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Summaries of Papers in this Issue

Electrochemical Studies of Strongly Chelating Anthraquinone Derivatives

Analytically important, strongly chelating anthraquinones and their derivatives were studied by d.c. polarography, cyclic voltammetry and microcoulometry to investigate their redox characteristics. All 18 substances were reduced in a two-electron reversible or quasi-reversible process in both aqueous and 75% ethanolic solutions. Depending on pH and the medium, single or double polarographic reduction waves appeared, which were diffusion controlled, although in some instances adsorption pre-waves were also observed. This behaviour is similar to the known behaviour of simpler quinone systems. The variation of the Ilkovič coefficient and half-wave potential with pH was studied in detail to investigate the acid - base behaviour of the species involved in the reduction process. As a result, it was possible to describe the reduction mechanism of the anthraquinones involved.

A number of new pK values were determined and others confirmed. Attempts to find linear free energy relationships were generally unsuccessful.

Keywords: *Anthraquinone derivatives*; *polarography*; *cyclic voltammetry*; *half-wave potentials*; *pK values*

G. ALI QURESHI, G. SVEHLA and M. A. LEONARD

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

Analyst, 1979, **104**, 705–722.

Direct Differential-pulse Polarographic Determination of Mixtures of the Food Colouring Matters Tartrazine - Sunset Yellow FCF, Tartrazine - Green S and Amaranth - Green S in Soft Drinks

Tartrazine and Sunset Yellow FCF can be determined directly in orangeade by differential-pulse (d.p.) polarography on the addition of pH 9 Britton - Robinson buffer and tetraphenylphosphonium chloride. The tetraphenylphosphonium chloride removes the large polarographic maximum obtained with tartrazine at pH > 4 and causes the d.p. polarographic peaks of the two colouring matters to be separated.

Tartrazine in limeade can be determined in a similar supporting electrolyte but these conditions are not suitable for the determination of Green S, which is usually present at a low concentration relative to the tartrazine and for which the d.p. polarographic peak is depressed by the addition of tetraphenylphosphonium chloride. Green S can be determined after adding pH 4 Britton - Robinson buffer and tetramethylammonium chloride to the limeade: the addition of tetramethylammonium chloride gives a better base line in the presence of tartrazine. The solution is then re-adjusted to pH 9 and tetraphenylphosphonium chloride is added in order to determine the tartrazine.

At pH 4 the sugar present in blackcurrant syrup gives a small d.p. polarographic peak at the same potential as Green S. At pH > 6 the peak of the sugar disappears but amaranth gives a broad polarographic maximum. This maximum is suppressed at pH 7.8 by the addition of tetramethylammonium chloride. Under these conditions the Green S peak is separate but the small concentrations of Green S normally present in blackcurrant drinks can only just be detected.

The procedures have been tested on soft drinks prepared with known concentrations of colouring matter.

Keywords: *Differential-pulse polarography*; *food colouring matters*; *tartrazine*; *Green S*; *amaranth*

A. G. FOGG and K. S. YOO

Chemistry Department, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU.

Analyst, 1979, **104**, 723–729.

Ion-selective Polymeric-membrane Electrodes with Immobilised Ion-exchange Sites

Part I. Development of a Calcium Electrode

A new type of ion-selective electrode is described in which ion-exchange sites are immobilised in a polymeric membrane by covalent bonding. Membranes were prepared by cross-linking a styrene - butadiene - styrene triblock copolymer with triallyl phosphate. After subsequent hydrolysis these membranes were evaluated as Ca^{2+} sensors. The electrodes formed exhibited an extended Nernstian response to Ca^{2+} (10^{-1}–10^{-6} M) and good selectivity over other alkaline earth and alkali metals. Such electrodes offer very fast response times, extended lifetimes and a wide pH working range. The possibilities of this new class of ion-selective electrode are also discussed.

Keywords: *Ion-selective electrode*; *calcium analysis*; *electroanalytical chemistry*; *polymeric membrane*; *immobilised ion-exchange groups*.

L. EBDON, A. T. ELLIS and G. C. CORFIELD

Department of Chemistry, Sheffield City Polytechnic, Pond Street, Sheffield, S1 1WB.

Analyst, 1979, **104**, 730–738.

High-frequency Microtitrimetric Determination of Acidic and Basic Constituents in Lubricating Oils

Part II. Determination of Total Base Number

A high-frequency microtitration method is described for the determination of total base number in new and used lubricating oils. The sample, dissolved in toluene - propan-2-ol - water (5 + 4.95 + 0.05), is titrated with standard alcoholic hydrochloric acid solution. A study of solvents, titrants, sample size and other experimental parameters is reported. This method provides sharp breaks at the end-points of the titration graphs and more repeatable and faster results than those obtained with the standard potentiometric method. The method can also be used for macrotitrations, with a commercially available oscillator, and can be suggested as an alternative to ASTM and IP methods for the determination of total base number.

Keywords: *Total base number determination*; *lubricating oils*; *high-frequency titration*

T. FERNÁNDEZ, J. M. ROCHA, N. RUFINO and A. GARCÍA LUIS

Compañia Española de Petróleos, S.A., Research Laboratory, Santa Cruz de Tenerife, Canary Islands.

and F. GARCÍA MONTELONGO

Department of Analytical Chemistry, University of La Laguna, Tenerife, Canary Islands.

Analyst, 1979, **104**, 739–749.

Continuous Solvent-extraction Method for the Spectrophotometric Determination of Cationic Surfactants

A rapid and automated spectrophotometric method for the determination of cationic surfactants, using the AutoAnalyzer, has been developed. This method is based on the continuous solvent extraction of the ion-pair complex formed in the reaction of Orange II with a cationic surfactant. Good traces and identical molar responses were obtained with seven different types of surfactant using methanol in the Orange II reagent. Fatty amines in mixtures of the amines and quaternary ammonium surfactants were determined by changing the pH of the aqueous phase. The proposed method was applied to the determination of cationic surfactants in several commercial products. The results agreed with those obtained by the two-phase titration procedure. The detection limit is 5 μM and the capacity is 10–20 samples per hour, with a relative precision of better than 1.5%.

Keywords: *Automatic analysis*; *cationic surfactant determination*; *fatty amine determination*; *spectrophotometry*; *Orange II*

JIRO KAWASE and MAKOTO YAMANAKA

Tochigi Research Laboratories, Kao Soap Co. Ltd., 2606 Akabane, Ichikai-machi, Tochigi, Japan.

Analyst, 1979, **104**, 750–755.

Automatic Emission Spectrometer for the Determination of Nitrogen-15

An automatic nitrogen-15 analyser, employing a novel use of a rhodium - platinum catalyst for the generation of nitrogen and capable of analysing 60 samples per hour, is described. Nitrogen compounds of biological origin are first converted into ammonium chloride by conventional Kjeldahl digestion and distillation methods. The ammonium chloride sample (about 5 μl containing about 10 μg of nitrogen) is injected into a soda-lime reactor at 590 °C through which flows a stream of purified helium. Ammonia that is released passes directly into the catalyst tube and the generated nitrogen and hydrogen are separated by passage through a gas-chromatographic column, which also retains the water.

After passing through a pressure restrictor the nitrogen flows in the helium stream through a Spectrosil discharge tube located in a microwave cavity. The emitted radiation is analysed by means of a specially constructed dual-wavelength monochromator and the intensities of the $^{14}N^{14}N$ (297.7 nm) and $^{14}N^{15}N$ (298.3 nm) bands are measured simultaneously by two photomultipliers. Amplified signals, proportional to the peak intensities, are fed through phase-sensitive detectors into a ratiometer, the output from which is fed to a digital voltmeter and printed out in terms of nitrogen-15 abundance. A peak detector indicates the total nitrogen content of each sample and actuates the nitrogen-15 print-out.

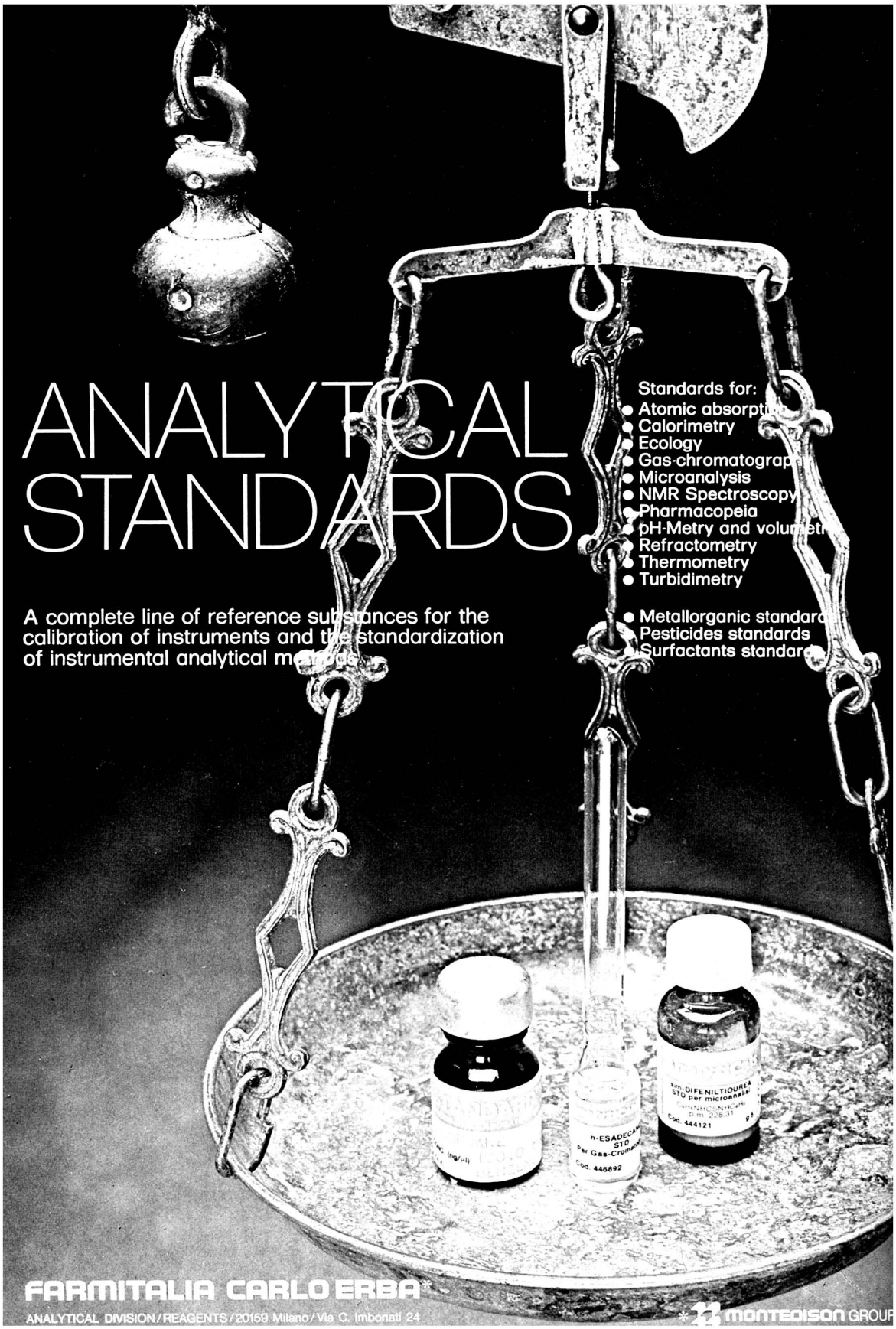
The response of the instrument is slightly curvilinear but may be regarded as linear over limited ranges. Calibration can therefore be achieved by running suitably chosen standards to fix upper and lower set points. Carry-over between samples is very small and is eliminated by running duplicates. Standard deviations of replicate measurements of natural abundance and enriched standards are less than 0.01 atom-%, while determinations of nitrogen-15 in biological samples were shown to be accurate to $\pm$0.01 atom-% by comparison with a Statron NOI-4 nitrogen-15 analyser.

Keywords: *Nitrogen-15 determination*; *catalytic nitrogen generation*; *automated emission spectrometer*

J. D. S. GOULDEN and D. N. SALTER

National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT.

Analyst, 1979, **104**, 756–765.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The Analyst

Electrochemical Studies of Strongly Chelating Anthraquinone Derivatives

G. Ali Qureshi,* G. Svehla and M. A. Leonard

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

Analytically important, strongly chelating anthraquinones and their derivatives were studied by d.c. polarography, cyclic voltammetry and microcoulometry to investigate their redox characteristics. All 18 substances were reduced in a two-electron reversible or quasi-reversible process in both aqueous and 75% ethanolic solutions. Depending on pH and the medium, single or double polarographic reduction waves appeared, which were diffusion controlled, although in some instances adsorption pre-waves were also observed. This behaviour is similar to the known behaviour of simpler quinone systems. The variation of the Ilkovič coefficient and half-wave potential with pH was studied in detail to investigate the acid - base behaviour of the species involved in the reduction process. As a result, it was possible to describe the reduction mechanism of the anthraquinones involved.

A number of new pK values were determined and others confirmed. Attempts to find linear free energy relationships were generally unsuccessful.

Keywords: *Anthraquinone derivatives*; *polarography*; *cyclic voltammetry*; *half-wave potentials*; *pK values*

Strongly chelating anthraquinones are selective and sensitive reagents suitable for the spectrophotometric determination of a number of metallic and non-metallic substances. Among them, alizarin fluorine blue [3-*NN*-di(carboxymethyl)aminomethyl-1,2-dihydroxyanthraquinone] was introduced first by Belcher *et al.*[1] for the determination of fluoride. Leonard and co-workers[2–11] have synthesised and studied a number of new reagents, all related to alizarin fluorine blue, and applied them for various analytical purposes. Because of their quinoidal structure, these substances display interesting electrochemical behaviour, which we undertook to study by polarography and cyclic voltammetry. Some anthraquinone derivatives (including alizarin) have been studied polarographically in the past,[12–19] but these did not involve most of the compounds studied by ourselves. In our study we tried to avoid buffers with complex-forming characters as far as possible, in order to be able to compare the polarographic behaviour of these anthraquinones with that of their complexes.

Experimental

Reagents

The compounds involved in this study are listed in Table I. Some of them (**I–XI** and **XIV**) were commercially available; others (**XII**, **XIII**, **XV** and **XVI**) were prepared by Mannich condensation of alizarin with the appropriate agent, and **XVII** and **XVIII** were prepared earlier by Al Ani[20] in this Department. The purity of each compound was checked by electrophoresis, thin-layer chromatography and by elemental analysis. Compounds which, when analysed, gave more than 3% divergences from the theoretical carbon or hydrogen values were rejected.

For the study 5×10^{-4} mol l^{-1} aqueous solutions were prepared of each compound; sometimes a few drops of 0.1 mol l^{-1} sodium hydroxide solution were added to assist rapid dissolution.

* Present address: Department of Analytical Chemistry, University of Uppsala, S-75121 Uppsala 1, Sweden.

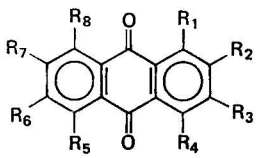
Studies were also made in solutions containing 75% *V/V* ethanol, as some anthraquinones were insoluble in water at certain pH values. In some instances special solutions were used; their composition will be mentioned in the appropriate section of the text.

All buffer solutions, supporting electrolytes and maximum suppressors were prepared from analytical-reagent grade reagents.

White-spot nitrogen gas was used for deaeration.

TABLE I

COMPOUNDS INCLUDED IN THE STUDY



No.	Name of compound	Substituents
I	1-Hydroxyanthraquinone	R_1 = OH
II	2-Hydroxyanthraquinone	R_2 = OH
III	Alizarin	R_1, R_2 = OH
IV	2,3-Dihydroxyanthraquinone	R_2, R_3 = OH
V	Alizarin-3-sulphonic acid (alizarin red)	R_1, R_2 = OH; R_3 = SO_3H
VI	Alizarin-5-sulphonic acid	R_1, R_2 = OH; R_5 = SO_3H
VII	3-Nitroalizarin (alizarin orange)	R_1, R_2 = OH; R_3 = NO_2
VIII	1,2,3-Trihydroxyanthraquinone (anthragallol)	R_1, R_2, R_3 = OH
IX	1,2,4-Trihydroxyanthraquinone (purpurin)	R_1, R_2, R_4 = OH
X	1,2,7-Trihydroxyanthraquinone	R_1, R_2, R_7 = OH
XI	1,2,5,8-Tetrahydroxyanthraquinone (quinalizarin)	R_1, R_2, R_5, R_8 = OH
XII	Alizarin-3-methylglycine	R_1, R_2 = OH; R_3 = CH_2–NH–CH_2COOH
XIII	Alizarin-3-methylsarcozine	R_1, R_2 = OH; R_3 = CH_2–N(CH_3, CH_2COOH)
XIV	Alizarin fluorine blue	R_1, R_2 = OH; R_3 = CH_2–N(CH_2COOH)$_2$
XV	Alizarin fluorine blue 5-sulphonic acid	R_1, R_2 = OH; R_3 = CH_2–N(CH_2COOH)$_2$; R_5 = SO_3H
XVI	Alizarin-3-methyldiethylamine	R_1, R_2 = OH; R_3 = CH_2–N(C_2H_5)$_2$
XVII	1,2-Dihydroxy-3-(2,4-dihydroxybenzeneazo)anthraquinone	R_1, R_2 = OH; R_3 = –N=N– (HO, OH)
XVIII	1-Hydroxy-2-(2,4-dihydroxybenzeneazo)anthraquinone	R_1 = OH; R_2 = –N=N– (HO, OH)

Apparatus

All polarographic studies were carried out with the Radiometer P04 polarograph, equipped with the E65 dropping-mercury electrode (DME) in conjunction with the drop life timer and current sampler unit DLT1.

Cyclic voltammetric measurements were made with an instrument, built in this Department, consisting of a Hewlett-Packard 3310B function generator, 1201B 100-μV dual-trace oscilloscope and a home-made potentiometric circuit with *IR*-drop compensation. A Radiometer P985B hanging-mercury electrode served as the working electrode, a platinum foil was used as the indicator electrode and a saturated calomel electrode as the common reference electrode. The three electrodes were mounted in such a way that the reference

electrode was shielded from the *IR* drop between the other two electrodes. In some instances the hanging-mercury electrode was replaced with an amalgamated platinum-pin electrode. Results were evaluated on the spot using the oscilloscope in its storing mode, but whenever the sweep rates allowed, voltammograms were also recorded with a Hewlett-Packard 7035B *X* - *Y* recorder.

For microcoulometric experiments a Manousek cell was used in conjunction with the P04 polarograph.

Current *versus* time curves (at constant potentials) were obtained with an instrument designed and built in the Department. The stabilised potential source had a tolerance of ± 1 mV and the current *versus* time curves were displayed on an oscilloscope and photographed. The currents were measurable with a relative standard deviation of less than 1%, and the system had a response time of a few milliseconds.

pH measurements were carried out with an EIL 7030 pH meter with its glass and saturated calomel electrodes.

Procedures

All polarographic measurements were carried out with a drop lifetime of 2 s with 70% blanking time and a 50-cm mercury column height (corrected for back-pressure), unless otherwise stated. The anthraquinones were mixed with the appropriate buffer and a few drops of 0.1% Triton X solution and water to yield a 2.5×10^{-4} mol l^{-1} concentration in the cell (unless otherwise stated). Solutions were deaerated with a stream of nitrogen for 10 min. Polarograms were recorded with the slowest possible speed between 0 and -1.8 V. The polarograms were evaluated by the "point method"[21] for both the half-wave potential and limiting current. The pH of each solution was measured after the polarographic experiment.

The cyclic voltammetric experiments were carried out with electrolytes similar to those used for polarography. Sweep rates were between 10 and 1000 mV s^{-1}, with current sensitivities adjusted appropriately. With a 10 mV s^{-1} sweep rate a full-scale sensitivity of 1 μA was usually sufficient.

Microcoulometric experiments were carried out on 2-ml solutions, which contained initially 5×10^{-4} mol l^{-1} of the alizarin together with the supporting electrolyte. The solution was placed in the Manousek cell and the capillary was positioned in such a way that its end was just below the surface of the solution. The deaerated solution was kept under a nitrogen atmosphere and was mixed after each 5 min by passage of a burst of nitrogen. A polarogram was then recorded every 30 min. The electrolysis was carried out at a constant potential corresponding to a well defined limiting diffusion current. After 20–25% conversion the electrolysis was discontinued and the solution replaced with the pure supporting electrolyte to measure the residual current, which was then used as a correction when evaluating the results. This was done both on the basis of Faraday's law directly, and with Gilbert and Rideal's logarithmic method.[22]

Current *versus* time curves were measured only if adsorption phenomena were suspected. In such instances the electrocapillary curve was first obtained by counting the number of drops falling naturally from a capillary per minute, at various electrode potentials. From the electrocapillary curve appropriate potential values were then selected, covering both distorted and undistorted regions, at which the current *versus* time curves were then measured. These curves were evaluated according to the guidelines given by Heyrovsky and Kuta.[23]

As the main task was to elucidate the electrochemical properties of strongly chelating anthraquinones, we studied compounds **XII–XVIII** in more detail than the others, which were examined first for the sake of comparison, mainly to investigate substitution effects. Our report will therefore be concentrated on the former group of substances.

Results

Polarography

The polarographic behaviour was investigated within the widest possible pH range for each compound. Depending on the medium and the pH of the medium and the pH of the solution, single, double or triple waves were obtained for all the substances involved. The

single waves were proved to be diffusion controlled. The double waves consisted either of two diffusion-controlled waves or of an adsorption pre-wave followed by a diffusion wave. The two diffusion-controlled waves usually appeared at higher pH values, and they were well separated (see Figs. 1 and 2). The adsorption pre-wave appeared at lower pH values and usually disappeared, or became less pronounced as the pH was increased, although in some instances it reappeared again at high pH values. This adsorption pre-wave always appeared close to the main diffusion wave. The triple waves always consisted of an adsorption pre-wave followed by two diffusion-controlled waves. Triple waves appeared only at high pH values (see Fig. 3).

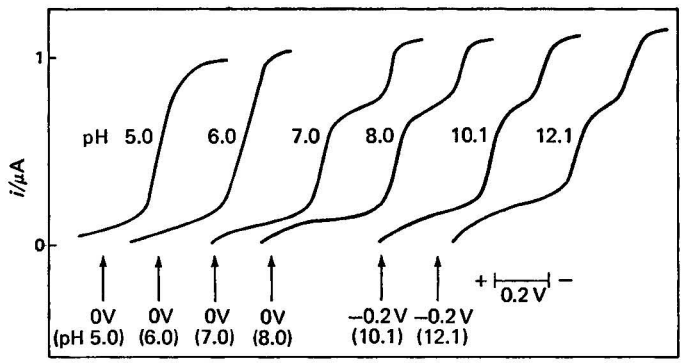


Fig. 1. Polarograms of 2.5×10^{-4} mol l^{-1} IX at various pH values in aqueous medium.

The polarograms of **VIII** in Fig. 3 display such waves at and above pH 4.6; in more acidic solutions the two waves consist of an adsorption pre-wave and a diffusion-controlled wave.

The over-all heights of the waves were found to be independent of the pH for most of the compounds. In some instances, however, significant variations were observed. Fig. 4 shows the variation of the Ilkovič κ coefficients (in amps per mole) for these anthraquinones. In certain instances (*e.g.*, **I**, **III**, **VI**, **XIII** and **XVI**) there was a significant drop in the coefficient as the pH of the solution increased and in other instances (**V**, **XIII**, **XIV** and again **XVI**) significant minima were observed on the κ *versus* pH curve, which cannot be attributed to inconsistencies in the evaluation of the polarograms. The κ coefficients depended, as expected, mainly on the medium—in aqueous solutions they averaged between 1 and 4×10^{-3} A mol^{-1} and in 75% ethanol between 4 and 7×10^{-3} A mol^{-1}.

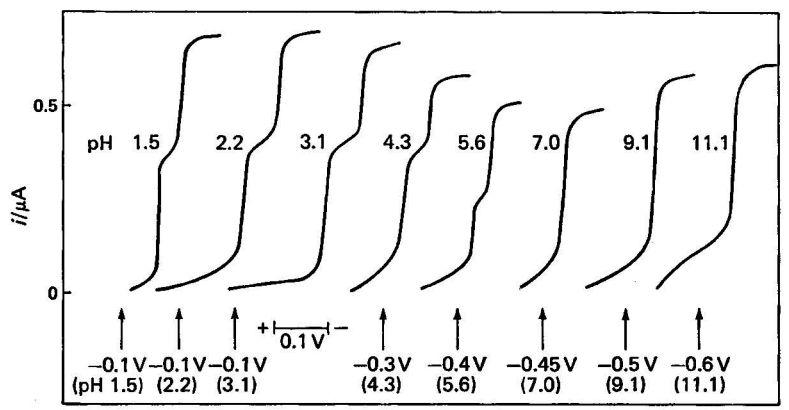


Fig. 2. Polarograms of 2.5×10^{-4} mol l^{-1} XV at various pH values in aqueous medium.

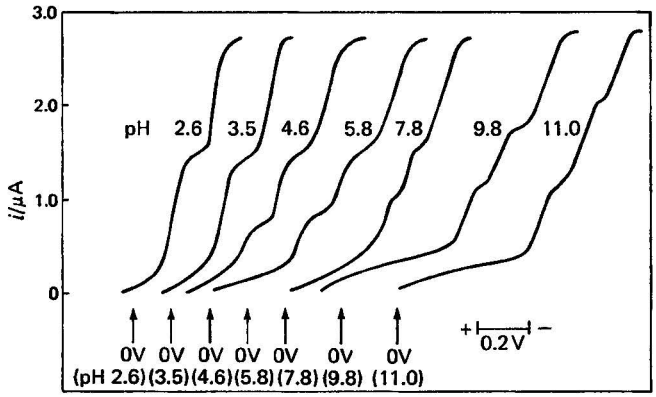


Fig. 3. Polarograms of 3 × 10^{-4} mol l^{-1} **VIII** at various pH values in 75% ethanol.

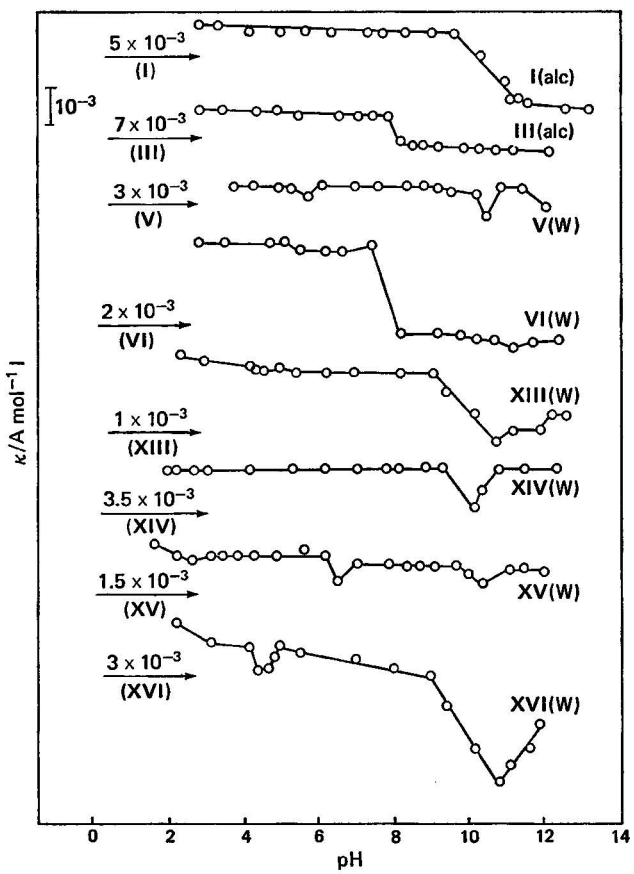


Fig. 4. Variation of the Ilkovič κ coefficient with pH for selected anthraquinone derivatives in aqueous (W) and 75% ethanol (alc) solutions.

The variation of half-wave potentials with pH was investigated with the greatest care, as these relationships provide valuable information on the acid - base equilibria in which the oxidised and reduced species are involved and, in an indirect way, also on the reduction process itself. Figs. 5 and 6 refer to aqueous solutions and Fig. 7 to alcoholic solutions. From the theoretical point of view the half-wave potentials themselves are not so important, but rather the slopes of the $E_{\frac{1}{2}}$ *versus* pH plots, as well as those pH values where these slopes change. The expected values for these slopes are simple multiples of the Nernstian prelogarithmic constant of 60 mV pH^{-1} with factors of 0, 0.5, 1 and 1.5, that is 0, 30, 60 and 90 mV pH^{-1}. Hence, the lines fitting the experimental points on these figures were drawn deliberately with such slopes—as is clear from the figures themselves, such lines do fit the experimental points reasonably well, although in some instances least-squares fits would produce slopes of different values. In the single instance of **XII** the experimental points could not be joined with a continuous set of lines.

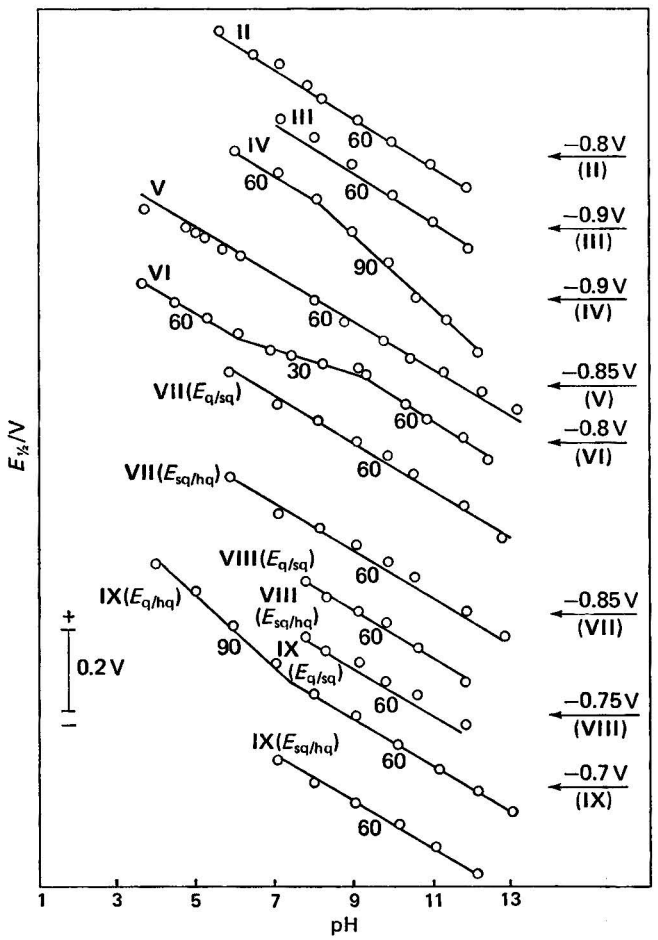


Fig. 5. Variation of the half-wave potentials of compounds **II–IX** with pH in aqueous medium.

The nature of diffusion-controlled waves has been verified by measurements taken at various mercury column heights and plotting the currents as the function of the square root of the (corrected) height. Such an operation was undertaken for each compound at several pH values, covering all sections of the $E_{\frac{1}{2}}$ *versus* pH curves. In addition to the linearity of these plots, we had various indirect evidence for diffusion control; thus, the absence of

irregularities on the electrocapillary curves and the shape of the instantaneous current *versus* time curves indicated clearly that diffusion currents were involved. Finally, waves were accepted to be diffusion controlled only when the current *versus* concentration plots proved the expected proportionality.

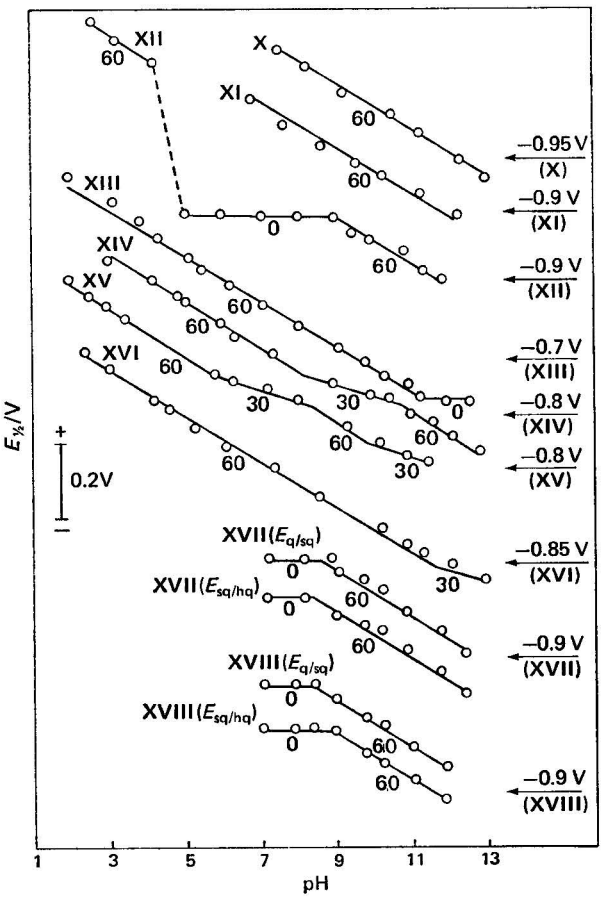


Fig. 6. Variation of the half-wave potentials of compounds **X–XVIII** with pH in aqueous medium.

The adsorption waves were investigated by several methods. Firstly, polarograms were obtained at different concentrations. Plotting the wave height against concentration a curve typical for adsorption currents was obtained (Fig. 8). Temperature effects were studied. As can be seen from Fig. 9, the over-all wave height increased with temperature, but the adsorption pre-wave disappeared gradually as the temperature was increased. Instantaneous current *versus* time curves were also studied whenever an adsorption wave was suspected. The oscillograms obtained at various potentials of the DME always showed distortions near to the half-wave potential of the adsorption wave (Fig. 10). Finally, electrocapillary curves were obtained in the presence of the depolariser. Plotting the average drop lifetime as a function of the potential of the DME, characteristic incisions occurred at the appropriate potentials, whereas in the absence of the depolariser the curve was smooth at these potentials (Fig. 11).

The cyclic voltammetric response of those substances which displayed adsorption waves was also interesting. In the first cycle, distortions were observed approximately at the expected potentials, but these disappeared when a second cycle was imposed immediately

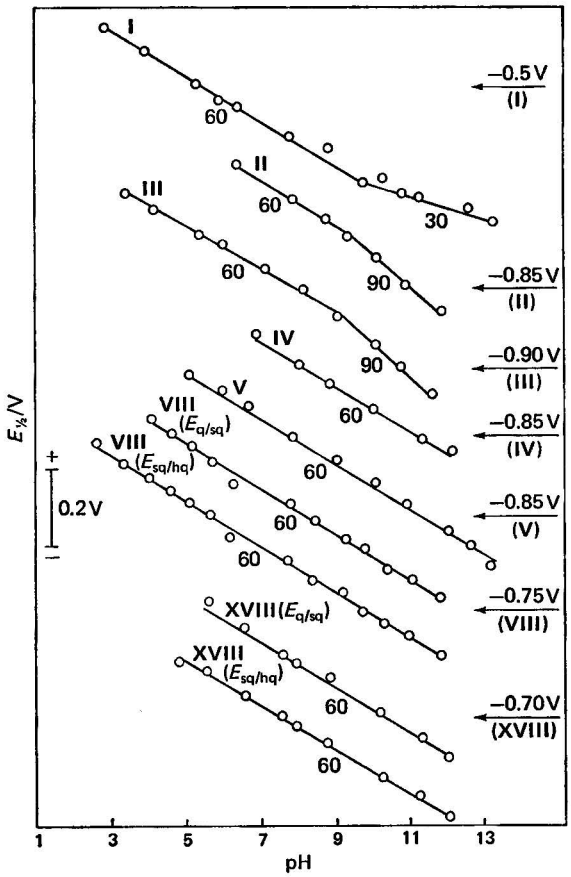


Fig. 7. Variation of the half-wave potentials of selected anthraquinones with pH in 75% ethanol.

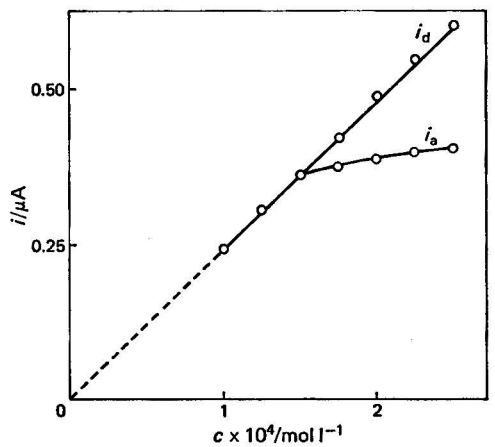


Fig. 8. Variation of the height of the total wave (i_d) and of the adsorption pre-wave (i_a) with concentration of XV at pH 4.30 in aqueous medium.

after the first. These adsorption waves displayed irreversible or, at very low scan rates, quasi-irreversible behaviour, to some extent contrary to expectations.[24] This unusual behaviour would merit a more detailed investigation and electrochemists with more theoretical outlooks than that of the present authors might find this a rewarding area of research.

