination of mercury by cold vapour atomic-absorption techniques. Certain clinical[1] and marine[2] samples (especially seaweed) contain large amounts of iodine, so that interferences in mercury analyses are encountered if techniques are not introduced to eliminate them.[3,4] This paper describes the use of this interfering effect for the determination of iodine.

Previous studies[4] have shown that (i) in highly acidic solutions, the threshold amounts of iodine that started to interfere in the determination of 25 ng of mercury(II) were less than or equal to 1 μg, whereas (ii) reduction of mercury(II), if carried out in solutions of low acidity (pH 2.5 after reduction), tolerated at least 5 mg of potassium iodide or iodate, and (iii) above pH 3 tin(II) chloride commenced to precipitate, destroying the determinations.

A pH of about 3 is therefore the lower limit of acidity at which it is still possible to reduce mercury(II) with tin(II) chloride in acidic solution. Some workers,[5,6] however, have generated mercury vapour from alkaline solutions by reducing mercury(II) with stannate(IV) ions. Even if the aim of their work was not to demonstrate the overcoming of iodine interference, it seems likely that the interference effect of iodine against mercury was at its minimum in alkaline solution. It can be calculated from the work of Kunert *et al.*[6] that their alkaline reduction of mercury(II) with stannate(IV) tolerated 25 times as much iodine as their corresponding reduction in acidic solution, which, it must be added, did not occur in extremely acidic solution but at an acidity of about 0.3 M.

On the basis of these results, it can be concluded that the magnitude of the iodine interference depends on the acidity of the solution where reduction of mercury(II) is carried out with tin(II) ions. In this work, this dependence on acidity was studied using nitric acid solutions of various concentrations. A method is proposed for the determination of iodine indirectly as an interferent in the cold-vapour determination of mercury.

* Presented at Euroanalysis IV, Helsinki/Espoo, Finland, August 23–28, 1981.

Experimental

Apparatus

Absorbance measurements were made using a Perkin-Elmer, Model 403, atomic-absorption spectrophotometer, equipped with the MHS–1 mercury - hydride system and a Perkin-Elmer mercury electrodeless discharge lamp (EDL). Readings were presented on a Perkin-Elmer, Model 056, strip-chart recorder. The instrumental parameters are listed in Table I. Erlenmeyer flasks (150 ml, Pyrex) with standard 29/32 taper joints were used as sample vessels.

TABLE I

INSTRUMENTAL PARAMETERS

Spectrophotometer parameters—	
Light source	EDL
Wavelength/nm	253.6
Slit (spectral slit width)/nm	3 (0.2)
Recorder full-scale/A	0.25
Recorder response (time constant)/s	2 (1)
Recorder parameters—	
Chart speed/mm min^{-1}	10
Range/mV	5
MHS–1 parameters—	
Programme	Hg II
Reduction solution/ml	2.5
Sample volume/ml	32
Temperature of silica cell/°C	250

Reagents and Solutions

All solutions were prepared from analytical-reagent grade chemicals using glass-distilled water.

Potassium iodide and potassium iodate stock standard solutions, 10 g l^{-1} of iodine. Potassium iodide (1.3081 g) and potassium iodate (1.6864 g) were dissolved in water and diluted to 100 ml. These solutions were diluted daily to obtain working standard solutions of suitable concentration.

Reducing agent. A 10% *m/V* solution of tin(II) chloride ($SnCl_2.2H_2O$) in 5% *V/V* hydrochloric acid was used. To free the solution of any contaminating mercury, argon was bubbled through it for 15 min.

Mercury(II) working standard solution, 0.1 mg l^{-1}. The solution contained 5% nitric acid and 0.01% *m/V* potassium dichromate as preservatives[7,8] and was prepared by suitable dilution of a stock solution (1000 mg l^{-1}; Titrisol, Merck).

Nitric acid solutions. Solutions containing 1.0, 2.0, 3.0, 4.0 and 5.0 mol l^{-1} of nitric acid were prepared from 65% acid (Merck) by appropriate dilutions with water and were used without standardisation.

Acid digestion mixture. Concentrated nitric acid - concentrated sulphuric acid (2 + 3) was used.

Gold(III) chloride solution, 100 μg ml^{-1}. This solution was prepared by dissolving the metal in aqua regia and diluting with water to give a solution containing 1% *V/V* aqua regia.

Choice of acid

Because solutions of relatively high acid concentrations were needed, studies were conducted in order to select an acid with the lowest mercury content.

Hydrochloric acid (Merck) contained too much mercury [105 p.p.b. (10^9)] to be used without purification. The mercury content of sulphuric acid (Merck) was found to be about 0.5 p.p.b. Because sulphuric acid, in addition, has been shown to have a significant concentration effect on the partition constant (*K*) of mercury,[9] its use in the present work was considered unsuitable.

K is the ratio of the reduced mercury concentrations in the gas and liquid phases after equilibration, *i.e.*,

$$K = \frac{[\mathrm{Hg}]_{\mathrm{gas}}}{[\mathrm{Hg}]_{\mathrm{liquid}}}$$

Increasing the concentration of sulphuric acid increases K.[9]

Nitric acid (Merck) did not affect the blank values in the concentration range 1.0–4.0 M and was therefore selected not only because of its low mercury content but also because it does not affect the partition constant of mercury when the acid concentration is varied.

Procedure

A 30.0-ml volume of one of the solutions indicated in Table II was dispensed into a reaction flask. Then, 1 ml of the mercury(II) working solution was added with an Eppendorf micropipette, followed by 1 ml of potassium iodide or iodate solution. The MHS–1 system was then immediately closed, and the programme[10] started (Table I). By varying the iodine concentrations, the depression of absorbance for 100 ng of mercury(II) was noted. Blank values were negligible.

Construction of the calibration graph

This was carried out in accordance with the *Procedure*, using 30 ml of 3.0 M nitric acid solution (90 mmol of nitric acid) and allowing increasing amounts of iodine (as potassium iodide or iodate) from 2.5 to 25.0 μg to interfere in the determination of 100 ng of mercury(II). Signal suppressions (Δabsorbance) were then plotted as a function of iodine concentration (Fig. 2). The sample volume was kept at 32 ml.

Procedure for the determination of iodine in seaweed

A 0.4-g amount of an air-dried (25 °C) and powdered sample (Std. NGU) was weighed into a 250-ml round-bottomed flask and 5 ml of acid digestion mixture were then added, using one drop of it (instead of silicone grease) to tighten the ground-glass joints, immediately followed by connection of the flask to an efficient reflux system consisting of two Davies-type condensers on top of each other. The sample was allowed to soak overnight at room temperature.

The next morning the flask was heated using a Gerhardt spiral heater, at first cautiously and then vigorously during the last quarter of the heating period of about 1 h. Ashing of the sample was considered to be complete when white fumes filled the flask. It was then allowed to cool to room temperature.

Whilst water-cooling the flask, 12.5 ml of 10.0 M sodium hydroxide solution were cautiously added through the top of the condenser, making the digest slightly alkaline. The sample pH was adjusted to 7–8 with dilute nitric acid and was then transferred quantitatively into a 100-ml calibrated flask and diluted to the mark with water. Aliquots of this solution were then taken for the determination of iodine according to the *Procedure*, keeping the nitric acid concentration and the sample volume as directed for the construction of the calibration graph.

To check the interference of metals in the iodine determination, 5-ml aliquots from the seaweed sample solution were analysed as for the iodine determination, but with the nitric acid concentration of the sample volume (Table I) being kept at approximately 0.03 M (0.90 mmol of nitric acid). At this low acidity, iodine from the sample aliquot does not suppress the absorbance of 100 ng of mercury(II), as shown in Table II. No signal suppression for mercury in these determinations was observed, which was taken as evidence of freedom from metal interferences against mercury(II) (see under Interferences). Theoretically, metals and species that are able to form more stable complexes with the available iodide or iodate than does the mercury(II) ion, would interfere with the proposed method, if present. Fortunately, this interference was not critical in the determination of iodine in seaweed, as shown by the results in Table IV, which are in good agreement with those obtained by neutron activation analysis.

Results and Discussion

Effect of Acid Concentration

The effect of changes in acidity on the interference of iodine against mercury(II) is illustrated in Table II and Fig. 1. The threshold amounts were determined according to the

TABLE II

THRESHOLD AMOUNTS OF IODINE THAT START TO INTERFERE WITH 100 ng OF MERCURY(II) AT VARIOUS ACIDITIES

	Nitric acid solutions					Sodium hydroxide solutions	
Acidity or alkalinity of the sample volume, before reduction/mol l^{-1}	4.0	3.0	2.0	1.0	0.03	0.02	0.04
Threshold amounts of iodine*/μg ..	0.8	1	2	10	3500	4500	5000
pH in reaction mixture after reduction			<0		1.2	1.5	2.1

* Masses of iodine (added as either potassium iodide or iodate) that suppress the signal for 100 ng of mercury(II) by approximately 3%.

Procedure for various acidities (Table II) and were defined as the masses of iodine that suppressed the absorbance of 100 ng of mercury(II) by approximately 3%.

These results show that the interference effect of iodine against mercury increases with increasing acidity. The reason for this is the increasing stability of the mercury iodide complexes such as HgI^+, HgI_2, HgI_3^- and HgI_4^{2-} with increasing acidity. This increase in the interference effect on mercury, however, is relatively small from 3.0 to 4.0 M nitric acid solution, as shown in Fig. 1 and Table II. Because of this small increase, it was decided to base subsequent work on a 3.0 M nitric acid solution, especially as the 5.0 M nitric acid solution interfered in these experiments, occasionally giving unexpectedly high absorbance values for mercury. This could be explained by broad-band absorption from the matrix, probably caused by nitrogen dioxide.

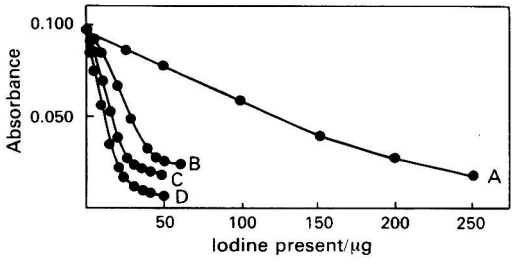

Fig. 1. Decrease in absorbance for 100 ng of mercury(II) with increasing amounts of iodine present in (A) 1.0, (B) 2.0, (C) 3.0 and (D) 4.0 M nitric acid solutions.

Linearity

The calibration graph of signal suppression (100 ng of mercury) *versus* iodine concentration (added as potassium iodide or iodate) provides a linear calibration graph from 2.5 to 25.0 μg of iodine (Fig. 2).

Precision and Accuracy

Precision is given in terms of the relative standard deviation of the absorbance due to 100 ng of mercury(II) in the presence of specified amounts of iodine (Table III) and in terms of the results of the determination of iodine in Std. NGU (Table IV).

Some accuracy data are shown in Table IV; the maximum accuracy will be obtained for about 10 μg of iodine. The analysed seaweed sample contained 850 p.p.m. of iodine according to the neutron activation analysis method. The proposed method gave 816 p.p.m. of

iodine as a mean of 15 determinations (five different aliquots were analysed in triplicate) with readings distributed over the whole of the straight line of the calibration graph. The standard deviation was 36 p.p.m. and the relative standard deviation was 4.4%.

Calibration graphs were constructed using both potassium iodide and iodate solutions, with agreement within experimental error. The iodate speciation, which is to be expected as a result of the oxidative digestion of seaweed, would not influence the accuracy.

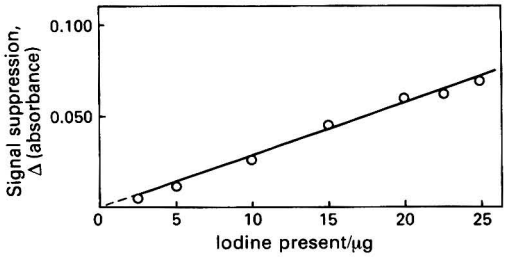

Fig. 2. Typical calibration graph for the indirect determination of iodine. Values on abscissa indicate the masses of iodine that were added as potassium iodide to 100 ng of mercury(II) in 3.0 M nitric acid solution just before determination.

Detection Limit and Sensitivity

The detection limit is defined as the amount of iodine that causes an average decrease in absorbance of 100 ng of mercury(II) equal to six times the standard deviation for measurements carried out in the absence of iodine. Defined in this way, the detection limit was 2.5 $\pm$ 0.7 μg of iodine, determined empirically according to the *Procedure*. The signal corresponding to the detection limit constitutes the first point on the linear part of the calibration graph for iodine (Fig. 2), thus indicating its linear dependence on the amount of the component to be detected. The degree of certainty applied to the detection of this least amount of iodine by the method is in accordance with the recent views of Liteanu and Říča.[11]

TABLE III

PRECISION DATA ON THE REPEATABILITY OF ABSORBANCE FOR 100 ng OF MERCURY(II) IN 3.0 M NITRIC ACID SOLUTION IN THE PRESENCE OF SPECIFIED MASSES OF IODINE

All measurements were carried out on the same day according to the procedure. Values are the means of five determinations.

Mass of iodine per determination (added as KI)/μg	Mean, absorbance units	Range,* absorbance units	Standard deviation, absorbance units	Relative standard deviation, %
0	0.098	0.002	0.0010	1.02
2.5	0.092	0.005	0.0022	2.39
5.0	0.085	0.004	0.0017	2.00
10.0	0.072	0.003	0.0013	1.80
15.0	0.053	0.004	0.0017	3.21
20.0	0.039	0.005	0.0022	5.64

* The difference between the greatest and least values.

The sensitivity (slope of the linear part of the calibration graph) was approximately 0.003 absorbance unit per microgram of iodine. The sensitivity, being a function of several variables, can probably be improved by varying some of these, such as the amount of mercury(II), the sample volume, the acidity and the concentration of the reductant. In the present work, only the acidity of the constant sample volume has been varied. By improving the sensitivity the detection limit will also be lowered, because the latter is proportional to the

reciprocal of the sensitivity of the method and to the standard deviation. It is hoped that the improvements resulting from the variation of these experimental parameters will be investigated at a later stage.

TABLE IV

RESULTS OF DETERMINATION OF IODINE IN A SEAWEED SAMPLE (Std. NGU) ANALYSED EARLIER BY NEUTRON ACTIVATION ANALYSIS

Std. NGU contained 850 p.p.m. of iodine (neutron activation method) and 34 p.p.b. of mercury (cold vapour method). The average absorbance of five runs for 100 ng of mercury(II) without added iodide is 0.098 (single determinations of 0.099, 0.097, 0.098, 0.097 and 0.098).

Aliquot of digested sample taken*/ml	Corresponding mass of iodine in Std. NGU (neutron activation)/μg	Measured absorbance	ΔAbsorbance (0.098 measured absorbance)	Mean Δabsorbance	Iodine content obtained for Std. NGU, p.p.m.
1	3.4	0.094	0.004	0.008	775
		0.088	0.010		
		0.089	0.009		
2	6.8	0.082	0.016	0.017	809
		0.080	0.018		
		0.080	0.018		
3	10.2	0.068	0.030	0.030	866
		0.068	0.030		
		0.067	0.031		
4	13.6	0.060	0.038	0.039	838
		0.057	0.041		
		0.059	0.039		
5	17.0	0.048	0.050	0.049	800
		0.052	0.046		
		0.046	0.052		
				Mean:	816
				Standard deviation:	36
				Relative standard deviation:	4.4%

* 0.4 g of Std. NGU was wet digested and diluted to 100 ml according to the procedure for the determination of iodine in seaweed. No corrections were made for the small contributions of mercury from the sample aliquots.

Interferences

In contrast to, for example, the iron(III) ion, the mercury(II) ion has a tendency to form complexes of increasing stability with ligand donor atoms of decreasing electronegativity. This behaviour of the mercury(II) ion produces a regular order of stability of its complexes with halogens [such as $HgI_n > HgBr_n > HgCl_n > HgF_n$ ($n = 1, 2, 3$ or 4)] where the order of electronegativities of the ligand donor atoms is reversed (F > Cl > Br > I). The degree of stability of the complex ions increases with increasing acidity.

This consideration of the chemistry of mercury is useful in understanding the interference effect of halogens in the determination both of mercury by cold vapour atomic-absorption spectrophotometry and of iodine by the described indirect method. In both methods chloride does not interfere and the interference from bromide is moderate; at least 5 mg of potassium bromide or bromate can be tolerated, even if the reduction is performed at high acidity.[4] At lower acidity (2 M) much more bromide and bromate could be tolerated with only a slight increase in the detection limit of iodine. This is consistent with the chemistry of the mercury(II) ion as presented above.

Cold vapour atomic-absorption methods have been reported to be generally free from interferences[1,3,4,9,12] with the exception of iodine.[1,3,4] Large amounts of elements such as copper and tellurium, which are easily reduced by tin(II) chloride, do interfere.[12] According to Koirtyohann and Khalil,[9] copper and lead at concentrations up to 20 p.p.m. have no effect on the mercury determination, but platinum, gold and silver do interfere when present at concentrations greater than about 1 p.p.m. The Std. NGU sample was analysed for gold by an electrothermal atomisation technique and found to contain 25 p.p.b. The relatively low content of heavy metals in *Ascophyllum nodosum* reported by the Norwegian Institute of Seaweed Research[13] makes it probable that interferences from metals will not normally occur. In addition, it is well known that many of the metals in question, and almost all of the noble metals, are quantitatively precipitated as hydrated oxides at a pH approaching neutrality. Thus, the final adjustment of the pH to 7–8 in the seaweed sample solution probably also removes the risk of interference from these metals in the indirect determination of iodine.

In this work the freedom from metal interferences was demonstrated for Std. NGU by carrying out the determination at low acidity (see Experimental). As can be seen in Table II and Fig. 1, the interference of iodine against mercury decreases with decreasing acidity, whereas the interference effect of gold(III) is virtually the same in both highly acidic and

slightly acidic solutions, as illustrated in Fig. 3. The same behaviour can be expected for other noble metals because of their similar chemistry (evidence for this will be presented in Part II).

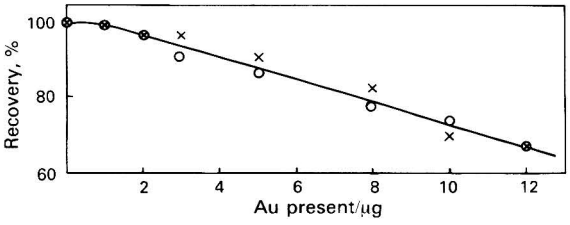

Fig. 3. Recovery for 100-ng mercury(II) runs in the presence of specified amounts of gold(III) in (×) 3.0 M nitric acid solution and (○) 0.03 M nitric acid solution.