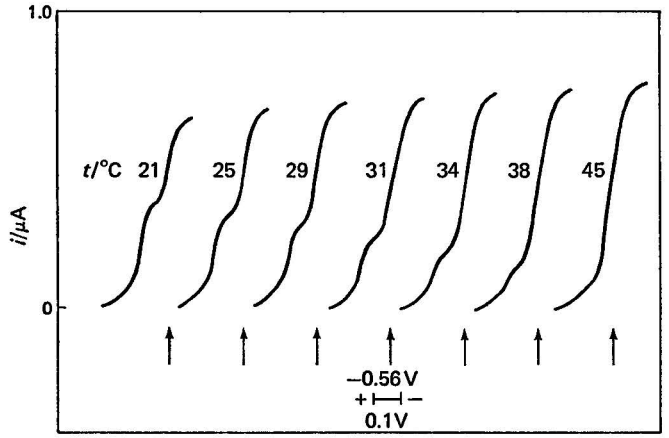


Fig. 9. Temperature dependence of the shape and height of the polarographic wave of a 2.5×10^{-4} mol l^{-1} solution of **XV** at pH 5.6 in aqueous medium.

Adsorption pre-waves were noted in connection with compounds **III, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI** and **XVIII**. As indicated before, their appearance is dependent on the experimental conditions, of which pH, concentration and temperature are the most important. Further details on these adsorption pre-waves are available in Qureshi's thesis.[25]

All purely diffusion-controlled, single or double waves were subjected to logarithmic analysis in order to calculate the value of the pre-logarithmic factor in the Nernst equation. To our surprise, but in good agreement with results obtained on simpler quinone - hydroquinone systems,[26] the number of electrons (for a coefficient $\alpha = 1$) was almost always found to be nearly unity, even with the single waves, where a value of 2 would be expected. This behaviour will be dealt with under Discussion.

Microcoulometry

To obtain a reliable value for the number of electrons taken up by a single molecule of the anthraquinones, we decided to carry out microcoulometric experiments. As the time

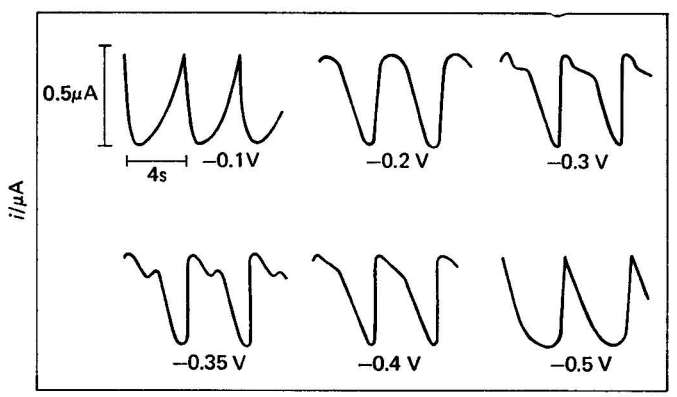


Fig. 10. Instantaneous current *versus* time curves of 2×10^{-4} mol l^{-1} solutions of **XV** at pH 4.30 in aqueous medium at different D.M.E. potentials.

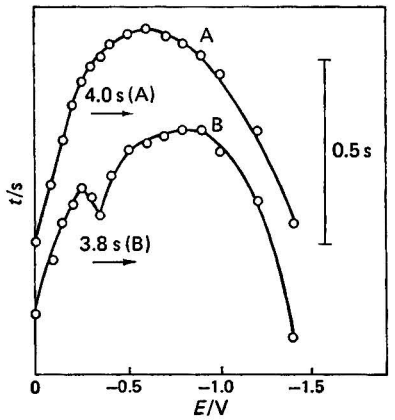


Fig. 11. Electrocapillary curve of mercury in a 10^{-1} mol l^{-1} acetate buffer (pH 4.30) in (A) the absence and (B) the presence of 2×10^{-4} mol l^{-1} **XV**.

duration of a single experiment is considerable, we omitted compounds **VII** and **IX**, carried out the measurement with most other compounds in neutral medium only, and only with the four key compounds, **XIII**–**XVI**, did we carry out three runs at three different pH values, involving acidic, neutral and basic media. With the three compounds displaying two separate reduction waves (**VIII**, **XVII** and **XVIII**), the pH was adjusted to 9, where the two waves are well established. A separate run was carried out for each wave. All results were evaluated both by graphical integration of the current *versus* time curves directly through Faraday's law, and also by using Gilbert and Rideal's equation.[22]

The two methods gave almost exactly the same results for the number of electrons: the difference between the corresponding figures was less than 0.5% in all instances. We feel that by evaluating results in both ways the figures are more reliable. The method based directly on Faraday's law eliminates the apparent weakness of the method of Gilbert and Rideal,[22] which takes into account only the initial and final current, but disregards the over-all shape of the current *versus* time curve. All of the compounds with single waves gave values between 1.97 and 2.30 for the number of electrons involved. The values for the double waves were in all instances higher than 1 (for each wave), but never exceeded 1.42. Fig. 12 shows the results obtained for **XV**. Through the points (which, theoretically, should

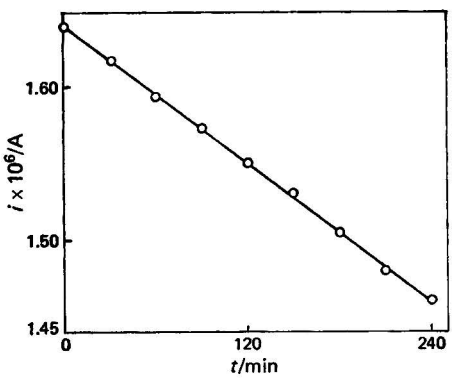


Fig. 12. Current *versus* time curve obtained during the microcoulometric reduction of 2 ml of 5×10^{-4} M **XV** solution.

follow an exponential function) a straight line was drawn and the graph was integrated as a trapezium. From this integration, a value of 2.100 is obtainable for the number of electrons, while Gilbert and Rideal's method gives 2.105.

Cyclic Voltammetry

The purpose of the cyclic voltammetric measurements was to find out whether the reduction of these compounds is reversible or not. Up to a sweep rate of 10 mV s^{-1} (which is 2–10 times higher than those used in the polarographic measurements) the reduction was either reversible or quasi-reversible, whereas, as pointed out, the adsorption was quasi-reversible at very low scan rates, or irreversible. In Fig. 13 three cyclic voltammograms are reproduced that show these different responses. All of these curves were obtained with a 10 mV s^{-1} sweep rate in buffered aqueous solutions at pH 10. Fig. 13(*a*) shows the cyclic voltammogram of 2.5×10^{-4} mol l^{-1} of **XIV**. The cathodic (positive) and anodic (negative) peak currents are virtually identical; the peak separation $[E_{p(c)} - E_{p(a)}]$ is 33 mV, not far from the theoretical value of 30 mV. Hence, one can say that the reduction of **XIV** is reversible under such conditions. A 10^{-3} mol l^{-1} solution of **VI** [Fig. 13(*b*)] displays quasi-reversible reduction, the cathodic and anodic peak currents differing by more than 10% and the peak separation being more than double the theoretical value. The adsorption peak, obtained in the first cathodic half-cycle, is barely distinguishable as a faint shoulder on the anodic half-cycle. The adsorption peak, obtained in the first cathodic half-cycle in a 3×10^{-4} mol l^{-1} solution of **VIII** [Fig. 13(*c*)], does not show up on the first anodic half-cycle, indicating that the adsorption, under these circumstances, is irreversible. However, the two reduction peaks are reproduced with much reduced definition on the anodic half-cycle, so the reduction of **VIII** is still a quasi-reversible process. Reduction peak potentials $[E_{p(c)}]$, contrasted with the polarographic half-wave potentials ($E_{\frac{1}{2}}$), together with a note on reversibility, are shown in Table II.

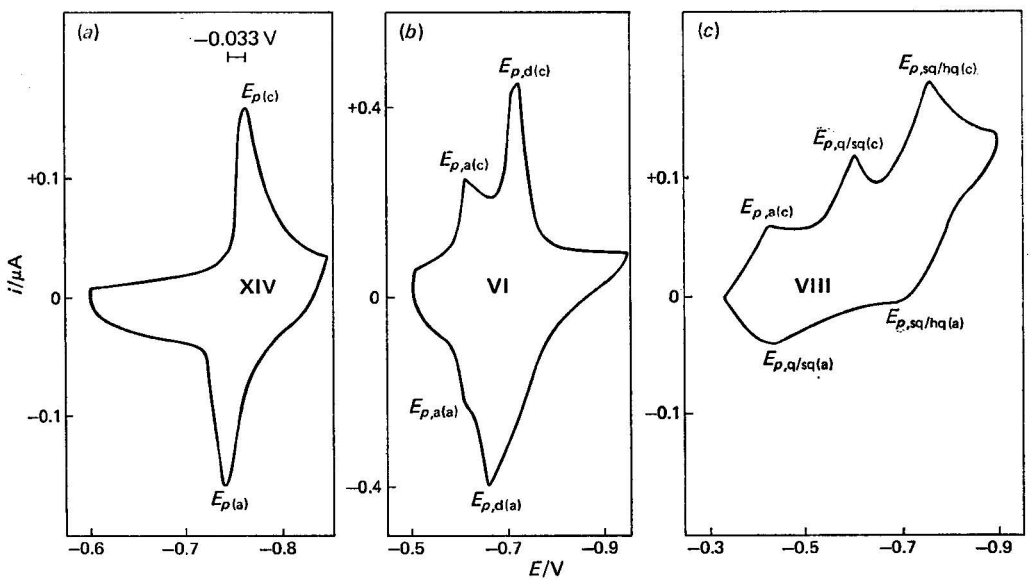


Fig. 13. Cyclic voltammograms of various anthraquinone derivatives. For explanation, see text.

Discussion

The purpose of this discussion is to explain the redox and acid - base processes in which the examined anthraquinones are involved, and to correlate these with the experimental facts.

Quinones (q), when reduced by taking up a single electron, form semiquinones (sq), while the uptake of a further electron results in the formation of hydroquinones (hq). In all of the redox processes, therefore, these three states of oxidation are expected to be involved.

TABLE II

CYCLIC VOLTAMMETRIC REDUCTION PEAK POTENTIALS [$E_{p(c)}$] AND POLAROGRAPHIC HALF-WAVE POTENTIALS ($E_{\frac{1}{2}}$) AT pH 10 OF ANTHRAQUINONES

Experimental conditions given in the text.

	$E_{p(c)}$/V			$E_{\frac{1}{2}}$/V			
Compound	q/hq	q/sq	sq/hq	q/hq	q/sq	sq/hq	Reduction†
I*	−0.77			−0.74			R
II	−0.78			−0.73			R
III	−0.85			−0.82			Q
IV	−0.85			−0.81			Q
V	−0.78			−0.74			Q
VI	−0.71			−0.67			R
VII		−0.60	−0.87		−0.56	−0.83	Q
VIII		−0.60	−0.76		−0.57	−0.72	Q
IX		−0.61	−0.81		−0.58	−0.78	Q
X	−0.87			−0.83			R
XI	−0.84			−0.81			Q
XII	−0.82			−0.79			Q
XIII	−0.81			−0.77			Q
XIV	−0.77			−0.73			R
XV	−0.78			−0.74			R
XVI	−0.79			−0.76			R
XVII		−0.77	−0.86		−0.73	−0.83	Q
XVIII		−0.76	−0.85		−0.73	−0.81	Q

* In 75% ethanolic solution.
† R = reversible; Q = quasi-reversible.

Each oxidation state, however, can attain different degrees of protonation; some of the substances involved in the study may contain as many as seven protons, which could, under certain circumstances, dissociate. Thus, the uptake of each electron may (or may not) be accompanied by the uptake of one or more protons.

Fig. 14 shows all of the possible oxidation and protonation states of the substances involved; boxes drawn with solid lines represent those states which were experimentally verified (the existence of the other states, in extremely acidic or alkaline media, cannot be ruled out, however). Capital, lower-case and Greek letters are used to denote different states of oxidation and protonation, and will be applied later to describe the processes involved.

Let us consider for example the reduction of the quinone H_5q (c). This may take place in one step (Cγ reaction):

$$H_5q + 2e^- \rightleftharpoons H_5hq^{2-} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

at the potential $E^\circ_{C\gamma}$ (which is close to the observed $E_{\frac{1}{2}q/hq}$ half-wave potential). As a result, a single wave with the uptake of two electrons is obtained.

The reduction may, of course, take place in two steps. First the semiquinone is formed (Cc reaction):

$$H_5q + e^- \rightleftharpoons H_5sq^- \quad .. \quad .. \quad .. \quad .. \quad (2)$$

to which a one-electron wave of $E_{\frac{1}{2}q/sq}$ half-wave potential corresponds. This is followed by a second reduction step (cγ reaction):

$$H_5sq^- + e^- \rightleftharpoons H_5hq^{2-} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

causing the occurrence of a second one-electron wave with $E_{\frac{1}{2}sq/hq}$ half-wave potential.

Whether under given experimental circumstances a single two-electron wave or two single one-electron waves are observed, depends on the stability of the semiquinone. Combining equilibria (1)–(3), one can describe the disproportionation (or dismutation) of the semiquinone with the equilibrium

$$2H_5sq^- \rightleftharpoons H_5q + H_5hq^{2-} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

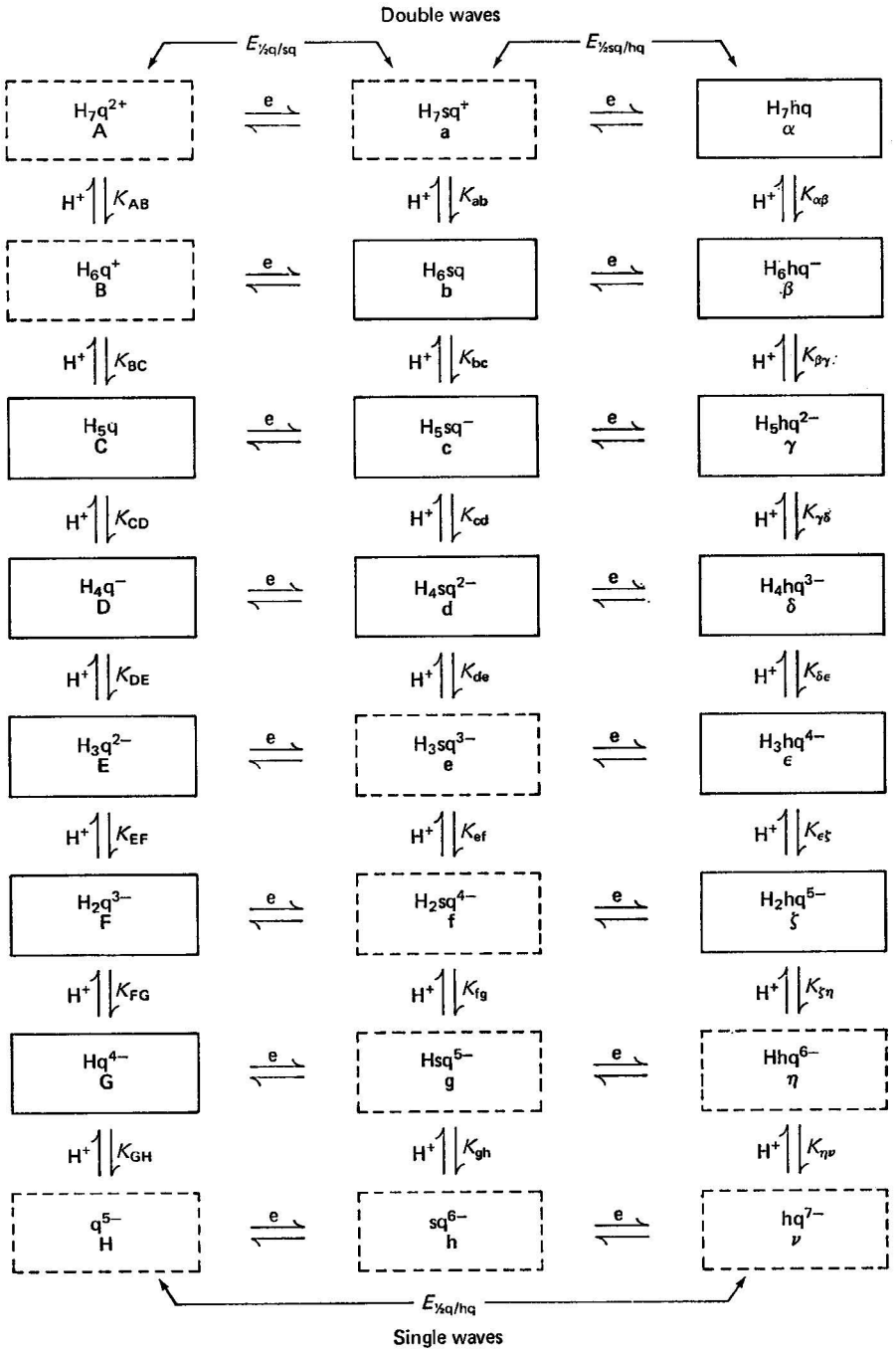


Fig. 14. Reduction and protonation of anthraquinones.

The stability of the semiquinone can be measured through the value of the semiquinone formation constant, K_{sq}, defined from reaction (4) as

$$K_{sq} = \frac{[H_5sq^-]^2}{[H_5q]\,[H_5hq^{2-}]} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Heyrovsky and Kuta[26] applied such considerations when describing the equation of the polarographic waves of quinones, and came to the conclusion that:

(*a*) if $K_{sq} = 0$ a single wave with a slope corresponding to a two-electron reduction is obtained;

(*b*) if $K_{sq} > 16$ two separate waves are obtained, each with a slope corresponding to a single-electron uptake; and

(*c*) if $0 < K_{sq} < 16$ a single wave is obtained, with a slope corresponding to anything between $\frac{2}{3}$ and 2 electrons per molecule.

These considerations explain the curious fact, mentioned under Results, that in many instances single waves were observed, for which the logarithmic analysis gave for the number of electrons a value close to 1, while microcoulometry always resulted in a number close to 2.

The reduction of quinones may or may not be accompanied by the uptake (and in some instances by the release) of one or more protons. Although individual polarographic waves do not reveal such processes, the evaluation of the half-wave potential *versus* pH curves makes it possible to find out about them. To see this clearly let us again examine the reduction of a quinone (H_5q). Let us suppose that $K_{sq} = 0$, so that there is no semiquinone formation; the reduction can therefore be described as

$$H_5q + 2e^- \rightleftharpoons H_5hq^{2-} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Depending on the pH of the medium, the quinone may undergo stepwise dissociation. For our purpose it will be sufficient to consider the first two steps only:

$$H_5q \rightleftharpoons H^+ + H_4q^- \quad .. \quad .. \quad .. \quad .. \quad (7)$$

and

$$H_4q^- \rightleftharpoons H^+ + H_3q^{2-} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

with the dissociation equilibrium constants

$$K_{CD} = \frac{[H^+]\,[H_4q^-]}{[H_5q]} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

and

$$K_{DE} = \frac{[H^+]\,[H_3q^{2-}]}{[H_4q^-]} \quad .. \quad .. \quad .. \quad .. \quad (10)$$

Further, the hydroquinone formed in process (6) may undergo stepwise protonation. Considering the first two of these steps, we can have

$$H_5hq^{2-} + H^+ \rightleftharpoons H_6hq^- \quad .. \quad .. \quad .. \quad .. \quad (11)$$

and

$$H_6hq^- + H^+ \rightleftharpoons H_7hq \quad .. \quad .. \quad .. \quad .. \quad (12)$$

With the symbols used in Fig. 14 these processes can be characterised with the following dissociation equilibrium constants:

$$K_{\beta\gamma} = \frac{[H^+]\,[H_5hq^{2-}]}{[H_6hq^-]} \quad .. \quad .. \quad .. \quad .. \quad (13)$$

and

$$K_{\alpha\beta} = \frac{[H^+]\,[H_6hq^-]}{[H_7hq]} \quad .. \quad .. \quad .. \quad .. \quad (14)$$

The oxidation - reduction potential of the system described in reaction (6) can be expressed as

$$E = E_{q/hq} - \frac{0.059}{2} \times \log \frac{[H_5hq^{2-}]}{[H_5q]} \quad .. \quad .. \quad .. \quad (15)$$

where $E^\circ_{q/hq}$ is the standard oxidation - reduction potential. The two concentrations included in the logarithmic term are in general not known, but can be calculated from the mass balance equations:

$$c_{hq} = [H_5hq^{2-}] + [H_6hq^-] + [H_7hq] \quad .. \quad .. \quad .. \quad (16)$$

and

$$c_q = [H_5q] + [H_4q^-] + [H_3q^{2-}] \quad .. \quad .. \quad .. \quad .. \quad (17)$$

where c_{hq} and c_q are the analytical concentrations of the hydroquinone and quinone species, respectively. Combining equations (9), (10) and (13)–(17) we can express the oxidation - reduction potential as

$$E = E^\circ_{q/hq} - \frac{0.059}{2} \times \log \frac{c_{hq}}{c_q} - \frac{0.059}{2} \times \log K_{\alpha\beta} K_{\beta\gamma} - \frac{0.059}{2} \times \log \frac{[H^+]^2 + K_{CD}\,[H^+] + K_{CD}\,K_{DE}}{[H^+]^2\,([H^+]^2 + K_{\alpha\beta}\,[H^+] + K_{\alpha\beta}\,K_{\beta\gamma})} \quad (18)$$

Provided that the diffusion coefficients of the oxidised and reduced forms are equal, the half-wave potential is measured at the point where the analytical concentrations of c_{hq} and c_q are equal. Thus, from equation (18) we can express the dependence of half-wave potentials on the hydrogen-ion concentration with the equation

$$E_{\frac{1}{2}} = E^\circ_{q/hq} - \frac{0.059}{2} \times \log K_{\alpha\beta} K_{\beta\gamma} - \frac{0.059}{2} \times \log \frac{[H^+]^2 + K_{CD}\,[H^+] + K_{CD}\,K_{DE}}{[H^+]^2\,([H^+]^2 + K_{\alpha\beta}\,[H^+] + K_{\alpha\beta}\,K_{\beta\gamma})} \quad (19)$$

The experimentally obtained $E_{\frac{1}{2}}$ *versus* pH curves should conform to this equation. The correlation of theory with experimental results is simple if one considers firstly such extreme cases where equation (19) can be drastically simplified. For example, in very acidic solutions we have

$$[H^+]^2 \gg K_{CD}\,[H^+] + K_{CD}\,K_{DE} \approx 0 \quad .. \quad .. \quad .. \quad (20)$$

and also

$$[H^+]^2 \gg K_{\alpha\beta}\,[H^+] + K_{\alpha\beta}\,K_{\beta\gamma} \approx 0 \quad .. \quad .. \quad .. \quad (21)$$

Applying such simplifications in equation (19) we arrive at the expression

$$E_{\frac{1}{2}} = E_{q/hq} - \frac{0.059}{2} \times \log K_{\alpha\beta} K_{\beta\gamma} - 0.059\text{pH} \quad .. \quad .. \quad .. \quad (22)$$

As long as the conditions described in the inequalities of (20) and (21) prevail, we therefore

expect the decrease in the half-wave potential with pH with a 60-mV slope. In other words, under these conditions the reaction

$$H_5q + 2H^+ + 2e^- \rightleftharpoons H_7hq \quad .. \quad .. \quad .. \quad .. \quad (23)$$

(using the symbols of Fig. 14, a c → α process) is taking place. If these conditions are not more valid, one can describe others that fit the case and with an analogous treatment one can calculate the expected slope of the $E_{\frac{1}{2}}$ *versus* pH curves. For the model chosen one can combine altogether nine different conditions (some of which are less likely to prevail in practice than others), which are shown in Table III.

TABLE III

SLOPES OF $E_{\frac{1}{2}}$ - pH CURVES AND REDUCTION PATHS FOR THE TWO-ELECTRON SINGLE-WAVE REDUCTION OF A QUINONE

2nd condition	1st condition: $[H^+]^2 \gg K_{CD}[H^+] + K_{CD}K_{DE}$	1st condition: $K_{CD}[H^+] \gg [H^+]^2 + K_{CD}K_{DE}$	1st condition: $K_{CD}K_{DE} \gg [H^+]^2 + K_{CD}[H^+]$
	increasing pH →		
$[H^+]^2 \gg K_{\alpha\beta}[H^+] + K_{\alpha\beta}K_{\beta\gamma}$	−60 mV pH^{-1}	−90 mV pH^{-1}	−120 mV pH^{-1}
	C → α	D → α	E → α
$K_{\alpha\beta}[H^+] \gg [H^+]^2 + K_{\alpha\beta}K_{\beta\gamma}$	−30 mV pH^{-1}	−60 mV pH^{-1}	−90 mV pH^{-1}
	C → β	D → β	E → β
$K_{\alpha\beta}K_{\beta\gamma} \gg [H^+]^2 + K_{\alpha\beta}[H^+]$	0 mV pH^{-1}	−30 mV pH^{-1}	−60 mV pH^{-1}
	C → γ	D → γ	E → γ

(increasing pH ↓)

According to such considerations, the $E_{\frac{1}{2}}$ *versus* pH curves should consist of linear sections with slopes of 0, −30, −60 or −90 mV pH^{-1} (the occurrence of a −120 mV pH^{-1} slope being most unlikely), for two-electron, single-wave processes. The experimental results, as shown in Figs. 5, 6 and 7, do in fact correspond to such predictions. It can also be shown that the pH values corresponding to the intersections of the linear portions with different slopes are in fact equal to the appropriate pK values of the substances involved in the process.

In a corresponding manner, one can involve other species with different degrees of protonation in such considerations, and carry out calculations in a similar fashion. Also, it is easy to extend such considerations to each of the double waves (when $K_{sq} > 16$) with one-electron uptake.

Based on such considerations, we evaluated all of the available $E_{\frac{1}{2}}$ *versus* pH curves and, taking into account all other experimental evidence and data obtained from the literature, have summarised our findings in Table IV. Our aim was to find (*a*) which species and how many electrons are involved in the electrochemical reduction and (*b*) what are the pK values of the quinones, semiquinones and hydroquinones involved. Table IV also contains information on the $E_{\frac{1}{2}}$ *versus* pH curves themselves and all pK values available in the literature. The symbols and abbreviations are the same as those used in Fig. 14. It is

TABLE IV

REDUCTION OF ANTHRAQUINONE DERIVATIVES

Compound	Wave	pH range	Parameters of the $E_{\frac{1}{2}}$ *vs.* pH line: Intercept/ V	Parameters of the $E_{\frac{1}{2}}$ *vs.* pH line: Slope/ V pH^{-1}	Reduction mechanism (*cf.*, Fig. 14)	pK values: Experimentally obtained	pK values: Taken from literature
(*a*) Aqueous solutions—							
II	q/hq	5.7–11.9	−0.125	−0.060	C → α		
III	q/hq	7.2–11.9	−0.220	−0.060	C → α		
IV	q/hq	5.9–8.35	−0.150	−0.060	C → α	pK_{CD} = 8.35	
		8.35–12.1	+0.095	−0.090	D → α		

TABLE IV (*continued*)

Compound	Wave	pH range	Parameters of the $E_{\frac{1}{2}}$ vs. pH line: Intercept/ V	Slope/ V pH^{-1}	Reduction mechanism (*cf.*, Fig. 14)	pK values: Experimentally obtained	Taken from literature
V	q/hq	3.6–~6	−0.140	−0.060	C → α	$pK_{\alpha\beta} \approx 6$	pK_{CD} = 6.67[27]; 5.60[28]
		~6–~11			D → β		
		~11–13.2			E → γ	$pK_{\beta\gamma} \approx 11$	pK_{DE} = 10.78[27]; 11.16[28]
VI	q/hq	3.5–6.0	−0.160	−0.060	C → α	$pK_{DE} = 9.1$	
		6.0–6.9	−0.340	−0.030	C → β		
		6.9–9.1	−0.065	−0.060	D → γ	$pK_{\alpha\beta} = 6.0$	pK_{CD} = 6.91[11]
		9.1–12.4			E → γ	$pK_{\beta\gamma} \approx pK_{CD}$	pK_{DE} = 11.02[11]
VII	q/sq	6.0–13.0	+0.045	−0.060	C → b		
	q/hq	6.0–13.0	−0.225	−0.060	b → α		
VIII	q/sq	7.7–11.9	+0.025	−0.060	C → b		
	sq/hq	7.7–11.9	−0.120	−0.060	b → α		
IX	q/hq	4.0–7.5	+0.245	−0.090	D → α	$pK_{\alpha\beta} = 7.5$	
	q/sq	7.5–13.1	+0.020	−0.060	D → C		
	sq/hq	7.5–12.2	−0.185	−0.060	C → β		
X	q/hq	7.4–12.9	−0.235	−0.060	D → β		
XI	q/hq	6.6–12.2	−0.195	−0.060	D → β		
XII	q/hq	2.4–4.0	−0.070	−0.060	C → α	$pK_{\alpha\beta} \approx pK_{\beta\gamma} \approx 4.5$	pK_{CD} = 6.25[30]
		4.9–6.25			C → γ		
		6.25–8.8	−0.720	0	D → δ	$pK_{DE} \approx pK_{EF} \approx 8.80$	pK_{FG} = 12.2[30]
		8.8–11.8	−0.190	−0.060	F → δ	$pK_{\gamma\delta} \approx pK_{CD}$	
XIII	q/hq	1.8–5.5	−0.165	−0.060	C → α	pK_{DE} = 11.2	pK_{CD} = 5.50[28]; 5.12[26]
		5.5–11.2			D → β	$pK_{\beta\gamma} \approx pK_{\gamma\delta} \approx$	pK_{DE} = 11.25[28]; 11.20[29]
		11.2–12.5	−0.845	0	E → ε	$pK_{\delta\varepsilon} \approx pK_{DE}$	pK_{EF} = 13.10[28]
XIV	q/hq	2.8–5.5 (0.75)			D → β	$pK_{\beta\gamma} \approx pK_{DE}$	pK_{CD} = 2.40[28]; 4.89[6]
		5.5 (or 7.5)–8.0	−0.185	−0.060	E → γ	$pK_{\gamma\delta}$ = 8.0	pK_{DE} = 5.54[28]; 7.55[6]
		8.0–10.7	−0.430	−0.030	E → δ	pK_{EF} = 10.7	pK_{EF} = 10.07[29]; 10.43[6]
		10.7–12.8	−0.110	−0.060	F → δ		pK_{FG} = 11.98[29]; 11.19[6]
XV	q/hq	1.6–2.5			D → β	$pK_{\beta\gamma} \approx pK_{DE}$	$pK_{BC} = 0.5^{10}$; $pK_{CD} = 1.4^{10}$
		2.5–5.6	−0.230	−0.060	E → γ	pK_{EF} = 5.6	$pK_{DE} = 2.5^{10}$; $pK_{EF} = 5.8^{10}$
		5.6–8.2	−0.400	−0.030	F → ε	$pK_{\gamma\delta} \approx pK_{\delta\varepsilon} \approx pK_{EF}$	$pK_{FG} = 10.0^{10}$; $pK_{GH} = 12.3^{10}$
		8.2–9.7	−0.155	−0.060	G → ε		
		9.7–11.4	−0.445	−0.030	G → ζ		
XVI	q/hq	2.2–4.67			C → α	$pK_{\gamma\delta}$ = 11.6	pK_{CD} = 4.67[28]
		4.67–10.65	−0.155	−0.060	D → β	$pK_{\alpha\beta} \approx pK_{CD}$	pK_{DE} = 10.65[28]
		10.65–11.6			E → γ		
		11.6–12.9	−0.500	−0.030	E → δ	$pK_{\beta\gamma} \approx pK_{DE}$	
XVII	q/sq	7.1–8.6	−0.640	0	C → c	$pK_{CD} = pK_{DE} = 8.6$	
		8.6–12.4	−0.125	−0.060	E → d	$pK_{cd} = 8.4$	
	sq/hq	7.1–8.4	−0.735	0	c → γ		
		8.4–11.9	−0.230	−0.060	d → γ		
XVIII	q/hq	7.0–8.4	−0.620	0	C → c	$pK_{CD} = pK_{DE} = 8.4$	pK_{CD} = 8.5[30]
		8.4–11.9	−0.120	−0.060	E → d	$pK_{cd} = 8.9$	
	sq/hq	7.0–8.9	−0.740	0	C → γ		
		8.9–11.9	−0.210	−0.060	d → γ		
(*b*) Solutions containing 75% ethanol—							
I	q/hq	2.8–9.8	−0.145	−0.060	C → α	$pK_{\alpha\beta} = 9.8$	$pK_{CD} = 10.8^{27}$
		9.8–13.3	−0.435	−0.030	C → β		
II	q/hq	6.3–9.6	−0.155	−0.060	C → α	$pK_{CD} = 9.6$	
		9.6–11.9	−0.130	−0.090	D → α		
III	q/hq	3.4–9.2	−0.240	−0.060	C → α	$pK_{CD} = 9.2$	pK_{CD} = 7.86[27]
		9.2–11.6	+0.045	−0.090	D → α		$pK_{DE} \approx 14^{27}$
IV	q/hq	6.9–7.95	−0.185	−0.060	C → α	$pK_{\alpha\beta} \approx pK_{CD}$	pK_{CD} = 7.95[27]; pK_{DE} = 13.31[27]
		7.95–11.7			D → β		
V	q/hq	5.1–13.2	−0.190	−0.060	C → α		
VIII	q/sq	2.6–11.9	−0.130	−0.060	C → b		
	sq/hq	2.6–11.9	−0.130	−0.060	b → α		
XVIII	q/sq	5.6–12.1	−0.085	−0.060	C → b		
	sq/hq	4.9–12.1	−0.225	−0.060	b → α		

worthwhile pointing out that the experimentally obtained, "polarographic" pK values agree well with the values taken from the literature, which were mostly obtained by spectrophotometry. The agreement is very good for the aqueous solutions of **XIII**, **XIV**, **XV** and

XVIII and is acceptable even with the alcoholic solution of **III**. Only with the aqueous solution of **VI** do the data differ substantially. In only these instances do we possess parallel polarographic and spectrophotometric data.

When evaluating these results we also tried to find linear free-energy relationships among certain derivatives. It must be said that the original Hammett[30] equation cannot be applied here (nor are the appropriate reaction and substituent constants available), and none of the more recent equations[31,32] would really be valid. Still, it seemed to be reasonable to attempt to find some correlation between the half-wave potentials (at a fixed pH) and the various pK values available. When doing so we hoped to extend the available polarographic free-energy correlation data for anthraquinones.[15,33,31] We constructed separate diagrams for the pK_{CD} and pK_{DE} values (only these are available in significant numbers) using the data in Table IV. For half-wave potential values we used the intercept figures (which correspond to the half-wave potential values extrapolated to pH 0). Although a number of points fall on a well defined straight line, as expected, we have not published such plots, because in both instances there were a number of unexpected deviations, which we were unable to explain. Also, there are parallel, sometimes greatly differing, values for the same pK value available in the literature, and a choice among them cannot easily be made. Although one can select a number of points that would show a reasonable straight-line relationship, such a selection would be arbitrary and not scientifically justified. A more detailed account of these attempts is available in a thesis.[25]

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Direct Differential-pulse Polarographic Determination of Mixtures of the Food Colouring Matters Tartrazine - Sunset Yellow FCF, Tartrazine - Green S and Amaranth - Green S in Soft Drinks

A. G. Fogg and K. S. Yoo

Chemistry Department, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU

Tartrazine and Sunset Yellow FCF can be determined directly in orangeade by differential-pulse (d.p.) polarography on the addition of pH 9 Britton - Robinson buffer and tetraphenylphosphonium chloride. The tetraphenylphosphonium chloride removes the large polarographic maximum obtained with tartrazine at pH > 4 and causes the d.p. polarographic peaks of the two colouring matters to be separated.

Tartrazine in limeade can be determined in a similar supporting electrolyte but these conditions are not suitable for the determination of Green S, which is usually present at a low concentration relative to the tartrazine and for which the d.p. polarographic peak is depressed by the addition of tetraphenylphosphonium chloride. Green S can be determined after adding pH 4 Britton - Robinson buffer and tetramethylammonium chloride to the limeade: the addition of tetramethylammonium chloride gives a better base line in the presence of tartrazine. The solution is then re-adjusted to pH 9 and tetraphenylphosphonium chloride is added in order to determine the tartrazine.

At pH 4 the sugar present in blackcurrant syrup gives a small d.p. polarographic peak at the same potential as Green S. At pH > 6 the peak of the sugar disappears but amaranth gives a broad polarographic maximum. This maximum is suppressed at pH 7.8 by the addition of tetramethylammonium chloride. Under these conditions the Green S peak is separate but the small concentrations of Green S normally present in blackcurrant drinks can only just be detected.

The procedures have been tested on soft drinks prepared with known concentrations of colouring matter.