This varying effect of acidity can be utilised to distinguish between metal interferences and the interference of iodine in the determination of mercury by cold vapour methods and to detect the interference of metals in determining the presence of iodine by the described method. Carrying out a determination at reduced acidity where a moderate amount of iodine does not interfere enables us to determine the interference effect of metals also.

Conclusion

The decrease in absorbance of mercury(II) caused by iodine in the cold vapour atomic-absorption mercury determination (Δabsorbance) depends on the acidity of the solution with which the reduction with tin(II) chloride is carried out and on the concentration of iodine. Using about 3–4 M nitric acid solution, Δabsorbance approaches its maximum (dependence on acidity), becoming virtually constant as regards the effect of changes towards higher acidity. The effect of changes in iodine concentration on Δabsorbance shows a linear dependence within certain limits, depending also on the acidity of the reduction medium and on the concentration of mercury(II).

The indirect method described here has been based mainly on these observations. It is rapid, simple and needs no special skill, and provides an alternative to existing methods for iodine determination, such as classical iodimetric, coulometric and neutron activation methods. Because chloride and bromide do not interfere in the determination, it is superior to the methods using iodide-selective electrodes. The interference from metals can be detected by carrying out the determination at reduced acidity where moderate amounts of iodine do not suppress the absorbance of mercury.

As a determination can be carried out within a few minutes, the speed and simplicity make it suitable for use in routine laboratory procedures.

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Effect of the Type of Organometallic Iron and Copper Compounds on the Determination of Both Metals in Petroleum Samples by Flame Atomic-absorption Spectroscopy

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The effect of the type of organometallic iron or copper compounds on the value of the absorbance signals of these metals in an air - acetylene flame and for iron in a dinitrogen oxide - acetylene flame is reported. The determination of iron and copper in xylene solutions of the asphaltene and resinous petroleum fractions was carried out using various organometallic compounds of both metals as standards and by both the calibration graph and standard additions techniques. There are serious limitations to the direct flame atomic-absorption spectrometric methods, owing to transport interferences. In addition, with iron, there are matrix interferences caused by the structure of the petroleum samples being analysed and also owing to the various forms of iron present in these samples. It is recommended that determination of these elements in heavy petroleum fractions should be carried out by dry mineralisation and flame atomic-absorption spectrometric analysis of aqueous solutions.

Keywords: *Iron determination*; *copper determination*; *atomic-absorption spectroscopy*; *petroleum*; *organometallic compounds*

Iron and copper are present in petroleum in the form of organometallic compounds of porphyrinic[1–4] or non-porphyrinic[5,6] character (with the donor atoms of the ligands being nitrogen, oxygen and sulphur, combined with the heaviest petroleum fractions. It is possible to find both of these metals in used lubricating oils in the metallic inorganic form, mostly as dissolved particles.

During previous developments of the application of flame atomic-absorption spectrometry to the determination of iron and copper in petroleum fractions directly after dilution in organic solvents, considerable attention has been paid to the problem of used oils. Several phenomena[7,8] have been discussed and it is apparent that comparisons of absorbance signals from samples in organic solvents with organometallic standards has not been found to be entirely satisfactory.[9]

It has been found that for some organometallic iron and copper compounds, in order to obtain the maximum absorbance, close agreement between the decomposition temperature of the complex and the boiling-point of the solvent is important.[10] The memory effect of the burner was explained[11] by the different volatilities of organometallic iron compounds in the air - acetylene flame. Significant effects caused by the different donor atoms (oxygen, nitrogen and above all sulphur) on the thermal stability of the chelates of iron and copper were established[12]; the structures of the chelates are hypothetically analogous to the structures of the non-porphyrinic iron and copper compounds present in heavy petroleum fractions.

These results, and the fact that with other transition metals (vanadium and nickel) the dependence of the analytical signal on the type of the organometallic compound used for calibration purposes has been confirmed,[13–15] provided an incentive for a more detailed evaluation of the direct determination of iron and copper in heavy petroleum fractions, which is described in this paper.

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Experimental

Apparatus

Measurements were carried out with a Varian Techtron, Model AA-6, atomic-absorption spectrometer equipped with an AB-51 burner for an air - acetylene flame and an AB-50 burner for a dinitrogen oxide - acetylene flame. A multi-element hollow-cathode lamp (ASL Atomic Spectra Lamps Ltd., Melbourne, Australia) was used as the primary radiation source. A background compensator (Varian, Model BC-6), with a hydrogen hollow-cathode lamp (ASL), was used for compensation of the non-specific absorption.

For the determination of iron the analytical line was 248.3 nm, spectral band width 0.2 nm and the lamp current was 8 mA. With the stoicheiometric air - acetylene flame, the flow-rate of the oxidant gas was 11.2 l min^{-1} and the fuel flow-rate 1.4 l min^{-1}; the flow-rate of oxidant gas in the oxidising flame was 13.8 l min^{-1} and fuel flow-rate 1.4 l min^{-1}. For the dinitrogen oxide - acetylene flame the flow-rate of the oxidant gas was 10.8 l min^{-1} and the fuel flow-rate was 5.4 l min^{-1}.

For the determination of copper in an air - acetylene flame the flow-rate of the oxidant gas was 11.2 l min^{-1} and that of the fuel 1.4 l min^{-1}. The analytical line was 324.7 nm, spectral band width 0.2 nm and the lamp current was 8 mA.

In all instances the solution aspiration rate was 2 ml min^{-1}.

Reagents

All organometallic iron and copper compounds were dried, prior to application, over phosphorus(V) oxide for 5 h in a vacuum, except for iron(II) benzoylacetonate, which was dried for 2 h at 110 °C. Reference stock xylene solutions containing 25 μg ml^{-1} of metal were prepared for iron and copper standards of Conostan (FeCON, CuCON) (Conostan Division, CONOCO Incorporation, Ponca City, OK, USA), iron(III) - acetylacetonate (FeAA) (K & K, Plainview, NY and Hollywood, CA, USA), copper(II) - cyclohexylbutyrate (BDH Chemicals Ltd., Poole, Dorset, CuCHB), copper(II) - benzoylacetonate (CuBA) and iron(II) - and copper-(II) - stearate (K & K, FeST, CuST) by slight heating of a xylene solution containing the corresponding amount of the standard. CuBA and copper(II) - acetylacetonate (CuAA) were prepared according to reference 16 and iron(III) - benzylacetonate (FeBA) according to reference 17. FeBA was dissolved in xylene after the addition of 2-ethylhexanoic acid (Fluka AG, Buchs, Switzerland), acetylacetonate (Laborchemie Apolda, Apolda, GDR) and dimethylaniline (Lachema, Brno, Czechoslovakia). CuAA is practically insoluble in xylene alone, so methanol was used to dissolve the compound and the methanol solution, with a copper concentration of 250 μg ml^{-1}, was then diluted with xylene, 1 + 9 V/V. For comparison, a stock solution of CuBA was prepared in the same way. Petroleum asphaltenes, with a known iron content, were also used as iron standards; the reference stock solutions in xylene, containing 25 μg ml^{-1} of iron, were prepared by slight heating of xylene solutions containing the corresponding amounts of asphaltenes. The iron and copper contents in all of the asphaltenes and standards used, except both the Conostan standards, were checked by flame atomic-absorption spectroscopy after mineralisation (dry process).

Preparation of Samples

The following petroleum samples were used: (1) Romashkino propane asphalt (USSR); (2) Saratov propane asphalt (USSR); (3) Morgane crude oil (Egypt); and (4) asphaltenes from the Romashkino crude oil.

For a dry mineralisation of the petroleum samples an appropriate amount of the sample (depending on the metal content) was weighed in a platinum dish and *p*-xylenesulphonic acid was added (0.1 g of the acid per 1 g of the sample). The lightest fractions of the sample were then evaporated off by heating on an electric plate and the remainder was burnt off in a muffle furnace at a temperature of 550 ± 30 °C, which was maintained for 5 h. The ash obtained was dissolved in 2.5 ml of dilute sulphuric acid (1 + 4 V/V) and 0.05 ml of hydrofluoric acid was added to decompose any silicates that may have been present. The contents of the platinum dish were transferred into a 10-ml calibrated flask and then diluted to the mark with re-distilled water.

The iron and copper contents of the samples were determined, after dry mineralisation, by flame atomic-absorption spectrometry, spectrophotometry and a polarographic square-wave method; the results are shown in Table I. The calculated averages of the measured values were considered as reference values of the metal contents.

For direct measurements in xylene solutions, appropriate amounts of the samples studied (not more than 0.2 g ml^{-1}) were dissolved, with slight heating, in xylene; the solutions were diluted, after cooling, with xylene to the required final volume. In no instance was a non-specific absorption detected.

TABLE I

DETERMINATION OF COPPER AND IRON IN PETROLEUM SAMPLES BY VARIOUS METHODS AFTER PREVIOUS DRY MINERALISATION OF THE SAMPLES

All results are in parts per million.

Sample No.	Method: FAAS* Fe	FAAS* Cu	Square-wave polarography Fe	Square-wave polarography Cu	Spectrophotometry Fe	Spectrophotometry Cu	Average value Fe	Average value Cu
(1)	161	3.65	160	—	159.6	3.52	160.0 ± 4.0	3.59 ± 0.2
(2)	91	—	95.8	—	95.5	—	94.1 ± 3.0	—
(3)	46.5	—	41.2	—	47.2	—	45.0 ± 3.8	—
(4)	335	4.82	334.5	—	334	4.77	334.5 ± 7.0	4.80 ± 0.3

* FAAS = flame atomic-absorption spectroscopy.

Results and Discussion

The stoicheiometric and oxidising air - acetylene flames, and the dinitrogen oxide - acetylene flame were used to measure the flame profiles of the organometallic iron compounds studied and the stoicheiometric air - acetylene flame was used to study the flame profiles of the copper compounds. No specific height above the burner top was found at which the absorbance signals of the metals in the standards studied and in the real petroleum samples were equal. The measuring position chosen for all three flames was just above the burner top, the maximum values of the iron and copper absorbance signals being obtained at that distance for all organometallic standards and real petroleum samples studied in this work.

Calibration graphs of the organaometallic iron and copper compounds, and also for iron in the petroleum asphaltenes, were measured in all the relevant flames. The calibration graphs for copper obtained in the air - acetylene flame are illustrated in Fig. 1, and the calibration

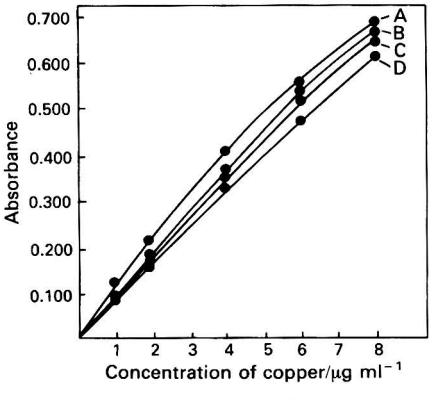

Fig. 1. Calibration graphs for copper obtained with reference solutions of different organometallic compounds in xylene with an air - acetylene flame: A, CuCON in xylene; B, CuCHB in xylene; C, CuBA in xylene and in a mixture of methanol - xylene; and D, CuAA in a mixture of methanol - xylene.

graphs for iron in the stoicheiometric air - acetylene and dinitrogen oxide - acetylene flames in Figs. 2(*a*) and (*b*). The dependence of the absorbance signals of copper and iron using a stoicheiometric air - acetylene flame on the type of compound used for calibration is evident from Figs. 1 and 2(*a*). With iron the same dependence was observed in the oxidising air - acetylene flame. The importance of this fact for the analysis of samples of unknown composition is evident. In the dinitrogen oxide - acetylene flame [Fig. 2(*b*)] this dependence is eliminated for pure organometallic compounds of iron. However, the calibration graph obtained for iron in asphaltenes was characterised by a substantially lower gradient compared with other organometallic iron compounds.

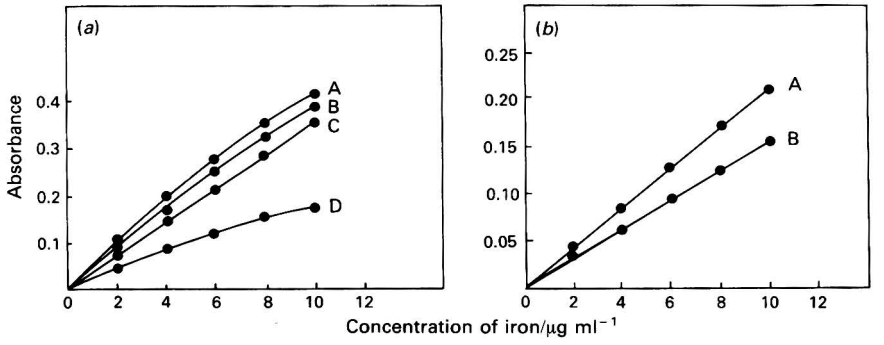

Fig. 2. Calibration graphs for iron obtained with reference solutions of different organometallic compounds and asphaltenes in xylene. (*a*) Stoicheiometric air - acetylene flame: A, FeST; B, FeCON; C, FeAA and FeBA; and D, asphaltenes. (*b*) Dinitrogen oxide - acetylene flame; A, FeST, FeCON, FeAA and FeBA; and B, asphaltenes.

For the selected petroleum samples, the iron and copper contents were determined by direct calibration and by the standard additions technique, using all of the organometallic compounds (and also for iron, asphaltenes) as standards. The results of the determinations of the copper contents are shown in Table II together with the reference values of the metal content, and the sample concentrations used in the diluted xylene solutions. In Tables III and IV are examples of results for the determination of iron in the oxidising air - acetylene flame and the dinitrogen oxide - acetylene flame, respectively. Serious errors in the results for the determinations of iron and copper using xylene solutions of the investigated samples are evident by comparison with the reference values.

TABLE II

DETERMINATION OF COPPER IN PETROLEUM SAMPLES

Sample No.	Concentration after mineralisation, p.p.m.	Concentration of sample in xylene/ mg ml^{-1}	Calibration graph technique, p.p.m.				Standard additions technique, p.p.m.			
			CuCON	CuCHB	CuBA	CuAA	CuCON	CuCHB	CuBA	CuAA
(1)	3.59 ± 0.2	164.4	0.73	0.85	0.94	1.21	1.16	1.29	1.35	1.46
(2)	4.80 ± 0.3	92.7	1.61	1.88	2.01	2.16	2.16	2.30	2.41	2.59

With copper (Table II) these errors can be attributed to both the interference of the sample matrix (the samples being characterised by a complex polycondensed aromatic structure) and to transport interferences[15] [for sample No. (1) in particular] associated with the concentration of the sample in the xylene solution being measured. Owing to the low copper content, it was impossible to work at lower sample concentrations. The application of the high temperature dinitrogen oxide - acetylene flame for the eventual reduction of the interferences of the sample matrix cannot be realised owing to the reduction in sensitivity of the determination of copper in this flame.

The dependence of the results of the determination of iron, obtained by the calibration graph technique in the oxidising air - acetylene flame (Table III), on the concentration of

TABLE III

DETERMINATION OF IRON IN PETROLEUM SAMPLES IN OXIDISING AIR - ACETYLENE FLAME

Sample No.	Concentration after mineralisation, p.p.m.	Concentration of sample in xylene/ mg ml^{-1}	Calibration graph technique, p.p.m.				Standard additions technique, p.p.m.			
			FeST	FeCON	FeAA and FeBA	Asphaltenes	FeST	FeCON	FeAA and FeBA	Asphaltenes
(1)	160.0 ± 4.0	18.3	76.5	82.2	92.9	177.6	127.0	128.7	131.1	340.1
		60.8	66.5	70.0	78.1	217.6	—	—	—	—
(2)	94.1 ± 3.0	17.4	51.7	57.6	66.1	114.9	68.3	70.3	73.2	178.0
		30.8	45.5	49.3	55.2	111.6	—	—	—	—
		57.8	45.0	48.2	53.6	105.5	—	—	—	—
		102.4	39.6	42.4	46.4	107.9	—	—	—	—
(3)	45.0 ± 3.8	61.4	16.3	18.1	22.0	37.5	23.3	23.2	24.4	62.3
		200.0	13.0	14.7	16.1	33.8	—	—	—	—
(4)	334.5 ± 7.0	7.7	135.4	160.3	181.8	349.0	207.8	210.2	214.3	—
		19.2	136.4	145.4	161.5	337.7	—	—	—	—

the sample in the measured solution, is evident from the comparison of these values with the reference values of the iron content. Even for very low concentrations of the samples in the xylene, *e.g.*, samples Nos. (1) (18.3 mg ml^{-1}), (2) (17.4 mg ml^{-1}) and (4) (7.7 mg ml^{-1}), accurate values of the iron content could not be obtained. The most probable explanation of the errors at these sample concentrations is either the considerable interferences of the heavy asphaltene and resinous petroleum fractions in the sample matrix, or the presence of colloid residues of inorganically combined iron. At higher sample concentrations in the xylene solution, *e.g.*, sample No. (2) (102.4 mg ml^{-1}), transport phenomena can also introduce errors, as is evident from the comparison of results obtained by the calibration graph and standard additions techniques for this sample. Practically the same situation was observed in the stoicheiometric air - acetylene flame.

Although the dinitrogen oxide - acetylene flame eliminated the dependence of the absorbance signal of iron on the type of compound used for calibration [Fig. 2(*b*)], its application is questionable for the analysis of petroleum samples. The results in Table IV show that matrix effects could not be eliminated for low sample concentrations in xylene [samples Nos. (2) and (4)]. Owing to the loss of response of the determination of iron in this flame, transport interferences appeared to an increased extent (owing to the lower dilution of the sample by the solvent). The results also confirm that, even in the dinitrogen oxide - acetylene flame, the interferences of the sample matrix cannot be eliminated by the standard additions technique.

TABLE IV

DETERMINATION OF IRON IN PETROLEUM SAMPLES IN DINITROGEN OXIDE - ACETYLENE FLAME

Sample No.	Concentration after mineralisation, p.p.m.	Concentration of sample in xylene/ mg ml^{-1}	Calibration graph technique, p.p.m.			Standard additions technique, p.p.m.		
			FeCON, FeST FeAA and FeBA	Asphaltenes	FeST	FeCON	FeAA and FeBA	Asphaltenes
(1)	160.0 ± 4.0	18.3	136.6	202.3	131.4	136.6	143.6	213.1
		60.8	141.4	185.8	—	—	—	—
(2)	94.1 ± 3.0	17.4	86.2	103.4	73.2	75.0	78.0	122.0
		30.8	77.9	103.9	—	—	—	—
		57.8	77.9	103.8	—	—	—	—
		102.4	71.3	100.6	—	—	—	—
(3)	45.0 ± 3.8	61.4	30.1	39.1	29.3	30.1	31.8	64.8
		200.0	27.9	37.6	—	—	—	—
(4)	334.5 ± 7.0	7.7	242.2	346.4	214.3	223.8	233.8	—
		19.2	214.3	332.5	—	—	—	—

In petroleum raw materials and their fractions, the iron and copper contents do not usually exceed the contents of sample No. (4) (asphaltenes), which are a concentrate of iron and copper contained in the crude oil. Not even for iron, with maximum (and in our opinion sufficient) dilution of the sample by the organic solvent, could agreement between the

reference iron content obtained after mineralisation and the content obtained by the determination in the xylene solution be attained. The structure of the asphaltene or resinous fractions therefore also affects the determination of both metals in crude oils containing these fractions.