Keywords: Differential-pulse polarography; food colouring matters; tartrazine; Green S; amaranth

The UK Statutory Instrument concerned with colouring matter in food[1] lists permitted colouring matters and gives specifications for purity of colouring matter samples; colour is to some extent self-limiting and no limits are set on the amounts of these colouring matters that can be added to foods. Most analytical publications on food colouring matters are concerned with the identification of colouring matters, usually by thin-layer chromatographic methods.[2–4] Spectrophotometric quantification can be applied after thin-layer chromatographic separation.[4]

The continuing need for the use of food colouring matters, together with some concern about their widespread use, has aroused interest in their determination in foods. Food colouring matters and their intermediates have been determined by liquid - solid, ion-exchange and steric-exclusion forms of high-performance liquid chromatography (HPLC),[4] but more recently better results have been obtained by using the newly introduced paired-ion chromatographic mode.[5,6] For thin-layer chromatographic - ultraviolet spectrometric or HPLC determination, the food colouring matters are first extracted from the foodstuff.[3] With beverages and water-soluble foods the colouring matters are first adsorbed on wool or polyamide and are then re-extracted into an organic solvent. Colouring matters in more intractable foods undergo a more rigorous extraction with an organic solvent or liquid ion-exchange resin, before being adsorbed on wool or polyamide.

This work was undertaken in order to assess the value of the differential-pulse polarographic method for the determination of food colouring matters. Most colouring matters are reducible at the dropping-mercury electrode and give distinct polarographic waves.[7] Clearly, samples of colouring matters obtained using the clean-up procedures described above could be determined by using a differential-pulse polarographic finish. The peak potential observed could be used as partial confirmation of the identity of the colouring matter. Colouring matters with the same reducible group, *e.g.*, the azo group, do tend to be reduced at similar potentials, however, so that identification cannot usually be made unequivocally and the analysis of mixtures of colouring matters can be difficult without prior separation.

The ion-pair extraction method has been used extensively in the determination of ionisable pharmaceutical compounds,[8] and is now being adapted increasingly to paired-ion HPLC.[9] We decided to try the ion-pair extraction approach with food colouring matters, and found that the acidic food colouring matters tartrazine and Sunset Yellow FCF could be extracted into chloroform from orange squash using tetraphenylphosphonium chloride. After evaporating the chloroform and dissolving the colouring matters in pH 9 Britton - Robinson buffer, two distinct polarographic waves were obtained. Subsequently, the extraction step was found to be unnecessary; the addition of tetraphenylphosphonium chloride to orange squash buffered at pH 9 alters the potential at which tartrazine is reduced. Whereas in the absence of tetraphenylphosphonium chloride the two colouring matters are reduced at similar potentials (E_p values: Sunset Yellow FCF −0.64 V; and tartrazine −0.73 V), in its presence two separate waves are obtained. Direct differential-pulse polarographic procedures for the determination of three mixtures of colouring matters have been developed.

Experimental and Results

Apparatus

Polarographic measurements were made with a PAR 174 polarographic analyser (Princeton Applied Research). For differential-pulse operation, the forced drop time was 1 s, the pulse height 50 mV and the scan rate 2 mV s^{-1}. Two-electrode operation was used with a dropping-mercury electrode and a saturated calomel reference electrode. A water-jacketed polarographic cell was used and the temperature was maintained at 25 °C.

Reagents and Samples

Britton - Robinson buffer (pH 1.9; 0.04 M in each constituent) was prepared by dissolving 2.47 g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid and then adding 2.7 ml of orthophosphoric acid and diluting to 1 l with water. The pH of the buffer was adjusted as required by means of 0.2 or 4 M sodium hydroxide solution.

Tetraphenylphosphonium chloride (0.01 M) and tetramethylammonium chloride (1 M) solutions were prepared from laboratory-grade reagents.

Samples of amaranth, Sunset Yellow FCF, tartrazine and Green S, and of a blackcurrant health drink syrup and a basic syrup, were kindly provided by Beecham Products Ltd.

The concentrations of colouring matters quoted in this paper assume that the samples of colouring matters are pure, *i.e.*, that they contain 100% *m/m* of the colouring matter. This assumption was adequate for carrying out the recovery tests made here, but for normal routine analytical use calibration graphs should be obtained by using assayed samples of the colouring matters.

Determination of Sunset Yellow FCF and Tartrazine in Sparkling Orangeade

An orangeade containing no colouring matter was prepared by diluting 15 ml of the basic syrup to 100 ml with distilled water that had previously been carbonated by using dry-ice. A blank polarographic solution was prepared by mixing 5 ml of this orangeade, 5 ml of 0.01 M tetraphenylphosphonium chloride and 20 ml of Britton - Robinson buffer (pH 1.9), adjusting the pH to 9 with sodium hydroxide solution and diluting to 50 ml. An aliquot (20 ml) of this solution was pipetted into the polarographic cell, deoxygenated for 10 min and polarographed. When carrying out development work, successive aliquots of concentrated solutions of Sunset Yellow FCF and tartrazine were added to the blank solution by means of a 100-μl syringe, the solution being deoxygenated for 1 min after each addition.

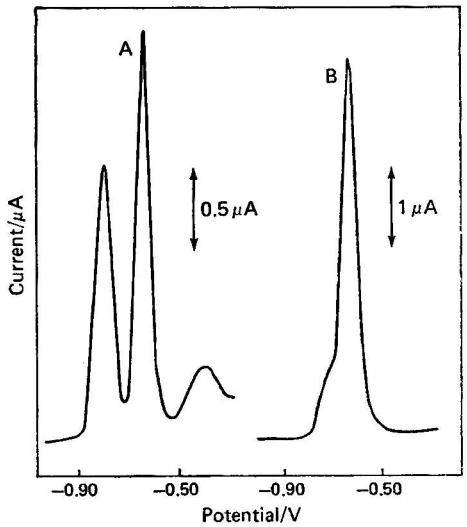

Fig. 1. Effect of tetraphenylphosphonium chloride on the d.p. polarogram of a mixture of Sunset Yellow FCF and tartrazine using the recommended procedure (A) with the addition of tetraphenylphosphonium chloride and (B) without its addition.

The effect of adding the tetraphenylphosphonium chloride on the polarograms of a mixture of Sunset Yellow FCF and tartrazine is shown in Fig. 1. The effect on the peaks of the individual colouring matters is illustrated by the results given in Table I. On the addition of small amounts of tetraphenylphosphonium chloride, the peak potential of tartrazine is shifted to a more negative potential and the peak current is increased by 140%. The peak potential of Sunset Yellow FCF remains unchanged but the peak current is decreased by 50%.

TABLE I

EFFECT OF TETRAPHENYLPHOSPHONIUM CHLORIDE (TPPC) CONCENTRATION ON THE D.P. POLAROGRAPHIC PEAKS OF TARTRAZINE AND SUNSET YELLOW FCF

Tartrazine concentration = 0.4 p.p.m.; Sunset Yellow FCF concentration = 1.84 p.p.m.

TPPC concentration, p.p.m. ..	0	9	20	40	60	90	100	380*	900	1200
i_p† (tartrazine)/μA	0.70	—	0.67	1.49	1.65	—	1.65	1.68	1.67	—
i_p‡ (Sunset Yellow FCF)/μA	5.4	4.5	3.20	1.60	2.10	2.50	—	2.60	—	2.56

* TPPC concentration used in recommended procedure.
† E_p = −0.73 V (in absence of TPPC) or −0.80 V (at all TPPC concentrations >0 p.p.m.).
‡ E_p = −0.65 V (in presence or absence of TPPC).

Calibration graphs for both colouring matters deviate slightly from rectilinearity at higher concentrations owing to adsorption effects at the dropping-mercury electrode but are not affected by the presence of the other colouring matter in the proportions normally found in soft drinks. The latter effect is illustrated in Fig. 2, which shows typical polarograms obtained to produce a calibration graph for tartrazine in the presence of Sunset Yellow FCF.

The recommended procedure for the determination of Sunset Yellow FCF and tartrazine in sparkling orangeade is as follows. Pipette 10 ml of 0.01 M tetraphenylphosphonium chloride solution into a 50-ml beaker. Add 5 ml of 0.01 M tetraphenylphosphonium chloride solution and 20 ml of pH 1.9 Britton - Robinson buffer. Adjust the pH to 9 with sodium hydroxide solution and dilute to 50 ml in a calibrated flask. Deoxygenate a portion of this solution in a polarographic cell and polarograph it between −0.3 and −1.0 V.

The procedure was tested by using a sparkling orangeade (42 p.p.m. of Sunset Yellow FCF and 20 p.p.m. of tartrazine) prepared from the basic syrup and the samples of colouring matter. The result obtained for ten determinations was 41.4 p.p.m. of Sunset Yellow FCF (coefficient of variation = 1.4%) and 20.5 p.p.m. of tartrazine (coefficient of variation = 1.0%).

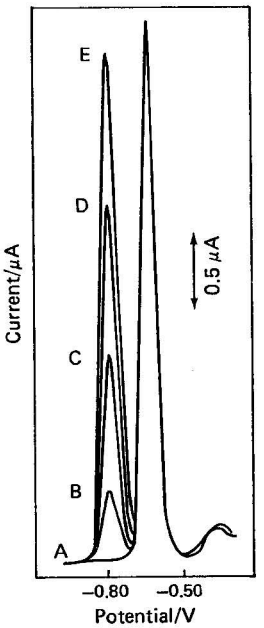

Fig. 2. D.p. polarograms produced in obtaining a calibration graph for tartrazine in the presence of Sunset Yellow FCF (12 p.p.m. in measured solution). Tartrazine concentration in measured solution: (A) 0, (B) 1.0, (C) 2.9, (D) 5.1 and (E) 7.5 p.p.m.

Determination of Tartrazine and Green S in Sparkling Limeade

Tartrazine is best determined under the solution conditions above for sparkling orangeade, *viz.*, at pH 9 in the presence of tetraphenylphosphonium chloride. Under these conditions Green S is reduced at a similar potential to tartrazine but fortunately, because only a small proportion of Green S relative to tartrazine is normally present in sparkling limeade and because the Green S wave is depressed in the presence of the phosphonium salt, interference of Green S in the determination of tartrazine is negligible.

Green S is best determined at pH 4 and the addition of tetramethylammonium chloride was found to improve the base line considerably. This improvement seems to arise owing to a partial suppression of the polarographic maximum of the tartrazine. Calibration graphs for Green S in the presence of tartrazine are rectilinear. Tartrazine can be determined after re-adjusting the pH to 9 and adding tetraphenylphosphonium chloride.

The recommended procedure for the determination of Green S and tartrazine in sparkling limeade is as follows. Pipette 10 ml of sparkling limeade into a 50-ml beaker. Add 5 ml of 1 M tetramethylammonium chloride and 10 ml of pH 1.9 Britton - Robinson buffer and adjust the pH to 4 with sodium hydroxide solution. Dilute to 50 ml in a calibrated flask. Deoxygenate a portion of this solution in a polarographic cell and polarograph it between −0.45 and −0.85 V.

Carefully re-adjust the pH of the solution in the cell to 9 with several drops of 4 M sodium hydroxide solution and add 10 mg of tetraphenylphosphonium chloride. Pass nitrogen through the solution to aid dissolution of the solid and to deoxygenate the solution, and polarograph the solution between −0.6 and −1.0 V.

The procedure was tested using a sparkling limeade (20 p.p.m. of tartrazine and 2 p.p.m. of Green S) prepared from the basic syrup and the samples of colouring matter. Typical polarograms are shown in Fig. 3.

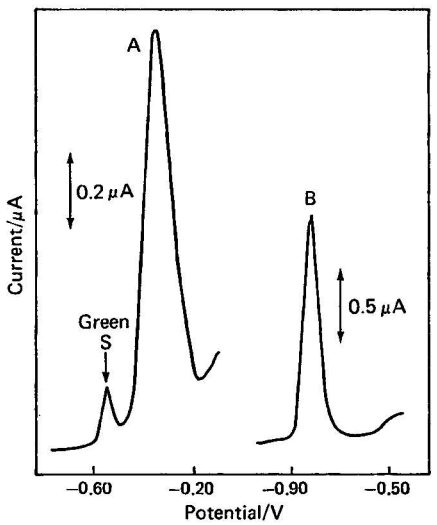

Fig. 3. Typical d.p. polarograms of Green S and tartrazine in limeade: (A) pH 4.1 and (B) pH 9.2.

The result of ten determinations was 2.1 p.p.m. of Green S (coefficient of variation = 3.4%) and 19.6 p.p.m. of tartrazine (coefficient of variation = 1.9%). At pH 9 the Green S gives a small peak at −0.75 V that is masked by the tartrazine peak. At the levels of Green S in the limeade this Green S peak is negligible; at very high concentrations of Green S a shoulder appears on the tartrazine peak.

Determination of Amaranth and Green S in Blackcurrant Health Drink

The sugar in the blackcurrant health drink syrup gives a small peak at pH 4 at the same potential as Green S. At pH > 6 this peak is absent. Amaranth gives a broad polarographic maximum at pH > 6 but this can be suppressed by the addition of tetramethylammonium chloride; tetraphenylphosphonium chloride, tetraethylammonium chloride and Triton X-100 do not suppress the maximum as effectively.

The recommended procedure for the determination of amaranth and Green S in blackcurrant health drink is as follows. Pipette 5 ml of blackcurrant health drink into a 50-ml beaker. Add 5 ml of 1 M tetramethylammonium chloride solution and 10 ml of pH 1.9 Britton - Robinson buffer. Adjust the pH to 7.8 with sodium hydroxide solution and dilute to 50 ml in a calibrated flask. Transfer a portion of this solution to a polarographic cell, deoxygenate it for 10 min and polarograph the solution between −0.30 and −0.80 V.

The procedure was tested using a blackcurrant health drink (250 p.p.m. of amaranth and 4 p.p.m. of Green S) prepared from blackcurrant health drink syrup and the samples of colouring matter. A typical polarogram is shown in Fig. 4. Clearly, the Green S is being polarographed near its detection limit for the amount of amaranth present but the procedure can be used as a limit test for this colouring matter. The result of ten determinations for amaranth was 245 p.p.m. with a coefficient of variation of 1.3%.

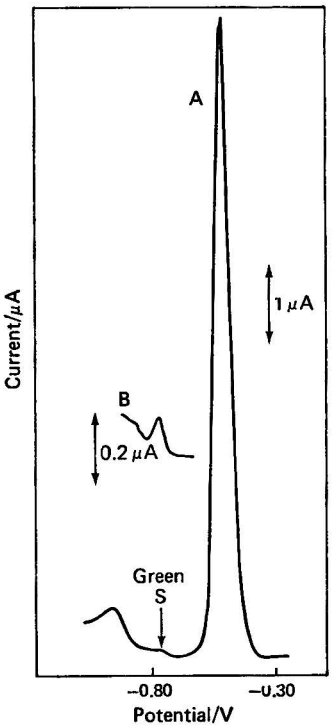

Fig. 4. (A) Typical d.p. polarogram for Green S and amaranth in blackcurrant health drink. (B) Polarographic peak of 0.6 p.p.m. of Green S in the measured solution in the presence of a smaller proportion of amaranth (amaranth concentration = 2.5 p.p.m.).

Discussion

The established method of identifying food colouring matters is thin-layer chromatography. This excellent method has the advantage of being inexpensive and the disadvantage of being difficult to quantify. Although relatively costly equipment is required, HPLC is now the nearest approach to an ideal method for the identification and determination of food colouring matters, combining efficient separations with precise quantification. Both thin-layer chromatographic and HPLC methods, however, generally require separation of the food colouring matters from even simple food matrices before application to the plate or column.

The differential-pulse polarographic procedures described here are applied directly to the soft drinks without the need for prior separation of the colouring matters on wool or polyamide. The differential-pulse polarographic peaks are sharp and afford some measure of identification. A 600-mV scan takes 5 min at 2 mV s^{-1} but a scan rate of 5 mV s^{-1} might be acceptable for routine use; deoxygenation takes about 10 min but can be effected on a batch basis in advance. Therefore, the procedures can be considered for routine use. Microprocessor-controlled polarographs are available that have full sample and data handling facilities after the solution-preparation step.

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Ion-selective Polymeric-membrane Electrodes with Immobilised Ion-exchange Sites

Part I. Development of a Calcium Electrode

L. Ebdon, A. T. Ellis and G. C. Corfield

Department of Chemistry, Sheffield City Polytechnic, Pond Street, Sheffield, S1 1WB

A new type of ion-selective electrode is described in which ion-exchange sites are immobilised in a polymeric membrane by covalent bonding. Membranes were prepared by cross-linking a styrene - butadiene - styrene triblock copolymer with triallyl phosphate. After subsequent hydrolysis these membranes were evaluated as Ca^{2+} sensors. The electrodes formed exhibited an extended Nernstian response to Ca^{2+} (10^{-1}–10^{-6} M) and good selectivity over other alkaline earth and alkali metals. Such electrodes offer very fast response times, extended lifetimes and a wide pH working range. The possibilities of this new class of ion-selective electrode are also discussed.

Keywords: Ion-selective electrode; calcium analysis; electroanalytical chemistry; polymeric membrane; immobilised ion-exchange groups

Ion-selective electrodes offer considerable advantages in analytical chemistry. They are simple and inexpensive to operate, sensitive and suited to on-line measurement. Thus, in addition to their application to laboratory analyses, applications to pollution monitoring, *in vivo* biological measurements and process control have been proposed. The major problems with ion-selective electrodes seem to be that certain of them lack selectivity and mechanical strength.

The range of ion-selective electrodes until about 10 years ago was limited to classical glass electrodes or electrodes using certain crystalline membranes. The introduction of electrodes based on liquid ion exchangers, *e.g.*, the calcium electrode first reported by Ross[1] in 1967, greatly extended the range of available electrodes. Ross used rather impure calcium didecyl phosphate (DDP) as sensor in conjunction with dioctylphenyl phosphonate (DOPP) solvent mediator supported on a Millipore filter. This type of electrode has been marketed commercially (*e.g.*, Orion 92–20). Such electrodes were recognised as a significant advance and were the first of a range of liquid ion exchanger ion-selective electrodes. In their design it was necessary to keep three liquids, the internal filling solution, the ion exchanger and the test solution, in electrolytic contact but to prevent mixing. This posed some constraints as did the limited lifetime of the electrodes, caused by leaking of the DDP and DOPP. This leaking also tends to raise the obtainable limit of detection. Severe interference from H^+ and some heavy metals and the expense of construction have also been cited as drawbacks of the Ross-type calcium ion-selective electrode.

In 1970, Moody *et al.*[2] reported a modification of the Orion system in which they used pure DDP and DOPP solvent mediator incorporated into a poly(vinyl chloride) (PVC) matrix. This convenient construction had the benefits of cheapness and ease of fabrication over the Ross system, but such electrodes still had a limited lifetime as the exchanger was entangled in the PVC matrix and so could still be leached out. The advantages of the PVC system were, however, obvious and it can be simply constructed[3] or purchased from commercial suppliers, finding widespread use for many applications.

Since this early work, the sensor and solvent-mediator systems have been the subject of much research, resulting in further improvements in the Ca^{2+} ion-selective electrode. In particular, it is now recognised that the solvent mediator performs an essential function in controlling the selectivity of the liquid ion exchanger electrodes. As an example, the use of calcium bis[di(2-ethylhexyl) phosphate] as sensor results in a Ca^{2+} ion-selective electrode if DOPP is used as solvent and as a divalent (water hardness) Ca^{2+}/Mg^{2+} test electrode if decan-1-ol is used.[4] From work carried out by Růžička *et al.*[5] and later by Moody *et al.*,[6] a better sensor for Ca^{2+} ion-selective electrodes seems to be calcium bis[di-4-(1,1,3,3-tetramethylbutylphenyl) phosphate] with DOPP. Work has also been carried out on the effects

of nitrating[7,8] the sensor and/or the mediator, but although giving useful information on the synergistic sensor - mediator system, nitration gave no real improvement in electrode performance.

In addition to the use of the organophosphoric acid type of sensor, good results have been reported for a Ca^{2+} ion-selective electrode using the non-cyclic neutral carrier *NN'*-di[(11-ethoxycarbonyl)undecyl]-*NN'*-4,5-tetramethyl-3,6-dioxaoctanediamide as sensor and 2-nitrophenoxyoctane as solvent mediator.[9] Although this system also shows high Ca^{2+} selectivity, it is also of the PVC matrix type and so the system will be leachable and subject to limited lifetimes, particularly when used in flowing systems.

A neutral carrier system has also been used in which the tetraphenylborate salts of a calcium adduct of poly(propylene glycol),[10] in conjunction with DOPP, is entangled in a PVC matrix. The resulting electrodes showed, in general, poorer Ca^{2+} selectivity than the phosphate systems and had shorter lifetimes.

It can be seen that there has been much work directed towards understanding and therefore improving the sensor exchanger - mediator system, but very little work has been carried out with different matrices. Schultz *et al.*[11] made an effort in 1968 to incorporate a dialkyl phosphate sensor into collodion, with little success, and in 1972 Griffiths *et al.*[12] reported poor electrode quality for some cellulose, collodion and pyroxylin entangled membranes. They suggested that the hydrophilicity of these materials is undesirable as leaching of the exchanger takes place very quickly. Schäfer[13] used poly(vinyl isobutyl ether) as a matrix material to prepare a divalent ion electrode, which showed no advantage over the PVC system and was again a polymer-entangled system from which the exchanger could be leached.

The advantages of PVC for electrode fabrication arise from its ready availability and lack of hydrophilicity. PVC is, however, stabilised by organometallic compounds that can block the ion-exchange sites and when these are removed its stability, *e.g.*, to ultraviolet light, is poor. More significantly, the ion exchanger in the membranes described above is not covalently bound. Thus, eventually the ion exchanger will migrate from the membrane and the resultant deterioration in electrode response as this leaching proceeds limits the useful lifetime of the membrane.

We are therefore studying different polymer systems and the possibility of immobilising ion-exchange sites into polymer matrices by covalent bonding. The immobilisation of the active ion-exchange groups should overcome the problems associated with leaching referred to above and lead to the development of ion-selective electrodes with extended lifetimes and sensitivity. Additionally, the careful selection of polymer systems should permit optimisation for enhanced mechanical and electrochemical properties. This new approach to the fabrication of ion-selective electrode membranes could lead to greater control over steric properties and hence to a significant improvement in selectivity.

Two other groups of workers have also directed attention towards covalently bonding ion-exchange groups to a polymer, although their work became known to us only after we had started the study described here. Keil *et al.*[14] phosphorylated a vinyl chloride - vinyl alcohol copolymer with decyl dihydrogen orthophosphate. The copolymer was then mixed with PVC in which DOPP was entangled, to produce a Ca^{2+} ion-selective electrode. Extended electrode lifetimes were not obtained in their study. Cutler *et al.*[15] bound ion-exchange groups to the ends of PVC chains both by the use of an amine as chain transfer agent during polymerisation and by the use of the $^{\bullet}SO_3^-$ radical anion as polymerisation initiator. The electrodes produced exhibited selectivity towards anionic or cationic surfactants and, although the electrode lifetimes were extended in comparison with the liquid ion exchanger surfactant ion-selective electrodes, the plasticiser used (tricresyl phosphate) was leached from the polymer and this limited the lifetime of the electrodes.

In the study reported here, membranes were prepared by cross-linking a styrene - butadiene - styrene (SBS) triblock copolymer with triallyl phosphate using free-radical initiation. After subsequent hydrolysis to give covalently bound pendant dialkyl phosphate exchanger groups, these membranes were evaluated as sensors for Ca^{2+} ion-selective electrodes.

Experimental

Chemicals

Tetrahydrofuran (THF) was freshly distilled from aluminium lithium hydride to dry it

and to remove stabilisers. Triallyl phosphate (TAP) was used as received (Aldrich Chemical Co.) but the initiator α,α-azobisisobutyronitrile (ABIN) was recrystallised from methanol. Analytical-reagent grade chemicals were used during electrode evaluation. The polymer used was Cariflex SBS 1101 (Shell Chemicals, London), which was purified by dissolving it in THF and re-precipitating it in cold, well stirred methanol. Gel permeation chromatography showed the number-average relative molecular mass ($\overline{M}_n$) as 9.8×10^4 g mol^{-1} with 70% SBS triblock poly(styrene-*b*-butadiene), 26% SB diblock and 4% homopolystyrene content. The polydispersity ($\overline{M}_w/\overline{M}_n$) was 1.51 and 100-MHz nuclear magnetic resonance spectroscopy indicated 27% *m/m* of polystyrene in the polymer, and hence 73% *m/m* as polybutadiene; 300-MHz nuclear magnetic resonance spectroscopy showed the butadiene units to be 90% 1,4- and 10% 1,2-configuration.

Preparation of Polymeric Membranes

Re-precipitated SBS (4 g) was dispersed in the solvent (40 cm^3) and allowed to dissolve overnight, after which the required amounts of TAP and ABIN were added with mixing. The polymer solution was then poured into a glass ring (100 mm i.d.) resting on a Cellophane sheet on a glass plate. A heavy weight and a front-silvered mirror were arranged such that a seal was maintained with the plate and ultraviolet radiation could be reflected normally on to the curing membrane. Membranes were usually cured after 6 h, depending on solvent volatility, forming strong, clear membranes about 1 mm in thickness. The membranes were removed from the Cellophane and portions hydrolysed to give the master membrane.

Evaluation of Electrodes

Discs, 10 mm in diameter, were cut from the master membranes and stuck to the end of clear, plasticised PVC tubing by using a cyanoacrylate adhesive (IS 12, Loctite, Dublin). Calcium chloride solution (10^{-1} M) saturated with silver chloride was used as the internal reference solution, the electrodes being mounted on modified pH electrodes in a similar fashion to PVC membrane electrodes.[3] The mounted electrodes were soaked overnight in 10^{-2} M Ca^{2+} solution in order to replace the Na^+ or K^+ form of the exchanger with the Ca^{2+} form. The time required for soaking was again dependent on the degree of cross-linking, but 12 h was the maximum required by any electrode. EMF measurements were made using the cell

$$Hg.HgCl_2 \mid KCl\ (sat.) \parallel \text{test solution} \mid \text{membrane} \mid CaCl_2\ (0.1\ M) \mid AgCl.Ag$$

using varying test solutions. A single-junction reference electrode (Orion 90-01, Orion Research Inc., Cambridge, Mass., USA) and a digital voltmeter (Orion 701) reading to ± 0.1 mV were used. The test solutions were stirred magnetically and maintained at 25 ± 0.5 °C. Calcium-ion activities were calculated from $a_{Ca^{2+}} = c\gamma$ where γ, the activity coefficient, was calculated using an extension of the Debye - Huckel equation:

$$-\log \gamma_{Ca^{2+}} = z^2\left(\frac{a\sqrt{I}}{1+\sqrt{I}} - 0.2I\right)$$

where I, the ionic strength, is given by $I = \frac{1}{2}\Sigma c_i z_i^2$. Potentiometric selectivity coefficients were determined by a mixed solution method using an interferent-ion level of 10^{-3} M and changing the calcium-ion concentration. Coefficients were evaluated using the IUPAC recommended method[16]:

$$k^{Pot}_{Ca,M} = \frac{a_{Ca^{2+}}}{(a_{M^{n+}})^{z/n}}$$

where $z = 2$ and $n =$ charge of interference ion M, $a_{M^{n+}} = 10^{-3}$ M and $a_{Ca^{2+}}$ is taken at the point when the mixed solution calibration differs from the extrapolated linear portion by $18/z$ mV (*i.e.*, 9 mV for Ca^{2+}).

Hydrogen-ion interference is expressed as the pH range over which there is no measurable change in electrode potential at a constant calcium-ion activity. The pH was changed by using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide solution in conjunction with a combination glass pH electrode (Activion Ltd., Halstead, Essex) and a pH meter (Pye, Model 290, Pye Unicam, Cambridge).

Results and Discussion

Incorporation of Ion-exchange Groups

The ion-exchange group chosen for this study was the classical dialkyl phosphate group, as this offered a well tried system that could be incorporated into a polymeric structure by a simple copolymerisation mechanism. A poly(styrene-*b*-butadiene) triblock elastomer (SBS) was chosen as the polymer because it contains the necessary C=C unsaturation for cross-linking, has mechanical behaviour similar to natural rubber vulcanisates without requiring cross-linking, can be dissolved in solvents and is easy to process. In order to incorporate the phosphate exchanger groups, the unsaturated C=C bonds are cross-linked by a free-radical initiated addition mechanism using triallyl phosphate (TAP). This mechanism can be represented by the scheme shown in Fig. 1.

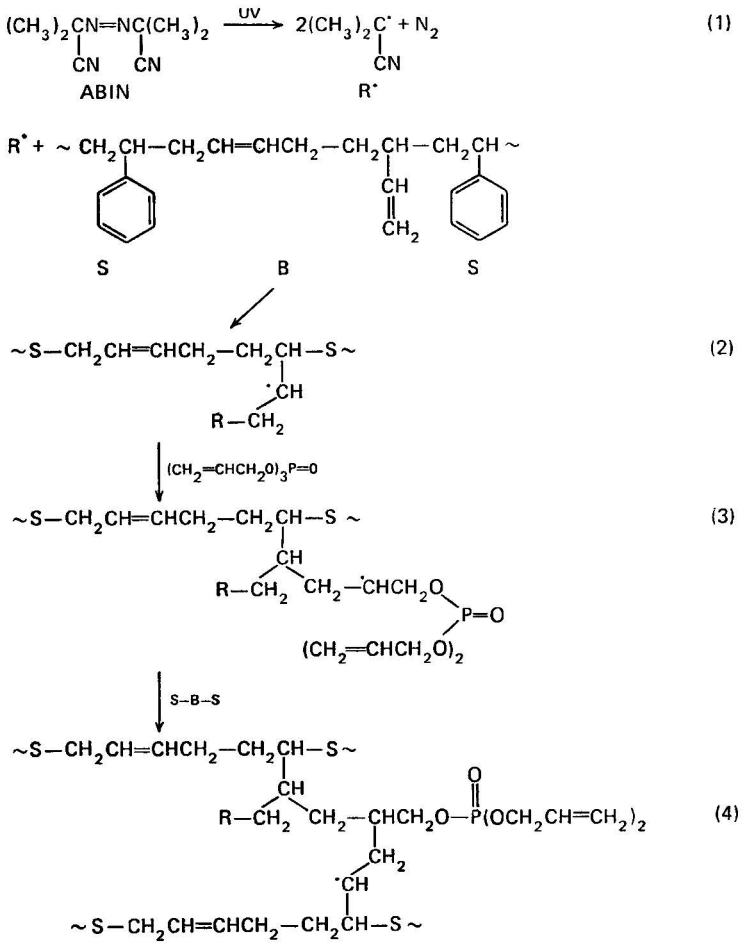

Fig. 1. Immobilisation of phosphate groups.

This simplified mechanism shows that initiation of SBS should occur [(1) and (2)], leading to incorporation of phosphate groups in the structure via covalent bonding (3) and cross-linking (4). In order to obtain the dialkyl phosphate sensor unit, it is necessary to hydrolyse the trialkyl phosphate system with alkali (Fig. 2). The stability of the resonance-stabilised dialkyl phosphate salt is such that further hydrolysis to remove other alkyl groups is very difficult and will not proceed under the conditions used. This leaves the dialkyl phosphate

group covalently bound to the polymeric membrane. Strong alkaline hydrolysis results in attack on the polymer itself, presumably at the residual unsaturation, and at a sodium hydroxide concentration of approximately 25% *m/m* the polymeric material breaks down completely. One disadvantage of this hydrolysis procedure is that the K^+ or Na^+ salt of the acid is formed and so the electrodes must be conditioned overnight in 10^{-2} M Ca^{2+} to create the Ca^{2+} form of the exchanger.

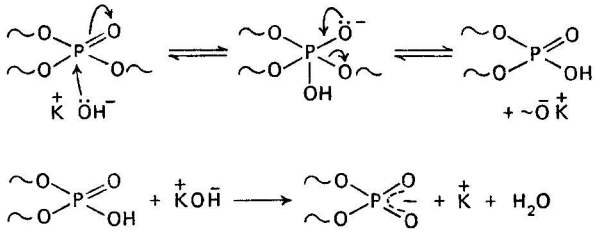

Fig. 2. Hydrolysis of trialkyl phosphate grouping.

The initial stage of this work was concerned with optimising the polymerisation and hydrolysis steps. The first consideration was the amount of cross-linking in the membrane, which is controlled primarily by the amount of TAP and ABIN present, with factors such as temperature and light intensity of lesser importance. Table I shows the effect of changing the amount of TAP and ABIN on the physical and electrochemical properties of the cast membrane. From these results, it can be seen that the best membrane (B) resulted from 5% *m/m* of TAP with 2.5% *m/m* of ABIN. High levels of TAP resulted in oily, non-functional membranes, and larger amounts of ABIN reduced the response and the clarity of the membrane.

TABLE I

EFFECT OF MEMBRANE COMPOSITION ON PHYSICAL AND ELECTROCHEMICAL PROPERTIES

Membrane	TAP, % *m/m*	ABIN, % *m/m*	THF solubility*	Physical properties of cast membranes	Electrochemical properties†
A	2.5	0	Soluble	Elastic, clear	Short linear range, low slope
B	5	2.5	Insoluble	Elastic, clear, slightly yellow	Functional membrane
C	2.5	5	Insoluble	Elastic, opaque	No response
D	5	10	Insoluble	Clear with opaque inclusions	No response
E	5	15	Insoluble	Bubbled, opaque inclusions	No response
F	15	2.5	Insoluble	Elastic, clear, yellow	Very short, linear range, low slope
G	50	5	Insoluble	Oily, yellow, rigid	Poor response
H	100	5	Insoluble	Thick, rigid with oily surface	Poor response

* Before hydrolysis.
† After hydrolysis for 5 h in 1% sodium hydroxide solution under reflux.

Table II shows the effects of both the hydrolysis conditions and the degree of cross-linking on the properties of the membrane. The use of mild aqueous alkaline conditions for hydrolysis gave membranes that showed near-Nernstian slopes but with short lifetimes. Those with longer lifetimes had significantly sub-Nernstian calibration slopes. Aqueous hydrolysis produced membranes that were more soluble in THF than the unhydrolysed membranes, presumably owing to further attack on the polymer structure in addition to hydrolysis of phosphate groups.

Hydrolysis with 5% *m/m* methanolic potassium hydroxide solution proceeded smoothly, resulting in an insoluble membrane with a Nernstian response and an extended lifetime (membrane 5). Reductions in the amounts of TAP and ABIN eventually gave membranes

TABLE II

EFFECT OF MEMBRANE COMPOSITION AND HYDROLYSIS CONDITIONS ON THE PHYSICAL AND ELECTROCHEMICAL PROPERTIES OF POLYMERIC ION-EXCHANGE ELECTRODES

Membrane	TAP, % *m/m*	ABIN, % *m/m*	Hydrolysis*	Solubility in THF	Slope/ mV decade^{-1}	Lifetime	Appearance
1	5.0	2.5	5 h, 1% aq. NaOH	Soluble	+16	>6 months	Milky, opaque
2	4.5	2.5	3 h, 1% aq. NaOH	Soluble (12 h)	+24.2	>6 months	Clear, becoming yellow - brown on hydrolysis
3	4.86	2.6	1 h, 1% aq. NaOH	Slightly soluble	+29.5	10 days	Mainly clear, yellow on hydrolysis
4	4.54	2.5	20 h, H_2O	Slightly soluble	+29	~3 days	Dark brown and brittle
5	4.4	2.5	5 h, 5% KOH - MeOH	Insoluble	+30	>6 months	Clear, pale yellow, tough and elastic
6	4.4	2.5	5 h, 10% KOH - MeOH	Slightly soluble	+35, decreasing to +17 after 20 d	20 days	Clear, yellow - brown
7	2.2	1.26	5 h, 5% KOH - MeOH	Insoluble	+24, decreasing to +15 after 3 d	5 days	Clear, pale yellow
8	2.2	1.26	5 h, 10% KOH - MeOH	Soluble	−19	3 days	Brown, becoming brittle
9	1.0	0.55	5 h, 5% KOH - MeOH	Soluble	+25	3 days	Clear and colourless
10	1.0	0.55	5 h, 10% KOH - MeOH	Soluble	−12	3 days	Brown
11	0	0	5 h, 5% KOH - MeOH	Soluble	−17	>3 months	Clear and colourless. Pure SBS membrane

* Conditions refer to reflux.
† After hydrolysis.

that, after hydrolysis under similar conditions to those used with membrane 5, did not appear to be strongly cross-linked and showed a calibration slope below Nernstian with a short lifetime. When the hydrolysis was more vigorous, a similar increase in solubility and a marked tendency to age became apparent (membrane 6). Membranes 8 and 10 showed negative calibration slopes, which may have been due to the passage of small anions through these membranes, which were not cross-linked. Porous membranes would be expected to respond to Cl^- ions because of the internal reference electrode. The properties of membrane 5 provide clear evidence for the immobilisation of the phosphate sensor in the polymer structure by covalent bonding. The insolubility of the polymeric material indicates that cross-linking has occurred and the presence of C–O–P and P=O absorption bands in the infrared region, using both transmission and attenuated total reflectance methods, shows the incorporation of phosphorus into the membrane. Quantitative phosphorus analysis was carried out by using acid digestion followed by a molybdenum blue spectrophotometric method. Tests on membrane 5 showed no leaching of phosphorus even after 10 d in THF, confirming the covalent binding of the phosphate group to the polymer structure.

Evaluation of Electrode Response

Table III shows the electrode responses using the membranes prepared. In all instances, the range of linear response is wide and in some instances extends below 10^{-6} M, although the use of diluted standards rather than a calcium-buffered system at this level is unlikely to give reliable results. This significantly extended performance on the low concentration side

TABLE III

RESPONSE AND SELECTIVITY DATA FOR POLYMERIC ION-EXCHANGE ELECTRODES

Membrane	Effective linear range/M	Slope at 25 °C/mV decade^{-1}	Static response time*/s	Selectivity coefficient,† $k^{Pot}_{Ca\ M}$				pH range
				Ba	Mg	K	Na	
1	10^{-5}–10^{-2}	+16	5		0.3	200	30	5–10
2	10^{-5}–10^{-1}	+24.2	5	0.80	0.82	0.35	0.17	4.5–10
3	10^{-5}–10^{-1}	+29.5	~120		0.4	~10^2	~10^2	
4	10^{-5}–10^{-1}	+29	5	Major interference from all cations				
5	10^{-5}–10^{-1}	+30	2	0.8	0.3	2.5	6	4–10
6	10^{-5}–10^{-1}	+35, decreasing to +17 after 20 d	2	0.65	0.2	12	6	4–10
7	10^{-5}–10^{-1}	+24, decreasing to +15 after 3 d	5		0.8	Initially negligible but increases with ageing		20 mV pH^{-1}
8	10^{-5}–10^{-1}	−19	5			Responding to anions		
9	10^{-5}–10^{-1}	+25	5		0.17	7	20	4.5–10
10	10^{-5}–10^{-1}	−12	5		Responding to anions			
11	10^{-5}–10^{-1}	−17	5		Responding to anions			

* For decade change of 10^{-5}–10^{-4} M Ca^{2+} concentration.
† Concentration of interferent ion 10^{-2} M.

compared with the Orion 92–20 type is thought to be due to the non-leachable ion-exchange groups and a calcium-buffered system might be expected to extend the range below 10^{-6} M. The range above 10^{-1} M Ca^{2+} has not been investigated, the range of concern in this study being 10^{-5}–10^{-2} M Ca^{2+}.

Some initial comments on the calibration slopes of some electrodes have been made above. The results obtained confirmed that optimum electrochemical properties were obtained with electrodes that were cross-linked and contained a high density of bonded phosphate groups in the membrane. It was not possible to increase the phosphate content indefinitely without producing membranes with poor physical characteristics. Early results showed that it was necessary to use a sufficient intensity of ultraviolet irradiation during polymerisation to ensure cross-linking. Membranes 1 and 2 gave electrode slopes of +16 and +24.2 mV decade^{-1}, and this was attributed to insufficient radiation, which resulted in poor initiator efficiency and hence lack of cross-linking. Membrane 11 was a pure SBS membrane and when treated under conditions identical with those used with membrane 5 gave a THF-soluble membrane with a negative calibration slope. Again, we would conclude that this pure SBS membrane is porous to small anions.

Response times were generally very short, with static times being in the region of 5 s for the range 10^{-1}–10^{-4} M Ca^{2+}, increasing to 15 s for the 10^{-5} and 10^{-6} M solutions. Dynamic response times were measured by making 10-fold changes in Ca^{2+} concentration by addition of concentrated calcium chloride solution from a piston syringe to 100 cm^3 of well stirred solution. The whole range from 10^{-6} to 10^{-1} M Ca^{2+} was covered in this manner and gave response times of 1 s for most solutions, only increasing to about 5 s for 10^{-6} M solutions. The reason for these fast response times compared with other organophosphorus-based electrodes is thought to lie in the mechanism of the electrode. In the liquid ion exchanger and PVC electrodes, the response relies on an ion-transport mechanism, whereas in this type of exchanger the mechanism might in some ways be analogous to the ion-exchange mechanism found in glass electrodes.

Selectivity studies were initially performed on a limited number of cations showing the general order of selectivity to be $Ca^{2+} > Ba^{2+} > Mg^{2+} > M^{+}$. Potentiometric selectivity coefficients for the divalent ions were $k^{Pot}_{Ca,Ba} = 0.8$ and $k^{Pot}_{Ca,Mg} = 0.3$, whilst interference from monovalent cations was less, thus making it possible to generate the K^+ or Na^+ form of the exchanger on hydrolysis and then to convert this into the Ca^{2+} form by soaking overnight in 10^{-1} M calcium chloride solution. These selectivity data are also presented, perhaps more meaningfully, in calibration graphs as in Fig. 3, where the interferent level is maintained at 10^{-3} M. The influence of increasing sodium concentration on $k^{Pot}_{Ca,Na}$ and electrode response is presented in Fig. 4, showing this type of electrode to be functional in the presence of 10^{-2} M Na^+, but with loss of Ca^{2+} response at the 10^{-1} M Na^+ level.

The H^+ interference was evaluated by varying the pH at a constant Ca^{2+} concentration (10^{-3} M) (see Fig. 5). Table III includes the pH range over which there is no measurable change in the electrode potential for different electrodes. With the electrodes reported

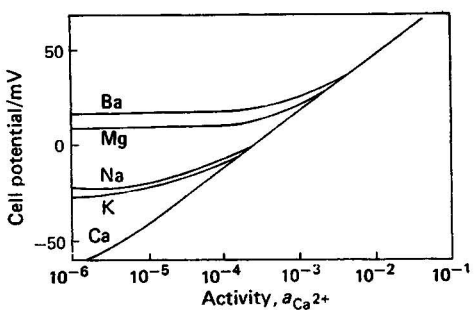

Fig. 3. Calibration graphs for a calcium ion-selective polymeric-membrane electrode with immobilised ion-exchange sites. The interferent effect of Ba^{2+}, Mg^{2+}, K^+ and Na^+ solutions (10^{-3} M as chloride in each instance) on this calibration is also shown.

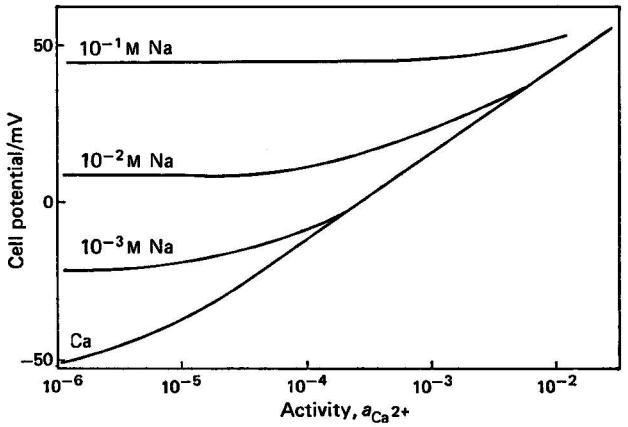

Fig. 4. Effect of Na^+ concentration on the calibration graph for a calcium ion-selective membrane electrode with immobilised ion-exchange sites.

here, for well cross-linked electrodes the pH range was wide (pH 4–10) and showed none of the "dips" seen with earlier organophosphate-based systems. This is perhaps to be expected as these "dips" were attributed to the solvent mediator and the system here has no such mediator. The useful alkaline end is limited analytically by the formation of calcium hydroxide, although the electrode may still be capable of giving the true Ca^{2+} activity. Poorly cross-linked membranes showed narrower pH working ranges and were unstable at low pH values; there was, however, no poisoning of the electrodes by H^+.

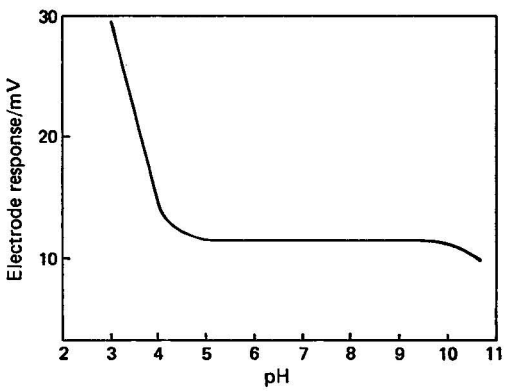

Fig. 5. Graph of electrode response against pH in 10^{-3} M calcium chloride solution.

In terms of general analytical behaviour, the electrodes yielded a reproducibility of ± 1 mV, but the poorly cross-linked membranes resulted in poorer reproducibility, especially when they also had a limited lifetime. Drifts with the better electrodes were of the order of 1 mV d^{-1}, but again the poorer cross-linked electrodes gave higher drifts (about 5 mV d^{-1}) and increased noise levels.

The lifetime of this type of electrode was found, as predicted, to be significantly longer than those of polymer entrapped liquid ion exchanger electrodes. Poorly cross-linked membranes lasted only a matter of days, but the well cross-linked membranes gave electrodes with lifetimes in excess of 6 months. These latter membranes showed no physical deterioration and the electrode response remained Nernstian with good selectivity. In the less cross-

linked membranes, shorter lifetimes of less than 1 month are accompanied by a noisy response, decreasing calibration slope and increasing monovalent cation interference. The lifetimes quoted are for electrodes stored in 10^{-2} M Ca^{2+} solution and calibrated at least once per week.

Conclusion

It is possible to form immobilised ion-exchange sites in an unsaturated polymer matrix by cross-linking SBS with triallyl phosphate and hydrolysing the resulting membrane to give pendant dialkylphosphoric acid exchange groups. The best membrane was prepared by cross-linking 4 g of SBS with 4.5% *m/m* TAP and 2.5% *m/m* ABIN initiator and hydrolysing the polymer with methanolic potassium hydroxide solution; the resulting membrane gave an ion-selective electrode of high physical strength and stability. This ion-selective electrode gave a Nernstian response to Ca^{2+} ions with selectivity over Ba^{2+}, Mg^{2+} and alkali metal cations. It showed a wide pH working range (pH 4–10) and a lifetime in excess of 6 months. Further work on the polymer system and ion-exchange sites is expected to result in a new family of ion-selective electrodes extending to other ions. Early indications are that this type could combine improved mechanical properties, such as robustness and lifetime, with advantageous electrochemical properties, including fast response speeds and lower limits of detection.

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High-frequency Microtitrimetric Determination of Acidic and Basic Constituents in Lubricating Oils

Part II.* Determination of Total Base Number

T. Fernández, J. M. Rocha, N. Rufino and A. García Luis
Compañia Española de Petróleos, S.A., Research Laboratory, Santa Cruz de Tenerife, Canary Islands

and F. García Montelongo
Department of Analytical Chemistry, University of La Laguna, Tenerife, Canary Islands

A high-frequency microtitration method is described for the determination of total base number in new and used lubricating oils. The sample, dissolved in toluene - propan-2-ol - water (5 + 4.95 + 0.05), is titrated with standard alcoholic hydrochloric acid solution. A study of solvents, titrants, sample size and other experimental parameters is reported. This method provides sharp breaks at the end-points of the titration graphs and more repeatable and faster results than those obtained with the standard potentiometric method. The method can also be used for macrotitrations, with a commercially available oscillator, and can be suggested as an alternative to ASTM and IP methods for the determination of total base number.

Keywords: Total base number determination; lubricating oils; high-frequency titration

In a previous paper the determination of the total acid number of lubricating oils by use of a high-frequency titrimetric method was described.[1] In this paper we report the application of the high-frequency titration method to the determination of the total base number of these oils.

The basicity of an oil is caused by nitrogen compounds, salts of weak acids (soaps), heavy metal salts and additives, such as inhibitors and dispersants. Basicity is an important property of lubricating oils as bases neutralise acidity, and therefore offset corrosion, which can be caused by oxidation of the oil during use.

The total base number (TBN) of an oil is defined as "the amount of acid, expressed in milligrams of potassium hydroxide, that is needed to neutralise all basic constituents present in one gram of sample."[2,3]

The ASTM and IP methods[2,3] of measuring TBN describe the potentiometric titration of a solution of the oil in toluene - propan-2-ol with a standardised solution of hydrochloric acid in propan-2-ol. If a manual instrument is used these methods are rather slow, because of the large number of points that must be recorded and the slow indication of the changing pH. At present, this situation is improved because most laboratories working with these methods use automatic titrators that do not require too much operator time. However, the graph obtained does not always show a satisfactory end-point, particularly with used oils. The use of a standard buffer solution, 2,4,6-trimethylpyridine, and hydrochloric acid in propan-2-ol, improves the results somewhat, but it is not certain that these results are meaningful and actually measure TBN as previously defined. Recently, potentiometric methods using glacial acetic acid as solvent have been introduced.[4,5] The acceptance of the results obtained is wider than for those obtained by other methods. However, they are not completely satisfactory for used oils.

In service, the replacement of a lubricating oil is determined by several factors, amongst which is the TBN. For heavily used oils the time for replacement may be difficult to determine because of errors in the measurement of TBN, if this is the only factor considered.

High-frequency techniques have been applied successfully to the titration of bases in non-aqueous media[6–9] and can help to solve the difficulties mentioned previously. In this connection a method for TBN determination has been described,[7] using a high-frequency

* For details of Part I of this series, see reference list, p. 749.

titrator adapted for use with a specially designed cell, but its specific design has created difficulties in its practical application.

This paper shows that the high-frequency titrators that are commercially available can be applied to macro- and micro-determinations of TBN. Procedures have been developed by using solvent and titrants similar to those used in the ASTM methods.[2–5] The proposed method reduces the time of analysis to about 10 min per sample, eliminates the contamination of electrodes, always shows sharp breaks at the end-point and provides greater precision. In addition, more meaningful results are obtained.

Experimental

Apparatus

High-frequency oscillator for microtitrations, digital ammeter, pH meter, electrodes and microburette have been described previously.[1]

An Oscimhometer OK-105 (Metallurgical Research Institute, Budapest),[10] equipped with a 500-ml (350 ml effective) inductive cell, was used for macro-titrations. This instrument was obtained from Metrimpex, Budapest 62 (P.O. Box 202) and the cost in 1977 of the manual instrument was about £450. According to reference 9 the Oscimhometer can be classified as being Group 1 response type, and although it is equipped with its own response meter, a Hewlett-Packard digital multimeter, Model 3465A, (1–100 μA $\pm$ 0.2%) measuring instrument was used.

Samples

The additives and trade lubricating oils are listed in each table. In order to prepare synthetic samples a paraffinic base oil [d_4^{15} 0.866 g ml^{-1}, kinematic viscosity 10 cSt (10^{-5} m^{-2} s^{-1}) at 100 °C], zero TBN value is used.

Reagents

Analytical-reagent grade chemicals were used throughout without further purification. In the following paragraphs volumes referring to macrotitrations are given in parentheses.

Titrants

Hydrochloric acid, 10^{-1} M solution in propan-2-ol. This solution is made up according to ASTM D-664.[2] Standardise it frequently by use of the high-frequency technique, using 50 μl (3 ml) of 10^{-1} M standard potassium hydroxide solution in propan-2-ol or 10^{-1} M Tris [2-amino-2-(hydroxymethyl)propane-1,3-diol] standard solution, and toluene - propan-2-ol - water as the solvent. The maximum deviation allowed must be less than $\pm$0.2% ($\pm 2 \times 10^{-4}$ M).

Perchloric acid, 10^{-1} M solution in glacial acetic acid. This is made up according to ASTM D-2896.[4] Standardise the solution frequently by use of the high-frequency technique, using 100 μl (3 ml) of standard potassium hydrogen phthalate solution in glacial acetic acid and toluene - propan-2-ol - acetic acid as the solvent.

Sodium acetate solution in glacial acetic acid, 10^{-1} M. This is made up according to ASTM D-2896.[4] Standardise the solution by using 100 μl (3 ml) of standard perchloric acid solution and toluene - propan-2-ol - acetic acid as the solvent.

Standard 10^{-1} M Tris aqueous solution.

Standard 10^{-1} M potassium hydrogen phthalate solution in glacial acetic acid. Dissolve the phthalate according to ASTM D-2896,[4] but diluting only with glacial acetic acid.

Titration solvents

Toluene - propan-2-ol - water (5 + 4.95 + 0.05). This is the main solvent, which is prepared according to ASTM D-664.[2]

Toluene - propan-2-ol - glacial acetic acid (3.5 + 3.5 + 3).

Toluene - ethanol - glacial acetic acid (4 + 3 + 3).

Procedure

Method 1. *Samples with TBN more than* 5. The test sample, 5–50 mg (1–5 g), is weighed

into the titration cell, dissolved with 6 ml (350 ml) of the toluene - propan-2-ol - water solvent mixture (5 + 4.95 + 0.05) and titrated with the 10^{-1} M alcoholic hydrochloric acid standard solution.

Method 2. *Samples with TBN less than* 5. The test sample, 50 mg (2–4 g), is weighed into the titration cell, 50 μl (1–2 ml) of standard 10^{-1} M sodium acetate solution are added, and the sample is dissolved with 6 ml (350 ml) of toluene - propan-2-ol - acetic acid solvent mixture (3.5 + 3.5 + 3). The solution is then titrated with the 10^{-1} M perchloric acid standard solution.

Volumes and masses given in parentheses are for macrotitration with the Oscimhometer. The working technique with this instrument is similar to that already reported for micro-titrations.[1] The titrant was added in 0.5-ml increments from a 10-ml burette, using about 3 ml of titrant to reach the end-point.

Calculation

The TBN value can be calculated from the equation

$$\text{TBN} = \frac{(A \pm B) \times M \times 56.1}{m}$$

where A is the volume, in microlitres (millilitres), of standard acid titrant used to reach the end-point minus the volume of acid titrant corresponding to the sodium acetate solution added (Method 2); B is the volume of basic titrant[1] or acid titrant, molarity M, used to titrate 6 ml (350 ml) of solvent mixture and is to be added in Method 1 and subtracted in Method 2 (the solvent of Method 1 may become slightly acidic and that of Method 2 slightly basic, see under *Sample size* below); M is the molarity of the acid standard solution; and m the mass of the oil sample in milligrams (grams).

Effect of the Experimental Parameters on the High-frequency Titration

In order to avoid duplication and presenting a study similar to that previously reported on the determination of TAN,[1] we prefer to discuss only the experiments carried out by use of microtitration. However, the main studies of TBN determination were also carried out by macrotitration. As will be seen later, the shape of experimental graphs, the results and the conclusions agreed well with those obtained by use of the micro-method.

Titrant standard solution

In order to use, as far as possible, the reagents specified in ASTM methods D-664 and D-2896, we used only solutions of perchloric acid in glacial acetic acid and hydrochloric acid in propan-2-ol as titrants for direct titrations and sodium acetate in glacial acetic acid for back-titrations.

Titration solvents

Solvents used as media for the titration of weak bases in petroleum products must dissolve the sample completely, enhance the basicity of the species to be titrated and have a low solvating power for cations. Further, the relative permittivity of the solvents used in conductimetric titrations should be less than 20,[8] but not too small as they must ionise compounds to be titrated, at least partially. Solvents such as nitromethane, 4-methylpentan-2-one or trifluoroacetic acid, which are widely used in the potentiometric determination of weak bases, cannot be used in conductimetric titrations because they do not satisfy some of the stated requirements. Acetic acid or toluene - alcohol - acetic acid mixtures give better results, but these solvents do not always improve the titration of weak bases. This can be related to the fact that the conductimetric end-points are generally located at the intersection of two straight, ascending lines.

In order to enhance the localisation of the end-point, we tried to alter the slope of the graphs by using mainly toluene - alcohol mixtures as solvents, with or without the addition of acetic acid. The behaviour of the selected solvents was studied by reference to the response graphs of the oscillator with the acidic titrants and verified later by use of the titration graphs.

Non-acidic solvent mixtures

In our first trials a number of binary mixtures, 1 + 1 toluene - X (X being ethanol, propan-2-ol, acetone, 1,4-dioxan, butan-2-one or 4-methylpentan-2-one), were used as representative solvents and tested in the titration of a synthetic sample with hydrochloric acid solution in propan-2-ol. Toluene - ethanol and toluene - propan-2-ol mixtures give the greatest slope change at the end-point. Further experience with such alcoholic solvents showed that a solvent with a relative permittivity of approximately 11 and a 40–60% alcoholic component should be the most satisfactory. The work was continued with toluene - ethanol (6.5 + 3.5), (6 + 4) and (5.5 + 4.5) and the solvent described in the ASTM method D-664, toluene - propan-2-ol - water (5 + 4.95 + 0.05), was chosen for comparative purposes.

Response graphs of the oscillator (Fig. 1) show that toluene - ethanol (6.5 + 3.5) is the best solvent because of its high sensitivity, but that the ASTM D-664 solvent, which has less sensitivity, can be recommended as it originates a more linear response graph over a wide range of titrant concentrations. Both solvents can dissolve up to 200 mg of sample under the experimental conditions.

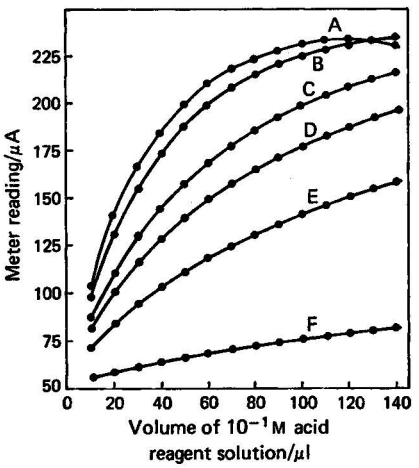

Fig. 1. Instrument response graphs for 10^{-1} M hydrochloric acid and several solvent mixtures: A, toluene - ethanol (6.5 + 3.5); B, toluene - ethanol (6 + 4); C, toluene - ethanol (5 + 5); D, toluene - ethanol (5.5 + 4.5); E, toluene - propan-2-ol - water (5 + 4.95 + 0.05), 10^{-1} M perchloric acid as titrant; and F, toluene - propan-2-ol - water (5 + 4.95 + 0.05).

Acidic solvent mixtures

Acetic acid was selected as the acidic component, because of its well known behaviour, as the solvent and species to be titrated are similar to those described in ASTM D-2896.

From the mixtures tried, after reference to their response graphs, toluene - ethanol - acetic acid (4 + 3 + 3) and toluene - propan-2-ol - acetic acid (3.5 + 3.5 + 3) were selected. Toluene - acetic acid (1 + 1), chlorobenzene - acetic acid (6.7 + 3.3) and the solvent specified in ASTM method D-2896 were also studied for comparative purposes. These solvents were further tested in the titration of a synthetic sample by direct and back-titration (Table I).

From the experimental graphs shown in Fig. 2, it can be deduced that toluene - propan-2-ol - acetic acid mixture is the best solvent for the direct titration and toluene - ethanol - acetic acid for the back-titration. Fig. 2 also shows that the solvent in the ASTM D-2896 method and toluene - acetic acid are not suitable for either titration method. Table I summarises the recommended solvent - titrant pairs.

TABLE I

SOLVENTS AND TITRANTS RECOMMENDED FOR THE DETERMINATION OF TBN VALUES BY HIGH-FREQUENCY TITRATION

Solvent	Titrant ($\sim 10^{-1}$ M)
Toluene - propan-2-ol - water (5 + 4.95 + 0.05)	Hydrochloric acid - propan-2-ol; perchloric acid - glacial acetic acid*
Toluene - propan-2-ol - acetic acid (3.5 + 3.5 + 3)	Perchloric acid - glacial acetic acid
Toluene - ethanol - acetic acid (4 + 3 + 3)	Sodium acetate - glacial acetic acid†

* Total volume added less than 60 μl.
† Perchloric acid previously added, back-titration.

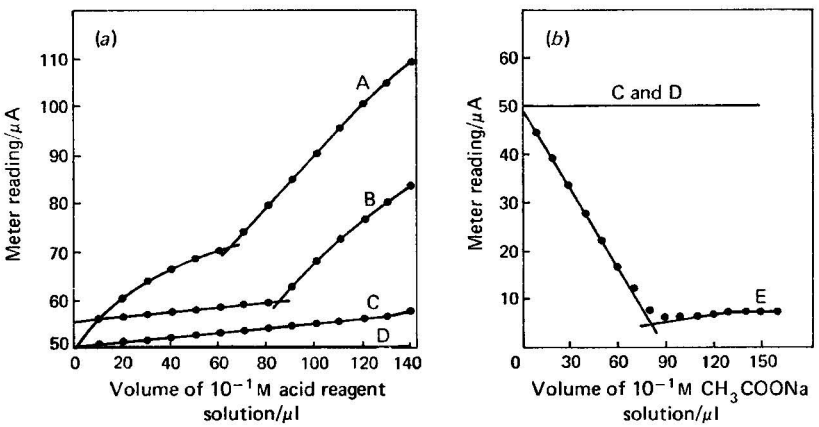

Fig. 2. Titration graphs for a synthetic sample of ECA-5830, TBN value 74, in several solvent mixtures. (*a*) Direct titration with 10^{-1} M perchloric acid; (*b*) back-titration with 10^{-1} M sodium acetate solution (150 μl of 10^{-1} M perchloric acid previously added). A, Toluene - propan-2-ol - acetic acid (3.5 + 3.5 + 3); B, toluene - propan-2-ol - water (5 + 4.95 + 0.05), 10^{-1} M hydrochloric acid as titrant; C, chlorobenzene - acetic acid (6.7 + 3.3); D, toluene - acetic acid (5 + 5); and E, toluene - ethanol - acetic acid (4 + 3 + 3).

Sample size

The response graphs showed that the total amount of titrant added should be limited to about 100 μl. The sample size chosen was adequate for the expected TBN value and the deviation of the results was studied. Table II shows the sample sizes recommended for different expected TBN values in micro- and macrotitration, and Table III shows the deviation obtained in titrating solutions of pure additives. The so-called pure additives are not pure substances but mixtures of different chemical species (*e.g.*, zinc dialkylphosphoro-

TABLE II

SAMPLE SIZE RECOMMENDED FOR VARIOUS EXPECTED TBN VALUES

	Microtitration		Macrotitration	
Expected TBN value	Approximate sample mass/mg	Sensitivity of weighing/mg	Approximate sample mass/g	Sensitivity of weighing/g
0–5	100	0.2	5–3	0.01
5–10	50	0.1	3–2	0.005
10–20	25	0.05	2–1	0.005
20–50*	20	0.02	1–0.5	0.002
50–100*	15	0.02	0.5–0.2	0.001

* Alternatively use a preparation of the lubricating oil in a base oil, which allows an amount in one of the first three ranges to be weighed out.

dithioate - basic barium dinonylnaphthalene sulphonate, polyamino monoalkenylsuccinimide - alkaline calcium petroleum sulphonate, barium thiophosphonate). Further experiences with a synthetic sample made from ECA-5830 and the base oil, TBN value 7.4, and amounts of sample ranging from 20 to 100 mg, showed that maximum deviation from the mean is 0.2 TBN unit (Table IV). This result is the same as was found for pure Lubrizol 864 solution, which has the same TBN value but a very different composition.

TABLE III

TBN VALUES DETERMINED FOR SAMPLES OF ADDITIVES

TBN value	Lubrizol 864	Anglamol 99	Lubrizol 6610
Determined	8.95	19.2	61.4
	8.91	19.3	62.3
	8.69	18.7	62.6
Mean	8.85	19.1	62.1
Maximum deviation (among results) ..	0.3	0.6	1.2
Maximum deviation (from mean) ..	0.2	0.4	0.7

TABLE IV

INFLUENCE OF SAMPLE SIZE ON THE DETERMINATION OF THE TBN VALUE FOR A SYNTHETIC SAMPLE OF ECA-5830 DISSOLVED IN BASE OIL

Sample mass/mg	10^{-1} M HCl added/μl	TBN value
24.13	33	7.67
40.09	54	7.56
57.3	77	7.54
67.5	90	7.48
76.7	101	7.39
92.7	120	7.26
111.6	143	7.19
Mean		7.44
Mean deviation (among results)		0.5
Mean deviation (from mean)		0.2
Standard deviation		0.2
Relative standard deviation		2.3%
Relative error[11]		2.1%

Similar experiments were carried out with the same synthetic sample, toluene - propan-2-ol - acetic acid solvent and perchloric acid in acetic acid as the titrant.

Fig. 3 shows the experimental graphs and Table V the results obtained. Fig. 4 relates

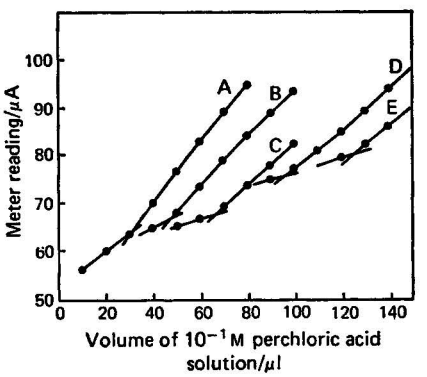

Fig. 3. Titration graphs for the determination of synthetic samples of ECA-5830 dissolved in base oil, according to Table V (graphs A, B, C, D and E correspond to titrations 1, 2, 3, 4 and 5 in Table V).

TABLE V

INFLUENCE OF SAMPLE SIZE ON THE DETERMINATION OF THE TBN VALUE FOR A SYNTHETIC SAMPLE OF ECA-5830 DISSOLVED IN BASE OIL, IN TOLUENE - PROPAN-2-OL - ACETIC ACID AS SOLVENT AND 10^{-1} M PERCHLORIC ACID AS TITRANT

Test	Sample mass/mg	Volume of 10^{-1} M perchloric acid added/μl	TBN value	
			Uncorrected	Corrected*
1	20.36	31	8.54	7.60
2	32.49	49	8.46	7.88
3	48.03	69	8.06	7.65
4	71.3	101	7.95	7.67
5	89.2	125	7.86	7.64
Mean value			8.17	7.69
Maximum deviation (from mean)			0.4	0.2
Maximum deviation (among results)			0.7	0.3
Standard deviation			0.3	0.1
Relative standard deviation			3.8%	1.4%
Relative error[11]			4.7%	1.8%

* 3 μl blank correction.

titrant consumptions to sample mass, and extrapolation reveals the need for a blank correction for the solvent, which must be taken into account in calculations. In this way the standard deviation and relative error are improved and the maximum deviation among results becomes less than 0.3 TBN unit. This correction can be related to the basicity of the acetate ions in the solvent.

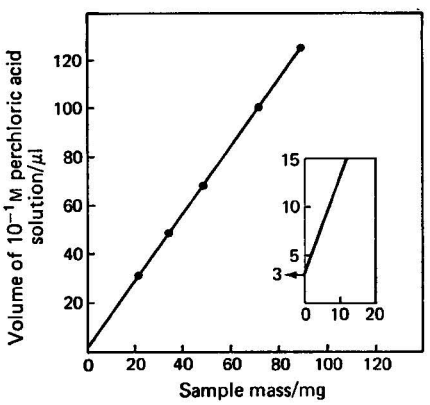

Fig. 4. Relationship between titrant consumption and sample mass according to Table V (on the magnified scale the blank value is shown).

Addition of sodium acetate or Tris

As increasing sample size above 200 mg leads to a loss in sensitivity and disturbs readings, the addition of a pure, typical, weak base to the sample was studied, in order to allow titration of those with a very low TBN value. Sodium acetate and potassium hydrogen phthalate were tested in the acidic solvent with perchloric acid as titrant. The results shown in Table VI suggested that the use of sodium acetate is better than potassium hydrogen phthalate. However, according to the results in Table VI, in a medium of toluene - propan-2-ol - water with hydrochloric acid in propan-2-ol as titrant, the addition of Tris can be convenient; this base must be used cautiously, keeping the amount added to a limit of 2.5 μmol. In all of the experiments the consumption of the 10^{-1} M acid titrant was kept below 150 μl so that the net consumption of titrant was about 30 μl.

TABLE VI

INFLUENCE OF THE ADDITION OF SODIUM ACETATE, POTASSIUM HYDROGEN PHTHALATE OR TRIS ON THE DETERMINATION OF THE TBN VALUE FOR A SYNTHETIC SAMPLE OF ECA-5830 DISSOLVED IN BASE OIL

Volume of 10^{-1} M base added/μl*	TBN value		
	Acetate†	Phthalate†	Tris‡
0	7.72	7.72	7.67
25	7.79	8.07	7.69
25	7.78	8.10	7.62
50	7.24	8.73	8.02
50	7.81	8.38	8.12
75	7.87	7.83	8.70
100	7.97	7.28	8.72

* Sample size ~20 mg.
† In toluene - propan-2-ol - acetic acid as solvent with 10^{-1} M perchloric acid as titrant (3 μl blank solvent, Table V).
‡ In toluene - propan-2-ol - water as solvent with 10^{-1} M hydrochloric acid as titrant.

Results and Discussion

In order to check the proposed methods, synthetic samples with compositions corresponding approximately to trade samples were prepared from a base oil and a set of additives widely used in the lubricating oil industry. The TBN values of the additives were known from the manufacturer or could be determined by the standard ASTM potentiometric method. Table VII summarises the TBN values obtained in the microtitration of several synthetic samples.

TABLE VII

TBN VALUES DETERMINED FOR SEVERAL SYNTHETIC SAMPLES OF ADDITIVES DISSOLVED IN BASE OIL

TBN value	Oloa	Lubrizol						
	851 219	4266	4084	6610 4084 3715	3882 1360	3882	56 1360	56
Determined	14.5	5.48	6.85	7.02	9.35	11.1	15.8	64.7
	14.6	5.46	6.81	6.96	9.38	11.4	15.7	63.8
	14.5	5.66	6.83	7.16	9.44	11.6	16.2	64.1
Mean	14.5	5.53	6.83	7.05	9.39	11.4	15.9	64.2
Expected value*	13.9	5.4	6.8	7.1	9.4	11.4	16.0	64.3
Potentiometric†		5.25 (5.11–5.47)		6.91 (6.63–7.20)		10.7 (10.3–11.3)	15.1 (14.8–15.4)	
Difference‡	+0.6	−0.13	+0.03	−0.05	−0.01	0.0	−0.1	−0.1

* Calculated from % *m/m* and TBN of additive added as known from the manufacturer.
† Determined by ASTM D-664, mean of three determinations; range given in parentheses.
‡ Difference: mean − expected value.

TABLE VIII

DETERMINATION OF TBN VALUES ON SAMPLES OF NEW CEPSA TRADE LUBRICATING OILS

	Sample trade name							
	Rodaje Chrysler	Extra HD-10	Multigrado 20-40W	Teseo S-3 SAE-30	Serie 3 SAE-30	Troncoil 1530	Régulo HD-50	Régulo Super 650
Determined	5.94	6.27	7.10	9.72	11.3	15.1	15.8	63.2
	5.72	6.21	6.96	9.68	11.4	15.2	15.9	64.6
	5.78	6.20	7.13	9.83	11.2	15.1	15.9	64.4
Mean	5.81	6.23	7.06	9.74	11.3	15.1	15.9	64.1
Expected value*	5.4	6.8	7.1	9.4	11.4	15.0	16.0	64.3
Potentiometric†		6.10 (5.92–6.27)		9.14 (8.99–9.34)		14.9 (14.6–15.3)		60.4 (59.8–61.5)
Difference‡	+0.41	−0.57	−0.04	+0.34	−0.1	+0.1	−0.1	−0.2

*, † and ‡ as in Table VII.

Other titrations were performed on two used oils from a Chrysler C-24S plant motor after

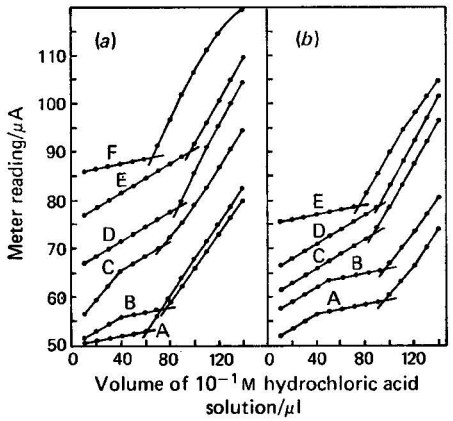

Fig. 5. Typical graphs obtained in the determination of TBN values of two used lubricating oils removed from a Chrysler C-24S plant motor. (*a*) Motor oil MIL-L-2104B after working time: A, 48 h (TBN 4.01); B, 96 h (TBN 2.79); C, 192 h (TBN 2.24); D, 312 h (TBN 1.26); E, 408 h (TBN 0.30); and F, 0 h (TBN 6.23). (*b*) Motor oil MIL-L-46152 after working time: A, 50 h (TBN 5.07); B, 100 h (TBN 3.97); C, 225 h (TBN 1.80); D, 300 h (TBN 1.36); and E, 0 h (TBN 7.06).

various periods of working (Figs. 5 and 6). Replicate determinations were also carried out on samples of new CEPSA trade lubricating oils [Table VIII and Fig. 7(*a*)].

Finally, the results obtained by macrotitration are shown in Fig. 7(*b*) and Tables IX and X; the latter table summarises TBN values determined on used oils from marine engines.

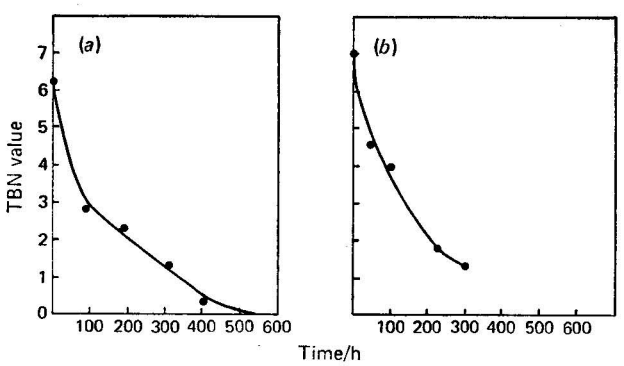

Fig. 6. Variation of TBN value with working time for two lubricating oils from a Chrysler C-24S plant motor: (*a*), motor oil MIL-L-2104B; (*b*), motor oil MIL-L-46152.