Conclusion

Experimental results for the determination of iron and copper in asphaltene and resinous petroleum fractions by flame atomic-absorption spectrometry in xylene solutions, discussed in this paper, point to the practical limitations of the method, resulting from the existence of transport and matrix interferences caused by the structure of these samples, and, for iron, by the presence of inorganic colloid iron. For obtaining accurate values of the iron and copper content in the asphaltene and resinous petroleum fractions, it is recommended that the determination of both metals should be carried out on solutions in water after previous mineralisation of the samples.

The iron and copper forms originally present in crude oils are removed during the preparation of light petroleum products and oils. The problem of the determination of iron and copper in these samples corresponds therefore to their determination in used oils, mentioned earlier in this paper.

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Effect of Surfactants on the Response of Ion-selective Electrodes with Poly(vinyl Chloride) Membranes

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The effect of cationic, anionic and non-ionic surfactants on the potentials of potassium-, calcium- and nitrate-selective electrodes has been studied. All electrodes investigated had either an internal reference solution or a solid-silver contact. The latter type was found to be less sensitive to surfactant interferences.

Keywords: *Surfactant effect*; *ion-selective electrodes*; *PVC membrane electrodes*; *potassium-, calcium- and nitrate-selective electrodes*

The widespread use of surfactants has stimulated the development of a number of new analytical methods for their determination. Those methods are, as a rule, not very selective, which is also the problem with methods based on ion-selective electrodes.[1] Another problem that arises is the influence of surfactants on the determination of other species using various methods. Such effects may, without doubt, interfere when ion-selective electrodes are used in water and sewage analysis.

Until now only a few papers have mentioned the effect of surfactants on ion-selective electrode measurements. Llenado[2] has investigated the behaviour of a calcium-selective electrode in the presence of cationic (Hyamine) and anionic (Sulframine) surfactants. The cationic surfactant competes with the calcium, with a selectivity coefficient about 1000, which enables the electrode to be used as a surfactant-selective electrode. The anionic surfactant, on the other hand, is extracted into the membrane phase, which shifts the standard potential of the electrode, owing to a change in the membrane composition.

Hamonds and Lambert[3] found that cetyltrimethylammonium bromide interferes with the response of a potassium-selective electrode. A similar effect was observed for *N*-alkyldiethanolamines when the amine molecule is sufficiently large and in a pH range where the amine is protonated. These workers found no effects for non-ionic or anionic surfactants.

In their paper, Craggs *et al.*[4] stated that the calcium-selective electrode is disturbed by anionic surfactants of the dodecylsulphate and alkylbenzenesulphonate type. They also found that the electrode responded directly to anionic surfactants at low concentrations and suggested that such behaviour depends on the composition of the membrane. This explanation is consistent with our views on the anionic interferences at calcium-selective electrodes.[5]

In this work, we aimed to investigate systematically the effect of various surfactants on cation-selective electrodes (K^+ and Ca^{2+}) and on an anion-selective electrode (NO_3^-). Because it is suspected that the electrode response may be influenced by the type of internal contact, the investigated electrodes were used with internal reference solutions or direct solid contact.[6,7]

Experimental

Electrodes and Apparatus

The potassium-selective membrane contained valinomycin and dioctyl adipate as a mediator in the poly(vinyl chloride) (PVC) film.

The calcium-selective membrane contained calcium dioctylphenylphosphate and dioctylphenylphosphonate as a mediator in the PVC film.

The nitrate-selective membrane contained tris(bathophenanthroline)nickel(II) nitrate and nitrophenyl octyl ether as a mediator in the PVC film.

The electrode body was composed of a PVC tube containing the correct reference solution for the electrodes with internal reference solutions, and the membrane disc was in direct contact with the solid-silver contact.[7]

Solid-state fluoride and chloride electrodes were used in complementary measurements.

A calomel electrode (K–401, Radiometer) and a silver - silver chloride electrode (Orion 90–02) with an electrolytic bridge containing 1 M sodium sulphate solution or 10% ammonium

nitrate solution were also used as reference electrodes for nitrate and cation measurements, respectively.

The potentials were recorded with an OP–208 (Radelkis) instrument.

Chemicals

Manoxol OT. Sodium dioctylsulphosuccinate (BDH Chemicals Ltd.) was used as an anionic surfactant.

Dodecyltrimethylammonium chloride (DTMC). Used as a cationic surfactant (Eastman).

Rokafenol N-8. $C_{10}H_{21}\cdot C_6H_4\cdot O\cdot(C_2H_4O)_8H$ (Organika-Rokita, Poland) was used as a non-ionic surfactant.

Brij 35 (polyoxyethylene lauryl ether). Also used as a non-ionic surfactant (Technicon).

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

Results and Discussion

Measurements with a Potassium-selective Electrode

In the absence of surfactants the calibration graph is rectilinear down to $\log[K^+] = -4.2$ with a slope of 53 mV pK^{-1}. The cationic surfactant acts as a typical competing substance with a selectivity coefficient of about 10^2 [Fig. 1(*a*)]. This is supported by experiments at a constant potassium concentration of 10^{-4} M and varying the DTMC concentration. Rectilinear dependence was obtained to concentrations below $10^{-5.3}$ M DTMC with a slope of 58 mV $pDTMC^{-1}$ (Fig. 2).

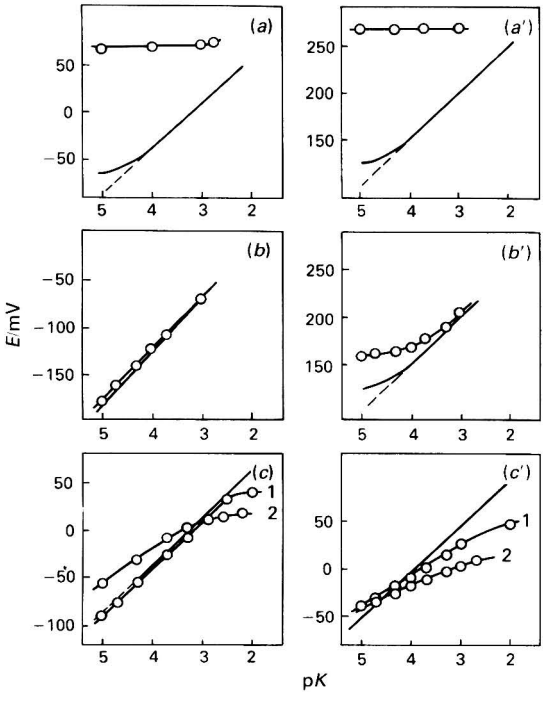

Fig. 1. Calibration graphs for potassium-selective electrodes with solid silver internal contact (*a,b,c*) and internal reference solution (*a',b',c'*) in the absence (lines without symbols) and in the presence of surfactants (lines with symbols). (*a,a'*), 10^{-4} M DTMC; (*b,b'*), 10^{-3} M Rokafenol N-8; (*c,c'*), 10^{-3} M (1) and 10^{-4} M (2) Manoxol OT.

The influence of non-ionic Rokafenol N-8 depends significantly on the type of internal contact. With a solid-silver contact electrode practically no effect was observed, in contrast to the internal reference solution electrode. In the latter, the surfactant modifies the electrode response in a similar manner to a cationic species with a selectivity coefficient of about 0.1 [Fig. 1(*b*)]. It is difficult to find a plausible explanation of this minor effect, in spite of its reproducibility.

The action of the anionic surfactant Manoxol OT is also more pronounced for the internal reference solution electrode than for the solid-silver contact electrode [Fig. 1(*c*)]. At a surfactant concentration of 10^{-4} M the latter is hardly disturbed, whereas the former shows a decrease in the slope of approximately 28 mV pK^{-1}. At a surfactant concentration of 10^{-3} M this effect is more pronounced and it also influences the response of the solid-silver contact electrode. The difference in electrode behaviour is similar to that observed earlier for lipophilic inorganic ions.[5] The concentration of cationic complexes in the membrane contacted from both sides with the solution of the main ion is higher than for the solid-silver contact electrode. This enhances the range and extent of the anion interference of the internal reference solution electrode. The lipophilicity of Manoxol OT (the interfering anion) is in this instance responsible for lowering the slope, and finally, in the region of high concentrations in the sample solution, for the reversed sign of the electrode characteristics.[8] The behaviour of the potassium electrode in solutions of constant potassium concentration and increasing levels of Manoxol OT (Fig. 3) shows, in the intermediate range, positive deviations, which may be attributed to the disturbance of the reference electrode; whereas at higher Manoxol OT concentrations a predominant, although irregular, anionic function develops.

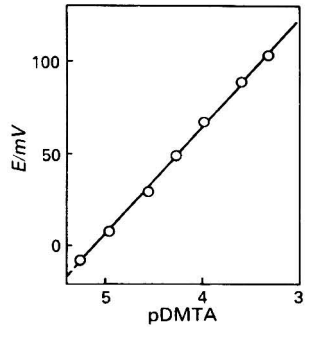

Fig. 2. Effect of DMTA on the response of potassium-selective electrode with silver internal contact in the presence of 10^{-4} M potassium nitrate.

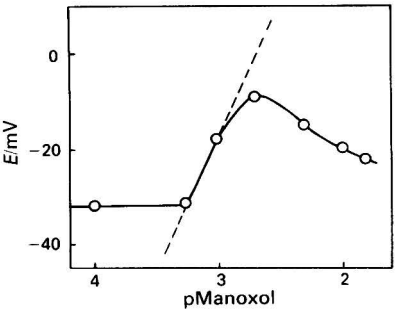

Fig. 3. Effect of Manoxol OT on the response of potassium-selective electrode with silver internal contact in the presence of 10^{-4} M potassium nitrate.

Measurements with a Calcium-selective Electrode

In the absence of surfactants the linear part of the calibration graph extends below 10^{-4} M Ca^{2+}. In the presence of a cationic surfactant (DTMC), it competes with the main ion drastically and even at the level 10^{-5} M the calcium measurements are impossible [Fig. 4(*a*)]. As might be expected, the non-ionic surfactant (Rokafenol N-8) has no effect on the electrode response [Fig. 4(*b*)]. This behaviour was observed for both types of electrode construction, which is in contrast to the response of the potassium-selective electrode of the solid-silver contact type.

The influence of the anionic detergent on the calcium-selective electrode is significant [Fig. 4(*c*)], which is in agreement with the results of previous studies.[2,4] Addition of Manoxol OT at a concentration of 10^{-4} M shifts the calibration graph to more negative values. Further increase of the Manoxol OT concentration seriously changes the shape of the graph. At low concentrations of calcium the slope increases up to 64 mV pCa^{-1} for the solid-silver contact electrode and to 37 mV pCa^{-1} for the internal reference solution electrode, suggesting that the monovalent species also contribute to the response of the electrode. At higher concentrations of calcium the slope of the calibration graph changes its sign. Such behaviour

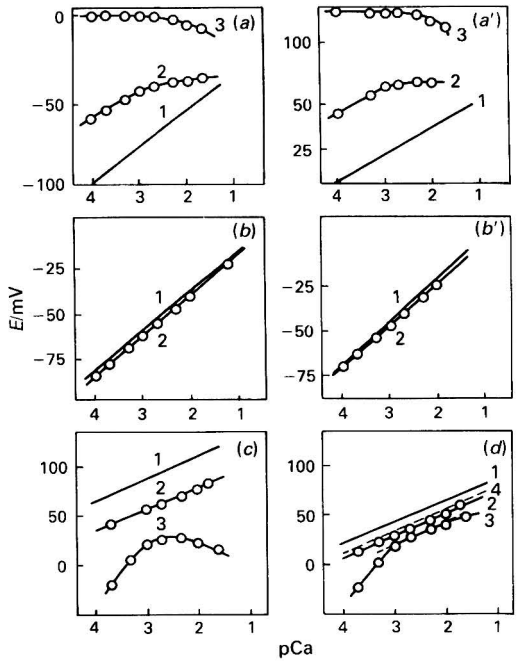

Fig. 4. Calibration graphs for calcium-selective electrodes with solid silver internal contact (*a*,*b*,*c*,*d*) and internal reference solution (*a'*,*b'*) in the absence (lines) and in the presence of surfactants (lines with symbols). (*a*,*a'*), 10^{-5} M (2) and 10^{-4} M (3) DTMC; (*b*,*b'*), 10^{-3} M Rokafenol N-8 (2); (*c*), first run in the presence of 10^{-4} M (2) and 10^{-3}M (3) Manoxol OT; (*d*), second run on the next day without surfactant (1) and in the presence of 10^{-4} M (2) and 10^{-3} M (3) Manoxol OT, and again repeated without surfactant (4).

seems to be attributed to several different phenomena. The negative shift of the response graph may result from a change in the membrane composition, as suggested by Llenado,[2] owing to extraction of the surfactant into the membrane phase. This is supported by the observation of the electrode response after a prolonged action of the surfactant on the membrane. Such treatment decreases the deviations from linearity of the calibration graph, even when the electrode functions in a solution containing a concentration of the surfactant of 10^{-3} M [Fig. 4(*d*)].

An interesting feature is the increase in the slope of the calibration graph at low calcium concentrations. This might be caused by partial complexation of calcium by the surfactant molecule. The concentration of the surfactant compared with the calcium concentration is insufficient to keep the complexation degree constant and its decrease when passing from pCa 4 to pCa 3 gives the net effect of a slope increase. Such an explanation is contradictory to the observations of Craggs *et al.*,[4] who on the basis of experiments in the presence of a strong ligand excluded the possibility of solution reactions. However, they used another surface-active compound, so their statement may be considered as not being valid in this instance.

The negative deviation at higher calcium concentrations has been suggested as being caused by anionic interferences, as previously investigated,[5] but undoubtedly some contribution of the reference electrode should be assumed.

Measurements with a Nitrate-selective Electrode

The effect of non-ionic and cationic surfactants is insignificant and only a shift of a few

millivolts towards negative values has been observed [Fig. 5(*a*) and (*b*)]. This is probably due to small impurities of chloride [or other anions] in the samples, which is particularly so at low nitrate concentrations. The most deleterious effect was, however, observed in the presence of the anionic surfactant. It competes with nitrate but surprisingly this effect is much stronger for the internal reference solution electrode than for the solid-silver contact electrode [Fig. 5(*c*)]. In the former, the potentiometric selectivity coefficient was estimated as being 10^4, whereas for the latter it was 10^2.

In addition, behaviour of both electrodes as functions of surfactant concentration is different (Fig. 6). The internal reference solution electrode shows a good surfactant response with a slope of above 50 mV p(Manoxol)$^{-1}$. The solid-silver contact electrode response is, in the presence of 10^{-4} M potassium nitrate solution, constant until the surfactant concentration approaches $10^{-4.8}$ M. This is in good agreement with the apparent values of the potentiometric selectivity coefficient given above. Further increase in the surfactant concentration decreases the potential and changes the slope. In the vicinity of a concentration of surfactant of 10^{-3} M, corresponding to the critical micelle concentration range, the response shows a positive deviation. The difference in behaviour of the two electrode types seems to be connected with the differences in transport through the membrane and with the additional equilibrium at the internal interface for the internal reference solution electrode.

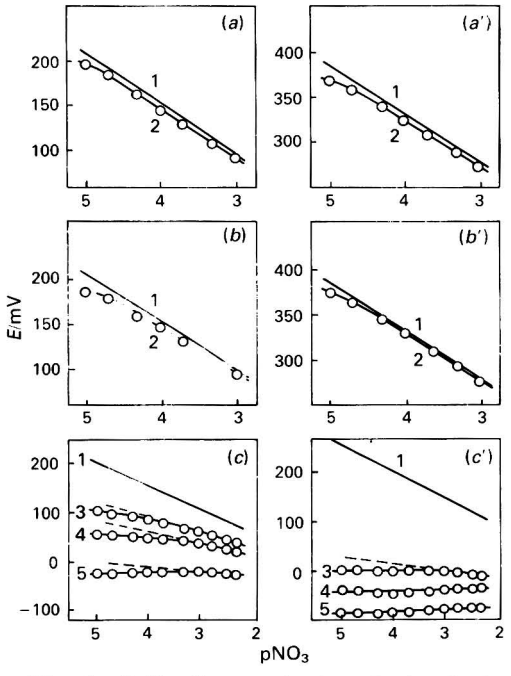

Fig. 5. Calibration graphs for nitrate-selective electrodes with solid silver internal contact (*a*,*b*,*c*) and internal reference solution (*a'*,*b'*,*c'*) in the absence (lines 1) and in the presence of surfactants: (*a*,*a'*), 10^{-3} M Rokafenol N-8 (2); (*b*,*b'*), 10^{-4} M DMTA (2); (*c*,*c'*), 10^{-5} M Manoxol OT (3), 2×10^{-5} M Manoxol OT (4) and 10^{-4} M Manoxol OT (5).

Investigation of the Reference Electrode Behaviour

It may be suspected that the presence of surfactants can influence not only the response of the indicator electrode but also the constancy of the reference electrode potential, including the liquid junction potential. Two series of experiments were performed. In the first, the response of a fluoride-selective electrode was measured *versus* the saturated calomel electrode and double junction reference electrode. In these experiments instead of Rokafenol N-8 as

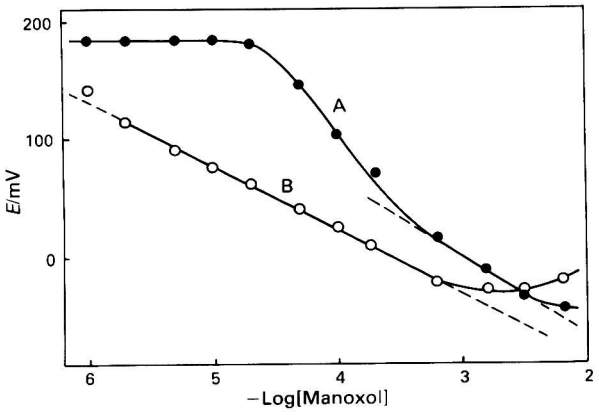

Fig. 6. Effect of Manoxol OT on the response of nitrate-selective electrodes with solid-silver contact (A) and internal reference solution (B) in the presence of 10^{-4} M potassium nitrate.

a non-ionic surfactant Brij 35 was used, which is also recommended for flow measurements with ion-selective electrodes.[9] A significant influence was only found for Manoxol OT, when its concentration exceeded $10^{-3.5}$ M (Fig. 7). The cationic and non-ionic surfactants exhibit an effect of minor importance at higher concentrations or even showed no effect.