The repeatability obtained (Tables VII and VIII) is very good, as the differences from the mean are less than 0.1 TBN unit; such values are obtained with the ASTM standard method only when the potentiometric graphs show good inflection points.[2]

The precision was established (Table IV) for a synthetic sample of ECA-5830, TBN value 7.4, and shows a standard deviation of 0.2 and 5.6% relative error (95% probability). The estimated accuracy of the method can be deduced from the results recorded in Table VII. In this wide range of TBN values (5–64), the differences between the obtained and expected values are less than or equal to 0.1, except for one sample. We must emphasise the need

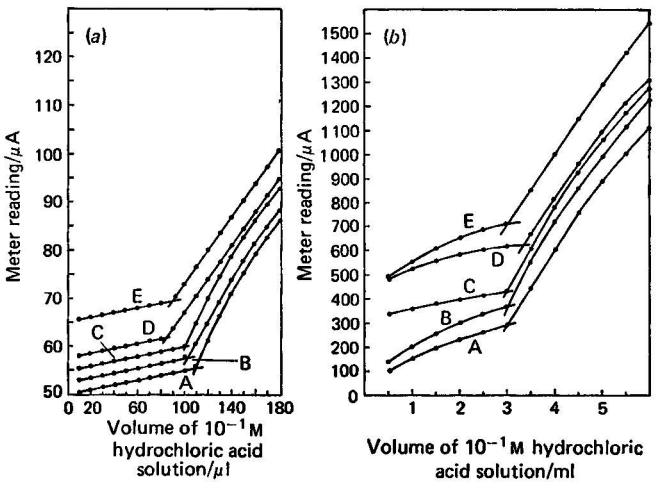

Fig. 7. Typical graphs obtained in the determination of TBN for synthetic, new and used lubricating oils by (*a*) micro- and (*b*) macrotitration: A, synthetic Lubrizol 56 (TBN 64.2–65.7); B, new Teseo S-3, SAE-30 (TBN 9.74–9.55); C, synthetic Lubrizol 56 + Lubrizol 1360 (TBN 15.9); D, used marine oil (TBN 13.0–14.2, vessel D, Table IX); and E, used marine oil (TBN 5.58–5.51, vessel A, Table IX).

for a good determination of the blank correction for the solvent, particularly with solvent mixtures containing acetic acid (Table V).

Samples with low TBN values can be resolved by cautious additions of Tris or, better, by adding sodium acetate. Nevertheless, this method is not totally satisfactory from the standpoint of the definition of TBN value according to ASTM method D-664, as basic constituents with a pK_b of about 10 cannot be titrated. It must be taken into account that the high-frequency conductimetric end-points are generally located at the intersection of two ascending lines. The slope of the titration line before the end-point becomes closer to the slope of the excess acid line as the basic strength of the sample decreases and the end-point becomes increasingly difficult to locate accurately. In this sense, the differences observed in titrating samples of different sizes can be related to their content in very weak species.

Table IX

Comparison of TBN values determined by macro- and microtitration on synthetic and new lubricating oils

Sample	TBN value	
	Microtitration	Macrotitration
Lubrizol 4266*	5.66	5.60
Lubrizol 56*	64.2	65.7
Lubrizol 56 + Lubrizol 1360*	15.9	15.9
Lubrizol 3882 + Lubrizol 1360* ..	9.39	9.40
ECA-5830*	7.96	7.99
Rodaje Chrysler†	5.81	5.96
Teseo S-3 SAE-30†	9.74	9.55

* Synthetic samples: additives dissolved in base oil.
† CEPSA samples, trade name.

In spite of the difficulties described, high-frequency titration appears to be a very encouraging technique, as by using mixed solvents it is possible to differentiate weak bases in mixtures.[6] A limitation in sample size can be avoided by use of the Oscimhometer[10]

TABLE X

DETERMINATION OF TBN VALUES FOR SAMPLES OF USED OILS FROM MARINE ENGINES

	Vessel A		Vessel B		Vessel C		Vessel D	
TBN value	*	†	*	†	*	†	*	†
Determined ..	5.61	5.50	6.99	6.51	6.55	6.77	13.0	14.4
	5.48	5.63	6.88	7.01	6.66	7.82	12.8	14.0
	5.64	5.42	6.94	6.89	6.98	6.47	13.1	14.1
Mean	5.58	5.54	6.94	6.80	6.73	7.02	13.0	14.2
New motor oil ..	8.00		9.74		15.13		25.64	

* Microtitration.
† Macrotitration.

according to the procedures described. In connection with this, Tables IX and X also report results obtained in tests performed by use of macrotitration using this oscillator, and one can see that the mean TBN values obtained with both techniques are virtually equal, except for one sample. Likewise, the shapes of the titration graphs are very similar [Figs. 7(*a*) and (*b*)]. With used oils, Table X suggests that the repeatability obtained by use of microtitration is better than that obtained with macrotitration. However, this suggestion cannot be substantiated firmly because there are too few results. Workers in CEPSA laboratories are now establishing a co-operative testing programme using macrotitration in order to solve this problem.

Compared with the standard potentiometric method, the use of high-frequency analysis avoids the possibility of electrode contamination and damage, as the electrodes are outside the titration cell. A sharp break is always obtained at the end-point of the titration graph, and no buffer is needed. Once the apparatus has been set up, a single TBN determination can be performed in about 10 min, instead of about 1 h, which is required for the potentiometric determination when a manual instrument is used.

The outstanding advantages of the high-frequency method of TBN determination are its good accuracy, the validity of its end-points and the smaller spread of results. As a comparison, four synthetic and four trade oil samples were analysed by the standard potentiometric method; results shown in Tables VII and VIII prove that TBN values obtained by use of the potentiometric method are not as consistent as those obtained with the high-frequency method. In back-titration, the high-frequency method (Table I) is an improvement on the equivalent potentiometric method, ASTM D-2896. The method developed for TBN determination can be suggested as an alternative to ASTM and IP methods.

A similar conclusion was reached in our paper on TAN determination by high-frequency titration.[1] This similarity can be used as an argument for the acceptance of high-frequency titration in both determinations.

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

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Continuous Solvent-extraction Method for the Spectrophotometric Determination of Cationic Surfactants

Jiro Kawase and Makoto Yamanaka

Tochigi Research Laboratories, Kao Soap Co. Ltd., 2606 Akabane, Ichikai-machi, Tochigi, Japan

A rapid and automated spectrophotometric method for the determination of cationic surfactants, using the AutoAnalyzer, has been developed. This method is based on the continuous solvent extraction of the ion-pair complex formed in the reaction of Orange II with a cationic surfactant. Good traces and identical molar responses were obtained with seven different types of surfactant using methanol in the Orange II reagent. Fatty amines in mixtures of the amines and quaternary ammonium surfactants were determined by changing the pH of the aqueous phase. The proposed method was applied to the determination of cationic surfactants in several commercial products. The results agreed with those obtained by the two-phase titration procedure. The detection limit is 5 μM and the capacity is 10–20 samples per hour, with a relative precision of better than 1.5%.

Keywords: *Automatic analysis*; *cationic surfactant determination*; *fatty amine determination*; *spectrophotometry*; *Orange II*

Recently, new kinds of commercial products containing cationic surfactants have been extensively developed, in addition to the well known hair rinse, fabric softener and sanitiser types. The determination of the cationic surfactants has hitherto been effected by two-phase titration procedures.[1–3]

We report here that the Orange II method,[4,5] which has hitherto been conducted only manually by spectrophotometry, can be automated successfully by means of the Auto-Analyzer.

Experimental

Reagents

Benzethonium chloride (A), hexadecylpyridinium chloride (B), benzylhexadecyldimethylammonium chloride (C) and trimethyloctadecylammonium chloride (D) were purchased from Tokyo Kasei Co. Ltd. Hexadecyl(2-hydroxyethyl)methyloctadecylammonium chloride (E) and hexadecylbis(2-hydroxyethyl)octadecylammonium chloride (F) were purchased from ICI Australia. Hexadecyldimethyloctadecylammonium chloride (G), hexadecylamine (H), octadecylamine (I), dialkylamine (R_1 and R_2 = C14–C18, relative molecular mass 499.83) (J) and alkyldimethylamine (R = C8–C18, relative molecular mass 293.69) (K) were produced by Kao Soap Co. Ltd.

These reagents were purified by recrystallisation from acetone or an acetone - ethanol mixture. Stock solutions (3 mM) of A–G were prepared by dissolving the purified compounds in distilled water and standardised according to the direct two-phase titration procedure[3] just before use. Amine surfactant (H–K) solutions (70 μM) were prepared by weighing purified compounds and acidifying the amines with an adequate amount of dilute sulphuric acid. All other reagents were of analytical-reagent grade.

Orange II reagent, pH 7.3. Dissolve 50 mg of Orange II, 3.78 g of potassium dihydrogen orthophosphate, 1.99 g of disodium hydrogen orthophosphate and 520 ml of methanol in 300 ml of distilled water, dilute the solution to 1 l with distilled water and adjust the pH to 7.3 with 1 N sodium hydroxide solution.

Orange II reagent, pH 1.6. Dissolve 50 mg of Orange II, 2.24 g of potassium chloride, 3 ml of concentrated hydrochloric acid and 520 ml of methanol in 300 ml of distilled water, dilute the solution to 1 l with distilled water and adjust the pH to 1.6 with 1 N hydrochloric acid.

Methylene blue solution. Dissolve 0.03 g of methylene blue, 50 g of sodium sulphate (anhydrous) and 12 g of concentrated sulphuric acid in 500 ml of distilled water and dilute the solution to 1 l with distilled water.

Standardisation of Cationic Surfactants[3]

To 10 ml of sample solution (approximately 3 mM), measured in a measuring cylinder, add 25 ml of the methylene blue solution and 15 ml of chloroform. Titrate the sample solution with 0.004 M sodium lauryl sulphate solution by the usual method. At the same time, test a blank by titrating 10 ml of distilled water in the same manner. The end-point is when the blue colour is completely discharged from the aqueous layer.

Method of calculation

$$C = (V_s - V_b) \times 0.004 \times 100$$

where C mmol is the concentration of the cationic surfactants, V_s ml is the volume of 0.004 M sodium lauryl sulphate solution used in the titration of the sample and V_b ml is the volume of 0.004 M sodium lauryl sulphate solution used in the titration of the blank.

Apparatus and Procedure

The apparatus is a Technicon AutoAnalyzer, Model II, consisting of the following components: automatic sampler, peristaltic pump, manifold, spectrophotometer equipped with a flow cell (light path length 15 mm) and 485-nm filter and recorder [Fig. 1(*a*)]. When necessary, a dilution loop [Fig. 1(*b*)] was used for analyses of commercial products.

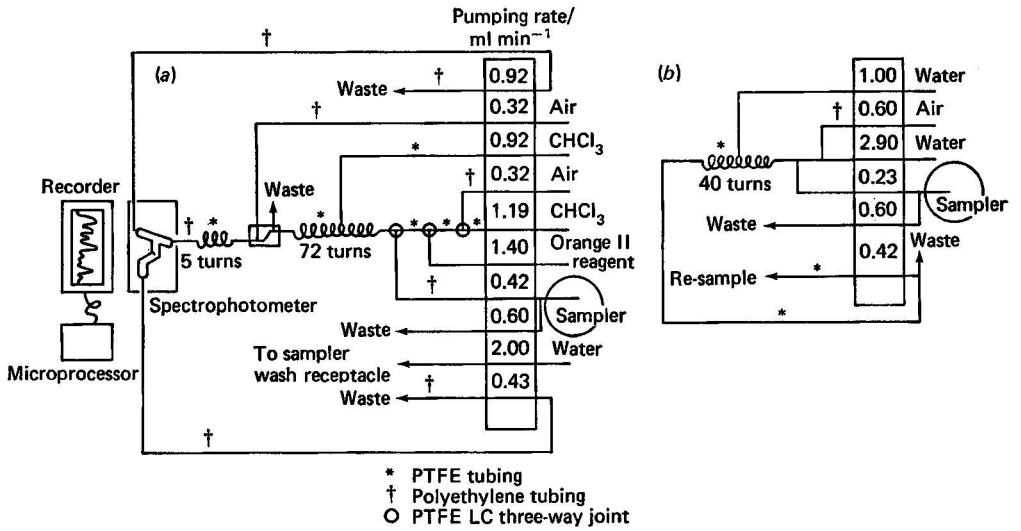

Fig. 1. (*a*), Diagram for extraction of cationic surfactants with Orange II. (*b*), Flow diagram of a dilution loop.

Except for Acidflex pump tubing, 2-mm bore PTFE tubing, 0.8-mm bore polyethylene tubing, mixing coils made of 2-mm bore PTFE tubing wound round a polyethylene pipe (25-mm diameter) and PTFE liquid chromatography (LC) three-way joints (Nihon Seimitsu JT02U) are used for lines that come into contact with chloroform. All connections, especially between the phase separator and the flow cell, should be as short as possible. Before beginning a run, the tubing should be washed out by pumping methanol through the lines used to introduce chloroform. To prevent the inflow of the aqueous phase into the flow cell, it was found necessary to have the chloroform phase present in the tubing prior to introducing the aqueous phase. As complex formation with fatty amines depends on the pH of

the aqueous phase,[5] Orange II reagent at pH 1.6 was used for the over-all determination of quaternary ammonium and amine surfactants, and that at pH 7.3 for the determination of quaternary ammonium surfactants alone.

The determination is effected automatically by passing a sample solution through a dilution loop, reacting the surfactant with the anionic dyestuff to form the ion-pair complex, extracting it into the chloroform phase and measuring the absorbance at 485 nm after phase separation. The sampling rate is 10–20 samples per hour. All calibration graphs were calculated with a custom-built microprocessor (Tachibana Electronic Co. Ltd.) connected to the recorder. The signal output of the recorder was monitored continuously at 1-s intervals over the run time. The absorbance, the equation of the straight line calibration graph, the regression coefficient and the calculated concentration of the sample were obtained from the print-out.

Results and Discussion

Effect of Methanol

The slow extractability of a cationic surfactant in the Orange II method is well known.[5] Ion-pair extraction in the continuous-flow analysis differs from that in the manual method, because it proceeds in the limited interfacial region between alternative aqueous and chloroform segments in a definite length of mixing coil, and is seldom allowed to reach equilibrium. Thus, in the continuous-flow analysis the relative molar extractability of the ion-pair complex would be influenced by the corresponding extraction rate, which differs slightly for each type of cationic surfactant. However, in practical analysis it is necessary to obtain identical molar responses that are independent of the type of surfactant.

The role of the solvating agent and its affinity for the ion-pair complex have been considered by Higuchi *et al.*[6] The addition of methanol to the water - chloroform extraction system in the continuous-flow analysis would be expected to enhance markedly the extraction process, through solvation of the ion pairs that are formed, and to be a powerful means of varying the relative molar extractability in the analysis.

Investigation has shown that the relative molar extractability of the ion-pair complexes formed by the reaction of Orange II with cationic surfactants A–G, in the continuous-flow analysis, differs slightly and varies with the methanol content of the Orange II reagent to different extents (Fig. 2). The rate of extraction of the complex is also different for each surfactant. It should be noted that the rate of extraction for the surfactants is much more rapid with a higher methanol concentration and that the molar extractability of the complex increases with increasing methanol concentration (up to 40% *V/V*).

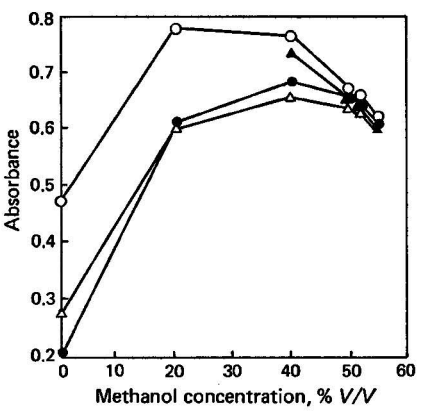

Fig. 2. Effect of methanol concentration on the absorbance measured at 485 nm, of: ○, surfactant B; △, surfactant C; ▲, surfactant D; and ●, surfactant G. Each cationic surfactant concentration was 200 μM. The pH of the Orange II reagent was 7.30.

There is also a distinct variation in the peak shapes with a change in the methanol concentration (Fig. 3). A longer period of steady state was attained with a higher concentration of methanol; the peak shape for each type of surfactant deteriorates as the hydrophilicity increases.

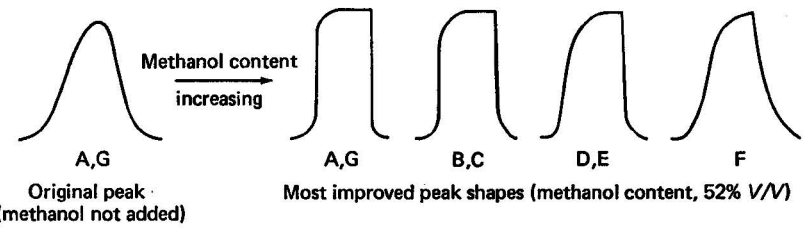

Fig. 3. Variation in the peak shapes with the methanol content and the type of surfactant, A–G. Sampling cam: 10 samples per hour, 3 parts of sample to 1 part wash.

The effect of methanol can be explained in terms of both solvating and dissolution effects. With increase in the concentration of methanol in the Orange II reagent, the methanol content of the chloroform phase also increases and this contributes to both the extraction rate and the extractability of the complex by its solvating effect. With 40% *V*/*V* or more of methanol in Orange II reagent, the ion-pair dissolution effect of methanol in the aqueous phase may predominate over the solvating effect and result in a decrease in the absorbance.

In this work, 52% *V*/*V* of methanol in the Orange II reagent was chosen as the best concentration to give good peak shapes and almost identical molar responses for the seven different types of surfactant (Table I).

TABLE I

RELATIVE MOLAR EXTRACTABILITIES OF CATIONIC SURFACTANTS

All values are normalised relative to the molar extractability of surfactant G.

pH of buffer solution*	Cationic surfactant†						
	A	B	C	D	E	F	G
7.30	103.3	102.0	100.0	98.9	101.1	97.9	100.0
1.60	103.3	102.0	100.2	99.1	101.6	98.2	100.0

* Methanol content of the Orange II reagent was 52% *V*/*V*.
† Each surfactant concentration was 200 μM.

Calibration Graph and Precision

The proposed method is suitable for determining up to 200 μM of cationic surfactant, and provided that the dilution loop [Fig. 1(*b*)] is used, a linear calibration graph covering the range up to 3 mM can be realised. The detection limit was found to be approximately 5 μM. The resulting calibration graphs for each cationic surfactant were linear at pH 7.30 and 1.60.

The precision of the method was evaluated by determining 1.5 mM of each cationic surfactant (A–G). The results proved to be within 1.5% of the given concentration with a coefficient of variation of 0.3–1.5%. Cationic surfactant D serves as a representative example (Fig. 4).

The cationic surfactant most frequently used in commercial products seems to be a dialkyldimethylammonium type, and therefore cationic surfactant G was used as a standard material for the commercial products analyses.

Interferents likely to be present in commercial products were examined. Inorganic salts, acids and non-ionic surfactants found in the usual formulations had only a small effect on the determination.

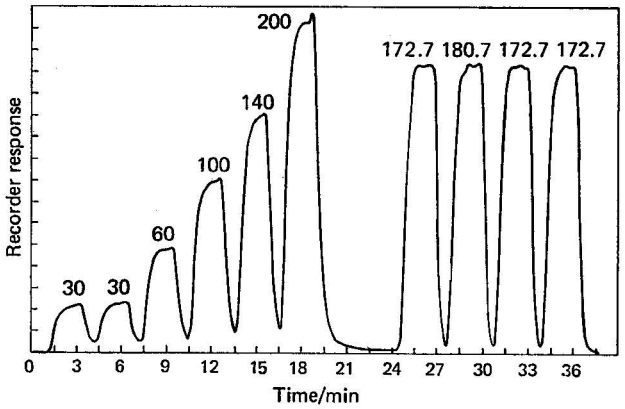

Fig. 4. Determination of surfactant D. Values on peaks represent the concentration in μM. Rate of sampling is 20 per hour.

Application to the Determination of Fatty Amines and Commercial Products Analyses

Solutions (70 μM) of the fatty amines (H–K) were analysed at pH 1.60 using the calibration graph for surfactant G. The results were satisfactory (Table II). Several commercial products containing cationic surfactants were analysed by the proposed method, after adequate dilution of the aqueous solutions. Orange II reagent at pH 1.6 was used for the over-all determination of quaternary ammonium and amine surfactants and that at pH 7.3 for the determination of quaternary ammonium surfactants alone. The results were compared with those obtained by the direct two-phase titration procedure,[3] which determines

TABLE II

RECOVERY OF FATTY AMINES

Amine	Present/μM	Found/μM	Recovery,* %
H	70.0	67.2	96.0
I	70.0	69.2	98.9
J	70.0	69.9	99.9
K	70.0	71.4	102

* The pH of the Orange II reagent was 1.60. The dilution loop [Fig. 1(*b*)] was not used.

TABLE III

COMMERCIAL PRODUCTS ANALYSES

	AutoAnalyzer method		
Sample*	pH 7.30: found/mM	pH 1.60: found/mM	Two-phase titration procedure[3]: found/mM
1	2.78	2.85	2.87
2	2.03	2.06	2.08
3	1.90	2.14	2.24
4	2.10	2.13	2.19
5	1.82	1.80	1.84
6	3.33	3.52	3.59
7	1.00	—	1.00
8	1.08	—	1.05
9	3.33	—	3.29

* Samples 1–3 were fabric softeners and samples 4–9 were hair rinses.

over-all cationic surfactants (Table III). Good agreement was obtained between the automated and conventional determinations.

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Automatic Emission Spectrometer for the Determination of Nitrogen-15

J. D. S. Goulden and D. N. Salter*
National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT

An automatic nitrogen-15 analyser, employing a novel use of a rhodium - platinum catalyst for the generation of nitrogen and capable of analysing 60 samples per hour, is described. Nitrogen compounds of biological origin are first converted into ammonium chloride by conventional Kjeldahl digestion and distillation methods. The ammonium chloride sample (about 5 μl containing about 10 μg of nitrogen) is injected into a soda-lime reactor at 590 °C through which flows a stream of purified helium. Ammonia that is released passes directly into the catalyst tube and the generated nitrogen and hydrogen are separated by passage through a gas-chromatographic column, which also retains the water.

After passing through a pressure restrictor the nitrogen flows in the helium stream through a Spectrosil discharge tube located in a microwave cavity. The emitted radiation is analysed by means of a specially constructed dual-wavelength monochromator and the intensities of the $^{14}N^{14}N$ (297.7 nm) and $^{14}N^{15}N$ (298.3 nm) bands are measured simultaneously by two photomultipliers. Amplified signals, proportional to the peak intensities, are fed through phase-sensitive detectors into a ratiometer, the output from which is fed to a digital voltmeter and printed out in terms of nitrogen-15 abundance. A peak detector indicates the total nitrogen content of each sample and actuates the nitrogen-15 print-out.

The response of the instrument is slightly curvilinear but may be regarded as linear over limited ranges. Calibration can therefore be achieved by running suitably chosen standards to fix upper and lower set points. Carry-over between samples is very small and is eliminated by running duplicates. Standard deviations of replicate measurements of natural abundance and enriched standards are less than 0.01 atom-%, while determinations of nitrogen-15 in biological samples were shown to be accurate to ±0.01 atom-% by comparison with a Statron NOI-4 nitrogen-15 analyser.

Keywords: *Nitrogen-15 determination*; *catalytic nitrogen generation*; *automated emission spectrometer*

The analysis of nitrogen-15 (^{15}N) by the classical method of Rittenberg[1] using a mass spectrometer is a tedious and demanding operation, which for many years severely limited the use of nitrogen-15 as a tracer in biological research. Subsequently, the technique of emission spectrometry was applied to the analysis of nitrogen isotopes by Broida and Chapman,[2] and the developments of Meier and Müller[3] and Faust[4] led to the introduction of reliable and relatively cheap commercial emission spectrometers. Further developments by Lloyd-Jones and co-workers[5–7] have resulted in improved accuracy and speed of analysis. The methods for the preparation of nitrogen for isotopic analysis have, however, continued to be time consuming and proved to be a limiting factor in nutritional studies.[8] The application of optical and mass-spectrometric techniques has been discussed in a recent review.[9]

In this paper an automatic nitrogen-15 analyser, capable of analysing 60 samples per hour, is described. The generation of pure nitrogen gas from a solution of ammonium chloride prepared from the test material is carried out automatically and the result printed out as nitrogen-15 atom per cent. Total nitrogen content of the sample can also be indicated simultaneously and displayed as a peak on a chart recording. The method is used routinely for samples containing 10 μg of nitrogen, and with some loss in accuracy may be used for samples containing as little as 2 μg of nitrogen. It is therefore ideally suited to the determination of nitrogen-15 in compounds available only in very small amounts, *e.g.*, amino acids prepared by ion-exchange chromatography of protein hydrolysates or physiological fluids. A preliminary report of this apparatus has been published.[10]

* To whom all correspondence should be addressed.

Principle of the Method

With the manual nitrogen-15 analyser (*e.g.*, the Statron NOI-4 or NOI-5), part of the emission spectrum of nitrogen in the 300-nm region is recorded and the peak heights for $^{14}N^{14}N$ and $^{14}N^{15}N$ in a series of replicate scans are measured. Nitrogen-15 isotopic abundance is then calculated from the mean ratio of these peak heights, after correction for background emission. In the National Institute for Research in Dairying (NIRD) automatic nitrogen-15 analyser, the two peaks at 297.7 and 298.3 nm are measured simultaneously, thus eliminating errors in the ratio due to variations of source intensity during the time needed for a wavelength scan.

For both the manual and the automatic methods the nitrogen-15 labelled biological material is first subjected to a Kjeldahl digestion; alkali is added to the digest and the ammonia released is steam distilled into dilute hydrochloric acid, from which ammonium chloride is recovered by evaporation. From this stage onwards analysis with the NIRD analyser is fully automatic apart from the manual injection of samples by syringe, avoiding the troublesome vacuum technique involved in the preparation of emission tubes containing nitrogen. Ammonium chloride solution (approximately 10 μg of nitrogen) is injected into a stream of helium and passes immediately through a heated reactor tube, the front portion of which contains soda-lime. Ammonia is generated and is dissociated by a catalyst in the rear portion of the tube. The generated gases are separated by a gas-chromatographic column, which also retains the water. They are then eluted sequentially through a flow restrictor into a discharge tube located in a microwave cavity. The emitted radiation is analysed by means of a dual-wavelength monochromator. Signals proportional to the intensities of the bands at 297.7 and 298.3 nm, measured simultaneously, are amplified and fed through a pair of phase-sensitive detectors into a ratiometer whose output is fed to a digital voltmeter and printed out as nitrogen-15 abundance in atom per cent. As the nitrogen flows through the discharge tube a peak detector actuates the printer and gives an indication of the total mass of nitrogen present in the sample.

Materials and Methods

The Statron NOI-4 emission spectrometer, the preparation of reagents and samples for analysis and the operation of the equipment have been described previously.[6] Standard nitrogen-15 labelled ammonium chloride samples (measured in a mass spectrometer) were obtained from C.Z. Scientific Ltd. For analysis in the NIRD automatic nitrogen-15 analyser, samples of biological material (normally containing 200–1 000 μg of nitrogen, but for amino acid fractions from a chromatography column sometimes as little as 20–50 μg of nitrogen) are digested with 3 ml of concentrated sulphuric acid (low nitrogen, Aristar grade, BDH, Poole, Dorset) and one tablet of mercury(II) oxide catalyst (Kjeltabs F.M., Thompson & Capper Ltd.), heating being continued for at least 1.5 times the clearing time. The ammonia released from the digest, by addition of an excess of 10 M sodium hydroxide solution containing 25 g l^{-1} of sodium thiosulphate, is distilled in a Markham's apparatus and collected in 10 ml of 0.025 M hydrochloric acid. Between distillations of different nitrogen samples, 20–25 ml of ethanol are distilled to remove trace amounts of adsorbed ammonia and prevent cross-contamination. The resulting ammonium chloride solution is evaporated to dryness at 70 °C and the solid re-dissolved in glass-distilled water to give a solution containing approximately 2 μg μl^{-1} of nitrogen. Alternatively, the microdiffusion method of Conway[11] can be used for samples of low nitrogen content Ammonia released from portions of the diluted digest with 10 M potassium hydroxide solution containing 100 g l^{-1} of sodium thiosulphate is collected in 1 ml of 0.05 N hydrochloric acid in the centre well of a 60-mm Conway unit. The nitrogen-15 labelled ammonium chloride solution is evaporated to dryness and reconstituted to approximately 2 μg μl^{-1} of nitrogen.

Components of the NIRD Nitrogen-15 Analyser

A block diagram of the main components of the automatic nitrogen-15 analyser is shown in Fig. 1.

Helium supply

Cylinder helium, first dried by passing through a molecular sieve (grade 5A), is purified by passing it through a BOC Rare Gas Purifier.

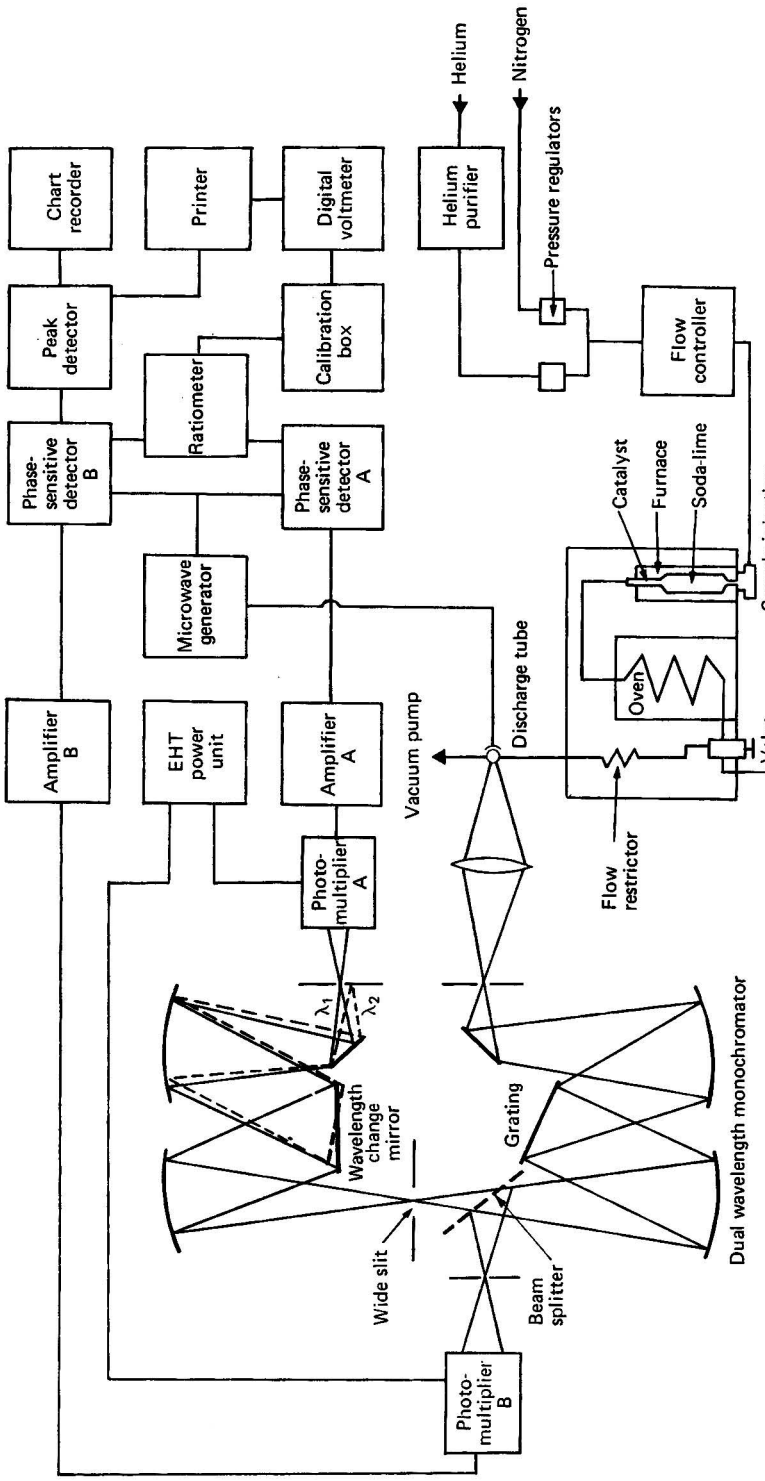

Fig. 1. Block diagram of main components of automatic nitrogen-15 analyser.

Reactor tube

In an earlier version of the NIRD nitrogen-15 analyser,[12] nitrogen was generated from the ammonium chloride solution by reaction with copper and copper(II) oxide, but a "memory effect" became apparent and produced serious cross-contamination of successive nitrogen-15 samples. Tests showed that the memory effect was most probably caused by the formation of oxides of nitrogen as by-products of the Dumas reaction. These reacted with water already adsorbed on the chromatographic column to produce nitric acid, which could take part in exchange reactions with nitrogen.

With the catalytic method of nitrogen generation, the front portion of a fused silica reactor tube (about 300 mm long, 14 mm o.d.) is packed with soda-lime (BDH, 10–16 mesh). The rear 100-mm portion (7 mm o.d.) of the tube is packed with about 1.4 g of the catalyst (Engelhard Industries Ltd., Chemical Group, Cinderford, Gloucestershire, Code No. 99966, 0.25% rhodium - 0.25% platinum coated on alumina spheres, diameter approximately 0.2 mm) and the whole reactor tube is enclosed in a furnace at 590 °C. As the alumina spheres on which the catalyst is deposited are known to sinter at temperatures above 600 °C, the furnace temperature is reduced to about 500 °C during overnight regeneration.

The sample of ammonium chloride solution (about 10 μg of nitrogen for normal operation) is injected manually through a silicone-rubber septum into the soda-lime tube and the liberated ammonia is dissociated. Because the dissociation reaction is endothermic, it is promoted by high temperatures. Ammonia dissociation is also accompanied by an increase in volume so that decomposition is favoured by high dilution in the helium carrier gas and small sample size. Exhaustion of the soda-lime (indicated by an erratic decrease in the intensity of the nitrogen peak on the chart record) leads to poisoning of the catalyst with the consequent presence of undissociated ammonia and the possibility of a memory effect between successive samples. Several thousand samples can be analysed before replacement is necessary.

Gas-chromatographic column

The column removes water and readily separates the nitrogen from attendant hydrogen. The nitrogen peak emerges 36 s after the hydrogen peak and has a pulse time of 2.4 s for half-peak maximum. The molecular sieve (13X, 30–60 mesh, Chromatography Services Ltd.) is packed into a stainless-steel tube 1.3 m long × 4 mm i.d., which has a water capacity equivalent to several hundred samples before regeneration. As water is absorbed the effective column length is reduced so that the elution time of about 1 min for nitrogen on a freshly regenerated column is progressively reduced during the analysis of a series of samples. The column is regenerated overnight by reversing the helium flow and setting the oven temperature surrounding the column to about 220 °C. Sintered stainless-steel filters are fitted at both column and reactor outlet connectors as a precaution against blockage of the flow restrictor.

Discharge tube

As the discharge operates at 10–15 Torr and the remainder of the preparation unit is at a positive helium pressure (approximately 30 p.s.i., which gave an elution time of just under 1 min for a 1.3-m column), it is necessary to introduce a throttle between the column exit and the discharge tube. This consists of a short stainless-steel tube flattened sufficiently to give the desired pressure drop.

To avoid fluorescence effects the discharge tube was made from Spectrosil, its dimensions being 250 mm over-all length × 3 mm i.d. Light output is affected only slightly by changes in the discharge tube internal diameter, operating temperature, microwave power and pressure. It is convenient to operate the discharge at room temperature at about 22 W.

Optical system

An optical diagram is shown in Fig. 1. A Rank-Hilger D331 double monochromator fitted with a 2400 lines per millimetre grating blazed for 300 nm was converted into a dual-wavelength monochromator. At 300 nm, the stated aperture and reciprocal dispersion were $f/5.5$ and 1.3 nm mm^{-1}, respectively. The transmitted beam enters a second compartment through a relatively wide slit that enables a narrow range of wavelengths to fall on the

wavelength change mirror. This mirror can be rotated through a small angle by a long lever working between a pair of adjustable stops.

Choice of optimum slit widths and electronic parameters proved to be particularly difficult as all of these factors are interrelated, and a compromise had to be made between optical resolution and electronic noise in relation to sample size. Satisfactory performance was obtained with 0.10-mm entrance and exit slits, giving a spectral band width of 0.13 nm. The intermediate slit was sufficiently wide to allow both the $^{14}N^{14}N$ and $^{14}N^{15}N$ bands to be transmitted. The nitrogen supply was used only for setting up the optical system.