In the next experiment the influence of Manoxol OT on the electrode system was noted: for fluoride and chloride solid-state electrodes no effect was found (Fig. 7). This gives the basis for the statement that the reference electrode with a liquid junction is sensitive to the anionic surfactant when its concentration exceeds $10^{-3.5}$ M. This fact must be taken into account when the influence of surfactants on measurements with ion-selective electrodes is considered.

Conclusions

The effect of surfactants on measurements with ion-selective electrodes depends on the charge type of the surface active agent and on the electrode system used. For PVC electrodes with a liquid ion exchanger or neutral ionophore the non-ionic surfactants are generally without any influence. For ionic species, their large lipophilic ions seriously interfere in the measurements and the electrodes show a preference, by several orders of magnitude, for the surfactant ions. This is in good agreement with previous studies on surfactant interferences[2–4] and has been practically exploited in electrodes sensitive to large organic lipophilic ions.[1] In specific instances it must also be kept in mind that the counter ion of the surfactant, being of the same charge sign as the main ion, may influence the electrode response. This is so when chloride ion is present in tetraalkylammonium salts, which may interfere in nitrate measurements.

A different mechanism of interference is observed in the presence of an anionic surfactant in measurements with potassium- and calcium-selective electrodes. For a neutral carrier potassium-selective electrode, the cationic carrier complexes favour, at higher concentrations, the anionic function of the membrane, which, as shown for Manoxol OT, is responsible for interferences in electrode behaviour. A similar net effect is observed for the ion-exchanger calcium-selective electrode. In the presence of lipophilic surfactant anions and on increasing the concentration of calcium as the main ion, the formation of a cationic calcium dioctylphenylphosphonate oxonium complex[5] promotes the anionic function of the electrode. This kind of interference is however sensitive to the history of the electrode and its pre-treatment, as the changes in membrane composition are occurring much slower than those in the solution.

Taking into account that this type of interference depends strongly on the composition of the membrane, it should be remembered that the disturbance of electrode response under the action of the same surfactant may be at variance with other types of PVC or proper liquid-state electrodes.

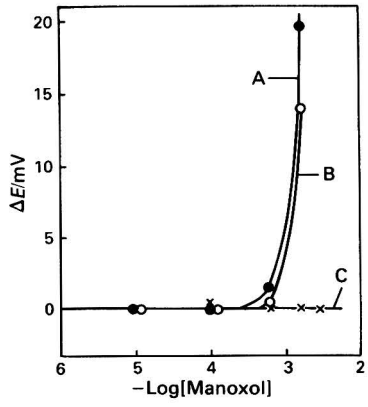

Fig. 7. Effect of Manoxol OT on the e.m.f. response in the presence of 10^{-4} M sodium fluoride for solid-state fluoride electrode and Radiometer K-401 S.C.E. (A), Orion 90–02 double-junction silver - silver chloride electrode (B) and silver - silver chloride electrode in the presence of 10^{-2} M potassium chloride (C).

A very important fact observed in this study is the different behaviour of the internal reference solution and solid-silver contact electrodes. This may be of practical utility in application of solid-silver contact electrodes to real analytical problems, which was not fully appreciated until now.

In all these studies, a non-specific interference must be also taken into consideration, which is connected with the change of the reference half-cell potential. This was observed for the anionic surfactant, Manoxol OT, but the obscure nature of this interference does not allow the acceptance of an unambiguous statement about what types of surfactants may show such an effect.

In interpreting these results, it must be remembered that for experiments carried out on various days the standard potential and slope of the electrode were not constant. This concerns, for example, the much more negative standard potential in Fig. 1(*c'*) compared with Fig. 1(*a'*) and (*b'*), and the slope of approximately 46 mV per decade in Fig. 1(*a*) compared with approximately 57 mV per decade in Fig. 1(*b*). Such differences were specifically observed when various electrodes of the same type were used. Therefore, each series of measurements was accompanied by a control calibration, which is usually shown on the same figure. The indicated potentials can be considered however as stable values under usual analytical conditions, *i.e.*, within several minutes.

It is expected that this study will give answers about some of the unexpected interferences in ion-selective measurements. However, some of the large variety of substances used as surfactants may, owing to their particular composition and structure, exhibit special behaviour.

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High-performance Liquid Chromatographic Determination of Vitamin D_3 in Foods with Particular Reference to Eggs

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A high-performance liquid chromatographic (HPLC) method for the determination of vitamin D_3 in foods is described. The method involves addition of vitamin D_2 to the sample as an internal standard followed by saponification and extraction of the unsaponifiable matter. Sterols are removed by precipitation and other interfering compounds by a thin-layer chromatographic clean-up procedure. Vitamins D_2 and D_3 are then separated using reversed-phase HPLC. Details of the accuracy and precision of the method are presented. This method has been applied successfully to the determination of vitamin D_3 in eggs, butter, milk and cheese.

Keywords: High-performance liquid chromatography; vitamin D_3 determination; foods

Several compounds found in foods possess vitamin D activity. Vitamin D_3 (cholecalciferol) occurs naturally in some foods, mainly dairy products, liver and some fish, whilst vitamin D_2 (ergocalciferol), a synthetic product that is chemically very similar and biologically equivalent in humans, is used in pharmaceutical preparations and some fortified foods, *e.g.*, dried milk. Various metabolites of vitamin D, *e.g.*, 25-hydroxycholecalciferol may also be present in some foods and, if total vitamin D activity of a food is required, the sum of vitamin D and any other metabolites that may be present must be measured. The determination of vitamin D in foods is difficult because even fortified foods contain very low levels and when the vitamin is extracted from a food it is accompanied by a large number of other compounds, some of which interfere with ultraviolet spectrophotometric methods of analysis and colorimetric determinations.

The traditional methods of analysis that were used until about ten years ago involved either time-consuming, imprecise and expensive biological assays[1] or colorimetric reactions, *e.g.*, that based on the modification by Nield *et al.* of the Carr and Price reagent.[2] Such colour reagents lacked specificity and therefore extensive purification was necessary to remove sterols, carotenoids, vitamin A and other interferences before vitamin D could be measured. In the 1960s gas - liquid chromatography was applied to the determination of vitamin D. One of the advantages of gas - liquid chromatography over colorimetric and biological assays was that it was possible to differentiate between vitamin D_2 and vitamin D_3. These two forms of vitamin D are chemically very similar and because it is rare to encounter both forms in a sample it is possible to use vitamin D_2 as an ideal internal standard when determining vitamin D_3 and *vice versa*. As with colorimetric determinations, extensive purification of extracts was needed before vitamin D could be determined by gas - liquid chromatography. Also, it was necessary to isomerise vitamins D_2 and D_3 to their more stable isotachysterols before analysis by gas - liquid chromatography because vitamin D undergoes thermal cyclisation if injected directly on to the gas - liquid chromatographic columns. The gas - liquid chromatographic methods developed in this laboratory[3,4] were applied successfully to the analysis of a large number of samples; however, the extensive clean-up and derivatisation procedures frequently took several days to complete.

The advent of high-performance liquid chromatography (HPLC) has led to continuing improvements in the assay procedure for vitamin D. Compared with gas - liquid chromatography, the technique requires less extensive clean-up procedures to remove interfering compounds. Also, the separation of vitamins D_2 and D_3[5] and their corresponding isomers, photoisomers[6,7] and metabolites[8] can be achieved rapidly. The HPLC technique was originally applied to the determination of vitamin D in pharmaceuticals[9,10] but more recently

it has been used to determine vitamin D in fortified foods.[11] It is now possible to determine naturally occurring levels of vitamin D_3 in those foods which are the main source of the vitamin.

The HPLC method originally developed in this laboratory[12] for the determination of vitamin D_3 in foods followed the earlier gas - liquid chromatographic procedure in many respects. It involved the addition of vitamin D_2 to the sample as an internal standard followed by saponification and extraction of the unsaponifiable matter. Sterols were removed by precipitation and it was then necessary to isomerise vitamin D_2 and vitamin D_3 to their corresponding isotachysterols before any remaining interferences could be removed on columns of activated alumina. The isotachysterols were separated on HPLC columns containing either C_{22} or C_{18} reversed-phase packing material. This method had considerable advantages over the gas - liquid chromatographic methods but it still suffered from several disadvantages that became apparent when a survey to determine the vitamin D_3 content of hens' eggs was undertaken. The efficiency of extraction of the vitamin was very poor, the extracts prepared for HPLC analysis contained large numbers of ultraviolet absorbing compounds that made it difficult to identify the isotachysterols and, in addition, the HPLC separation of the isotachysterols of vitamin D_2 and D_3 was very difficult to achieve.

These difficulties were overcome by developing an improved procedure for the saponification and extraction of vitamin D and also an alternative procedure for purifying the sample extracts so that it was no longer necessary to isomerise vitamin D. Ascorbic acid was added as an antioxidant and nitrogen was also bubbled through the solution during saponification to reduce oxidation and to ensure a thorough mixing of the sample. Multiple ether extractions of the unsaponifiable matter which contained the vitamin D were carried out in order to improve the efficiency of extraction and saturated salt solution was used to wash the extracts in preference to water to minimise emulsion formation.

Various procedures for removing interfering compounds from sample extracts prior to HPLC have been reported including sterol precipitation,[13] conventional column chromatography,[14] thin-layer chromatography[15] and preparative HPLC.[16] Sterol precipitation was found to be useful as a preliminary clean-up procedure, but additional systems for cleaning up the extracts were also necessary. Various procedures were considered including the use of normal phase HPLC columns and Waters Associates Sep-pak cartridges. It was found that the HPLC column became contaminated very quickly and the cartridges were too small to cope with the relatively large volume of sample extract that had to be purified. A thin-layer chromatographic procedure was eventually found that gave satisfactory results. The main advantage of this approach is that it is possible to chromatograph a standard in parallel with the sample, thus permitting location of the very narrow band containing the vitamin D. Several thin-layer chromatographic systems were investigated but the system described by Johnson and Vickers,[17] involving silica-coated plates and hexane + dibutyl ether + butan-2-one (34 + 6 + 7) as the developing solvent, was found to give the most efficient separations when applied to the clean-up of egg extracts. This system has also been applied to the clean up of extracts from samples of butter, cheese and milk. Thin-layer chromatographic systems have been used for the isolation of vitamin D by many workers who report 95–100% recoveries, provided that the necessary precautions are taken to minimise degradation: the thin-layer plates are developed immediately the sample extracts have been applied to the plates and the vitamins are removed from the plates immediately they have been located.

Full details of the method for the determination of vitamin D_3 in eggs are presented below, together with an evaluation of the accuracy and precision of the method.

Experimental

Apparatus

All glassware must be rinsed thoroughly with ethanol before use.

Saponification and extraction

Heated water-bath.
Separating funnel, 1 l.
Conical flasks fitted with side arms, 500 ml.
Rotary evaporator and 500-ml *florentine flasks.*

Sterol precipitation

Porosity number 2 sintered glass funnel.

Thin-layer chromatography

Lined TLC tank.
Ultraviolet lamp for locating vitamins.

High-performance liquid chromatography

The HPLC system consisted of a solvent reservoir, a high-pressure pump, a stop-flow injection valve, a stainless-steel column (250 × 4 mm i.d.) packed with C_{22} reversed-phase packing (5 μm), a flow-through ultraviolet detector and a recorder. A Perkin-Elmer LC 75 stop-flow ultraviolet scanning and variable wavelength detector was used whenever possible for identification and checking on the purity of sample components as they eluted from the column.

Reagents

Saponification and extraction

Ascorbic acid. Analytical-reagent grade.
Ethanolic potassium hydroxide solution. Dissolve 42 g of potassium hydroxide in 143 ml of absolute ethanol and 7 ml of water.
Light petroleum, 40–60 °C boiling range. Glass distilled.
Diethyl ether. Glass distilled.
Phenolphthalein solution. A 1% solution in ethanol.
Saturated sodium chloride solution.

Sterol precipitation

Methanol + water (90 + 10). Use glass-distilled methanol.

Thin-layer chromatography

Silica coated TLC plates. Merck, Kieselgel 60 F_{254}, stored at 40 °C then run in developing solvent before applying the sample extract.
Developing solvent. Hexane + dibutyl ether + butan-2-one (34 + 6 + 7) containing 0.5% butylated hydroxyanisole (BHA) as antioxidant.

High-performance liquid chromatography

Standard solutions of vitamin D_2 and vitamin D_3. Prepared from pure standards (Sigma). Calculate the concentrations by ultraviolet absorption measurements, using the following extinction coefficients: vitamin D_2 $E^{1\%}_{1cm}$ 475 l mol^{-1} cm^{-1} at 265 nm in ethanol; and vitamin D_3 $^{1\%}_{1cm}$ 485 l mol^{-1} cm^{-1} at 265 nm in ethanol.
Mobile phase. Methanol + water (either 95 + 5 or 90 + 10). An efficient column could be used with methanol + water (95 + 5) although a less efficient column may require methanol + water (90 + 10), which will increase the retention time and resolution of the vitamins D_2 and D_3.

Method

Use vitamin D_2 as an internal standard if vitamin D_3 is to be determined and *vice versa*. Initially confirm the absence of the proposed internal standard from the sample and also ensure that no other compounds elute from the HPLC system with the same retention time. Concentrate samples of eggs (approximately 75% water content) and milk (approximately 90% water content) by freeze drying.

Saponification and Extraction

Weigh approximately 50 g of a representative sample into a 500-ml conical flask fitted with a side arm. Add an accurately measured amount of internal standard solution approximately equal to the expected level of vitamin D in the sample, 1 g of ascorbic acid and

150 ml of ethanolic potassium hydroxide solution. Saponify for 30 min under reflux on a boiling water-bath while bubbling a stream of nitrogen through the solution via the side arm of the flask. Cool the solution and transfer quantitatively into a 1-l separating funnel using 250 ml of water to aid the transfer. Extract twice with two 500-ml portions of light petroleum (boiling range 40–60 °C) + diethyl ether (1 + 1) shaking each time for at least 3 min. Wash the separate extracts with 250-ml portions of salt solution until the salt solution is neutral to phenolphthalein. (Gentle shaking and the presence of salt helps to prevent the formation of emulsions. However, during the washing of the first extract an emulsion may be formed and if so, it is advisable to extract this aqueous phase with another 500 ml of mixed solvent.) Transfer the combined washed extracts into a suitable rotary-evaporator flask using a few millilitres of ethanol to aid the transfer and evaporate to dryness. If the residue after evaporation appears cloudy, add a few millilitres of ethanol and re-evaporate to remove the water causing the effect.

Sterol Precipitation

Dissolve the extract in approximately 90 ml of methanol, warming if necessary, and add 10 ml of water. Cool the solution to 0 °C for 2–3 h and remove the precipitated sterols by filtration through a porosity No. 2 sintered glass funnel, washing the precipitate with a few millilitres of ice-cold methanol + water mixture (90 + 10). If the concentration of vitamin D in this extract is sufficiently high (greater than 5 μg per 100 ml), the extract may be divided. (Store one half at −20 °C for further analysis as necessary.) Transfer the other half into a suitable rotary-evaporator flask and evaporate to dryness for thin-layer chromatographic and then HPLC analysis.

Thin-layer Chromatographic Procedure

Dissolve the extract in approximately 500 μl of light petroleum (boiling range 40–60 °C) and apply approximately 450 μl of this solution in the form of a narrow band along 80% of the 10-cm lower edge of the silica plate. Add a solution containing vitamin D_2 and vitamin D_3 to the remaining 50 μl of the sample extract and apply as a narrow band on either side of the sample band. Develop in diethyl ether for a few minutes to concentrate the sample bands and then develop the plate in a solvent of hexane + dibutyl ether + butan-2-one (34 + 6 + 7) containing 0.5% of BHA as an antioxidant. When the solvent front has moved to the top of the plate, remove the plate from the tank and before the solvent evaporates locate the vitamin D in the spiked sample bands with an ultraviolet lamp. Limit the use of the lamp to prevent the decomposition of the vitamins. Remove the corresponding band of silica containing the vitamin D from the plate and extract the silica three times with 15-ml portions of HPLC-grade methanol. Combine the extracts and evaporate to dryness. At this stage the extract is ready for HPLC analysis; however, it may be necessary to repeat the thin-layer chromatographic procedure if the HPLC analysis of the extract shows that not all interfering compounds have been removed.

Transfer the extract from the thin-layer chromatographic procedure into a small specimen tube with light petroleum and evaporate the solvent on a dry block at 40 °C using a stream of nitrogen. Dissolve the extract in approximately 200 μl of HPLC-grade methanol containing 0.1% of BHA.

NOTE—

When applying the extract to the plate care must be taken to avoid contamination of the sample extract with the standard vitamin D solutions. The use of disposable glass capillary tubes is recommended.

HPLC Analysis

Chromatographic conditions

Use a stainless-steel column packed with Magnusil $C_{22}5X$, which will separate vitamins D_2 and D_3 and satisfy the system suitability test below, with the following conditions: mobile phase, methanol + water (either 95 + 5 or 90 + 10 depending on the efficiency of the HPLC column); flow-rate, 1–2 ml min^{-1}; chart speed, 5 mm min^{-1}; and detector, 265 nm, 10 mV output.

An HPLC column packed with Spherisorb S5 ODS2 and a mobile phase of either methanol - water or acetonitrile - chloroform has also been used successfully in this laboratory.

System suitability test

Inject a standard solution containing equal amounts of vitamins D_2 and D_3 dissolved in methanol on to the column. Set the chart speed so that the vitamin D peaks are about 2 cm wide at the base (see Fig. 1, a chromatogram of a standard solution of vitamin D_2 and D_3). The concentration of vitamins D_2 and D_3 should be similar to the concentration expected in the sample extracts.

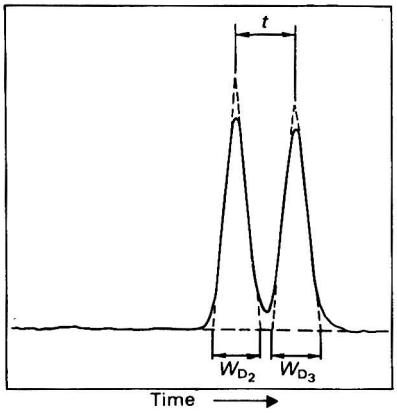

Fig. 1. System suitability test, chromatogram of a standard solution of vitamins D_2 and D_3. Stationary phase, Magnusil C_{22}; stainless-steel column (250 × 4 mm i.d.); mobile phase, methanol - water (95 + 5); flow-rate, 1 ml min^{-1}; ultraviolet detector at 265 nm.

Measure t, the distance between the two vitamin D peak apexes, W_{D_2} and W_{D_3}, the widths of the D_2 and D_3 peaks at the base line and calculate the resolution, R, of the peaks.

$$R = \frac{2t}{W_{D_2} + W_{D_3}}$$

The system is suitable if the resolution is equal to or greater than 0.8.

Response factor, Rf

Measure the peak heights of vitamin D_2 and D_3 in the system suitability test and calculate the response factor Rf:

$$Rf = \frac{\text{peak height } D_2}{\text{mass of } D_2} \times \frac{\text{mass of } D_3}{\text{peak height } D_3}$$

It is important to measure Rf regularly throughout the analyses in order to monitor any changes in chromatographic conditions.

Analysis of sample solution

Inject a suitable volume of the sample solution on to the column and record the chromatogram. Measure the peak heights of vitamin D_2 and vitamin D_3.

$$\text{Vitamin } D_3 \text{ content } (\mu g \text{ per } 100\ g) = \frac{M_1 \times H_1 \times Rf \times 100}{H_2 M_2}$$

where M_1 = mass (μg) of D_2 added as an internal standard;
M_2 = mass (g) of sample;
H_1 = height of the D_3 peak; and
H_2 = height of D_2 peak.

A Perkin-Elmer LC 75 ultraviolet scanning and variable wavelength detector was used whenever possible for identification of sample components as they eluted from the HPLC column. Base line corrections were performed by stopping the solvent flow at the base of the peak prior to scanning and calibrating the detector between 210 and 280 nm. Flow was then commenced until the peak maximum was reached when either ultraviolet scan or absorbance ratios were obtained. Identification was confirmed by comparison with pure standards and peak purity was confirmed by scanning at various times during the elution of the peak.

Assessment of Accuracy

The internal standard, either vitamin D_2 or D_3, is added to the sample at the beginning of the analysis. The accuracy of the method is therefore dependent on how accurately the internal standard is added to the sample and whether the ratio of vitamin D_2 to D_3 remains constant throughout the analysis. A series of experiments were conducted in order to confirm that the ratio of vitamin D_2 to vitamin D_3 does not vary.

(*i*) Standard solutions containing different ratios of vitamin D_2 and D_3 were prepared, aliquots were retained for HPLC analysis and the remaining solutions were subjected to saponification and extraction as described in the standard method. Results are shown in Table I.

TABLE I

HPLC DETERMINATION OF STANDARD SOLUTIONS BEFORE AND AFTER EXTRACTION

Height D_3/height D_2 before extraction	Height D_3/height D_2 after extraction	Recovery of D_3 relative to D_2, %
1.947	1.959	101
1.955	1.965	101
0.965	0.973	101
0.964	0.976	101
0.498	0.495	99
0.493	0.490	99

(*ii*) An aliquot containing a standard solution of vitamin D_2 and D_3 was also subjected to the TLC clean-up procedure and analysed by HPLC. Results are as follows: height D_3/height D_2 before thin-layer chromatography, 1.460; height D_3/height D_2 after thin-layer chromatography, 1.442; and the recovery of D_3 relative to D_2, 99%.

Assessment of Precision

Five replicate determinations of vitamin D_3 in a sample of freeze-dried egg were carried out in order to assess precision. Results are shown in Table II.

TABLE II

REPLICATE DETERMINATIONS OF VITAMIN D_3 IN A SAMPLE OF FREEZE-DRIED EGGS

	Vitamin D_3 content of whole egg/μg per 100 g
A	1.8
B	1.9
C	1.6
D	1.5
E	1.3
Mean	1.6
Standard deviation	0.2

Discussion

This improved method of vitamin D determination has been applied successfully to the analysis of a series of egg samples as part of a survey to determine the average vitamin D_3 content of eggs, to monitor any seasonal variations of this level and to determine any differences between the vitamin D_3 content of free-range and intensively produced eggs. Fig. 2

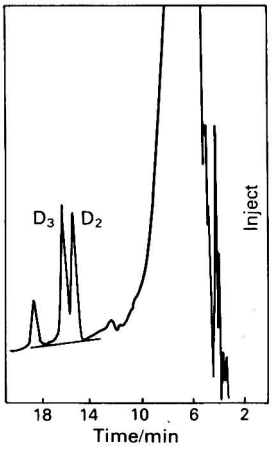

Fig. 2. HPLC analysis of egg extract. Conditions as in Fig. 1.

shows a typical chromatogram of the HPLC analysis of an egg extract. This is the first time that this technique has been applied to the determination of vitamin D_3 in eggs, most previously reported data was obtained nearly 40 years ago using biological assays.[18] Full details of these results will be presented in a separate report.[19] In this survey only vitamin D_3 concentration was measured. If total vitamin D activity had been required, the sum of vitamin D_3, 25-hydroxy-vitamin D_3 and any other vitamin D active metabolites present in the sample would have been measured.[20] A preliminary investigation has shown that a C_{22} reversed-phase column will separate 25-hydroxy-vitamin D_3 from vitamins D_2 and D_3.

This method has also been used successfully for the determination of vitamin D_3 in several samples of butter (0.8–2.0 μg per 100 g), two samples of milk (0.03 μg per 100 g) and a sample of cheese (0.5 μg per 100 g).

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Simple and Sensitive Technique for Investigation of Desorption Properties

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The technique of thermally stimulated pressure (TSP) is described and has been applied to study the state of bound water on crystallised lysozyme at hydration levels of 0–24 mg of water per gram of protein. If it is assumed that for the low densities used the molecules of water are bound to independent sites in the macromolecule of lysozyme, first-order kinetics can be used to fit the experimental pressure *versus* temperature graphs. The activation energy is 37.63 kJ mol^{-1}.

Keywords: *Bound water*; *lyszoyme*; *thermally stimulated pressure*; *activation energy*; *desorption*

Since the middle of the nineteenth century,[1] biologists have studied the state of water molecules in biological systems. In 1855 Nägeli[2] pointed out that some of the water in extruded cytoplasm from plant and animal tissues is bound to the constituent macromolecules. More recently several techniques and properties have been used to study the state of water in macromolecules and membranes: cryoscopy,[3] specific conductivity,[4] differential scanning calorimetry,[4] nuclear magnetic resonance spectroscopy,[5] dielectric relaxation,[6,7] X-ray spectrometry,[8] thermally stimulated depolarisation current,[9,10] sorption of water vapour[11,12] and others.[13–16] In spite of the existence of so much literature on the subject, few data on the energies involved in the process have been reported.

We describe here the technique "thermally stimulated pressure" (TSP), which consists in the measurement of the water vapour pressure, in a fixed volume, as a function of temperature while the sample is subjected to a linear heating rate. Graphs of pressure of water vapour *versus* sample temperature at different hydration levels are presented that give information about the activation energy and the corresponding relaxation time of the desorption process.

Theoretical

We assume that the water molecules are bound to different classes of sites, j, in the macromolecule, which are independent at these hydration levels (from 0–25 mg of water per gram of protein). Any physical quantity relative to a class will be denoted by the subscript j, and the main characteristics of a class of sites are its free energy of interaction with the water molecule, denoted by ΔF_j, and the transition probability, W_j, from the bound to the free state. The rate of desorption of water molecules from class j at a given temperature T, assuming first-order kinetics and a linear heating rate, *i.e.*, $dt = (1/b)dT$, where b is the heating rate will be

$$\frac{dN_j(T)}{dT} = -\frac{1}{b} W_j(T)\, N_j(T) \qquad (1)$$

We assume that the Arrhenius activation law[15] is valid:

$$W_j(T) = W_{j0}\exp(-\Delta F_j/kT) \qquad (2)$$

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where W_{jo} is the characteristic frequency of the desorption process, k the Boltzmann constant and

$$\Delta F_j = \Delta E_j - T\Delta S_j \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

ΔE_j is the activation energy and ΔS_j is the activation entropy. At the initial state of the process ($T = T_o$), all molecules are bound. At a given temperature, $T > T_o$, the conservation law gives

$$N_j(T) + N_{tj}(T) = N_{oj} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where $N_{tj}(T)$ is the number of free water molecules and N_{oj} the total number of water molecules. The solution of equation (1), with the law (2), is

$$N_j(T) = N_{oj} \exp\left[-\frac{1}{b} \cdot W_{jo} \int_{T_o}^{T} \exp(-\Delta F_j/kT')\, dT'\right] \quad .. \quad .. \quad (5)$$

The pressure $P(T)$ measured inside the flask is given by

$$P_j(T) = \frac{N_{tj}(T)}{V} .kT_w \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where V is the volume of the flask and T_w is the temperature of the wall of the flask (the temperature of the water vapour). Solving equation (4) for N_{tj}, using (5), we obtain

$$P_j(T) = P_{tj}\left\{1 - \exp\left[-\frac{1}{b} .W_{jo} \int_{T_o}^{T} \exp(-\Delta F_j/kT')\, dT'\right]\right\} \quad .. \quad (7)$$

where $P_{tj} = \frac{N_{oj}kT_w}{V}$ is the final pressure when all molecules of class j are free. The measured pressure, $P(T)$, will be a sum over j of expressions such as equation (7):

$$P(T) = \sum_j P_j(T) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

Differentiating equation (7) with respect to T, and considering equation (3), we obtain, for one class of sites

$$L = \left[\frac{b}{P_{tj} - P_j(T)}\right] \frac{dP_j(T)}{dT} = W_{jo} \exp(\Delta S_j/k) \exp(-\Delta E_j/kT) \quad .. \quad (9)$$

By plotting the logarithm of the left-hand side of equation (9) against $1/T$, the activation energy can be determined. On extrapolating to infinite temperature we obtain $W_{jo}\exp(\Delta S_j/k)$.

Experimental

Commercial lysozyme (Grade 1) was obtained from Sigma (crystallised three times, dialysed and lyophilised) and was used as received. Tablets, approximately 1 × 13 mm diameter, were made by compressing the powder in a Carver, Model C, laboratory press. The best tablets were obtained with a density of 0.9 g cm^{-3}. To obtain a better result it was necessary to dehydrate the powder before and during the pressing. The main reason for choosing lysozyme powder was its high resistance to thermal denaturation and for continuous cycles of sorption and desorption of water molecules.[14] The dry state of the tablets was obtained by extrapolating to zero time the graphs of mass *versus* the hydration time. Before being weighed, the samples were left to dehydrate under vacuum for 24 h at 70 °C. The "humid" state was obtained by leaving the tablets to hydrate on the balance plate.

The tablet support and the heater system are shown in Fig. 1. The heater system consisted of a resistance (*ca.* 700 Ω) inserted in a porcelain cylinder, connected to a variable a.c. source with a suitable voltage (25–50 V) to obtain a constant heating rate of 0.10 K s^{-1}. The

tablet support consisted of a hollow cylinder, in which the heater system was inserted. The upper part (185 mm high) and the walls (1 mm thick) were made of stainless steel; the lower part (35 mm high) had a parallelepiped shape and was made of copper to facilitate heat conduction. The tablet support was inserted in a cylindrical glass plate (100 mm diameter, 20 mm high) for access to the wiring (see Fig. 2). The system, as shown in Fig. 2, will rest on the mouth of a Duran 50, Schott Mainz Jena ER Glas, flask (5 l). The mouth of the flask was ground and a flat brass ring with the same diameter, possessing a circular guide for insertion of a rubber ring to prevent leaks, was attached to it with Araldite epoxy resin. The whole flask was immersed in a solid thermostat maintained at 200 °C to prevent sorption of water molecules on to the walls of the flask.

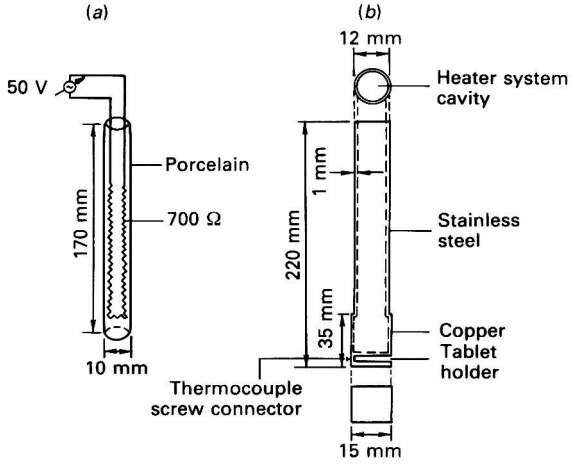

Fig. 1. (*a*) Heater system, single resistance inside heater system cavity to vary temperature. (*b*) Tablet support, holder chosen to minimise any temperature gradient in sample.

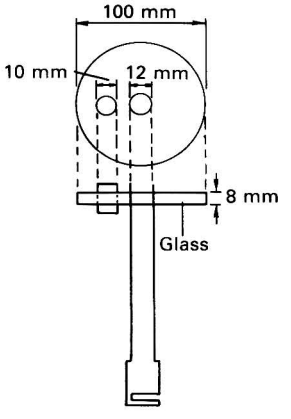

Fig. 2. Top of 5-l flask that contains sample. Walls of the flask kept at 200 °C to avoid adsorption of water molecules.

The pressures at sample temperatures were recorded with a Hewlett-Packard 700 4B *X* - *Y* recorder. The flask had a lateral exit on its neck which was connected through a rubber tube to a stainless-steel T-connector, one end of the connector being coupled to a mechanical pump and the other to a Varian NRC 531 thermocouple vacuum gauge which was energised by a Varian 856-A source. This system was previously calibrated with a Stockes McLeod gauge. There were two more thermocouples (copper - constantan thermocouples, Leeds & Northrup Co.): one was connected through a screw to the tablet support (see Fig. 1) and allowed measurement of the temperature of the sample; the other was left free inside the flask to measure the temperature of the water vapour, which was identical with that of the wall of the flask. Both were connected to Philips DC PM2436 micrometers.

Results and Discussion

The first molecules adsorbed on a dehydrated macromolecule of lysozyme (bound-water molecules) are connected by hydrogen bonds to hydrophilic amino acids on the surface. These bonds are:

$$\text{O—H} \cdots \text{O}$$

$$\text{N—H} \cdots \text{O}$$

Lysozyme, like the globulins, conforms to the principle of "hydrophobic in, hydrophilic out" or the oil-drop model of a protein. After completing the bonds with the macromolecules, the water molecules start to form monolayers[13]; 300 mg of water per gram of protein are sufficient to cover the surface of the lysozyme molecule with a single monolayer of water.[8]

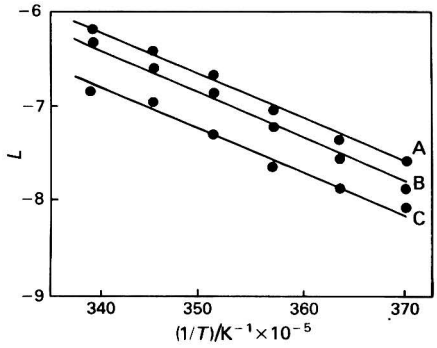

Fig. 3. Representation of equation (9). A, $h = 9.0$; B, $h = 14.0$; and C, $h = 24.0$ mg of water per gram of protein.

Fig. 3 shows the graph of L *versus* $1/T$ [equation (9)], from which the parameters to be used in equation (7) can be calculated. Solving equation (7) then gives the solid points shown in Fig. 4 together with the experimentally measured graphs. It is important to note, however, that equation (9) can be applied only to monomolecular kinetic processes with one activation energy and one corresponding relaxation time, which means one class of sites. In the general case, with several classes of sites, the adjustment of the parameters is more complicated and a numerical programme is needed in order to minimise the mean square errors in an iterative method using the general equation (8).

With linear heating of the hydrated samples (250-370 K), the hydrogen bonds break. In Fig. 4, the continuous lines show the experimental pressure of water vapour formed by desorption of bound water molecules *versus* the sample temperature.

The energy obtained, 9.03 kcal mol^{-1}, agrees with the Monte Carlo simulation of Hagler and Moult[15] for waters in close contact with the protein and within 10% of the average energies for six water molecules inside the lysozyme enzyme calculated by Clementi *et al.*[16] Finally, we should point out that the ratio of the hydrations must be equal to the ratio of final pressures for any pair of curves, $h_i/h_j = P_{t_i}/P_{t_j}$. This is true within an error of 4%.

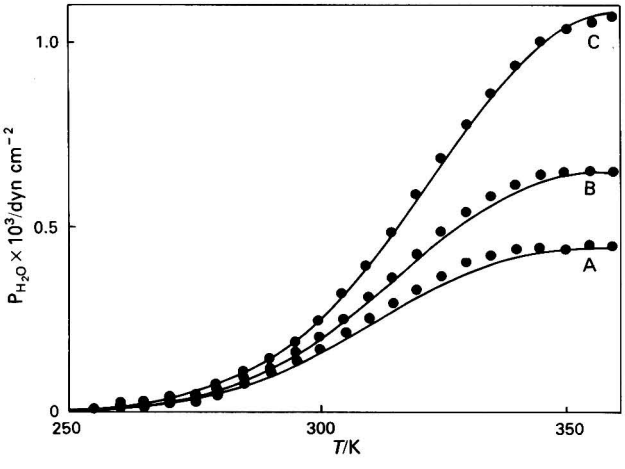

Fig. 4. Vapour pressure of water *versus* sample temperature. Full lines show experimental curves. The circles show the results obtained by the present model, which uses one class of sites. The agreement is very reasonable. A, $h = 9.0$; B, $h = 14.0$; and C, $h = 24.0$ mg of water per gram of protein.

These results were compared with thermogravimetric analysis (TGA) by application to the same system. This technique consists in the continuous measurement of the mass of a sample as a function of the linear increase in temperature. Owing to the high sensitivity of the thermogravimetric scale (Rigaky type, Cat. No. 8076D1) it is possible to detect the variations observed in TSP measurements.

Using the same theoretical basis we have from the sample mass

$$N(T) = N_0[m(T) - m_f] \quad .. \quad .. \quad .. \quad .. \quad (10)$$

where N_0 is Avogadro's number, $m(T)$ is the sample mass at temperature T and m_f the final mass when the sample is dehydrated. For only one class of sites, substituting $N(T)$ given by equation (10) in equation (1), we obtain

$$W(T) = b\frac{d}{dT}\left\{\ln[m_f - m(T)]^{-1}\right\} \quad .. \quad .. \quad .. \quad (11)$$

Therefore, by applying this expression to TGA curves, the energy and corresponding relaxation time can be determined. The results obtained with TGA agree with those of TSP, as shown in Table I.