Electronics system

The electronics system was assembled from commercially available units with a minimum amount of modification. A Microtron 200 Mk III [Electro-Medical Supplies (Greenham) Ltd.] microwave generator is used with about 80% depth of modulation at approximately 1 kHz to excite the discharge. To avoid troublesome earth-loop problems, the screen on the modulation lead from the case is disconnected, and the $3\lambda/4$ cavity (Electro-Medical Supplies, Type 215L) insulated from the monochromator chassis. A pair of EMI 9789 QB photomultipliers (EMI, Hayes, Middlesex) are fitted into Rank-Hilger mounts and the electronics altered for a.c. operation with the cathode earthed, supplied by an EMI Type PM 25A EHT unit. The measuring system consists of a pair of precision amplifiers (Brookdeal Electronics, Model 9452), and a pair of phase-sensitive detectors (Brookdeal Electronics, Model 9411) supplying the two input channels of a ratiometer (Brookdeal Electronics, Model 9547). The A lead (signal beam, see Fig. 1) is connected directly to the ratiometer whilst the B lead (reference beam) is connected through a potentiometer circuit (not shown), which serves as a fine control for balancing the two signals. An adjustable signal is taken off this circuit to operate a 1-mV chart recorder by means of which the approximate nitrogen concentration of the sample is monitored. A specially designed electro-mechanical peak detector built into the recorder actuates the printer at the peak maximum.[13]

The output of the ratiometer supplies the input to the printer (Anadex DP 501) via a calibration box (providing variable scale expansion and back-off) and a digital voltmeter (Fenlow Type 701-BCD). This allows the output to be printed as either millivolts or atom per cent. nitrogen-15 with appropriate adjustments for the slope and intercept of the straight-line calibration relationship.

Temperature control

For satisfactory reproducibility it was found necessary to house the nitrogen-15 analyser in a temperature-controlled room. A number of different components contribute to the temperature sensitivity of the instrument, *e.g.*, wavelength shifts at the monochromator, photometric imbalance due to the differing temperature coefficients of the photomultipliers and temperature drifts of the electronic units. Tests have shown that provided the monochromator temperature is held to ± 0.2 °C, the errors in recorded nitrogen-15 are less than ± 0.01 atom-%. Regular temperature checks and occasional adjustments of the setting of the room thermostat enabled the temperature to be held within these limits without difficulty.

Vacuum and pressure system

The vacuum pump is not required to have a high performance and even a small rotary pump, such as the Edwards ISP 30, needs several feet of 2-mm bore tubing to restrict pumping capacity.

Calibration and Correction for the Background Effect

Even with no nitrogen present, the discharge shows some emission in the 300-nm region. Increasing the helium flow-rate, however, reduces the background emission, possibly owing to a change in pressure in the discharge zone. When the total amount of nitrogen in the sample decreases, it is necessary to increase the amplifier gain or photomultiplier EHT. This leads to an increase in the relative error due to background emission. The signal at 298.3 nm ($^{14}N^{15}N$ band) was backed off by a voltage corresponding to the background

emission. This enables a two-wavelength measurement to provide results that are substantially independent of the nitrogen content.

The theoretical basis for this technique is illustrated in Fig. 2. In the diagram the heights of the $^{14}N^{14}N$ and $^{14}N^{15}N$ bands relative to the signal with an opaque shutter are represented by x and y, respectively. The corresponding background levels are a and b. The true intensity ratio (R) is given by $R = (x - a)/(y - b)$ and hence $x = Ry - Rb + a$. The signal ratio (R') observed during normal signal measurement is given by

$$R' = \frac{x}{y} = R - \frac{Rb}{y} + \frac{a}{y} = R - \frac{(Rb - a)}{y}$$

and for R' to approach the true value R, the condition to make uncorrected background errors negligible is therefore that $y \gg (Rb - a)$. This does not necessarily hold at low nitrogen-15 enrichments when R is fairly large, hence the need to make a background correction by subtracting a constant signal (c, the back-off) from each channel. The measured ratio then becomes $R' = (x - c)/(y - c)$. By making the back-off equal to the background emission (b) at the wavelength of the $^{14}N^{15}N$ peak, which is the smaller signal at low nitrogen-15 enrichments, we minimise errors, and $R' = (x - b)/(y - b)$. At low nitrogen-15 enrichments $b \ll x$ and $b \approx a$, so that $R' = (x - a)/(y - b) = R$. In a practical test it was found that signals a and b were 0.6 and 0.5%, respectively, of the signal x for a natural abundance sample. At very high nitrogen-15 abundance, *i.e.*, if $y \gg x$, it may be more accurate to put $c = a$, but this is seldom necessary in practice.

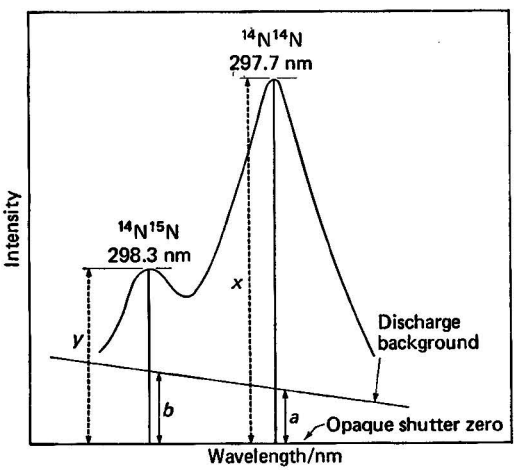

Fig. 2. Theoretical basis of correction for background effect.

For normal operation, a 5-μl sample of an ammonium chloride solution containing 10 μg of nitrogen is injected into the helium stream and the EHT adjusted so that the nitrogen peak on the recorder reaches about 70% of full-scale deflection. Tests have shown that using the zero off-set procedure, errors in nitrogen-15 atom per cent. for the low enrichment samples are within ± 0.01 when the peak height is within the range 60–90% deflection. Adjustment of the volume injected from the 10-μl syringe enabled sample concentrations within the range 1–4 $\mu g\ \mu l^{-1}$ to be accommodated. Samples containing as little as 2 μg of nitrogen may be measured if the photomultiplier EHT is increased and the instrument calibration is set up at this level, but the error then increases to about 0.1 atom-%.

The calibration procedure is carried out using a known low nitrogen-15 abundance sample (usually natural abundance) and one other of known enrichment chosen in relation to the enrichment of the samples of interest. For normal use, the instrument is first conditioned

by injecting 10 samples of ammonium chloride solution each containing 10 μg of nitrogen in 5 μl. After checking EHT, zeros and balance, the low nitrogen-15 sample is injected and the intercept (back-off) potentiometer adjusted to show the correct reading. The high nitrogen-15 sample is then injected and the slope (variable scale expansion) potentiometer adjusted to give a correct reading. These two potentiometers are housed in the calibration box (Fig. 1). When the upper set point is fixed (usually at 1.88 atom-%) and the lower at natural abundance (0.365 atom-%), readings between 0.365 and 2 atom-% are correct to $\pm$0.01 atom-% nitrogen-15. As the true relationship between millivolts and nitrogen-15 atom per cent. is curvilinear, print-out values for samples above 2 atom-% can be taken as approximate, and a first-order correction made using an error curve. More accurate results for such samples can be obtained by running the samples with an appropriate series of standards and carrying out a regression analysis on the printed nitrogen-15 readings. Alternatively, if a number of samples to be run are expected to have high enrichments, the instrument may be calibrated with two standard nitrogen-15 samples with values on either side of the mean expected, using the lower enrichment to set the intercept. The results can then be taken directly from the print-out.

Repeatability and accuracy

The results of measurements of isotopic enrichments in three standard [^{15}N] ammonium chloride solutions are shown in Table I. Six samples from each solution were measured in

TABLE I

REPEATABILITY OF MEASUREMENTS OF STANDARD [^{15}N]AMMONIUM CHLORIDE SAMPLES WITH THE NIRD AUTOMATIC NITROGEN-15 ANALYSER

Enrichment of standard used for calibration,* atom-% nitrogen-15	Measured nitrogen-15, atom-%					
	NIRD automatic			Manual emission spectrometer		
	Values	Mean	S.D.†	Values	Mean	S.D.†
0.37	0.364	0.374	0.009	0.398	0.385	0.023
	0.373			0.393		
	0.386			0.352		
	0.379			0.395		
	0.378			0.411		
	0.361			0.360		
1.44	1.430	1.430	0.006	1.468	1.421	0.029
	1.441			1.433		
	1.427			1.430		
	1.432			1.400		
	1.424			1.389		
	1.424			1.404		
2.54	2.536	2.542	0.009	2.556	2.553	0.036
	2.541			2.566		
	2.545			2.525		
	2.527			2.523		
	2.548			2.618		
	2.554			2.530		

* Supplied by C.Z. Scientific Ltd.
† S.D. = Standard deviation.

the Statron NOI-4 and a further six samples from each of the same solutions were measured in the NIRD automatic nitrogen-15 analyser. These results show that the automatic analyser gave acceptable values for the standards (within 0.01 atom-%) and also indicate a higher reproducibility using the automatic method. The accuracy of the NIRD automatic nitrogen-15 analyser is illustrated in Table II, which shows the results of measurements of nitrogen-15 in samples of the duodenal contents of a steer that had been given a feed containing [^{15}N]urea. In each instance two samples of the biological material were digested

TABLE II

ACCURACY OF MEASUREMENT OF NITROGEN-15 IN BIOLOGICAL SAMPLES

The nitrogen-15 enrichment of the same ammonium chloride solutions prepared from various samples of duodenal contents and rumen bacteria from a steer were measured both in the NIRD automatic analyser and in the Statron NOI-4 emission spectrometer.

Sample	Sample No.	NIRD automatic		Manual emission spectrometer	
		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2
Duodenal contents	1	0.59	0.58	0.59	0.55
	2	0.79	0.81	0.79	0.76
	3	1.11	1.12	1.10	1.10
	4	1.40	1.38	1.36	1.37
	5	1.67	1.67	1.73	1.76
	6	1.51	1.52	1.52	1.55
Rumen bacteria, C375/1 ..	1	0.37	—	0.39	—
	2	0.93	—	0.92	—
	3	1.81	—	1.83	—
	4	2.06	—	2.06	—
	5	1.93	—	1.94	—
	6	1.69	—	1.68	—

and a sample of the ammonium chloride prepared from each was measured, and also measured again using the manual emission spectrometer. Results from the two instruments agreed well, the automatic analyser showing less variation between duplicates than the manual analyser. The standard deviation obtained when 10 measurements were made with the NIRD analyser on a sample with a mean nitrogen-15 enrichment of 0.608 atom-% was 0.006 atom-%, similar to that obtained with standard ammonium chloride solutions.

Memory effect

The memory effect with the NIRD automatic analyser is very small and for practical purposes can be ignored. To test the effect four natural abundance samples, injected at 1-min intervals, were followed by four samples of exceptionally high nitrogen-15 abundance (19.5 atom-%) and these were followed by five samples of natural abundance. The results (Table III) show that even under these extreme conditions an increase in the natural abundance sample immediately following the highly enriched sample was only just detected.

TABLE III

TEST OF THE MEMORY EFFECT IN NIRD AUTOMATIC NITROGEN-15 ANALYSER

Four natural abundance samples of ammonium chloride were injected at 1-min intervals and immediately followed by four samples containing 19.5 atom-% nitrogen-15 and a further five samples of natural abundance, at the same rate. Each sample contained 10 μg of nitrogen.

Sample No.	Nominal atom-% ^{15}N	Print-out value atom-% ^{15}N
1	0.365	0.365
2	0.365	0.359
3	0.365	0.364
4	0.365	0.360
5	19.5	19.519
6	19.5	19.513
7	19.5	19.413
8	19.5	19.514
9	0.365	0.407
10	0.365	0.372
11	0.365	0.362
12	0.365	0.362
13	0.365	0.364

Discussion

The most important feature of the NIRD automatic nitrogen-15 analyser is the high rate of analysis that is possible (one sample per minute) compared with manually operated optical emission spectrometers. This can be achieved without sacrificing the high chemical sensitivity obtainable manually, and with at least as high accuracy ($\pm$0.01 atom-% in the normal operating range). It is also simple to operate, completely dispensing with time-consuming and tedious vacuum techniques.

The essential development that has enabled this high throughout is the use of a rhodium - platinum catalyst for the generation of pure nitrogen. At the time when this work was started the use of such a catalyst in nitrogen-15 analysis had not been reported. Subsequently, however, a report of the use of a rhenium-filament catalyst for the batchwise conversion of ammonia to nitrogen for the mass-spectrometric determination of ^{14}N to ^{15}N ratios has appeared.[14] Under the conditions maintained in the analyser this method does not suffer significantly from interfering side-reactions that hinder the use of traditional methods such as that of Dumas, using copper - copper(II) oxide, or that of Rittenberg, using alkaline hypobromite solution. The latter method has been used in an East German automatic nitrogen-15 analyser (Statron Isonitromat), which was announced after the work reported here was started, and the procedures required to correct for the memory effect in this instrument result in a slower rate of analysis (18 samples per hour). It is quoted to have a similar reproducibility ($\pm$0.01 atom-%) to that of the NIRD analyser. However, in the Statron system, measurements are made at three different wavelengths, enabling an automatic correction to be made for background emission. The use of triple wavelength measurement in conjunction with the NIRD nitrogen preparation system might reduce background drift. This drift is probably caused by changes in the background level due to emission from residual water molecules. As the effective length of the molecular sieve column becomes reduced, owing to saturation with water molecules, the drift tends to increase towards the end of a run. Similarly, a further improvement in stability with a consequent extension of the limits of accuracy below $\pm$0.01 atom-% might be achieved by replacement of the double photomultiplier measurement system with a single photomultiplier and suitably modified electronics to allow simultaneous measurements at three wavelengths. Within the limits used, however, the gradual downward drift observed in calibration is not a serious problem, as it shows up as a change in intercept and, to a first approximation, affects all readings equally. Correction can be readily made either by adjustment of the intercept potentiometer or by analysing a natural abundance sample at intervals throughout a run and applying the correction to the printed results.

Although mass spectrometry remains the most accurate means of measuring nitrogen-15, with a reproducibility of $\pm$0.001 atom-% possible in modern apparatus, automatic optical emission spectrometry offers a number of advantages. Not least among these are the ease of operation and the much higher rate of analysis possible over extended periods. The very small amount of nitrogen required (10 μg), compared with the mass spectrometer (200–2000 μg for highest accuracy), permits measurement of nitrogen-15 in nitrogen metabolites obtainable only in small amounts.

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Studies in Gas Chromatography - Chemical Ionisation Mass Spectrometry of Some Silicate Anions

J. B. Addison

Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada, B3H 3Z1

Chemical ionisation mass spectrometry of trimethylsilyl derivatives of the anions SiO_4^{4-}, $Si_2O_7^{6-}$, $Si_3O_9^{6-}$ and $Si_3O_{10}^{8-}$ has been studied using isobutane as the reagent gas. The protonated molecular ion was observed in all recorded spectra. Also, the chemical ionisation spectra showed many fewer fragment peaks when compared with the corresponding electron impact spectra.

Keywords: Chemical ionisation mass spectrometry; silicate anions

A method for the gas-chromatographic separation of silicate anions was first described by Lentz,[1,2] and involved the formation of volatile trimethylsilyl (TMS) derivatives of the silicate anions. This TMS method was improved upon by Wu *et al.*[3] in order to obtain total separation of SiO_4^{4-}, $Si_2O_7^{6-}$ and $Si_3O_9^{6-}$ anions and partial separation of higher anions such as $Si_3O_{10}^{8-}$ and $Si_4O_{12}^{8-}$.

High-resolution mass spectrometry has been used to identify the chromatographically separated TMS derivatives,[3] but the molecular ion was either of very low abundance or not detected. This work reports the chemical ionisation mass spectrometric analysis of the TMS derivatives of silicate anions to yield protonated molecular ions in very high abundance and to facilitate easy identification of such anions.

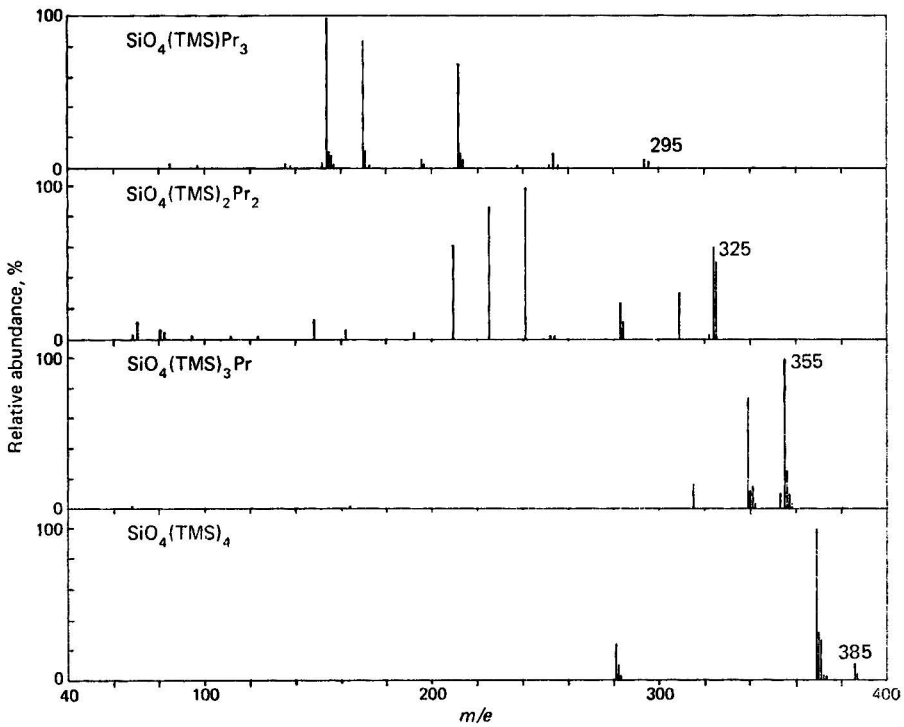

Fig 1. CI mass spectra of TMS derivatives of SiO_4^{4-} anions.

Experimental

The gas chromatographic - mass spectrometric analyses were performed on a Finnigan 4000 mass spectrometer, interfaced via an all-glass jet separator to a Finnigan 9610 gas chromatograph. Chromatographic separation was made on a 12 ft × ⅛ in stainless-steel column, packed with ultraphase 3% SE-30 on 80–100-mesh Gas-Chrom W. The gas-chromatographic conditions were as follows: column temperature, programmed from 60 to 240 °C at the rate of 6 °C min^{-1}; injection port temperature, 200 °C; helium flow-rate, 20 ml min^{-1}; and the separator and transfer line temperatures, maintained at 280 and 230° C, respectively.

The mass spectrometer parameters were: electron energy, 70 eV; ion source temperature, 270 °C; source pressure, 8 × 10^{-5} Torr; emission current, 0.35 mA; and scan speed, 3 s per scan. Automatic repetitive scanning was applied in the recording of mass spectrometer data and a Finnigan Incos data system was subsequently used to process the data.

Isobutane was used as the reagent gas for gas chromatography - chemical ionisation mass spectrometry and was used as a make-up gas with helium as the carrier gas; the isobutane source pressure used was 0.45 Torr. An enhancement technique[4] that performs an automatic background subtraction was applied to all chemical ionisation (CI) spectra reported. Perfluorokerosene was the calibration substance on which the mass assignments were based. For the detailed preparation of the TMS derivatives, see reference 3.

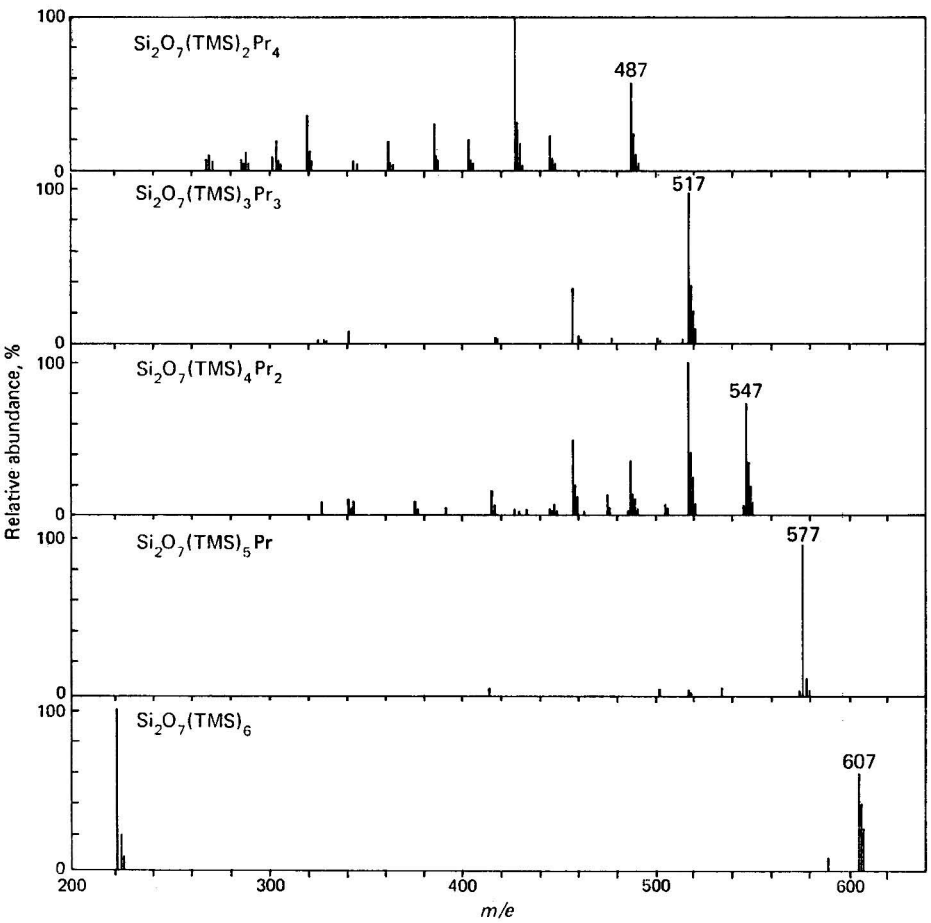

Fig. 2. CI mass spectra of TMS derivatives of $Si_2O_7^{6-}$ anions.

Results and Discussion

Figs. 1–4 illustrate the CI and electron impact (EI) mass spectra of the derivatives that were clearly separated by gas chromatography. Fig. 5 shows a typical total ion current

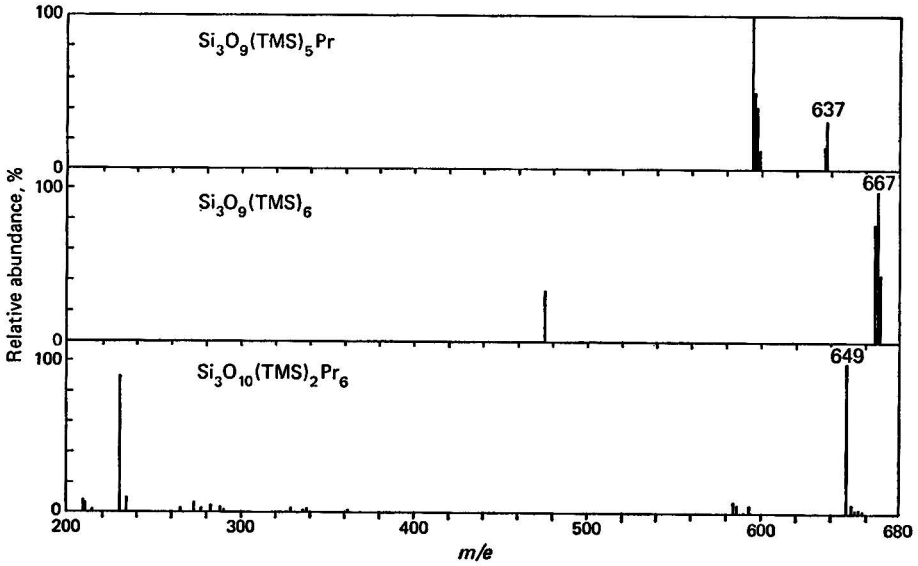

Fig. 3. CI mass spectra of TMS derivatives of $Si_3O_9^{6-}$ and $Si_3O_{10}^{6-}$ anions.

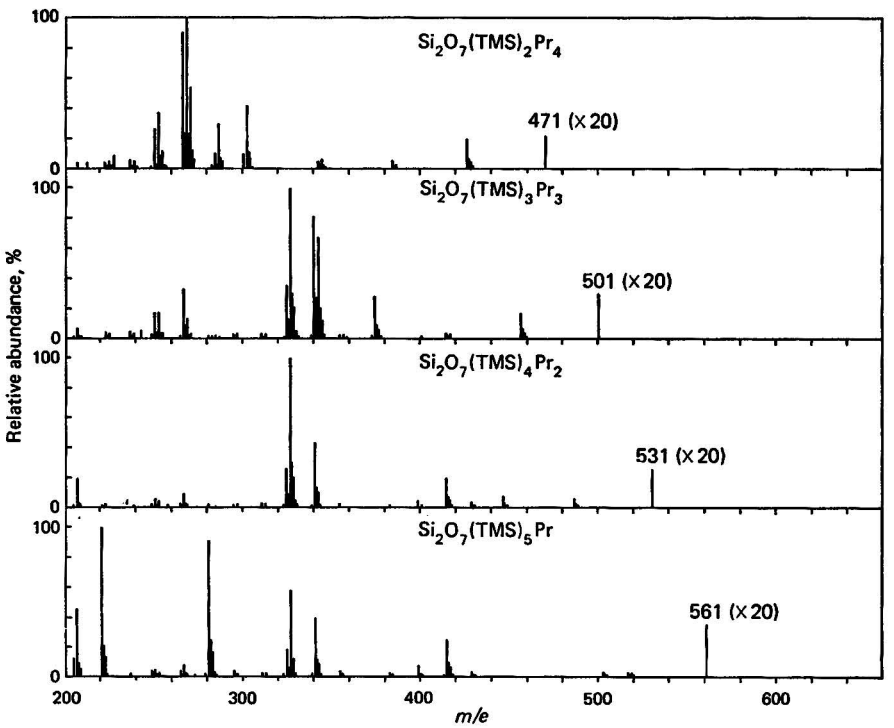

Fig. 4. EI mass spectra of TMS derivatives of $Si_2O_7^{6-}$ anions.

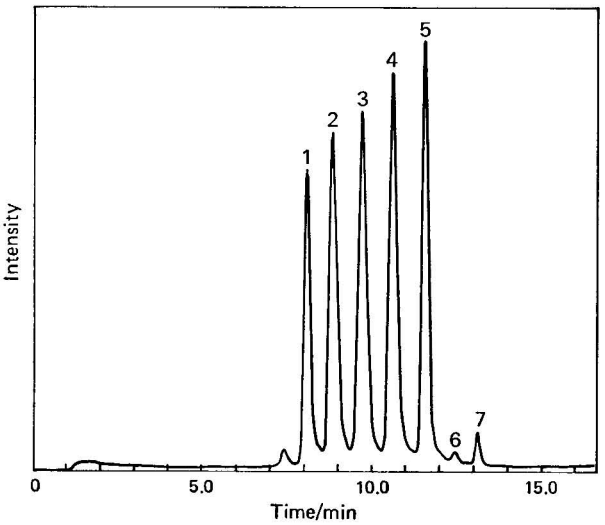

Fig. 5. Typical total ion current chromatogram of TMS derivatives of silicate anions. Peaks: 1, $Si_3O_7(TMS)_3Pr_4$; 2, $Si_2O_7(TMS)_3Pr_3$; 3, $Si_2O_7(TMS)_4Pr_2$; 4, $Si_2O_7(TMS)_5Pr$; 5, $Si_2O_7(TMS)_6$; 6, $Si_3O_9(TMS)_5Pr$; and 7, $Si_3O_9(TMS)_6$.

chromatogram, which is a plot of the accumulated ion current from the mass spectrometer source of the gas chromatograph effluent *versus* time.

The $[M + 1]^+$ ions, the protonated molecular ions, are present in all CI spectra. Comparisons of CI and EI spectra indicate that CI spectra have fewer fragment peaks, higher relative abundances and contain sufficient information for the identification of silicate anions present.

Table I lists the compounds identified by mass spectrometry and the relative abundances of the protonated molecular ions. The CI spectra show an enhancement of abundances of ions in the molecular ion region; no molecular ions were present in the EI spectra for identification, instead the $[M - 15]^+$ ions seem to be the major structurally significant ions for identification, although they display low abundances. The EI spectra also show the usual characteristic peaks obtained by Butt and Rainey[5] using gas chromatography - mass spectrometry, *i.e.*, *m/e* 73 $[Si(CH_3)_3]^+$, 75, 147, 207 and the $[M - 15]^+$ peak. The reported spectra represent the mass range from 200 to 680 AMU.

The $[M - 15]^+$ ions were due to the loss of a methyl radical from the TMS derivatives of

TABLE I

COMPARISON OF RELATIVE INTENSITIES OF $[M + 1]^+$ AND $[M - 15]^+$ IONS IN CI AND EI SPECTRA, RESPECTIVELY

Compound	Relative intensity, %		*m/e*, $[M + 1]^+$
	CI, $[M + 1]^+$	EI, $[M - 15]^+$	
$SiO_4(TMS)Pr_3$	4.25	22.78	295
$SiO_4(TMS)_2Pr_2$	53.13	13.73	325
$SiO_4(TMS)_3Pr$	100.00	4.17	355
$SiO_4(TMS)_4$	11.78	1.48	385
$Si_2O_7(TMS)_2Pr_4$ ..	58.70	1.11	487
$Si_2O_7(TMS)_3Pr_3$ ..	100.00	1.51	517
$Si_2O_7(TMS)_4Pr_2$ ..	73.00	1.27	547
$Si_2O_7(TMS)_5Pr$	100.00	1.76	577
$Si_2O_7(TMS)_6$	64.00	1.57	607
$Si_3O_9(TMS)_5Pr$	32.26	0.99	637
$Si_3O_9(TMS)_6$	100.00	2.08	667
$Si_3O_{10}(TMS)_2Pr_6$ ..	100.00	—	649

the corresponding silicate anions, as a result of direct electron-impact ionisation. On the other hand, the more gentle ionisation of these derivatives yielded the protonated molecular ions; for example, CI involved electron impact of the isobutane reagent gas, resulting in the ionisation of the reagent gas and followed by transfer of a proton to a silicate anion.

All of the TMS derivatives studied, except $SiO_4(TMS)Pr_3$, gave intense $[M + 1]^+$ peaks. The reagent used in this work was a good Brönsted acid with respect to the above silicate anions, and transfer of a proton to the anions was very efficient. The low relative abundance of the $[M + 1]^+$ ion for the $SiO_4(TMS)Pr_3$ anion may be due to the greater difference in proton affinities between this anion and the reagent gas, with the result that there is a transfer of a greater amount of energy to this anion from the reagent gas, followed by a high degree of fragmentation of the anion and less efficient protonation. The high degree of fragmentation is supported by the high relative abundance of the $[M - 15]^+$ ion present in the EI spectrum of this particular anion.

It is worth mentioning that apart from the formation of completely derivatised TMS products, incomplete TMS derivatisation yielded tetramethylsilylpropyl $[(TMS)_xPr_y]^+$ products. These products are formed by the replacement of $SiMe_3$ groups with propyl (Pr) groups during derivatisation in propan-2-ol and have been reported elsewhere.[6–8] The CI mass spectra of these $[(TMS)_xPr_y]^+$ anions also show a very high relative abundance of the protonated molecular ion $[M + 1]^+$ and identification is fairly straightforward.

Conclusion

Isobutane chemical ionisation mass spectrometry offers a very useful approach to the identification of silicate ions. The production of the protonated molecular ions of very high relative abundance and the formation of fewer fragment peaks with enough chemical formation for characterisation make this CI technique far superior to the EI technique, which gives the $[M - 15]^+$ ion as the most significant peak for identification and no molecular ion.

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Internal Standardisation and its Value in the Assessment of the Suitability of the Column for Quantitative High-performance Liquid Chromatography

R. A. Moore

Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey, GU20 6PH

The requirement that the best precision be obtained for a pharmaceutical raw material assay using automated high-performance liquid chromatography, with valve injection, over long runs, suggested that the inclusion of an internal standard would be beneficial in allowing an additional method for the calculation of results. The accurate volume injection of the valve and loop system thus permitted standard calibration and subsequent sample evaluation by the peak-height ratio method as well as from absolute peak heights alone. After a number of experiments, column deterioration rendered the assays invalid, and retrospective analysis of the data demonstrated the merits of internal standard related parameters in showing the decline in performance of the column.

The major benefits derived from the approach adopted were criteria for judging the acceptability of the assay results, without excessive replication for a particular sample, and prior warning of the need to re-pack the column.

Keywords: Quantitative high-performance liquid chromatography; internal standardisation; column assessment; pharmaceutical analysis; automatic injection

In contrast to the development of gas - liquid chromatography (GLC), the more recent technique of high-performance liquid chromatography (HPLC) has grown in an environment where the options for quantitation, via a particular means of detection, are fairly numerous. Sample valves for HPLC enable accurate volumes of liquid to be injected and therefore an internal standard may appear to be of much less value than in GLC procedures. Peak heights or areas can be measured, either manually or by other means such as with an integrator or computer.

In the study described here, to aid the appraisal of quantitative HPLC, using an automatic injector with a sample valve, for the routine assay of high-purity samples (>95%) of a pharmaceutical raw material, an internal standard was also included to allow an alternative method of calculation, and manual peak-height measurements were made. The column had previously been used in several similar applications.

The method resolved the drug compound of interest, an aromatic piperazine derivative with only tertiary nitrogen atoms, from potential process impurities; in the absence of a suitable related compound phenyl 1-adamantyl ketone was chosen as the internal standard, eluting in a typically "clean" region of the chromatogram.

Previous reports[1,2] have established the effects of variation of the major factors, namely mobile-phase composition and flow-rate, sample load and temperature, on the precision of HPLC. In the work reported here, temperature was the only factor not routinely controlled, such that a significant effect on the chromatography was to be expected (during a 12-h overnight run a variation from 23 to 12 to 15 °C might be obtained), and frequent standard injections were therefore made. One of the five experiments was also performed under temperature-controlled conditions.

Experimental

Reagents

The water used was previously distilled, and methanol and sodium dihydrogen orthophosphate dihydrate were of AnalaR quality.

Drug standard. The standard used was a specially purified batch characterised at 99.7%

purity (by thin-layer chromatography, differential scanning calorimetry, normalised GLC, normalised HPLC and water and solvent level determinations), and this value was used in assay calculations.

Internal standard solution. A 10 mg ml^{-1} solution of phenyl 1-adamantyl ketone (one peak only by HPLC) in methanol.

Eluent. A 0.05 M solution of sodium dihydrogen orthophosphate dihydrate in methanol - water (70 + 30), de-gassed under vacuum for about 1 min before use.

Apparatus

Solutions were sampled by a microprocessor-operated automatic injector with a motorised sample valve and a 10-μl sample loop (Micromeritics, Model 725). A dual-piston reciprocating pump with a solvent inlet filter (Milton Roy, Constametric II) delivered a constant flow of 1.0 ml min^{-1} (requiring approximately 950 lb in^{-2}) to a 125 $\times$ 4.9 mm i.d. stainless-steel column packed with 5-μm octadecylsilyl-bonded silica (Spherisorb S50DS, Phase Separations Ltd.). The column was prepared by upwards packing from a propan-2-ol slurry using a stirred-slurry column packer (Micromeritics, Model 705) and a pneumatic amplifier pump (Haskel MCP-110) at a pressure of 4500 lb in^{-2}. The column end-fittings included stainless-steel mesh discs of 8-μm pore diameter (two discs at the outlet end) to retain the packing. Detection was effected by ultraviolet absorption using a variable-wavelength detector with a 10-μl flow cell, set at 240 nm and 0.5 absorbance unit full-scale deflection (f.s.d.) (Cecil 212). Chromatograms were recorded at 10 mV f.s.d. (Servoscribe RE 541.20).

Procedure

General procedure

Duplicate sample masses (90 or 45 mg for experiment 1 or 3, respectively) were transferred into calibrated flasks, dissolved in a pipetted amount of internal standard solution and diluted to volume with methanol - water (70 + 30) to give solutions with nominal concentrations of 0.45 mg ml^{-1} of drug and 0.50 mg ml^{-1} of internal standard. Three standard masses, corresponding approximately to the sample mass (m), 1.1 m and 0.9 m, were treated in a similar manner to the samples.

Before each run, repeated injections of a standard solution at 12-min intervals were used to check for system equilibration; during the run standards were injected frequently between samples, triplicate injections being taken from each vial. All experiments were allowed to run overnight, about 12 h being necessary. For each vial the three peak heights of drug and internal standard were measured and the values averaged to give a mean value for both components. The mean peak-height values were subsequently used to construct standard calibrations for both peak height and peak-height ratio, and to calculate sample assays.

Over the course of each experiment a series of the three standard solutions was injected four or five times to allow adequate standardisation of all test solutions. A calibration graph was drawn for each series of standards, resulting in four or five graphs for peak height and an identical number for peak-height ratio. Sample assays were calculated by reference to the relevant graphs for peak height and peak-height ratio.