TABLE I

PARAMETERS OBTAINED BY TSP AND TGA TECHNIQUES

Hydration/mg of water per gram of protein	Heating rate/ K s^{-1}	ΔE/eV		$(1/W_{10})\exp(-\Delta S_1/k)$/s $\times 10^{-4}$
		TSP	TGA	
9.0	0.101	0.388		1.15
14.00	0.103	0.388		1.43
24.00	0.100	0.388		2.05
40.00	0.035		0.380	3.00
60.00	0.034		0.340	3.20

In conclusion, the technique of thermally stimulated pressure can clearly provide valuable information on the dehydration properties of biological materials. The sensitivity of the technique is high and it can be readily carried out using simple apparatus at a relatively low cost.

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SHORT PAPERS

Differential-pulse Polarographic Determination of Titanium in Steel

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Keywords: Steel analysis; titanium determination; trace metal analysis; differential-pulse polarography

The widespread use of titanium in steel alloys makes an accurate and sensitive method for its analysis very desirable for production control.

Numerous organic reagents are known for the spectrophotometric determination of titanium,[1] but as the major constituents of steel alloys (for instance iron, chromium, nickel and vanadium) can also form coloured compounds, a time-consuming operation is often necessary to remove the interferents or isolate the titanium.

In order to improve sensitivity, both atomic-absorption spectrometry[2–4] and inductively coupled plasma[5,6] spectrometry have been applied, but titanium lines suffer serious interference from many elements, so a suitable means of eliminating or suppressing them must be found.

Voltammetric techniques have also been applied for the determination of titanium in steels[7,8] but no advantage was taken of the catalytic activity of the titanate ions in acidic oxalate solution containing chlorate. When using this type of supporting electrolyte, titanium(IV) gives an exceptionally high polarographic current, owing to its catalysed electro-reduction, and the well shaped polarographic wave is suitable for trace analysis.

The method described here is based on the dissolution of the steel alloy in a mixture of hydrofluoric and nitric acids, which are then removed by evaporation to dryness. Sulphuric acid is added to the residue and electrolysis over a mercury cathode is performed in order to remove iron. The other constituents of the supporting electrolyte are then added and the differential-pulse polarographic determination is carried out.

Experimental

Apparatus

An AMEL (Milan, Italy), Model 472, Multipolarograph was used. A forced drop time of 2 s was imposed on the dropping-mercury electrode (D.M.E.). An Ingold, Model Pt–805/NS, platinum-ring counter electrode and an Ingold, Model 303/NS, saturated calomel reference electrode (S.C.E.) were used. The differential-pulse polarographic conditions used were a pulse height of 100 mV and a scan-rate of 2 mV s^{-1}.

The solutions were de-aerated with pure nitrogen for 5 min before the polarographic analysis. All measurements were carried out at 25.0 $\pm$ 0.1 °C.

The steel samples were dissolved in PTFE test-tubes, fitted with T 29/32 sockets, by means of a sample dissolution device (Berghof, Tubingen, FGR) that consists of an aluminium heating block (in which the PTFE test-tubes can be inserted) whose temperature is programmed by means of a TP-regulator. The device is also fitted with T 29/32 cones adapted for passing a stream of filtered air into the test-tubes.

The electrolysis over a mercury cathode was performed in a Metrohm EA 1044–20 polarographic cell, connected to a suitable d.c. power supply, at 40 $\pm$ 1 °C.

Reagents

Erbatron electronic-grade chemicals were used. The potassium chlorate was supplied by Merck (Darmstad, GDR).

Normal precautions for trace analysis were taken throughout.

Reagents and solutions were stored in polyethylene bottles; polyethylene calibrated equipment was used throughout in order to prevent leaching. PTFE vessels were filled with 1 + 1 sulphuric acid, left overnight and rinsed thoroughly with doubly distilled water before use in order to remove every trace of titanium.

In order to avoid pollution, it is advisable to keep a set of equipment to be used for titanium determination only and to wash it after use with running distilled water until required again.

Working standards were preparated daily by diluting a stock titanium(IV) solution (1000 μg ml^{-1}) prepared by dissolving titanium(IV) chloride in 2 M hydrochloric acid (Normex, atomic-absorption standard; Carlo Erba, Milan).

Study of Polarographic Working Conditions

A few papers, dealing with the catalytic activity of titanate ions in acidic oxalate solution containing chlorate were found.[9–11]

In order to define the best working conditions, with regard to the composition of the supporting electrolyte, the effect of the oxalic acid and potassium chlorate concentrations and the pH on the shape on the catalytic reduction wave were examined. It was found that the reduction potential of the titanate ion in acidic oxalate solution is not influenced by the presence of chlorate and the reduction wave takes place at $E_{\frac{1}{2}} = -0.26$ V (*versus* S.C.E.), as is the case in the absence of chlorate. The above observations suggest that the reduction of the titanate ion - oxalate complex is catalysed by the chlorate ion. Potassium chlorate alone in the same supporting electrolyte does not exhibit any wave in this region.

The peak current was found to be linearly dependent on the titanate ion concentration at the part per billion (10^9) or the low part per million level.

TABLE I

VARIABILITY OF THE DIFFERENTIAL-PULSE POLAROGRAPHIC RESPONSE AS A FUNCTION OF POTASSIUM CHLORATE ADDITION

Supporting electrolyte: 0.2 M oxalic acid - 0.25 M sulphuric acid. Polarographic conditions as reported in the procedure.

Potassium chlorate added/M	0	10^{-4}	10^{-3}	10^{-2}	5×10^{-2}	7.5×10^{-2}	10^{-1}	2.5×10^{-1}	5×10^{-1}
Diffusion current increase*	1	1.0	1.9	6.5	16.8	22.8	24.3	23	14

* Peak height with $KClO_3$ addition/peak height without $KClO_3$.

The peak height increase depends on the potassium chlorate concentration. Table I shows the variability of the differential-pulse polarographic peak of titanium(IV) in 0.2 M oxalic acid - 0.25 M sulphuric acid when variable amounts of potassium chlorate are added. The chlorate effect is evident, 0.1 M potassium chlorate solution giving the best results. Above this concentration the peak seems to decrease and eventually splits. Table II shows the variability of the differential-pulse polarographic peak of titanium(IV) in 0.1 M potassium chlorate - 0.25 M sulphuric acid when variable amounts of oxalic acid are added. An oxalic

TABLE II

VARIABILITY OF THE DIFFERENTIAL-PULSE POLAROGRAPHIC RESPONSE AS A FUNCTION OF OXALIC ACID ADDITION

Supporting electrolyte: 0.1 M potassium chlorate - 0.25 M sulphuric acid. Polarographic conditions as reported in the procedure.

Oxalic acid added/M	0	10^{-4}	10^{-3}	10^{-2}	4×10^{-2}	10^{-1}	2×10^{-1}	3×10^{-1}
Diffusion current increase*	1	2.0	3.1	4.5	6.0	8.1	10.0	10.0
$E_{\frac{1}{2}}$/V	−0.41	−0.41	−0.35	−0.29	−0.28	−0.27	−0.26	−0.26

* Peak height with $KClO_3$ addition/peak height without $KClO_3$.

acid concentration of 0.2 M is sufficient to give the best results; 0.3 M is the highest concentration possible, owing to the limited solubility of oxalic acid in acidic potassium chlorate solutions.

Table II also shows the reduction potential change as a function of the oxalic acid concentration.

Other complexing agents, such as ethylenediamminetetraacetic acid (EDTA) or citric acid, formed less sensitive complexes with titanium(IV).

The pH value produces a marked effect on the titanium(IV) - oxalic acid complex. As shown in Fig. 1, a pH value of 0.9, corresponding to 0.25 M sulphuric acid in 2×10^{-1} M oxalic acid solution, is the best choice.

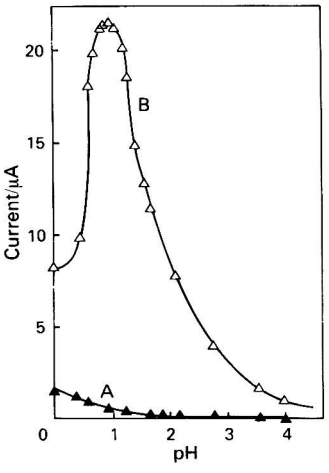

Fig. 1. Effect of pH on the Ti(IV) - oxalic acid complex. Supporting electrolyte, 0.1 M potassium chlorate - 0.2 M oxalic acid. A, Blank; and B, 0.5 μg ml^{-1} of Ti(IV).

Effects of Diverse Metals

Nineteen elements, aluminium, antimony, arsenic(III) and -(V), bismuth, chromium(III) and -(VI), cobalt, copper, iron, lead, manganese, molybdenum, nickel, niobium, phosphorus, silicon, tin, tungsten, vanadium and zinc, which are commonly encountered in steel, were tested. Table III shows the effect of the interfering ions. No interferences were observed in the presence of over 200-fold excesses by mass of the other metals mentioned above.

Only iron, as a major constituent of the sample, will normally be present in greater excesses than those considered. It was found that when overcoming a 1500-fold excess of iron there was positive interference in the titanium determination. Therefore, only steel alloys having a titanium content greater than 0.1% can be analysed directly. When the titanium content is less than 0.1%, the large excess of iron, and possibly other interfering ions, can be removed easily by electrolysis over a mercury cathode.

TABLE III

INTERFERENCES DUE TO FOREIGN SPECIES

Ion	Excess by mass over titanium (1 μg)	Error, %
Antimony(III)	2.5	+11
Molybdenum(VI)	12.5	+6
Copper(II)	30	+4
Chromium(III) and -(VI)	150	−3

Separation of Titanium by Electrolysis

When steels require an accurate chemical analysis it is often necessary to separate the complex steel matrix to avoid interaction effects. Among the separation methods commonly available, electrolysis over a mercury cathode appears to be the best. This technique is particularly suitable for separating iron, cobalt, nickel, copper, chromium, *etc.*, from elements such as aluminium, titanium and vanadium. Electrolysis also prevents the contamination effects due to precipitation reactions or batch solvent extraction procedures and it permits solution volumes to be kept small.

A simple electrolysis cell is quite satisfactory and a current density at the cathode of about 0.2 A cm^{-2} is sufficient to give a high deposition rate without causing an undue temperature rise in the solution.

Mercury cathode electrolysis is performed in 1.25 M sulphuric acid; after its completion suitable amounts of potassium chlorate and oxalic acid solutions are added and polarographic measurement is carried out.

The sulphuric acid concentration proved not to be critical; at the value chosen it prevented titanium hydrolysis.

Procedure

Place 10–100 mg of the steel in a PTFE test-tube; add 2 ml of 65% nitric acid and 5 ml of 12 M hydrofluoric acid. The reaction starts spontaneously. Plug the test-tube with the PTFE cone adapted for passing filtered air and insert the tube into the sample dissolution device. Bring the temperature to 100 °C and wait for 10–15 min in order to complete the sample dissolution, then pass a stream of filtered air through the tube for 2 h. Only a small residue remains.

Add 25 ml of 1.25 M sulphuric acid and shake well in order to dissolve any residue, then transfer the solution into a cell fitted with a mercury cathode and a platinum-disc anode.

Carry out the electrolysis for 30 min, with a current density of 0.2 A cm^{-2}, then without switching off the electrolysis current transfer 5 ml of the solution into a polarographic cell. Add 10 ml of 0.25 M potassium chlorate solution and 10 ml of 0.5 M oxalic acid solution, then de-aerate for 5 min and record the polarogram from 0 V to −0.7 V (*versus* S.C.E.).

Record another polarogram using the same procedure, with the same amount of sample, to which a suitable amount of titanium had been added before the dissolution. The amount of the standard titanium solution added to the sample should be such that the addition approximately doubles the titanium content of the weighed sample. The titanium content of the unknown sample can be calculated as in the standard addition method.

Results

The described procedure was tested on several certified standard steels. Some results are shown in Table IV. As can be seen, the proposed procedure allows good accuracy for samples covering a wide range of titanium concentrations. When the prescribed conditions are used, the proposed procedure permits the determination of titanium at concentrations as low as 2–5 $\mu g\ g^{-1}$, with relative standard deviations of about 4–5%. The calibration graph is linear and reproducible up to titanium concentrations of at least 1 $\mu g\ ml^{-1}$.

As wide differences may occur in steel alloy composition, it was preferred to add known amounts of titanium directly to the sample in order to have an internal standard subjected

TABLE IV

ANALYSIS OF STEELS

Sample	Titanium, % Certified	Titanium, % Found	No. of determinations	Relative standard deviation, %
BCS 321 (mild steel)	0.13	0.128	20	2.0
BCS 451 (mild steel)	0.096	0.098	20	2.5
BCS 453 (mild steel)	0.016	0.015	20	4.0
NBS 19g (acid open-hearth steel)	0.027	0.025	20	4.0
NBS 348 [Ni 26–Cr 15 (A286)]	2.24	2.24	20	1.7
NBS 361 (AISI 4340 steel)	0.02	0.019	20	4.2

to the same procedure as the sample. In such a way, any effect due to highly refractory compounds that may be formed due to matrix characteristics is avoided.

When steels are dissolved in hydrofluoric - nitric acid mixtures, some carbides can remain undissolved: the addition of some potassium permanganate helps to oxidise these compounds without affecting the method. In general, the dissolution procedure chosen should depend on the type of steel to be analysed.

Owing to the catalytic nature of the electrochemical process and the enhanced sensitivity peculiar to the differential-pulse polarography the method seems suitable for trace analysis. The procedure is simple and suitable for routine analysis.

The authors thank Professor F. Zignani for helpful discussions and appreciation is expressed to the Accademia Nazionale dei Lincei for a Fellowship held by one of us (D.F.).

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High-performance Liquid Chromatographic Determination of Oxprenolol Hydrochloride and Metoprolol Tartrate in Tablets and Injections

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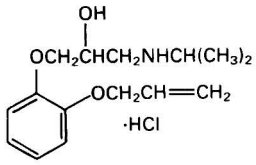
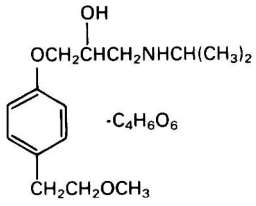
Keywords: *Oxprenolol hydrochloride determination*; *metoprolol tartrate determination*; *tablets*; *injections*; *high-performance liquid chromatography*

Oxprenolol and metoprolol are beta-adrenoceptor blocking agents widely accepted in the treatment of hypertension, angina and cardiac arrhythmias. They lower blood pressure by diminishing or preventing beta-adrenergic stimulation produced by such factors as exertion or emotion. Despite their widespread use there are no rapid, simple and precise methods for their quality control. There is no monograph on metoprolol tartrate in the current edition of the British Pharmacopoeia.[1] A British Pharmacopoeia monograph on Oxprenolol Hydrochloride Tablets BP involves a lengthy double extraction procedure followed by evaporation of organic solvent, dissolution of the residue in dilute hydrochloric acid and spectrophotometric determination. The reported gas-chromatographic procedures for metoprolol[2,3] and oxprenolol[4] and the high-performance liquid chromatographic (HPLC) procedure for oxprenolol[5] are lengthy as they are meant for their determinations in biological fluids. This paper describes a simple HPLC procedure for determining these drugs in tablets and injections.



Oxprenolol hydrochloride



Metoprolol tartrate

Experimental

Materials and Reagents

Oxprenolol hydrochloride and metoprolol tartrate drug substances were received from Ciba-Geigy Ltd. Amethocaine Hydrochloride BP, analytical-reagent grade methanol and ammonia solution (sp. gr. 0.88) and distilled water were also used.

Apparatus

A reversed-phase column, 250 × 5 mm i.d., 10-μm Bondapak C_{18} (Waters Associates Ltd.), was used in conjunction with a septumless injector (Waters, Model U6K), a variable wavelength detector (Waters, Model 450), a pump (Waters, Model 6000A) and a recorder (Smiths Servoscribe, Model RE 571–20).

Procedure

Samples were diluted with a solution of an internal standard (IS) to give an expected concentration of 0.1% of the drug. A 0.014% *m/V* solution of Amethocaine Hydrochloride BP in methanol - water (50 + 50 *V/V*) was used as the internal standard.

Oxprenolol

Tablets. Weigh and powder 20 tablets. To an amount of the powder equivalent to 20 mg of drug, add 20.0 ml of internal standard. Shake well for few minutes and centrifuge. Use the supernatant liquid for HPLC analysis.

Injection (dry ampoule). Dissolve the contents of 10 ampoules in 20 ml of internal standard solution.

Metoprolol

Tablets. Weigh and powder 20 tablets. To an amount of the powder equivalent to 25 mg of drug add 25.0 ml of internal standard solution. Shake well for few minutes and centrifuge. Use the supernatant liquid for HPLC analysis.

Injection. Mix the contents of 10 ampoules. Dilute 2.0 ml of the contents with 2.0 ml of internal standard solution. (In this instance the expected concentration of the drug will be 0.05%.)

HPLC conditions

Column, 250 × 5 mm i.d., 10-μm Bondapak C_{18}; mobile phase, methanol - water (75 + 25 *V/V*) containing 0.3% *V/V* ammonia for oxprenolol or 0.2% *V/V* ammonia for metoprolol; flow-rate, 2.0 ml min^{-1}; injection volume, 10 μl; chart speed, 120 mm h^{-1}; wavelength, 273 nm; detector range, 0.1 a.u.f.s.; temperature, ambient.

Standard solutions of drugs were prepared by dissolving 20.0 mg of the drug substance in 20.0 ml of internal standard solution. In preparing a standard solution for metoprolol injection, 2.0 ml of the sample were replaced by 2.0 ml of water containing 2.0 mg of the drug substance. Standards were analysed in a similar way to the samples. Two injections were made of each sample. The results were calculated as follows.

$$\text{Drug (\%)} = \frac{\text{average peak-height ratio of drug to IS in sample}}{\text{average peak-height ratio of drug to IS in standard}} \times \text{concentration of standard} \times \text{dilution factor}$$

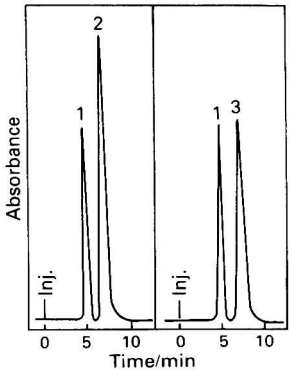


Fig. 1. Chromatograms of tablet sample extracts: 1, amethocaine hydrochloride; 2, oxprenolol hydrochloride; and 3, metoprolol tartrate.