Experiment 1

Duplicate masses from each of four sample batches gave eight test solutions, T1 to T8, which were arranged with the three standard solutions (STD1 to STD3) in the following injection sequence:

STD1, STD2, STD3, T1, T2, STD1, STD2, STD3, T3, T4, STD1, etc.

Series 1 Series 2

Assays were evaluated based on weighted averages from the surrounding calibration graphs, *e.g.*, the mean peak-height value for T1 was converted into an active content from the peak-height calibrations from both the Series 1 and Series 2 standards.

The correct intermediate value was then calculated on the assumption that the peak height for each standard solution had changed linearly with time over the period including

both series of standards. The weighted value was used for calculation of the T1 assay via peak height. A similar procedure was then followed to obtain the peak-height ratio assay. However, the time difference between the first injection of STD 1 in Series 1 and the third injection of STD 3 in Series 2 was nearly 5 h and a significant drift resulted between calibration graphs; therefore, an alternative arrangement of standard and test solutions was considered to be desirable in successive experiments.

Experiment 2

The solutions from experiment 1 were re-run with a new vial sequence:

Series 2 Series 4

STD1, T1, STD2, T2, STD3, T3, STD2, T4, STD1, T5, STD2, T6, STD3, T7, STD2, T8, STD1

Series 1 Series 3

Assays were calculated using the particular standard series bracketing the sample, *e.g.*, the active contents of T2 and T3 were read from Series 1 and Series 2 standard calibration graphs, respectively.

Experiment 3

One of the previously used sample batches and three new batches were assayed, duplicate masses being taken through the procedure as in experiment 2, except that the chromatography was performed in a room controlled at 25 ± 1 °C. Fresh standard solutions were also prepared.

Experiment 4

The solutions from experiment 3 were re-injected without control of the laboratory temperature.

Experiment 5

After re-packing the front end of the column, the procedure in experiment 4 was repeated.

Results and Discussion

In each experiment, for each sample batch, the mean assay result and standard error of the mean (S.E.M.) were calculated from the four constituent assay values (peak height and peak-height ratio methods of calculation on the duplicate sample masses). The results are given in Table I.

TABLE I

MEAN SAMPLE ASSAYS AND S.E.M. (%)

Sample batch	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
A	97.6 (±0.2)	97.0 (±0.3)	—	—	—
B	98.7 (±0.4)	97.9 (±0.5)	—	—	—
C	97.9 (±0.4)	96.3 (±0.2)	—	—	—
D*	98.3 (±0.5)	96.9 (±0.5)	98.6 (±0.8)	100.8 (±1.4)	98.4 (±0.4)
E	—	—	96.2 (±0.5)	94.7 (±0.8)	96.8 (±0.9)
F	—	—	97.0 (±0.7)	96.9 (±0.9)	96.0 (±0.1)
G	—	—	97.4 (±0.8)	96.2 (±1.1)	96.0 (1 soln. only)

* Sample D is re-mixed sample B.

Close inspection of the chromatograms revealed a decline in resolution of the two peaks from almost base-line separation in experiment 1 to the situation existing in experiment 4,

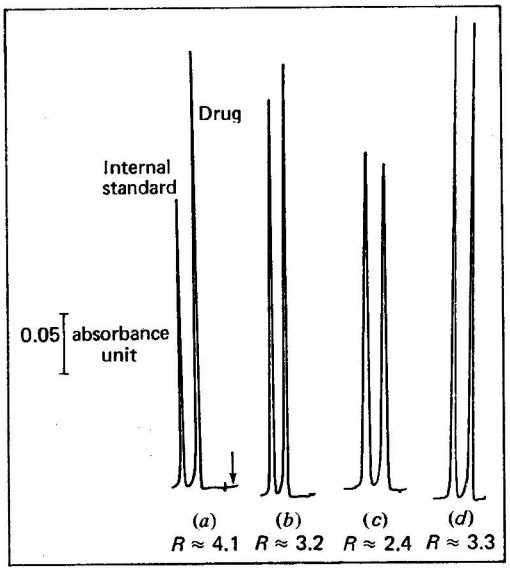

Fig. 1. Typical chromatograms obtained in (*a*) experiment 1, (*b*) experiment 3, (*c*) experiment 4 (equilibration injection) and (*d*) experiment 4 (end of run).

where the first few equilibration injections resulted in distorted, split or shouldering peaks and later equilibration injections yielded "normal" peaks with increased inter-peak valley heights above the base line. Fig. 1 illustrates some typical chromatograms obtained during the lifetime of the column, together with the resolution values (R) for the two peaks.

At the end of experiment 4, removal of the column front-end fitting revealed an approximately 1 mm deep depression in the packing, and this was re-filled manually with a fresh, stiff slurry of the packing material. Subsequent equilibration injections showed that the column was again giving good resolution and peak shape and experiment 5 was performed.

The column settling effect, as observed above and previously in this laboratory, is likely to be attributable to one or more of three factors: (i) poor initial packing; (ii) chemical attack on the bonded phase or the silica matrix of the packing; and (iii) expulsion of packing from the column or fracture of the particles, or a combined effect.

A previous exercise to compare the packing of reversed-phase columns from propan-2-ol or aqueous - methanolic sodium acetate slurries produced columns of similar efficiency and with similar settling rates in use; the latter method was recommended by the packing manufacturer to overcome electrostatic attraction between particles to aid dispersion. In the work described here, the pressure used to pack the column was far greater than that used in assay work. Consequently, the first factor above was not considered to contribute towards the settling.

Chemical attack should be considered unlikely with an eluent of pH approximately 5.4, although other workers have reported settling of reversed-phase columns with phosphate buffers.

According to the supplier's instructions, two 8 μm mesh discs were used for retention of the packing, but may have allowed gradual loss of particles, especially any "fines," and the third factor is expected to yield the major contribution to column settling.

In a current investigation, 2 μm mesh discs are being used for retention of the column packing in place of 8 μm mesh discs to compare column lifetimes. Comparative results should shed light on the processes responsible for column settling, including any contribution from the second factor mentioned above.

Table II lists the parameters that best illustrate the over-all quality of the chromatography.

Mean range of triplicates

The values should show little temperature effect, being dependent on measurements made over only a 0.5-h period. The results reflect the reproducibility of volume delivery of the automatic injector as well as chromatographic variation. The peak-height measurements for the drug show that very good precision is obtainable; the corresponding precision for the internal standard was not usually as good, as can be seen from the peak-height ratio values.

In experiment 4, where the quality of the chromatography was low, agreement between triplicate results for peak-height measurements was poor. The less precise peak-height results for triplicate determinations in experiment 5, compared with that obtained initially, is attributed to decreasing performance of the automatic injector, as a short time later the sample valve had to be freed of a blockage.

TABLE II

PARAMETERS DEPENDENT ON CHROMATOGRAPHIC PERFORMANCE

PH = peak height; PR = peak-height ratio.

Expt. No.	Mean range of triplicates (%)		Range of standard calibrations, %*		Mean difference (MD)† between methods	Mean bias (MB)‡ between methods
	PH_{drug}	PR	PH_{drug}	PR		
1	0.6	1.5	3.6	1.5	0.8	−0.4
2	1.0	1.8	3.5	2.0	1.0	+0.05
3	0.8	0.8	1.2	3.3	2.1	−0.3
4	3.3	3.8	2.9	7.0	2.5	+1.1
5	2.3	2.3	4.5	1.4	0.3	−0.1

* Range of values measured at standard mass *m*.

† $MD = \frac{\Sigma|\text{PH assay} - \text{PR assay}|}{\text{number of solutions}}$, *i.e.*, ignoring signs.

‡ $MB = \frac{\Sigma\text{PH assay} - \text{PR assay}}{\text{number of solutions}}$, *i.e.*, +ve or −ve.

Range of standard calibrations

Good straight-line calibration graphs were obtained, peak-height ratio graphs giving better fits to the points than peak-height graphs. The peak-height graphs displayed an irregular sequence with time, whereas those for peak-height ratio were in regular ascending or descending order. Thus, while peak-height values must be affected by fluctuating temperature, which would determine the actual masses of solutes being injected, peak-height ratio values for two adjacent resolved peaks should be much less temperature dependent. The range of peak-height values therefore illustrates "chromatographic drift" and "temperature drift" over the duration of an experiment, while the range of peak-height ratio values reflects largely a "chromatographic drift" factor that increases as the column deteriorates. It is therefore seen that:

(i) In experiment 3 control of the temperature results in a 1.2% peak-height range compared with 3% and above for the other experiments, while the peak-height ratio range continues the increasing trend shown from experiments 1 and 2.

(ii) In experiment 4, where the peak-height range is comparable to those of experiments 1 and 2, the peak-height ratio range is in keeping with the degraded state of the column.

(iii) Renewal of the column front end yields a peak-height range again of the expected order, with a low peak-height ratio range typical of a stable column.

Mean difference (MD) and mean bias (MB)

These terms are determined solely from assay differences between the two methods of calculation on a particular solution (either ignoring or using positive and negative signs), and are therefore virtually independent of sample and standard weighing errors. For a particular experiment these terms will provide equivalent information on the chromatography irrespective of the combination of samples and batches used.

The two parameters in combination readily demonstrate the acceptability of results from a given experiment.

Thus, the MD for experiment 3 suggests a wider assay variation than is desirable, although

no strong bias between methods is shown. Further column deterioration in experiment 4 leads to unacceptably high values for MD and MB, correlating with the unreliable nature of the assays.

Examination of assay S.E.M. values for each experiment (Table I) reveals the same trend as with MD values, although the former will also include variation due to weighing operations.

It is useful to note that column efficiency measurements, with respect to the two components of interest, are of limited worth in the evaluation of the column for quantitation purposes. The column efficiencies (plate numbers), calculated from peak width at half-height measurements, are given in Table III. A gradual decline in column performance was found between experiments, but only in experiment 4 was a significant change shown during the run, allowing for an estimated measurement error of $\pm 10\%$.

TABLE III

COLUMN EFFICIENCIES

Expt. No.		Plate number: Drug	Plate number: Internal standard
1		1600	4000
2		1400	3300
3		1400	2800
4	(a) First equilibration injections	<1000	<1000
	(b) Start of run proper	1200	1900
	(c) End of run	1400	2600
5		1400	3200

The efficiency for the internal standard is much more sensitive to chromatographic changes than for the drug and approximately 3000 plate numbers for the internal standard might appear to be a criterion for acceptability of the column for quantitative analysis. By this method of assessment the column is of marginal acceptability for quantitation purposes in experiment 3, a little better for experiments 2 and 5 and much better for experiment 1. However, the previously described methods of evaluation demonstrate the significantly better chromatographic stability and precision of results in experiments 2 and 5, compared with experiment 3, and even suggest superior stability for experiment 5 compared with experiment 1. Column efficiency for the internal standard is therefore an insensitive gauge for the measurement of the suitability of columns for quantitation purposes.

Excluding the results from experiment 4, which are shown to be invalid, over-all sample assays with corresponding values via peak height and peak-height ratio are given in Table IV.

TABLE IV

OVER-ALL MEAN SAMPLE ASSAYS AND S.E.M. (%)

PH = peak height; PR = peak-height ratio.

Sample batch	Grand mean	Mean via PH	Mean via PR
A	97.3 (±0.2)	97.2 (±0.2)	97.3 (±0.4)
B	98.3 (±0.3)	98.7 (±0.4)	97.8 (±0.5)
C	97.1 (±0.3)	96.8 (±0.5)	97.4 (±0.5)
D*	98.1 (±0.3)	97.5 (±0.4)	98.6 (±0.4)
E	96.5 (±0.5)	96.0 (±0.6)	96.9 (±0.7)
F	96.5 (±0.4)	96.6 (±0.7)	96.4 (±0.4)
G	96.9 (±0.6)	97.7 (±0.8)	96.1 (±0.8)

* Sample D is re-mixed sample B.

Application of Snedecor's *F*-test and Student's *t*-test shows that for each sample the individual peak height and peak-height ratio assays belong to the same population. In each instance the grand mean assays are within 1% of the sample purity predicted by the other techniques used to characterise the standard.

Chromatographic precision was also assessed from repeated equilibration injections of a standard solution, yielding typical coefficients of variation of peak height and peak-height ratio of 1.4% and 0.6%, respectively, from six injections in experiment 2, and 0.4% and 0.4% from nine injections in the temperature-controlled experiment 3. These results compare favourably with literature values.[1,3]

With the exception of one sample (D), all assay results were obtained from only two weighings per sample, and therefore any statistical treatment of the results must be viewed with caution. However, the results obtained suggest that in a simple analytical procedure in which the error is mainly chromatographic, a high precision of results should be readily achieved in a single run with a good column, and adequate control as above using three or four weighings per sample; the use of an internal standard permits the assessment of the column and evaluation of the chromatographic variation of assays.

Complete automation of the procedure, including measurement and calculation, would minimise the tedium associated with the regular standardisation necessary in the absence of adequate temperature control.

Conclusions

An internal standard is not essential for precise quantitation in HPLC, corroborating the findings of other workers.[3,4] When the greatest accuracy and precision are required, however, and for repetitive assays, particularly over long runs, the inclusion of an internal standard is recommended, in order to allow calculation via both peak-height ratio and peak height alone, because of the major advantages accrued.

Unless strict temperature control is observed, the variation of peak-height values with time for a given solution may be erratic, while the corresponding variation of peak-height ratio measurements gives an estimate of "chromatographic drift." When experimental parameters other than temperature are controlled, the "chromatographic drift" evaluates the column stability, which is the important factor governing the precision of quantitative analyses.

For a routine assay a maximum variation of peak-height ratio with time may be established for acceptable column stability. Likewise, the "mean difference" and "mean bias" factors based on comparative peak height and peak-height ratio assays for each sample solution (preferably eight or more) also assess the acceptability of assay results and give an objective prior warning of the need to re-pack a column. Thus, values of MD up to 1.5% and MB up to 0.5% might be regarded as indicating acceptable assays. Until column packing methodology, materials and equipment are much more standardised, such a means of following column lifetimes should prove of great help in quantitative HPLC.

It is important to monitor the complete duration of an experiment for over-all appraisal of assay precision; calculations based on initial repeated injections of a solution are insufficient, as can be seen in experiment 3 where very precise equilibration injections were observed, although the peak-height ratio range and MD values showed continuing deterioration of the column and borderline acceptability of results. A mean assay difference of about 1.5% between duplicate weighings of the four samples by peak height or peak-height ratio calculation methods also indicated the poor precision.

The application of both peak height and peak-height ratio calculations therefore provides more meaningful information on column behaviour and assay precision than can otherwise be obtained by either technique alone on a given number of samples.

Mr. G. F. Snook is thanked for helpful discussions.

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Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

Determination of Small Amounts of Selenium in Organic Matter

Methods for determining small amounts of selenium in organic matter have been examined. Determination first requires oxidative destruction of organic matter. The usual oxidising systems employing sulphuric acid initially, with hydrogen peroxide, nitric and/or perchloric acids, gave very low recoveries of selenium when high proportions of fats were present in the samples. Continuous combustion and oxygen flask methods were also unsatisfactory. A wet-oxidation procedure in which nitric and perchloric acids (5 + 1) were followed by nitric and sulphuric acids gave satisfactory results. It was important to ensure that selenium was present as selenium(IV) by boiling the solution with hydrochloric acid after the oxidation. Colorimetric, gas - liquid chromatographic, fluorimetric and atomic spectroscopic methods were considered for the selenium determination; of these, the last two were selected for collaborative trials. For the fluorimetric finish, the solution, after oxidation, was treated with 2,3-diaminonaphthalene reagent, the complex was extracted with cyclohexane or dekalin and the fluorescence in the organic phase was measured in a spectrofluorimeter with excitation at 369 nm and emission reading at 525 nm. Atomic-absorption or fluorescence measurements were made after hydrogen selenide generation and atomisation. The precisions of analyses for samples with selenium contents from less than 0.1 to 10 $\mu g\ g^{-1}$ by the fluorimetric and hydride generation methods are illustrated.

Keywords: *Selenium determination*; *wet oxidation*; *fluorimetry*; *atomic spectroscopy*

The Analytical Methods Committee has received and approved for publication the following Report from its Metallic Impurities in Organic Matter Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this report was: Dr. L. E. Coles (Chairman until 1978), Mr. C. A. Watson (Chairman from 1978), Mr. W. Cassidy, Mr. P. N. Coleman (Sub-Committee Secretary from 1977), Mr. R. E. Collier, Dr. W. H. Evans, Mr. M. T. Friend (from 1978), Mr. S. Greenfield, Mr. W. H. Hill, Mr. B. E. Pearce, Mr. W. L. Sheppard (resigned 1977) and Dr. J. M. Skinner, with Mr. P. W. Shallis as Secretary (until 1977) and Dr. N. W. Hanson as Secretary (from 1977).

Introduction

Preliminary investigations by the Sub-Committee indicated that of a number of methods that have been described for the determination of small amounts of selenium, two methods in particular merited further investigation. The fluorimetric method as described by Hall and Gupta[1] appeared to possess sufficient sensitivity, while the numerous methods based on reduction to the hydride followed by its decomposition to selenium atoms and atomic spectroscopic measurements were also thought to cover the range of interest. Before any of these methods of determination could be employed it was necessary to find a method of sample preparation that would remove the organic matrix and leave the selenium in a suitable state for fluorimetric or atomic spectroscopic determination.

Experimental

Sample Preparation

It is usually convenient to wet oxidise organic matter prior to the determination of trace metals and several Sub-Committee members undertook preliminary studies using dried milk as the substrate.

Early results using previously recommended procedures[2] based on sulphuric acid - hydrogen peroxide, sulphuric acid - nitric acid and sulphuric acid - nitric acid - perchloric acid were not encouraging, very low recoveries being obtained in the presence of samples that contained high proportions of fats. The situation was not improved by the substitution of a Bethge apparatus for the Kjeldahl flasks that were originally used. A radiochemical study by one Sub-Committee member demonstrated that the selenium had not been lost from the system, but was present in a state in which it was not determined by the fluorimetric method. Similar results were also obtained by Levesque and Vendette.[3]

TABLE I

DETERMINATION OF SELENIUM AFTER SAMPLE OXIDATION IN THE OXYGEN FLASK

Test sample	Final measurement method*	Se recovery, %†
Milk + 10 p.p.m. of Se (0.1-g sample)	Hydride/AAS	60–80
Condensed milk + 1 p.p.m. of Se (1-g sample)	Automatic hydride/AAS	20–40
10 μg of Se	Hydride/AAS	41–68
10 μg of Se	Fluorimetric	86–95
Wheat + 40 p.p.m. of Se (0.25-g sample)	Hydride/AAS	48–62
Wheat + 40 p.p.m. of Se (0.25-g sample)	Fluorimetric	95
Milk powder + 40 p.p.m. of Se (0.25-g sample)	Hydride/AAS	48–78
Milk powder + 40 p.p.m. of Se (0.25-g sample)	Fluorimetric	96
Milk powder + 20 p.p.m. of Se (0.5-g sample)	Automatic hydride/AAS	75–92
Condensed milk + 1 p.p.m. of Se (0.3-g sample)	Fluorimetric	71

* AAS = atomic-absorption spectrometry.

† In Tables I–III, a single figure is the result obtained by one laboratory. A range represents the extreme results obtained by the collaborating laboratories.

One of the collaborating laboratories investigated the use of a continuous combustion apparatus, but the results were still unsatisfactory. Three laboratories investigated the use of oxygen flask combustion with various samples and the results from these investigations are shown in Table I. As can be seen, these results were encouraging but still erratic and the Sub-Committee was of the opinion that, as no obvious advantage had been demonstrated by these other methods of sample preparation and as they were not so widely available as wet-oxidation techniques, further efforts should be devoted to producing a satisfactory method employing the wet-oxidation technique.

A sample of condensed milk that had been spiked with 1 p.p.m. of selenium was analysed by using a number of sample preparation techniques and an automated hydride generation - atomic-absorption finish. The results of this investigation are reported in Table II.

TABLE II

DETERMINATION OF SELENIUM AFTER VARIOUS METHODS OF SAMPLE OXIDATION

All determinations were carried out on a 1-g condensed milk sample containing 1 p.p.m. of selenium.

Method of oxidation	Se recovery, %
Oxygen flask	20–40
Oxygen bomb	<20
Wet oxidation - hydrogen peroxide/sulphuric acid	<8
Wet oxidation - nitric acid/sulphuric acid	4–16
Wet oxidation - perchloric acid/nitric acid	91–96*
Wet oxidation - perchloric acid/fuming nitric acid	86–91*
Wet oxidation - nitric acid/perchloric acid/sulphuric acid, Kjeldahl flask	75–80
Wet oxidation - nitric acid/perchloric acid/sulphuric acid, block digestion	85–88

* These results are artificially high as the presence of small but variable amounts of nitric acid in the digest causes an enhancement of up to 30%.

Interpretation of Table II requires care, as the results obtained when using perchloric - nitric acid or perchloric - fuming nitric acid are artifically high by about 30% owing to an enhancement effect that occurs with the subsequent selenium determination when some nitric acid remains in the digest. The results obtained when using the nitric - perchloric - sulphuric acid digestion were thought to be sufficiently good to warrant a collaborative trial and a procedure based on that used by the Association of Official Analytical Chemists in Washington,[4,5] with the subsequent modification,[6] was employed. The particular feature of this method, compared with those previously tried, was that sulphuric acid was added only after most of the sample had been oxidised by treatment with nitric and perchloric acids.

The recovery of selenium from condensed milk is reported in Table III; some of the results were obtained after the procedure had been modified to include a stage in which the digest was boiled with hydrochloric acid to ensure that all selenium was present as selenium(IV). This modification was used throughout the subsequent exercise, although examination of the results in Table III fails to show any statistically significant improvement. It should be noted that the hydrochloric acid concentration must not exceed 6 N, as selenium is volatilised as selenium(IV) chloride from boiling concentrated hydrochloric acid solutions.[7] Recent work by Bunton *et al.*[8] has confirmed the importance of the selenium being present as selenium(IV) and they ensured this by treating the digest with hydrogen peroxide after removal of the perchloric acid.

TABLE III

DETERMINATION OF SELENIUM IN CONDENSED MILK SAMPLES USING A PROCEDURE BASED ON THAT GIVEN IN REFERENCES 4–6

All determinations were carried out on a condensed milk sample containing 1 p.p.m. of selenium.

Laboratory	Finish*	Se recovery, %
A	Fluorimetric	75–83
B	Fluorimetric	91–111
C	Hydride/AAS	90–108
D†	Automatic hydride/AAS	80–88
E†	Hydride/AFS	87–98
B†	Fluorimetric	102–110

* AAS = atomic-absorption spectrometry; AFS = atomic-fluorescence spectrometry.

† Digests boiled with 4 N hydrochloric acid to ensure conversion of all selenium into selenium(IV).

Selenium Determination

The Sub-Committee examined several methods for selenium determination, including a colorimetric procedure[9] and a gas - liquid chromatographic (GLC) procedure.[10,11] It became clear, however, that only two techniques would provide the sensitivity required for the analysis of foodstuffs and additives, combined with the required degree of reliability. These were the fluorimetric method described by Hall and Gupta[1] and methods based on reduction to the hydride followed by decomposition to selenium atoms in either a heated tube or a flame and measurement of the atomic absorption or fluorescence produced by suitable irradiation of the atom cloud.

Because of the wide range of sensitivities of fluorimeters and spectrofluorimeters it was not possible to recommend precise conditions for making a given determination, as the calibration range and hence the mass of sample required vary from instrument to instrument. For methods based on hydride generation it was even more difficult to provide a recommended procedure, as not only do the sensitivities of flame spectrometers vary greatly, but the type of atom cloud produced also depends on the design of the reduction vessel, carrier gas flow-rate, flame temperature or tube temperature and the method of sample introduction. Although in the Sub-Committee's experience single-injection atomic-absorption measurements were difficult to perform reliably, the use of instruments with more sophisticated data handling than were available during this work might enable these methods to yield satisfactory results routinely.

One member of the Sub-Committee used an automated procedure based on that proposed by Vijan and Wood,[12] which has the advantage of producing steady rather than transient signals, leading to good detection limits and better precision than could be obtained by the various "single-injection" methods when using a simple atomic-absorption spectrometer. Another member of the Sub-Committee found that the non-atomic absorption signal produced, caused by the large volume of hydrogen and consequent acid carry-over that occurred owing to the high acidity necessary to reduce selenium successfully, was difficult to correct for and he obtained better results by the use of atomic fluorescence. The hydride was decomposed in an argon - hydrogen diffusion flame with irradiation from an electrodeless discharge lamp operated at 40 W in a Broida-type cavity. The relative detection limits obtained by various Sub-Committee members using a variety of techniques are reported in Table IV and may be useful as a guide when deciding calibration ranges and sample masses.

TABLE IV

RELATIVE DETECTION LIMITS FOR SELENIUM BY VARIOUS METHODS

Laboratory	Method	Detection limit/ng*	Notes†
A	Fluorimetric method with single-beam spectrofluorimeter	10	1
B	Fluorimetric method with single-beam spectrofluorimeter	20	1
C	Fluorimetric method with filter fluorimeter	100	1
C	Hydride generation/AAS in flame-heated silica tube	10000	2
D	Automatic hydride generation/AAS in electrically heated silica tube	2	3
E	Hydride generation/AFS in argon - hydrogen diffusion flame	2	2
F	Fluorimetric method with single-beam spectrofluorimeter	7	1
G	Fluorimetric method with single-beam spectrofluorimeter	30	1

* Detection limits are calculated from the standard deviation of the lowest levels determined by each laboratory and for the purposes of this comparison are defined as twice the standard deviation of these determinations.

† Notes—

1. With fluorimetric methods it is possible to use the whole of the digest, hence for a 1-g sample mass these figures represent the detection limit in parts per billion (10^9).
2. With hydride generation methods the detection limit for a sample depends on the mass of sample that it is possible to take, the final volume of the digest and the proportion of the digest used in the analysis.
3. The automatic method uses a pumped flow of the digest at a rate of 3.9 ml min^{-1}. The detection limit reported is the concentration of this sample solution in ng ml^{-1}.

The procedures recommended in the Appendix are of necessity very general and the analyst must select sample masses and final volumes of digest to suit the measuring system. Using the recommended sample preparation procedures and making measurements by the various methods outlined above, a number of samples of organic material were analysed. The results of these experiments are given in Tables V–X. In each instance the mean is reported, together with the range, although the latter is replaced by the standard deviation where sufficient results are available.

Examination of Tables V–X indicates that similar results are obtained regardless of the method of end determination. In no instance is the mean of the fluorimetric results statistically significantly different from the results obtained by hydride generation. In general, the agreement between laboratories using hydride generation was slightly better than those using the fluorimetric finish, although the number of results is insufficient to allow any conclusions to be drawn regarding the superior precision of either method. However, if Fig. 1, which illustrates the relationship of relative standard deviation to selenium content of the various samples, is examined, it would appear that for levels below 100 ng g^{-1} there may be some advantage in the use of the hydride generation procedures. (The results illustrated in Fig. 1 are based on an inter-laboratory comparison of the data and it must be emphasised that the number of laboratories using each technique was small, particularly for the lower levels of selenium when hydride generation was used.) It is worth noting that although these results were obtained with a wide range of sample matrices, the precision appears to be concentration dependent rather than sample dependent, particularly for the fluorimetric method, where all the results fall close to a single smooth curve. The precision - concentration relationship for the hydride generation method is less well defined than that

TABLE V

DETERMINATION OF SELENIUM IN SELENIUM YEAST

Laboratory	Finish	Selenium content, p.p.m.*
A	Fluorimetric	135 (±20%, 3 results)
B	Fluorimetric	124 (±4%, 3 results)
C	Hydride/AAS	144 (RSD 3%, 6 results)
D	Hydride/AAS	149 (±3%, 3 results)
E	Hydride/AFS†	147 (±6%, 3 results)
	Hydride/AFS‡	137 (±5%, 3 results)
G	Fluorimetric	126 (RSD 10%, 5 results)

* In Tables V–X, figures in parentheses represent the total range found; where no range is given only a single result was reported. In most instances insufficient results were available to calculate the standard deviation although this is reported when four or more results were available. Pooling the data for the various methods did not indicate a significant statistical difference between the hydride and fluorimetric methods. RSD = relative standard deviation.

† Calibration graph method.

‡ Standard additions method.

TABLE VI

DETERMINATION OF SELENIUM IN CATTLE NUTS

Laboratory	Finish	Selenium content, p.p.b.
A	Fluorimetric	183 (±10%, 3 results)
B	Fluorimetric	237 (RSD 3.3%, 4 results)
C	Fluorimetric	247 (±13%, 2 results)
D	Hydride/AAS	180 (±0%, 2 results)
E	Hydride/AFS	230 (±8%, 3 results)
G	Fluorimetric	200 (1 result)

TABLE VII

DETERMINATION OF SELENIUM IN KALE

Laboratory	Finish	Selenium content, p.p.b.*
A	Fluorimetric	110 (±0%, 4 results)
B	Fluorimetric	125 (±8%, 3 results)
C	Fluorimetric	150 (1 result)
D	Hydride/AAS	125 (±4%, 3 results)
E	Hydride/AFS	106 (±6%, 3 results)
G	Fluorimetric	100 (1 result)

* The mean value reported for this sample by other workers is 121 p.p.b.

TABLE VIII

DETERMINATION OF SELENIUM IN DRIED MILK, SAMPLE A

Laboratory	Finish	Selenium content, p.p.b.*
A	Fluorimetric	50 (±10%, 2 results)
B	Fluorimetric	73 (RSD 14%, 4 results)
C	Fluorimetric	121 (±10%, 2 results)
D	Hydride/AAS	80 (1 result)
E	Hydride/AFS	91 (RSD 5%, 4 results)
F	Fluorimetric	70 (1 result)

* Result obtained by neutron-activation analysis was 150 p.p.b. with a standard deviation of 30 p.p.b.

TABLE IX

DETERMINATION OF SELENIUM IN DRIED MILK, SAMPLE B

Laboratory	Finish	Selenium content, p.p.b.*
A	Fluorimetric	70 (±7%, 2 results)
B	Fluorimetric	99 (RSD 16%, 4 results)
C	Fluorimetric	125 (±5%, 2 results)
D	Hydride/AAS	90 (1 result)
E	Hydride/AFS	103 (±6%, 3 results)
F	Fluorimetric	90 (1 result)

* Result obtained by neutron-activation analysis was 110 p.p.b. with a standard deviation of 30 p.p.b.

TABLE X

DETERMINATION OF SELENIUM IN DRIED MILK, SAMPLE C

Laboratory	Finish	Selenium content, p.p.b.*
A	Fluorimetric	30 (±0%, 2 results)
B	Fluorimetric	75 (RSD 17.3%, 4 results)
C	Fluorimetric	100 (±3%, 2 results)
D	Hydride/AAS	60 (1 result)
E	Hydride/AFS	75 (±10%, 2 results)
F	Fluorimetric	90 (1 result)

* Result obtained by neutron-activation analysis was 50 p.p.b.; the precision was not stated for this sample.

for the fluorimetric method, but has been represented as a single curve in Fig. 1. Nevertheless it is still apparent that selenium concentration, rather than sample type, is the predominant factor in determining the precision. This suggests that the recommended procedures will produce satisfactory results for the range of samples examined, provided that samples of suitable size are taken.

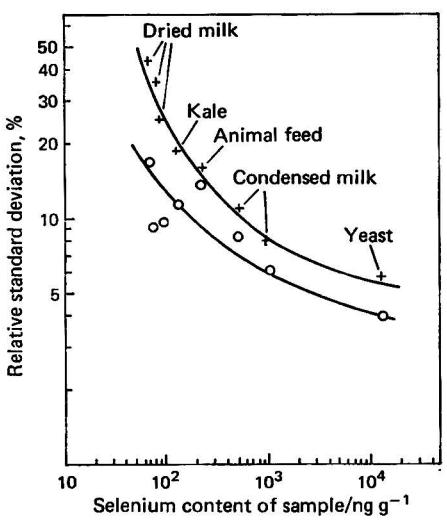

Fig. 1. Precision of analysis for samples with various selenium contents: +, fluorimetric method; and ○, hydride generation method.

Recommendation

The Sub-Committee recommends the methods described in the Appendix as suitable for the determination of selenium in the range 0.1–10 $\mu g\ g^{-1}$ in samples of organic matter.

APPENDIX

Recommended Methods for the Determination of Selenium

The organic matter is destroyed by wet oxidation with nitric, perchloric and sulphuric acids. Wet-oxidation methods can be hazardous, particularly when digestions of unfamiliar materials are carried out for the first time, and users are urged to familiarise themselves with previous reports on this subject.[2] The selenium is converted into selenium(IV) by boiling with 4 N hydrochloric acid and determined either by the fluorimetric method, using 2,3-diaminonaphthalene (DAN) as reagent, or by hydride generation, atomisation and atomic spectroscopic measurement.

Reagent Blank

Carry out a blank test by the entire procedure, using the exact amounts of reagents as used in the test and omitting only the sample.

Procedure for Oxidation of the Sample and Reduction of Selenium

Reagents

When possible "low in metals" grade reagents should be used.
Nitric acid, sp. gr. 1.42 (71% *m/m*).
Hydrochloric acid, sp. gr. 1.18 (36% *m/m*).
Sulphuric acid, sp. gr. 1.84 (98% *m/m*).
Perchloric acid, sp. gr. 1.70 (72% *m/m*).
Nitric acid - perchloric acid solution (5 + 1 *V/V*).
Nitric acid - sulphuric acid solution (1 + 1 *V/V*).

Apparatus

Kjeldahl flasks (100 ml) were used, with means of heating at a controlled rate and with facilities for the extraction of perchloric acid fumes. Alternatively, a programmable "block digestor" may be found to be advantageous, particularly when large numbers of samples have to be processed.

Destruction of Organic Matter

Place a sample of suitable size (see Note 1) with several acid-washed glass beads into a 100-ml Kjeldahl flask. Add 30 ml of nitric acid - perchloric acid (5 + 1) solution and either allow the sample to digest overnight at room temperature or heat cautiously until the initial foaming subsides and the sample is solubilised. Gradually increase the temperature until steady boiling is achieved, taking care to avoid loss of sample by bumping or excessive frothing. When the volume has been reduced by approximately half, cool and add 10 ml of nitric acid - sulphuric acid (1 + 1) solution. Return the flask to the heater and continue to heat through the perchloric acid oxidation stage, characterised by vigorous surface reaction and evolution of white fumes, and *ensuring that, if the yellow digest begins to darken, charring is prevented by the cautious addition of* 1-ml *increments of nitric acid.* Continue to heat past the stage at which the digest develops an intense greenish yellow colour, until a clear colourless or pale yellow solution and dense fumes of sulphur trioxide are produced, and then allow to cool.

Reduction of Selenium

Dilute the digest with water and add sufficient hydrochloric acid to produce a solution 4 N in hydrochloric acid and boil gently for 5 min; allow to cool, and dilute to a suitable known volume with water or hydrochloric acid (see Note 2).

NOTES—

1. The mass of sample chosen is determined by two factors, the first being the composition of the material and the second the expected selenium content of the sample relative to the sensitivity of the measuring system. For the volumes of reagents given above, samples low in fats and sugars and containing 50% or more of water can be digested in amounts up to 10 g, while amounts up to 5 g are suitable for samples containing 10–50% of water and 3-g amounts of most drier materials can be handled. Samples with a high fat or sugar content should be limited to 1–2 g only, owing to the danger of violent reaction with perchloric acid. Larger amounts of suitable samples can be handled if small amounts of additional nitric acid are added to prevent any charring during the initial boiling stage.
2. The volumes of water and hydrochloric acid used must be chosen such that the final volume and acidity are consistent with the measuring system being employed. When deciding on these volumes, both the effect on sensitivity and the optimum acidity for a given hydride generation system (if used) must be considered.

Fluorimetric Measurement of Selenium

Reagents

Ammonia solution, approximately 17% *m/m.* Dilute 500 ml of ammonia solution (sp. gr. 0.880) to 1 l with water.

Formic acid, 50% *V/V.*

Cyclohexane or dekalin.

Hydrochloric acid, approximately 4 M. Dilute 360 ml of hydrochloric acid (sp. gr. 1.18) to 1 l with water.

Hydrochloric acid, approximately 0.1 M. Dilute 10 ml of hydrochloric acid (sp. gr. 1.18) to 1 l with water.

2,3-*Diaminonaphthalene* (*DAN*) *reagent.* Add a few drops of 0.1 M hydrochloric acid to 1.0 g of DAN and stir to form a paste. Transfer into a 250-ml separating funnel with 200 ml of 0.1 M hydrochloric acid and shake for approximately 30 s. Extract the solution three times with 20-ml portions of cyclohexane or dekalin, dilute to 1 l with 0.1 M hydrochloric acid and filter through a wetted filter-paper into an amber-glass bottle. Add sufficient cyclohexane or dekalin to give a protective layer 2–3 mm in depth and store in a cool, dark place (see Note 3). This solution is stable for 2–3 weeks.

Hydroxylammonium chloride - EDTA solution. Dissolve 0.3 g of the disodium salt of EDTA and 25 g of hydroxylammonium chloride in 1 l of water and mix well.