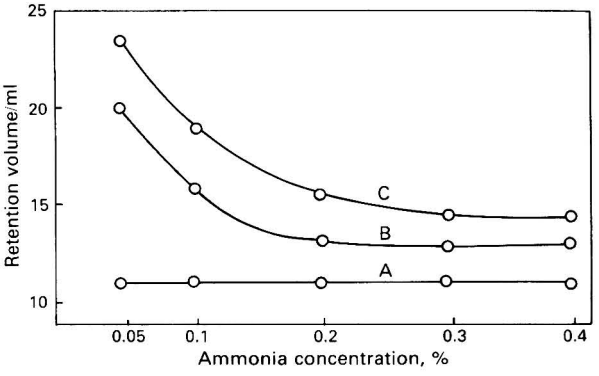


Fig. 2. Effect of ammonia concentration on elution volumes of A, amethocaine hydrochloride; B, metoprolol tartrate; and C, oxprenolol hydrochloride.

From this equation the amount of drug per tablet was calculated, the sample mass and the average mass of a tablet being taken into consideration.

Results and Discussion

The conditions applied in the experiment gave base-line peaks for the drug and internal standard. This is shown in Fig. 1. The retention times for amethocaine hydrochloride, oxprenolol hydrochloride and metoprolol tartrate were 300, 450 and 450 s, respectively. The change in concentration of ammonia from 0.05 to 0.4% V/V in the mobile phase had a significant effect on the elution of the drugs but not on the internal standard (Fig. 2).

TABLE I

DETERMINATION OF OXPRENOLOL HYDROCHLORIDE AND METOPROLOL TARTRATE IN TABLETS AND INJECTIONS

Sample	Drug content, %* Proposed method	BP method
Oxprenolol hydrochloride—		
Tablets		
20 mg	102.0	101.0
40 mg	100.0	98.0
80 mg	99.9	99.0
160 mg	100.0	99.0
160 mg slow release	98.0	101.0
160 mg†	98.0	99.0
Injection		
2-mg dry ampoule	100.0	—‡
Metoprolol tartrate—		
Tablets		
50 mg	97.5	—‡
100 mg	97.9	—‡
100 mg§	101.0	—‡
100 mg¶	99.5	—‡
200 mg	101.0	—‡
Injection		
1 mg ml^{-1}	98.6	—‡

* With respect to the content claimed; average of two determinations.
† Contains 0.25 mg of cyclopenthiazide.
‡ No BP methods available for these products.
§ Contains 12.5 mg of hydrochlorothiazide.
¶ Contains 12.5 mg of chlorthalidone.

Retention times and volumes were measured from the point of injection. The oxprenolol tablets were also analysed by the BP[1] method for comparison. The combined results are shown in Table I.

For both drugs the sample to internal standard peak-height ratios were found to be rectilinear in the concentration range 0.02–0.2% *m/V* with regression coefficients of 0.999. Reproducibility experiments were performed on 20-mg oxprenolol hydrochloride and 50-mg metoprolol tartrate tablets. In each instance ten separate determinations gave a coefficient of variation of 1.1%. In each formulation the sample chromatogram correlated very well with the standard chromatogram. When 40-mg oxprenolol hydrochloride and 100-mg metoprolol tartrate tablets were replaced by matching placebos, no peaks from non-active ingredients were observed at the retention times of the drugs or internal standard. The thiazide diuretics commonly used in combination with these drugs (Table I) do not interfere with the analysis. They are present in very small amounts in comparison with the drugs in question and elute with the solvent peak. Portions of aqueous tablet extracts (without internal standard) of both drugs were heated at 100 °C for 2 h and 75 °C for 48 h. The solutions were cooled, brought to the original volume with water and chromatographed along with the unheated solutions. No new peaks in the heated samples nor any changes in the original peaks were detected. The presence of ammonia in the mobile phase did not cause a reduction of the column efficiency. For each drug the time required for the chromatography is less than 10 min. No lengthy sample preparation is required and the results for oxprenolol tablets show no significant difference between the proposed and the BP[1] method at the 95% confidence level. As few manipulations are required, the method may be automated.

The author thanks Ciba-Geigy Ltd. for kindly providing oxprenolol hydrochloride and metoprolol tartrate drug substances and placebo tablets, Astra Pharmaceuticals Ltd. for metoprolol tartrate placebo tablets and Mr. B. Midcalf for his interest in the project.

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Selective Complexometric Determination of Palladium Using Pyridine as Releasing Agent

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Keywords: *Selective complexometry*; *palladium determination*; *pyridine release*

Existing complexometric methods[1–6] for palladium have mostly dealt with its determination in pure solution or in the presence of platinum group metals, which do not normally react with EDTA. Determination of palladium in the presence of common metal ions, using some selective masking agent, could be useful in the analysis of its alloys with copper and nickel, which are widely used as contact materials, and those with rare earths, which are used as magnetic materials. However, no work on these lines was carried out until a few years ago

when we proposed the use of dimethylglyoxime[7] for the selective masking of palladium in the presence of various foreign metal ions. Subsequently we employed 1,2,3-benzotriazole,[8] 1,10-phenanthroline[9] and thiourea[10] for the same purpose. The present paper describes the use of pyridine for the quantitative release of EDTA from the palladium - EDTA complex in a weakly acidic medium. The liberated EDTA is titrated against standard lead(II) nitrate solution with xylenol orange as indicator. A large number of metal ions do not interfere.

Experimental

Reagents

All of the chemicals used were of analytical-reagent grade. A stock solution of palladium was prepared by dissolving 1 g of palladium chloride (Johnson - Matthey Chemicals) in the minimum amount of hydrochloric acid (sp. gr. 1.18) and diluting to 1 l. This solution was standardised before use. EDTA solution (0.01 M), lead(II) nitrate solution (0.01 M), xylenol orange (0.1%) and pyridine (50%) solutions were made from BDH analytical grade reagents. Sodium acetate - acetic acid buffer was prepared in the usual way. Suitable salts of other metal ions were used as a source of these ions in solution.

Procedure

Determination of palladium in the presence of other cations

To a solution containing 3–30 mg of palladium and various amounts of foreign metal ions, which is placed in a 250-ml conical flask, add excess of 0.01 M EDTA solution and dilute the mixture to about 100 ml with distilled water. Adjust to pH 4–5 with dropwise addition of dilute sodium hydroxide solution and finally to 5–5.5 with sodium acetate - acetic acid buffer. Add a few drops of xylenol orange indicator solution and back titrate the excess of EDTA with 0.01 M lead(II) nitrate to a sharp, yellow to red, colour change. Add 1–10 ml of 50% pyridine solution (0.5 ml of pyridine for each 3 mg of palladium) to the flask and heat the contents on a water-bath at 60 °C for 10 min. Cool and add 5 ml of 10% acetic acid. Titrate the liberated EDTA with 0.01 M lead(II) nitrate solution to the same colour change as in the first titration. The results are given in Table I.

Determination in alloys

Dissolve 0.1–0.2 g of alloy sample in the minimum volume of aqua regia and dilute to 100 ml in a standard calibrated flask. Pipette a suitable aliquot into a conical flask, add excess of 0.01 M EDTA solution and dilute to 100 ml. Adjust the pH to 5–5.5 and proceed with the determination of palladium as was described above. The results for alloys are given in Table II.

Results and Discussion

Kragten[11] recently reported the stability constant of a palladium(II) - EDTA complex as being 26.4. Palladium(II) is known to form a strong complex, having a composition $Pdpy_2Cl_2$, with pyridine.[12] Stability constant data for this complex is not given in the available literature; however, from the fact that pyridine releases EDTA from a palladium(II) - EDTA complex, it is evident that palladium(II) forms a stronger complex with pyridine. Common metal ions, on the contrary, form very weak complexes with pyridine.[13] The strongest among these is that with copper(II), its stability constant being 6.54. Thus, the EDTA complexes of other metal ions are more stable than their respective pyridine complexes, whereas, with palladium, the reverse is true and hence EDTA is selectively released by pyridine from palladium(II) - EDTA. Derivatives of pyridine such as picoline, lutidine, collidine and piperidine are likely to yield a similar effect. Some of them may prove to be superior to pyridine and their use is being investigated.

From the initial experiments, carried out to evaluate the optimum conditions for the quantitative decomposition of the palladium - EDTA complex, it was noted that 0.5 ml of pyridine effected a quantitative release of EDTA from 3 mg of palladium. The addition of pyridine caused an upward shift in pH, which for most of the solutions lay between 6 and 6.5. From a set of experiments it was found that the pH could be brought back to 5–5.5, for the second titration, by adding 5 ml of 10% acetic acid.

The results given in Table I show that the present titration is selective for palladium in the presence of copper(II), nickel(II), zinc(II), lead(II), cadmium(II), cobalt(II), aluminium(III), iron(III), bismuth(III), tin(IV), titanium(IV), zirconium(IV) and the rare earths.

TABLE I

DETERMINATION OF PALLADIUM IN PRESENCE OF FOREIGN METAL IONS

Foreign ion	Amount/mg	Palladium/mg Taken	Palladium/mg Found	Error, %
Cu(II)	10.00	3.00	3.03	+1.00
	20.00	30.00	30.15	+0.50
	40.00	6.00	5.96	−0.67
Ni(II)	20.10	7.20	7.18	−0.28
	30.15	15.00	15.00	—
	20.10	24.00	24.10	+0.42
Zn(II)	10.05	18.00	17.98	−0.11
	25.13	6.60	6.56	−0.61
Pb(II)	10.50	30.00	30.10	+0.33
	21.00	9.00	9.05	+0.56
Cd(II)	15.30	9.00	9.05	+0.56
	5.10	18.00	18.05	+0.28
Co(II)	10.00	24.00	24.00	—
	20.00	12.0	11.96	−0.33
Mn(II)	3.00	6.00	5.96	−0.67
	5.00	12.00	12.08	+0.67
Al(III)	5.00	9.60	9.58	−0.21
	25.00	15.00	15.11	+0.73
Fe(III)	10.00	7.80	7.77	−0.38
	25.00	30.00	29.96	−0.13
Bi(III)	10.20	3.00	2.96	−1.33
	20.40	24.00	24.05	+0.21
Ti(IV)	10.00	9.60	9.68	+0.83
	15.00	12.00	11.96	−0.33
Zr(IV)	5.20	12.00	11.96	−0.33
	15.60	18.00	18.05	+0.28
Sn(IV)	6.00	3.00	3.03	+1.00
	12.00	19.20	19.15	−0.26
La(III)	24.00	6.60	6.65	+0.76
	6.00	18.00	18.09	+0.50
Y(III)	18.00	9.00	8.99	−0.11
	10.00	12.00	12.02	+0.17
Sm(III)	24.50	15.00	15.09	+0.60
	12.25	24.00	23.95	−0.21
Gd(III)	23.10	6.00	6.01	+0.17
	10.50	21.00	20.95	−0.24
Ce(III)	19.60	12.00	12.08	+0.67
	9.80	24.00	24.05	+0.21
Rh(III)	5.90	9.00	8.99	−0.11
	11.80	12.00	12.08	+0.67
Ru(III)	12.40	9.60	9.68	+0.83
	6.20	12.00	11.96	−0.33
Os(VIII)	6.00	6.00	6.01	+0.17
	12.00	15.00	15.00	—

Manganese(II) gives some trouble in the end-point detection in both back titrations if more than 5 mg are present. Palladium forms strong complexes with nitrogen-containing ligands and hence cannot be titrated in the presence of hexamine or buffers containing ammonia. Sodium acetate - acetic acid is the most suitable buffer for the titration of palladium and the same is used in the present work. Although pyridine does not have any releasing effect on the mercury(II) - EDTA complex, the metal ion yields a highly protracted end-point in the presence of acetate buffer and thus interferes. As other platinum group metals do not react with EDTA in the cold, palladium can be determined in their presence by use of the first back titration. However, when some common metal ion is additionally present it is necessary to heat the solution with pyridine and to carry out the second titration. Under such conditions iridium(III) and platinum(IV) affect the titration adversely. Rhodium(III), ruthenium(III) and osmium(VIII) do not interfere in any instance.

Table II contains the results of a typical series of determinations on synthetic solutions and three solid alloy samples. This table shows that the results by the present method are in good agreement with those obtained by an accepted gravimetric method using ascorbic acid.[14] It is also seen from these results that except for 3 mg of palladium, the error in no case exceeded 0.83%. However, for 3 mg, the lowest amount of palladium studied, the errors varied from 1.00 to 1.33%.

TABLE II

DETERMINATION OF PALLADIUM IN SYNTHETIC SOLUTIONS AND SOLID ALLOY SAMPLES

Alloy*	Palladium/mg	
	Taken	Found†
40% Cu - Pd	30.00	29.89 30.03‡
43% Cu - Pd§	17.10	17.11 17.05‡
40% Ni - Pd	22.50	22.49 22.55‡
39% Ni - Pd§	18.30	18.35 18.33‡
35% Co - Pd	24.35	24.38 24.40‡
40% La - Pd	15.00	15.06 15.05‡
64% Sm - Pd§	16.00	15.99 15.95‡

* Synthetic samples unless otherwise mentioned.
† Mean of three determinations.
‡ By gravimetric method using ascorbic acid.
§ Solid alloy sample.

Among masking agents investigated earlier, dimethylglyoxime and benzotriazole gave precipitates, making the handling of more than 15 mg of palladium difficult. Although 1,10-phenanthroline yielded a soluble complex, it suffered from the interference of various cations and had limited applications. Thiourea was free from the above limitations; however, copper(II) originally interfered during masking with this reagent. This difficulty was obviated by cooling the solution to 8 °C and by using a minimum excess of the masking agent. Use of a minimum excess of the reagent could cause a problem when handling an unknown sample and can be viewed as a serious limitation from the viewpoint of practical application, particularly when it is applied to a copper - palladium alloy which is probably the most widely used alloy of palladium. With the present method, no such manipulation of conditions is necessary for any of the foreign ions.

Grateful thanks are due to Dr. V. S. Arunachalam, Director, Defence Metallurgical Research Laboratory, Hyderabad, for permission to publish this paper. Thanks are also due to Dr. P. Subramaniam and Mr. B. V. Rao for their keen interest in this work.

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Gravimetric Determination of Nickel as the Ternary Nickel - Pyridine - Picrate Complex

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Keywords: *Nickel determination*; *gravimetry*; *nickel - pyridine - picrate complex*

A study of ternary complex formation between nickel, pyridine and picrate ion has been made. The metal - pyridine complex reacts with picrate to give an insoluble, stable, green precipitate. Conditions of formation, solubility and other properties of the complex have been studied, together with its composition. Above 105 °C a stable compound, $Ni(py)_2(pic)_2$, with a high formula mass, is formed that is suitable for use in the gravimetric determination of nickel.

Experimental

Reagents

All reagents were of analytical-reagent grade. De-ionised, distilled water was used to prepare all solutions.

Sodium picrate stock solution, 0.1 M. Prepared by dissolving the appropriate amount of purified picric acid in water and then neutralising the solution with standard sodium hydroxide solution using a potentiometric method.

Pyridine, sp. gr. 0.98. This was diluted as required.

Nickel and other metal solutions. These were prepared from the pure salts and standardised complexometrically with EDTA.

Methods

Nickel was determined at varying pyridine concentrations, and conditions for quantitative precipitation and interferences by metal ions were examined. Experiments to determine the organic and metal contents of the precipitate were performed.

Determination of nickel in a solution containing nickel alone

To a solution containing at least 0.12 mg ml^{-1} of nickel add 0.2 M pyridine solution (nearly four times the amount required for a 1:4 molar ratio of the cation to the reagent), then add excess of 0.1 M sodium picrate solution. Under these conditions the pH is near neutral. Allow the green precipitate to stand for 30 min, then centrifuge it or filter it through a weighed sintered-glass crucible of porosity G4. Wash the precipitate with 0.1 M pyridine solution, dry it at 105 °C for 2 h and weigh it.

Conditions for quantitative precipitation

Solutions containing 6 mg of nickel and excess of sodium picrate were transferred into separate beakers and to each was added pyridine with a total concentration varying between 0.05 and 6 M.

Keeping constant the amount of nickel and the pyridine and picrate concentrations, the pH was varied between 2 and 10. The precipitate was filtered off and weighed as described above.

Interferences

The effect of various cations on the precipitation of nickel was examined.

If only chromium was present as an interferent, an excess of iron(III) ion must be added for complete coprecipitation.[1] Separation of zinc from nickel was effected in an moniacalam medium at 70 °C with thioacetamide solution.[2] Cadmium was eliminated with the same

reagent at pH 2 or lower and at 90 °C.[3,4] Before precipitation of nickel the solution was boiled and the pH adjusted nearly to neutral. Copper, on the other hand, forms a soluble complex with 2 M pyridine solution.

Results and Discussion

In nearly neutral solutions containing pyridine (0.1–2 M) nickel can be quantitatively precipitated by means of sodium picrate solution. The concentration of pyridine must be kept between these limits; at lower or higher concentrations incomplete precipitation or re-dissolution occur, respectively.

Quantitative precipitation can be effected in the pH range 6–8, and the determination of nickel in solutions containing different amounts of nickel can be carried out satisfactorily under these conditions (Table I).

TABLE I

GRAVIMETRIC DETERMINATION OF DIFFERENT AMOUNTS OF NICKEL USING PYRIDINE AND SODIUM PICRATE

Mass of nickel taken/mg	Calculated mass of $Ni(py)_2(pic)_2$/mg	Mass of precipitate obtained/mg*	Difference/ mg	Error, %
0.12	1.4	1.5	+0.1	+7.14
0.6	6.9	6.7	−0.2	−2.89
2.4	27.5	27.0	−0.5	−1.82
4.2	48.2	48.5	+0.3	+0.62
5.4	61.9	61.5	−0.4	−0.65
18.0	206.3	207.2	+0.9	+0.44
30.0	343.9	341.9	−2.0	−0.58

* All the results are the average of five determinations.

The composition of the dried nickel precipitate was calculated from elemental analysis, with the following results: C, 40.26%; H, 2.17%; N, 16%. The percentage of oxygen was calculated to be 33.14% after nickel determination. A concentration of 8.43% of nickel was found by EDTA titration using a mercury indicator electrode and pyridine as solvent. These results indicate that the precipitate has a formula mass of 673.1, the gravimetric conversion factor of $C_{22}H_{14}N_8O_{14}Ni$ to nickel being 0.08722. Such a relative molecular mass agrees well with a calculated formula in which the nickel:pyridine:picrate ratio is 1:2:2.

No other nickel precipitate shows such a high formula mass, including recent gravimetric nickel determinations.[5–7] This high relative molecular mass makes gravimetric determinations possible even in samples that contain as little as 0.12 mg of nickel and the volume of the test solution required is small, being not more than 1 ml. The sensitivity and precision are better than those of conventional methods.

TABLE II

RESULTS FOR ANALYSIS OF MONEL METAL ALLOY

Monel metal: Ni 60, Cu 33, Fe 6.5%, Mn, C, Si and S 0.5%.

Copper			Nickel		
Amount present in the sample/mg	Amount found/ mg	Error, %	Amount present in the sample/mg	Amount found/ mg	Error, %
8.2	8.3	+1.21	15.0	15.2	+1.33
16.5	16.4	−0.61	30.0	30.3	+1.00

Unfortunately, the level of interference is higher. Elements that interfere by precipitation at pH 6–8 (aluminium, iron and chromium) or by coordination through the nitrogen atom of the pyridine to form complexes whose precipitation and re-dissolution occur under the same conditions as nickel (cadmium and zinc) must be eliminated. The recovery of nickel is not affected by common anions.

An interesting feature is the fact that copper is not precipitated with 2 M pyridine. Copper and nickel often interfere mutually when they are present in mixtures. Simultaneous determinations of both elements were performed. Nickel was separated and determined gravimetrically. The copper content in the soluble pyridine complex was determined by potentiometry using EDTA. Good results were obtained in the analysis of Monel metal alloy (Table II).

The authors express their gratitude to Dr. Franz Neugebauer of the Max Planck Institute for carrying out the elemental analysis and for valuable suggestions. They also thank the Scientific and Artistic Development and International Cooperation Services for support.

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Book Reviews

MASS SPECTRAL CORRELATIONS. Second Edition. By FRED W. MCLAFFERTY and RENGACHARI VENKATARAGHAVEN. *Advances in Chemistry Series*, 40. Pp. viii + 124. American Chemical Society. 1982. Price $24.95. ISBN 0 8412 0702 X.

The Second Edition of Mass Spectral Correlations represents a considerable expansion of the First Edition published in 1963, mainly owing to the extensive use of computerised data banks to produce statistical information concerning the occurrence of the various ions up to *m/z* 150. The lists are laid out in blocks of ascending relative molecular mass, each block comprising a single integral mass (*e.g.*, *m/z* 58). Each block is sub-divided into groups comprising the actual ions (*e.g.*, C_3H_8N, *m/z* 58.0656 and C_3H_6O, 58.0418) and the probability for each ion occurring in the data base of 32830 compounds is given. The complex lists of statistical numbers associated with each ion are explained in detail in the introduction and briefly on the front-end papers. Beyond *m/z* 150 accurate masses and sub-formulae are given, but no computer-aided statistics are shown.

This book is meant to be a handbook for those mass spectroscopists who, presented with an unknown spectrum, have to assign possible structures. Today this probably represents a small percentage of scientists working in this field; most spectroscopists have at least some idea of the types of compound they are analysing. However, by showing the sub-structures that may give rise to a particular ion it is possible that several structures could be derived for an unknown. Examining the book it is obviously meant for the specialist. A considerable number of the compounds in the data base used contain more than one halogen atom, *e.g.*, Cl or Br. However, the isotopic patterns associated with such atoms are ignored. For the novice such patterns due to the isotopes may not be familiar. Thus a weakness in the list, possibly owing to the computerised approach used, is the lack of isotope information which aid spectral interpretation.

The lists themselves are biased towards low mass ions (not surprising considering the spectra are obtained from 70 eV electron bombardment ionisation). Above *m/z* where the data base is insufficient to make any correlations few entries are made and many people may feel some of their favourite ions have been ignored. For instance, in petroleum research 231 (4-methyl steroids) or 259 (ring C aromatic steroids) are not listed. Indeed the listing of 259 lists $C_4H_3{}^{81}BrCl_2F_3$, $C_5Cl_3{}^{37}ClF_3$, $ClC_4H_8O(Cl)$-phenyl-$C(CH_3)_2$ as sub-structures, which is a reflection of the data base used to compile these lists, which seems to contain an abundance of halogenated compounds.

I think the book will have limited appeal to a specialist audience. It is unlikely that it will be an everyday reference for the routine laboratory. The authors point out that "the user must remember that there is a finite possibility that the correct assignment for a peak in an individual unknown spectrum is not represented in this compilation." That possibility is likely to be quite large and this book should be used with care. However, it does represent an attempt to put mass spectral interpretation on the same basis as infrared or NMR interpretation. Although it is only partially successful in this aim it is a first step in this direction. N. J. HASKINS

HOST GUEST COMPLEX CHEMISTRY II. Edited by F. VÖGTLE. *Topics in Current Chemistry, Volume* 101. Pp. viii + 203. Springer-Verlag. 1982. Price DM88; $41. ISBN 3540 11103 4.

As a sequel to Volume 98 of *Topics in Current Chemistry*, this publication again presents four review papers by various authors, which examine different aspects of the structure and properties of the guest - host complexes formed by poly- and oligodentate ligands.

The capability of ionophores to form stable lipophilic complexes with hydrophilic species, and consequently provide a mechanism for the transfer of such species across membranes, is of considerable importance to biochemists, and provides the subject matter for two of the papers. The first of these deals with the structure of ionophores and their complexes, and the way in which coronands, cryptands and podands can act as synthetic analogues to those that occur naturally; particular emphasis being placed upon stereochemical considerations. The second paper examines the dynamics of membrane transfer. Although neutral macrocyclic species are briefly considered, the larger part of this paper deals with carboxylic ionophores and emphasises the role that conformational change plays in determining membrane transport mediating properties.

R. M. Kellogg's contribution is a fascinating review of the way in which it is possible to design and then synthesise molecules to perform specific functions. Biochemical modelling of small enzyme systems is described, and again the use of synthetic analogues to mimic the behaviour of naturally occurring species is outlined.

The final paper deals with phase transfer catalysed reactions, in which complexes are used to transfer active species across phase boundaries. Whilst much of the paper concentrates on quaternary ammonium complexes, there is some discussion of the potential role of coronands, cryptands and podands.

Overall, a detailed and well referenced book that biochemists in particular may find of value, although it is probable that it will prove of limited direct interest to the majority of analytical chemists.

I. C. DOWNING

UNDERGRADUATE INSTRUMENTAL ANALYSIS. Third Edition, Revised and Expanded. By JAMES W. ROBINSON. Marcel Dekker. Pp. xx + 550. 1982. Price SWFr75. ISBN 0 8247 1530 6.

This book is compulsive reading, but for all the wrong reasons. By the time the first three chapters have been read (Concepts of Analytical Chemistry, 28 pp., Introduction to Spectroscopy, 34 pp., and Concepts of Spectroscopy, 19 pp.), a variety of emotions, mainly disbelief, will have been experienced that will compel the knowledgeable reader to continue the experience if only to confirm his suspicions. The story continues with chapters on NMR (35 pp.), Infrared (49 pp.), Ultraviolet Molecular Absorption (43 pp.), AAS (28 pp.), Spectrophotometry, Colorimetry and Polarimetry (25 pp.), Flame Photometry (26 pp.), Emission Spectrography (31 pp.), X-ray Spectroscopy (38 pp.), Chromatography (63 pp.), Thermal Analysis (21 pp.), Mass Spectrometry (29 pp.) and Electrochemistry (75 pp.). Although the topics included must reflect the author's view as to what "the more common instrumental methods" are it is odd that neither "radiochemical methods" nor "automation" is included. There is no mention of electron spectrometries or surface analysis. Some of the existing chapters are a little unbalanced (HPLC is only given one page, out of 63 on chromatography, and there is no mention of size exclusion chromatography; there is no mention of differential-pulse polarography or of PVC membrane ion-selective electrodes in the chapter on electrochemistry) for a book dated 1982 which claims to be "revised" and "expanded."

Classification of the errors in this book has proved somewhat difficult, but is based on the classification most familiar to analytical chemists, random, systematic and gross. There are at least 110 printing (random) errors, a good many of which would need a scientific proof reader to spot, so perhaps the publishers cannot be blamed for all of these. In this category are included some that might be labelled systematic (p. 117 "the wavelength of visible light falls between 450 μm and 750 μm") or even gross error (p. 402 "refracted index," p. 500 "perchloride"). In addition, there are at least 11 scientists' names spelled incorrectly. In the category of systematic error are the occasions on which explanations are doubtful, oversimplified or misleading; these total some 54 instances. (On p. 250, UV radiation is referred to as "black light"; the photometric error curve is dealt with twice, pp. 59–60 and pp. 247–249, the results do not agree with each other and the curve shapes, Figs. 8.10 and 2.25, are different; in the second explanation the maths are nonsense and the legend for Fig. 8.10 is incorrect.) Also in this category are the cross-reference errors; on at least 18 occasions a reference to a previous section or equation is wrong. Finally, the gross error category; there are at least 20 diagrams that contain mistakes and 50 statements or explanations that are definitely incorrect (p. 51, A mole of solution contains the same number of grams of solute as the molecular weight of the solid. p. 72, By continuously rotating the monochromator . . . the complete spectrum can be scanned. p. 177, . . . By dissolving a sample in a non-polar solvent such an alcohol. p. 280, . . . Excited potassium atoms pass on their energy to unexcited sodium atoms. p. 323, Since the L shell has three sub-levels, we can see three K lines, which are respectively $K\alpha_1$, $K\alpha_2$ and $K\alpha_3$. p. 349, Long wavelengths means high frequency and therefore high energy radiation. p. 518, . . . The potential necessary to continue oxidation will steadily increase until water is reduced). Fig. 12.29, which is obviously a GC trace (urinary volatiles, temperature programme from 30 to 210 °C), is described in the text, p. 409, as a typical capillary liquid chromatogram. Paper chromatography is classified as liquid - solid, p. 396. The DC plasma jet is classified as an inductively coupled plasma, p. 311;

and so the list goes on. The index is adequate apart from classifying non-aqueous titrations and non-dispersive IR as sub-sections of NMR.

Overall the book appears not to have been checked by anyone who knows anything about maths, physics or analytical chemistry. The author and publisher ought to be exremely embarrassed. J. F. TYSON

PROCEEDINGS OF THE FOURTH INTERNATIONAL SYMPOSIUM ON CAPILLARY CHROMATOGRAPHY HELD IN HINDELANG/ALLGÄU, GERMANY, MAY 3–7, 1981. Edited by R. E. KAISER. Pp. 939. Hüthig. 1981. Price DM92.60. ISBN 3 7785 0743 5.

The symposium papers are here gathered into a soft-backed book of over 900 pages, printed from typescript, with corrections where necessary and addenda resulting from discussion. An Index is included and the contents pages are arranged in alphabetical order of speakers.

The 45 papers were given by speakers from Japan, Czechoslovakia, the Netherlands, Sweden, USA, Switzerland, South Africa, United Kingdom, Belgium, Germany, Austria, Italy and China. They range in subject from the use of a "home computer" in capillary chromatography to laser-induced fluorescence detection techniques. The fields of application investigated include polymers, drugs and their metabolites, pesticides, polycyclic aromatic hydrocarbons, amino acids, carbohydrates and alkaloids. Many of the papers cover theoretical aspects and the parameters of the technique.

Many of the authors are eminent in the field and the volume as a whole contains a wealth of information on all aspects of capillary chromatography as at present developed. In the reviewer's opinion it is among the better recent compilations of symposia papers and without doubt the next best thing to actually being present and taking part in the symposium. D. SIMPSON

COMPREHENSIVE TREATISE OF ELECTROCHEMISTRY. VOLUME 2: ELECTROCHEMICAL PROCESSING. Edited by J. O.'M. BOCKRIS, BRIAN E. CONWAY, ERNEST YEAGER and RALPH E. WHITE. Pp. xxii + 616. Plenum. 1981. Price $57.50. ISBN 0 306 40503 2.

This volume on electrochemical processing covers the use of electrochemistry in industrial production. It deals with the main topics of interest in this area as follows: electrolytic production of hydrogen, production of chlorine, inorganic electrosynthesis (including chlorate, hypochlorite, perchloric acid, bromate, iodate, periodate, peroxydisulphate) electro-organic syntheses, electrometallurgy of aluminium, electrolytic refining and winning of metals, electroplating, electrochemical machining, theory of the structure of ionomeric membranes, electrodeposition of paint and mineral flotation. It covers the effect of overvoltages in various situations and the cells that are commercially available in each instance. The chapter on electro-organic synthesis does not attempt to compete with Baizer's book but rather to give a comprehensive and concise survey of the different reaction types together with some molecular orbital theory background.

Of what use is this book to the analytical chemist? Possibly not a lot unless the analyst is involved in these process industries. Nevertheless it is a good general background for the electroanalytical chemist. A. G. FOGG

THEORY AND MATHEMATICS OF CHROMATOGRAPHY. By ABDEL SALAM SAID. Pp. x + 210. Hüthig. 1981. Price DM75; $38. ISBN 3 7785 06016 1.

The first 50 or so pages of this book represent an introduction to the kinds of mathematical problems encountered in chromatographic theory. It is clear and concise and attractively presented with examples. This will be very useful to beginners, even in other fields of physical chemistry. The remainder of the volume spells out most of chromatographic theory as so far developed and, it too, is nicely presented and informative. The practical implications of the various equations derived are brought out, illustrative numerical problems are solved and a few are left for the reader to solve.

This is an interesting and useful volume that should find a considerable readership. It seems a pity, therefore, that, first, the price is so high and, secondly, that so little of the bibliography is devoted to other than the author's own published work. J. H. PURNELL

RECENT ADVANCES IN CAPILLARY GAS CHROMATOGRAPHY, VOLUMES 2 AND 3. Edited by W. BERTSCH, W. G. JENNINGS and R. E. KAISER. Pp. xii + 592. Hüthig. 1981. Price DM75; $38. ISBN 3 7785 0711 7.

Capillary column systems are nowadays used for a wide variety of gas-chromatographic separations of both a special and a routine nature. They bring with them a number of technical difficulties extra to those associated with packed column systems and there is no doubt that the capillary chromatographer must bring a high level of art and artisanship to his work. This book fully recognises this and is clearly aimed at the practical man; of the 50 papers, no more than two or three make any significant reference to theory or its implications. But the remainder really attempt to cover practice in all its aspects. One learns first how, in this day and age, one should make capillaries, how to deactivate them and how to coat them. Subsequently, there are accounts of how to overcome the various problems of connecting them into the system, of injecting the sample and whether to split it or not. Finally, we are provided with examples of recent improvements in application, predominantly in the analysis of complex mixtures of relatively involatile species.

The volume of information provided is evidently considerable, but it is this very feature that detracts from the merits of the volume. This is no general manual for the laboratory; indeed the wide diversity of views represented and clear signs in a number of the articles of rather less than objective reasons for recommending practical approaches would be as likely to confuse as to inform. The title is, in fact, apt; this volume is all about new and often unestablished, advances in technique and it will be most valuable to those already familiar with established practice. For them, at the price, it will be a good investment.

J. H. PURNELL

Effect of Surfactants on the Response of Ion-selective Electrodes with Poly(vinyl Chloride) Membranes

The effect of cationic, anionic and non-ionic surfactants on the potentials of potassium-, calcium- and nitrate-selective electrodes has been studied. All electrodes investigated had either an internal reference solution or a solid-silver contact. The latter type was found to be less sensitive to surfactant interferences.

Keywords: *Surfactant effect*; *ion-selective electrodes*; *PVC membrane electrodes*; *potassium-, calcium- and nitrate-selective electrodes*

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Analyst, 1982, **107**, 1356–1362.

High-performance Liquid Chromatographic Determination of Vitamin D_3 in Foods with Particular Reference to Eggs

A high-performance liquid chromatographic (HPLC) method for the determination of vitamin D_3 in foods is described. The method involves addition of vitamin D_2 to the sample as an internal standard followed by saponification and extraction of the unsaponifiable matter. Sterols are removed by precipitation and other interfering compounds by a thin-layer chromatographic clean-up procedure. Vitamins D_2 and D_3 are then separated using reversed-phase HPLC. Details of the accuracy and precision of the method are presented. This method has been applied successfully to the determination of vitamin D_3 in eggs, butter, milk and cheese.

Keywords: *High-performance liquid chromatography*; *vitamin D_3 determination*; *foods*

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Analyst, 1982, **107**, 1363–1369.

Simple and Sensitive Technique for Investigation of Desorption Properties

The technique of thermally stimulated pressure (TSP) is described and has been applied to study the state of bound water on crystallised lysozyme at hydration levels of 0–24 mg of water per gram of protein. If it is assumed that for the low densities used the molecules of water are bound to independent sites in the macromolecule of lysozyme, first-order kinetics can be used to fit the experimental pressure *versus* temperature graphs. The activation energy is 37.63 kJ mol^{-1}.

Keywords: *Bound water*; *lysozyme*; *thermally stimulated pressure*; *activation energy*; *desorption*

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Analyst, 1982, **107**, 1370–1374.

Differential-pulse Polarographic Determination of Titanium in Steel

Short Paper

Keywords: *Steel analysis*; *titanium determination*; *trace metal analysis*; *differential-pulse polarography*

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Analyst, 1982, **107**, 1375–1379.

High-performance Liquid Chromatographic Determination of Oxprenolol Hydrochloride and Metoprolol Tartrate in Tablets and Injections

Short Paper

Keywords: *Oxprenolol hydrochloride determination*; *metoprolol tartrate determination*; *tablets*; *injections*; *high-performance liquid chromatography*

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Analyst, 1982, **107**, 1379–1382.

Selective Complexometric Determination of Palladium Using Pyridine as Releasing Agent

Short Paper

Keywords: *Selective complexometry*; *palladium determination*; *pyridine release*

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Analyst, 1982, **107**, 1382–1385.

Gravimetric Determination of Nickel as the Ternary Nickel - Pyridine - Picrate Complex

Short Paper

Keywords: *Nickel determination*; *gravimetry*; *nickel - pyridine - picrate complex*

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Analyst, 1982, **107**, 1386–1388.

A201 for further information. See page x

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THE ANALYST **November, 1982**

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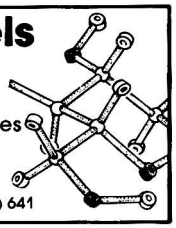

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ANALYTICAL DIVISION — SPECIAL TECHNIQUES GROUP

SYMPOSIUM ON UNUSUAL AND DIFFICULT SAMPLES

December 8th 1982

This symposium and the AGM of the Special Techniques Group will be held on Wednesday December 8th, 1982 in the Read Lecture Theatre, Sherfield Building, Imperial College, London.

The symposium will include lectures on the following techniques: MOLE, Raman and Raman microprobe spectroscopy, Fourier transform spectroscopy and radioimmunoassay techniques.

For further information and registration forms please contact:

John Huddleston.
Instrument & Applied Physics Division
AERE Harwell, Oxfordshire OX11 0RA
Tel: Abingdon (0235) 24141 Ex. 2030.

A204 for further information. See page x

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