Standard selenium solution A. Add 10 ml of nitric acid (sp. gr. 1.42) to 1.000 g of selenium and warm to dissolve. Dilute to 1 l with water and mix well.

1 ml of solution ≡ 1 mg of Se.

Standard selenium solution B. Dilute 10.0 ml of standard selenium solution A to 1 l with 0.1 M hydrochloric acid.

1 ml of solution ≡ 10.0 μg of Se.

Standard selenium solution C. Dilute 10.0 ml of standard selenium solution B to 1 l with 0.1 M hydrochloric acid.

1 ml of solution ≡ 0.10 μg of Se.

Preparation of Calibration Graph

Transfer into a series of 150-ml beakers 0.0, 2.0, 4.0 and 6.0 ml of standard selenium solution C (see Note 4). Add 1 ml of perchloric acid to the contents of each beaker and heat on a hot-plate until the first appearance in each beaker of fumes of perchloric acid. Cool, add 5 ml of 4 M hydrochloric acid to each and boil gently for 5 min. Remove the beakers from the hot-plate, cool and add to each in turn 20 ml of water, 5 ml of formic acid and 10 ml of hydroxylammonium chloride - EDTA solution. With the use of a pH meter adjust the pH of each solution to 1.8 with ammonia solution, add 5 ml of DAN solution and transfer the beakers to a water-bath maintained at 50 °C.

After 30 min, remove the beakers from the water-bath, cool and transfer the solutions with a minimum of water washings to separate 250-ml separating funnels fitted with PTFE

stopcocks. Adjust the volumes to about 70 ml with water and extract each with 10 ml of cyclohexane or dekalin. Allow the layers to separate, discard the lower aqueous layer and wash the organic layers each with 25 ml of 0.1 M hydrochloric acid. Allow the layers to separate and discard the lower acid layer. Measure the fluorescence of each cyclohexane or dekalin phase in a spectrofluorimeter with excitation at 369 nm and reading the emission at 525 nm (see Note 5). Correct the readings for the blank containing no added selenium and plot a calibration graph from the net values.

Preparation and Measurement of the Sample Solution

Take the sample solution from the Destruction of Organic Matter above, or a suitable aliquot containing not more than 0.6 μg of selenium, and transfer it into a 150-ml beaker with the aid of 20 ml of water and continue as outlined under Preparation of Calibration Graph from ". . . 5 ml of formic acid and 10 ml of hydroxylammonium chloride - EDTA solution . . ." to ". . . with excitation at 369 nm and reading the emission at 525 nm (see Note 5)."

Correct the fluorescence reading for the reagent blank, and determine the selenium content of the sample by reference to the calibration graph.

NOTES—

3. The procedure involving the formation of the DAN - selenium complex and subsequent handling of the solutions should be carried out in diffused light.
4. The calibration range can be adjusted to suit the particular fluorimeter or spectrofluorimeter being used, provided that it is compatible with the sample mass or the aliquot of it taken for analysis.
5. When using instruments that do not produce corrected spectra, the apparent excitation and emission wavelengths are influenced by the instrument optics and the detector response; it is therefore necessary to scan around the quoted wavelengths to find the maxima for a given instrument.

Measurement of Selenium by Hydride Generation, Atomisation and Atomic Spectroscopy

Principle of Method

The diluted acidic digest is reduced by sodium tetrahydroborate(III) solution to yield hydrogen selenide, which is carried by a stream of carrier gas to a heated area where it is atomised and the concentration of atoms produced is measured by atomic spectroscopy.

Because of the wide range of apparatus available for applying this family of techniques, it is not possible to give instructions as to how the determination should be performed. The following comments may, however, be helpful.

1. The detection limits for selenium obtained by these methods varied by at least three orders of magnitude according to the technique employed by Sub-Committee members. The choice of sample mass and final digest dilution volume must be compatible with the useful working range of the given generation - spectrometer apparatus available.
2. If a single injection system is used for atomic-absorption measurements, the detection limit is very poor unless simultaneous background correction is employed.
3. An automatic evolution procedure such as that employed by Vijan and Wood[12] requires the minimum of labour and produces good detection limits and precision.
4. For every individual apparatus, the optimum acidity, carrier gas flow-rate, sample volume, sodium tetrahydroborate(III) volume and concentration will vary. These parameters must be optimised before attempting to obtain useful measurements.
5. The atom reservoir for making atomic-absorption measurements is preferably an electrically heated silica tube furnace operating at a temperature of at least 800 °C. Satisfactory results may be obtained by using a silica tube of approximately 10 mm i.d. with a central side-arm for "hydride" introduction, heated with an air - acetylene flame.
6. An alternative method of making atomic spectral measurements is to use the technique of atomic fluorescence. When using this technique an argon - hydrogen-entrained air flame is a suitable atom reservoir.
7. When discrete sample injection is used, the experience of the Sub-Committee is that only syringes or injectors of all-plastic construction are suitable for sample handling.
8. It should be noted that optimum performance in the absence of interfering ions may

be obtained either by injection of the sample to the generator already containing the sodium tetrahydroborate(III) or *vice versa*, according to the system employed. This situation may be modified in the presence of certain ions that inhibit hydride generation.[13]

9. Optimum performance of a given system may be found to vary according to whether or not the carrier gas inlet to the generator is above or below the aqueous reactants.

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SHORT PAPERS

Hydrocarbon Analysis of Naphtha Using Capillary-column Gas Chromatography Under Isothermal Conditions

Pradeep Kumar, S. L. S. Sarowha and P. L. Gupta
Indian Institute of Petroleum, Dehra Dun-248005, India

Keywords: *Hydrocarbon analysis*; *naphtha*; *capillary-column gas chromatography*; *Kovát's retention indices*

Several attempts have been made,[1–6] with varying degrees of success, to carry out a detailed individual hydrocarbon analysis of petroleum naphtha and its fractions, which find extensive use as feedstocks for various petrochemical processes and products. Because of the complexity and occurrence of a large number of hydrocarbon isomers, gas chromatography with long capillary columns and special facilities such as temperature and pressure programming has been tried. Such procedures are difficult to apply to routine analyses.

This paper reports a method of detailed hydrocarbon analysis using a squalane capillary column (150 ft × 0.01 in) without prior separations or temperature or pressure programming. At room temperature fairly good resolution of various isomers in naphtha fractions boiling up to 150 °C is achieved. For the separation of some selected compounds, however, running of the chromatogram at another temperature is necessary. From a study of the variation of Kovát's retention indices with temperature for different hydrocarbon types, it is shown that a reasonably good separation of various hydrocarbons can be achieved at 55 °C. Data from two chromatograms, at room temperature and at 55 °C, are combined to give the final results, showing good repeatability and therefore suggesting that the procedure is promising for the routine analysis of naphtha samples.

Experimental

The gas chromatograph used for this study was basically a Philips Instrument, Model PV 4000 series, with a flame-ionisation detector modified to accommodate capillary columns by incorporating a Hamilton inlet splitter. The capillary column was a stainless-steel (150 ft × 0.01 in) WCOT type, coated with squalane and supplied by Perkin-Elmer.

Peaks in the chromatogram are identified by measuring Kovát's retention indices, confirming selected hydrocarbons with the help of available reference compounds or using literature data whenever available (assuming that a reproducibility of ± 1 for the retention index could be achieved in practice). Air-peak distances (calculated by using Peterson and Hirsch's method[8]) have been subtracted from retention distances for the calculation of Kovát's indices.

Results and Discussion

The chromatogram of a typical naphtha (boiling range 40–150 °C) run at room temperature is shown in Fig. 1. From Fig. 1 and Table I it is evident that peaks differing by two or more retention index units are generally well separated, and that for the same difference in retention index values, separation appears to improve as the retention index values increase. Thus, at the beginning of the chromatogram components differing by two or more retention index units are not well resolved, *e.g.*, cyclopentane, 2,3-dimethylbutane and 2-methylpentane, but towards the end of the chromatogram even components differing by less than two retention index units show good separation. Hence, it appears that the peak width (measured in terms of retention indices) increases at a slower rate than the increase in retention index.

TABLE I

KOVÁT'S RETENTION INDICES OF IDENTIFIED HYDROCARBONS USING A SQUALANE CAPILLARY COLUMN

Hydrocarbon	Peak number from Figs. 1 and 3	Measured Kovát's retention indices of peaks from chromatograms 25 °C	55 °C	$10 \times \frac{\Delta I}{\Delta t}$ from literature[8-12]	Concentration in a typical naphtha, % m/m
Butane	3	400.0	400.0	0.00	0.8
2-Methylbutane	4	473.0	—	0.07	1.6
Pentane	5	500.0	500.0	0.00	1.9
2,2-Dimethylbutane	6	534.6	—	0.82	0.1
Cyclopentane	7	562.0	569.9	1.38	0.6
2,3-Dimethylbutane	8	564.6	569.9	0.90	0.1
2-Methylpentane	9	568.3	569.9	0.17	2.0
3-Methylpentane	10	582.6	585.0	0.45	1.3
Hexane	11	600.0	600.0	0.00	2.2
2,2-Dimethylpentane	12	624.7	626.1	0.65	3.7
Methylcyclopentane	12	624.7	626.1	1.52	
2,4-Dimethylpentane	13	629.0	626.1	0.34	0.1
Benzene	14	633.3	641.2	2.47	2.5
2,2,3-Trimethylbutane	15	636.3	—	1.44	Trace
3,3-Dimethylpentane	16	655.4	660.6	1.32	0.1
Cyclohexane	17	657.6	662.3	2.22	5.4
2-Methylhexane	18	665.4	664.8	0.17	1.4
1,1-Dimethylcyclopentane	19	669.1	675.6	1.95	0.6
2,3-Dimethylpentane	19	669.1	672.6	0.86	0.6
3-Methylhexane	20	674.8	675.6	0.31	1.4
1-*cis*-3-Dimethylcyclopentane	21	678.9	683.5	1.63	1.0
1-*trans*-3-Dimethylcyclopentane	22	682.9	686.8	1.65	0.9
3-Ethylpentane	23	685.3	686.8	0.64	0.3
1-*trans*-2-Dimethylcyclopentane	23	685.3	689.5	1.59	1.4
2,2,4-Trimethylpentane	—	—	—	1.08	Trace
Heptane (n-C_7)	24	700.0	700.0	0.00	2.2
1-*cis*-2-Dimethylcyclopentane (1-c-2-DMCP)	25	716.4	722.8	2.10	0.3
2,2-Dimethylhexane (2,2-DMC_6)	26	721.3	720.4	0.52	0.1
1,1,3-Trimethylcyclopentane (1,1,3-TMCP)	26	721.3	724.7	1.91	0.2
2,2,3,3-Tetramethylbutane (2,2,3,3-Tet.MC_6)	26	721.3	726.6	2.39	11.4
Methylcyclohexane (MCH)	26	721.3	726.6	2.43	
2,5-Dimethylhexane (2,5-DMC_6)	27	727.0	726.6	0.22	0.4
Ethylcyclopentane (ECP)	28	730.0	735.5	1.83	0.5
2,4-Dimethylhexane (2,4-DMC_6)	29	730.0	732.9	0.42	0.4
2,2,3-Trimethylpentane (2,2,3-TMC_5)	30	—	737.6	1.52	Trace
1-*trans*-2-*cis*-4-Trimethylcyclopentane (1-t-2-c-4-TMCP)	31	737.0	742.5	1.63	0.4
3,3-Dimethylhexane (3,3-DMC_6)	32	740.5	743.7	1.32	0.2
Toluene	32	740.5	747.6	2.42	5.5
1-*trans*-2-*cis*-3-Trimethylcyclopentane (1-t-2-c-3-TMCP)	33	744.1	749.1	1.65	0.6
2,3,4-Trimethylpentane (2,3,4-TMC_5)	34	748.8	—	1.27	0.2
2,3,3-Trimethylpentane	35	754.9	759.9	2.02	0.1
1,1,2-Trimethylcyclopentane	36	758.1	764.5	2.32	0.4
2-Methyl-3-ethylpentane	36	758.1	—	1.00	0.2
2,3-Dimethylhexane	36	758.1	759.9	0.58	
2-Methylheptane	37	764.2	764.5	0.15	1.2
4-Methylheptane	38	765.0	766.8	0.15	0.1
3,4-Dimethylhexane	39	768.1	—	1.00	0.1
1-*cis*-2-*trans*-4-Trimethylcyclopentane	39	768.1	772.1	2.18	0.1
3-Methyl-3-ethylpentane	39	768.1	—	1.95	—
2,2,4,4-Tetramethylpentane	39	768.1	—	1.90	—
3-Ethylhexane	40	771.0	772.1	0.68	1.0
3-Methylheptane	40	771.0	772.1	0.31	
1-*cis*-2-*trans*-3-Trimethylcyclopentane	41	774.1	779.8	2.27	Traces
2,2,5-Trimethylhexane	41	774.1	776.6	0.65	
1-*cis*-3-Dimethylcyclohexane	42	779.9	786.4	2.42	2.9
1-*trans*-4-Dimethylcyclohexane	42	779.9	783.3	2.77	
1,1-Dimethylcyclohexane	42	779.9	786.4	2.91	
1-Methyl-*cis*-3-ethylcyclopentane	43	783.2	788.3	1.93	0.2
Cycloheptane	44	786.3	794.7	3.54	—
1-Methyl-*trans*-3-ethylcyclopentane	44	786.3	791.6	2.02	0.6
2,2,4-Trimethylhexane	44	786.3	—	1.35	
1-Methyl-*trans*-2-ethylcyclopentane	44	786.3	791.6	2.14	
1-Methyl-1-ethylcyclopentane	45	788.4	794.7	—	0.1
1-*trans*-2-Dimethylcyclohexane	46	795.5	803.3	2.87	1.2
1-*cis*-2-*cis*-3-Trimethylcyclopentane	46	795.5	803.3	2.77	
1-*cis*-4-Dimethylcyclohexane	47	800.0	806.5	2.95	0.7
1-*trans*-3-Dimethylcyclohexane	47	800.0	806.5	2.61	
Octane	47	800.0	800.0	0.00	1.7
Isopropylcyclopentane	48	806.8	—	2.61	0.2
2,2-Dimethylheptane	49	813.7	—	0.45	0.1
1-Methyl-*cis*-3-ethylcyclopentane	50	815.8	—	2.54	0.2
2,2,3-Trimethylhexane	51	819.0	—	1.38	0.3
2,4-Dimethylheptane	—	—	—	0.20	
1-*cis*-2-Dimethylcyclohexane	52	822.6	829.3	3.07	0.2
2-Methyl-4-ethylhexane	52	822.6	—	0.55	0.9
Propylcyclopentane	53	825.6	830.1	2.03	0.9
2,6-Dimethylheptane	53	825.6	—	0.25	—
Ethylcyclohexane	54	829.3	834.6	2.69	2.3
Ethylbenzene	54	829.3	834.6	2.80	1.3
2,5-Dimethylheptane	55	832.0	—	0.30	0.6
3,5-Dimethylheptane	55	832.0	—	0.55	
3,3-Dimethylheptane	—	—	—	1.15	—
1,1,3-Trimethylcyclohexane	56	834.7	840.3	2.82	—
2,3,3-Trimethylhexane	57	835.5	841.3	1.85	—
1,1,4-Trimethylcyclohexane	58	837.2	843.8	—	—
1-*cis*-3-*cis*-5-Trimethylcyclohexane	59	840.4	847.0	—	—
p-Xylene	60	843.9	849.5	2.68	1.4
2,3,4-Trimethylhexane	60	843.9	—	1.60	0.1
m-Xylene	61	846.3	852.4	2.64	4.2
2,3-Dimethylheptane	62	—	853.5	0.65	0.9
4-Ethylheptane	63	—	856.7	0.45	0.1
1-*cis*-2-*cis*-3-*trans*-4-Tetramethylcyclopentane	64	—	859.5	—	—
3,4-Dimethylheptane	65	—	860.2	1.05	0.1
4-Methyloctane	66	—	861.5	0.30	0.8
2-Methyloctane	67	—	863.3	0.15	0.5

TABLE I (*continued*)

Hydrocarbon	Peak number from Figs. 1 and 3	Measured Kovát's retention indices of peaks from chromatograms 25 °C	55 °C	$10 \times \frac{\Delta I}{\Delta t}$ from literature[9–12]	Concentration in a typical naphtha, % *m/m*
3-Ethylheptane ..	68	—	865.7	0.45	0.2
3-Methyloctane ..	69	—	869.3	0.35	
1-Methyl-*trans*-3-isopropylcyclopentane	69	—	869.3	—	0.7
1-Methyl-*cis*-3-isopropylcyclopentane	69	—	869.3	—	
o-Xylene	70	—	870.9	3.10	1.5
1-*trans*-3-Dimethyl-*cis*-2-ethylcyclopentane	71	—	877.0	—	—
3,3-Diethylpentane ..	72	—	880.6	2.90	0.1
1-Methyl-1-propylcyclopentane ..	73	—	882.3	—	—
1-Methyl-*trans*-2-propylcyclopentane	73	—	882.3	—	—
1-Methyl-*cis*-3-propylcyclopentane	74	—	883.7	—	—
1-Methyl-*trans*-3-propylcyclopentane	75	—	885.1	—	—
1-*cis*-2-*cis*-4-Trimethylcyclohexane	76	—	886.5	—	—
1-*cis*-2-*trans*-4-Trimethylcyclohexane	77	—	889.6	—	—
1-*trans*-2-Diethylcyclopentane ..	78	—	891.7	—	—
Isobutylcyclopentane	78	—	891.7	—	—
1-Methyl-1-ethylcyclohexane ..	79	—	894.3	—	—
1-*cis*-3-Diethylcyclopentane ..	79	—	894.3	—	—
1-*trans*-3-Diethylcyclopentane ..	79	—	894.3	—	—
Isopropylbenzene ..	80	888.6	895.3	2.50	—
Nonane	81	900.0	900.0	0.00	—

Table I shows retention index values measured from the chromatograms. For overlapping peaks a slight error in retention index values is possible owing to difficulties in correctly locating individual peak maxima. Temperature coefficients of the indices ($\Delta I/\Delta t$) have also been reported in the literature.[9–12] By increasing the column temperature naphthenes and aromatics are retained longer than alkanes; hence, preferential separation of naphthenes and aromatics from alkanes could be achieved. With small changes in the column temperature, the separation of naphthenes from aromatics is difficult, as the $\Delta I/\Delta t$ values are only marginally different for these two types of hydrocarbons. For very large temperature changes, however, the retarded naphthenes and aromatics may start interfering with the alkanes of higher carbon number. As an example, 3,3-dimethylhexane (3,3-DMC_6) and toluene elute together at room temperature; at 55 °C they are separated fully, but if the temperature is increased to 75 °C, it is found that 3,3-dimethylhexane merges with 1-*trans*-2-*cis*-4-trimethylcyclopentane (1-*t*-2-*c*-4-TMCP) and toluene starts to interfere with 1-*trans*-

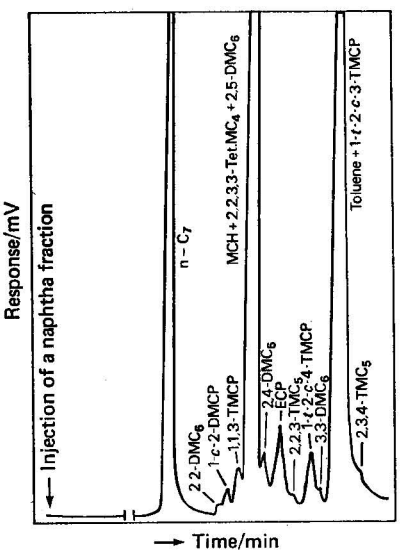

Fig. 2. Section of a chromatogram at a column temperature of 55 °C.

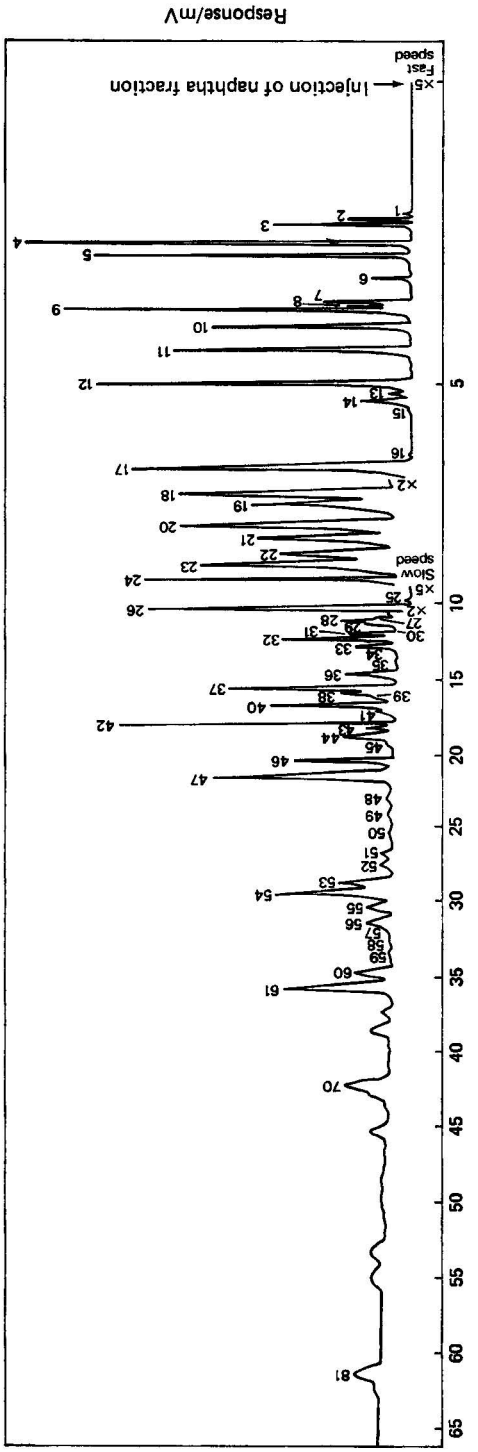

Fig. 1. Chromatogram of a typical naphtha fraction on a squalane capillary column (150 ft × 0.01 in) at ambient temperature.

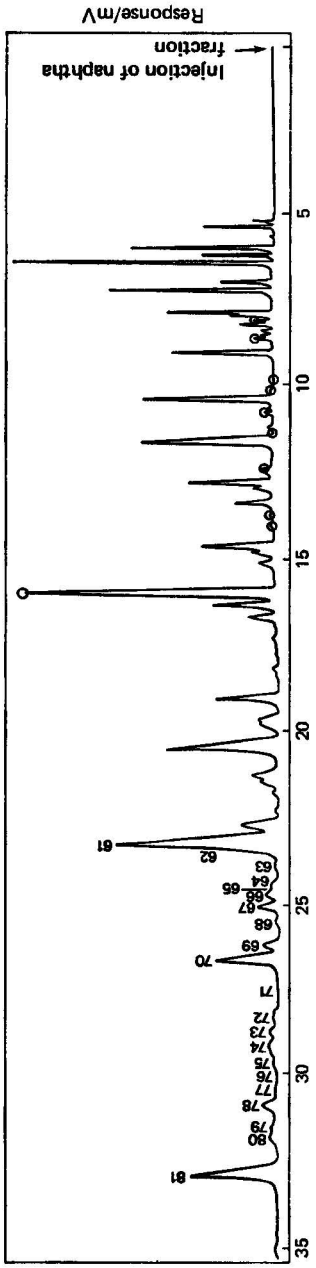

Fig. 3. Chromatogram of a typical naphtha fraction on a squalane capillary column (150 ft × 0.01 in) at 55 °C.

2-*cis*-3-trimethylcyclopentane (1-*t*-2-*c*-3-TMCP). With the aim of separating the maximum number of pairs (that elute together at room temperature) and avoiding interference from neighbouring components, a compromise temperature of 55 °C has been selected.

The separations possible by judicious selection of column temperature are illustrated further in Fig. 2, where a section of the chromatogram run at 55 °C is shown. At 25 °C methylcyclohexane elutes with 2,2-dimethylhexane (2,2-DMC_6), 1,1,3-trimethylcyclopentane (1,1,3-TMCP) and 2,2,3,3-tetramethylbutane (2,2,3,3-Tet.MC_4). At a column temperature of 55 °C, 2,2-dimethylhexane and 1,1,3-trimethylcyclopentane are separated, as shown in Fig. 2, but methylcyclohexane (MCH) and 2,2,3,3-tetramethylbutane are still not separated from each other as the $\Delta I/\Delta t$ values for these compounds differ only slightly. Some typical separations are given in Fig. 3, which shows a complete chromatogram of a naphtha sample at 55 °C. Components not separated from others at room temperature but separated at 55 °C are marked in the chromatogram with circles. Kovát's indices of the hydrocarbons at 55 °C are also shown in Table I for ready identification. Separated components are measured from this chromatogram; the other compounds interfering at room temperature can be estimated by subtraction. The composition of a typical straight-run naphtha fraction (40–150 °C) up to C_8 hydrocarbons is shown in Table I (last column) as an illustration. The concentrations reported were calculated from normalisation of peak areas (measured by triangulation). The only significant pair remaining unresolved is ethylcyclohexane and ethylbenzene. If need be, ethylbenzene can be determined by analysing the sample on a polar column, such as 7,8-benzoquinoline.

Hence, the proposed method, utilising isothermal chromatography at two temperatures, shows reasonably good separation.

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The Impact Cup: A Simple Aid in Flame Spectrometric Analysis at High Analyte Concentrations

Malcolm S. Cresser

Department of Soil Science, University of Aberdeen, Meston Walk, Aberdeen, AB9 2UE

Keywords: Impact cup; flame spectrometry; high analyte concentrations; pneumatic nebuliser

The upper useful concentration limit in any flame spectrometric method of analysis is imposed by the onset of a high degree of curvature in the calibration graph. A number of approaches may be adopted in order to extend the working range. If an absorption, emission or fluorescence line is available that exhibits poorer sensitivity, this line can be used in place of the more sensitive line. In some instances useful alternative wavelengths are not available,

however, or the change in sensitivity may be so drastic that the alternative wavelength is of limited value. In atomic-absorption spectrometry burner rotation or a separate, short-path burner can be used to increase the sensitivity for 1% absorption and thus extend the working range. In the author's experience, this approach often leads to poorer precision, because of the increase in background noise caused by the greater relative importance of fluctuations in molecular absorption and/or emission at the edges of the flame. Moreover, both of the above approaches suffer from the serious drawback that the incidence and extent of interferences increase significantly when solutions with high concentrations of analyte element are nebuilised, because of the larger size of the residual solid particles after evaporation of the solvent.[1–4]

An alternative approach to the problem is to take steps to decrease either the nebulisation rate or the nebulisation efficiency. The nebulisation rate can be reduced by restricting the nebuliser capillary diameter or by increasing its length. Unfortunately, this may lead to a greatly increased tendency towards nebuliser clogging with only small changes in sensitivity. The nebulisation rate can also be reduced by the addition of a miscible, high-viscosity additive, which involves a time-consuming sample preparation step, or by employing a peristaltic pump to reduce the rate of solution uptake. It has also been demonstrated[5] that the useful working range can be extended by nebulisation of discrete aliquots of sample that are sufficiently small for the nebuliser not to reach its normal equilibrium working conditions. All of these approaches considerably decrease the throughput of samples for a given instrument.

This short paper reports a novel approach to the problem of increasing the useful working range of flame spectrometric determinations. The impact bead of a conventional atomic-absorption spectrometer is replaced with a PTFE impact cup, which serves to reduce considerably the nebulisation efficiency.

Experimental

Reagents

Stock solutions containing 1000 μg ml^{-1} of analyte or interfering element were prepared from analytical-reagent grade salts, and diluted as appropriate. The stock magnesium solutions ($MgCl_2.6H_2O$ and $MgSO_4.7H_2O$) were standardised by complexometric titration.

Apparatus

Measurements were made using a Baird A3400 atomic-absorption spectrometer operated under normal conditions for routine analysis. When appropriate the impact bead was replaced with a PTFE cup made as shown in Fig. 1 to fit on the manufacturer's impact bead support arm. The interior of the cup was made with sloping sides and PTFE was chosen as the construction material to minimise the possibility of sample solution being retained inside the cup, which might have produced memory effects.

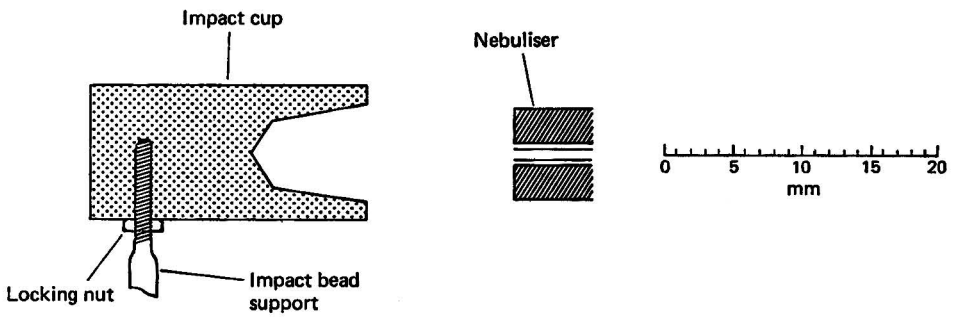

Fig. 1. Design of the PTFE impact cup.

Results and Discussion

It was found that the use of the impact cup in place of the usual impact bead caused a 12-fold change in nebulisation efficiency, which in turn caused an approximately 12-fold change in sensitivity in both atomic-absorption and -emission spectrometry for readily atomised elements. Thus the absorbances from 1 μg ml^{-1} solutions of cadmium, copper, magnesium and zinc nebulised into an air - acetylene flame were reduced 11.5-, 12.0-, 12.5- and 12.2-fold, respectively. The emission signals from 1 μg ml^{-1} solutions of potassium and sodium were reduced 13.8- and 14.8-fold, respectively. The differences between these factors are attributable to curvature in the calibration graphs, particularly that due to ionisation in the instances of sodium and potassium.

To investigate the effect of use of the impact cup on precision, 0.2 and 0.5 μg ml^{-1} magnesium solutions were nebulised repeatedly into a 130-mm air - acetylene flame, using the impact bead, and the absorbances measured. A 1 μg ml^{-1} magnesium solution was nebulised periodically and the scale expansion adjusted if necessary to give an arbitrary reading of 0.8 for this solution. The coefficients of variation were found to be 0.028 and 0.012 at 0.2 and 0.5 μg ml^{-1} of magnesium, respectively. This procedure was then repeated using the impact cup, but for 2.4 and 6.0 μg ml^{-1} magnesium solutions, using a 12 μg ml^{-1} standard solution to adjust the scale expansion. The coefficients of variation were found to be 0.035 and 0.013, respectively. Thus the precision when the impact cup is used is comparable to that when the impact bead is used at 12 times lower concentrations.

As the use of the impact cup appears to be similar in effect to a 12-fold dilution, significant improvements should be observed in the linearities of calibration graphs. Typical graphs for the determination of sodium in an air - acetylene flame by emission spectrometry at 589 nm are shown in Fig. 2, using 130- and 10-mm flames for the impact bead and a 130-mm flame

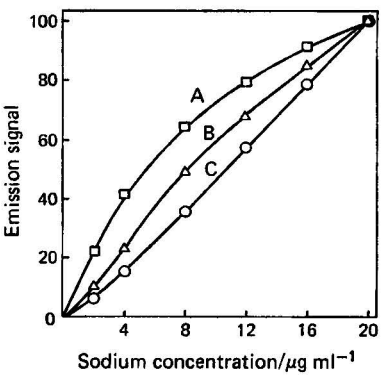

Fig. 2. Flame emission calibration graphs for sodium at 589 nm. A, Impact bead, 130-mm flame; B, impact bead, 10-mm flame; and C, impact cup, 130-mm flame.

for the impact cup. The photomultiplier voltage was adjusted so that identical arbitrary readings were obtained from a 20 μg ml^{-1} sodium solution in each instance. The curvature of the calibration graph is eliminated over the range 6–20 μg ml^{-1} when the cup is used, although curvature due to ionisation is observed over the range 0–6 μg ml^{-1}. Similar ionisation-induced curvature is normally observed with the impact bead over the range 0–0.5 μg ml^{-1} for the 130-mm flame if a calibration graph is plotted over the range 0–1 μg ml^{-1}. It can be eliminated by the addition of an ionisation buffer. The calibration graph for the range 0–240 μg ml^{-1} of sodium using the impact cup and a 130-mm flame is identical, within the limits of experimental error, with the graph for the range 0–20 μg ml^{-1} using the impact bead. If the impact cup is used in conjunction with the small flame, the useful working range extends to more than 1000 μg ml^{-1}. No significant memory effects were observed.

Typical atomic-absorption calibration graphs for magnesium, as both the chloride and the sulphate, are shown in Fig. 3. The degree of scale expansion was adjusted to give an arbitrary reading of 0.8 for 20 μg ml^{-1} of magnesium as the chloride in each instance. A slight curvature is observed over the range 0–8 μg ml^{-1} when the impact cup is used with the 130-mm flame. This curvature is normally observed over the range 0–0.7 μg ml^{-1} using the impact bead and the 130-mm flame. Using the impact cup and the small flame, the calibration graph for magnesium, as the chloride, over the range 0–240 μg ml^{-1} was similar to that obtained using the small flame and impact bead over the range 0–20 μg ml^{-1}. It is interesting to note that, whereas magnesium as the chloride and as the sulphate gave identical calibration graphs over the range 0–20 μg ml^{-1} when the impact cup and the 130-mm flame were used, the calibration graphs for the two magnesium species were different (see Fig. 3) when the impact bead and the 10-mm flame were used. The elimination of this interference over the range 0–20 μg ml^{-1} is almost certainly attributable to the fact that only extremely fine droplets reach the flame when the impact cup is utilised, so that the smaller residual solid particles are completely volatilised.[3,4]

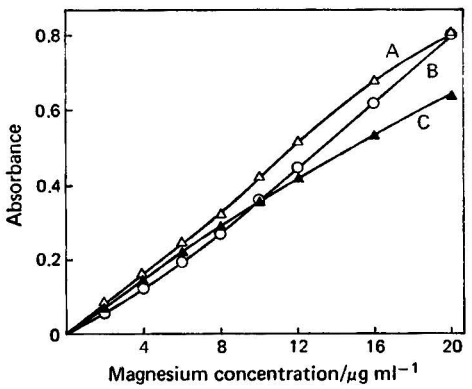

Fig. 3. Flame atomic-absorption calibration graphs for magnesium at 285 nm. A, $MgCl_2$, impact bead, 10-mm flame; B, $MgCl_2$ and $MgSO_4$, impact cup, 130-mm flame; and C, $MgSO_4$, impact bead, 10-mm flame.

To see if the reduction in interference was general, the effect of various amounts of phosphorus on the determination of 100 μg ml^{-1} of calcium in a stoicheiometric air - acetylene flame was investigated. The results, shown in Fig. 4, clearly demonstrate that a substantial

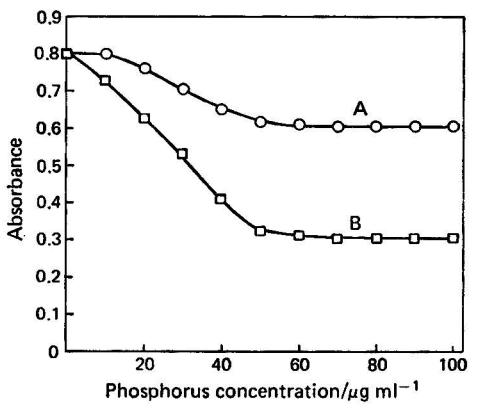

Fig. 4. Effect of phosphorus on the absorbance from 100 μg ml^{-1} of calcium. A, Impact cup, 130-mm flame; and B, impact bead, 130-mm flame.

reduction in the degree of interference is observed when the impact cup is used, providing further support for the hypothesis that the fraction of spray reaching the flame when the impact cup is used consists only of very fine droplets. A similar reduction in the degree of interference may be achieved by 12-fold dilution using the impact bead.

Conclusions

The use of an impact cup in conjunction with a pneumatic nebuliser produces an effect that in every respect is equivalent to 12-fold dilution for flame spectrometric analysis on the system used. Use of the impact cup therefore increases the useful working range by a factor of 12 without the usual problems associated with flame spectrometric determinations at high analyte concentrations such as increased incidence and extent of interferences and/or reduced sample throughput. If the impact cup is used in association with the techniques usually employed to extend the working range, much greater extensions become possible. If the impact cup and impact bead are fitted side-by-side on two arms of an inverted Y, it is possible to change from one to the other in a few seconds, without even extinguishing the flame.

The author is indebted to George Wilson for the manufacture of the impact cup and to Fiona Mitchell for assistance with the experimental work.

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Book Reviews

ANNUAL REPORTS ON ANALYTICAL ATOMIC SPECTROSCOPY, REVIEWING 1977. Volume 7. Edited by J. B. DAWSON. Pp. x + 291. London: The Chemical Society. 1978. Price £17.50; $35 (CS Members £13).

Under the editorship of J. B. Dawson the reviewing team have once again lived up to the expectations created by the earlier volumes in this series. In his foreword the editor comments that the first aim of the reviewers is to provide the reader with up to date information on world wide developments in the field of analytical atomic spectroscopy. This is nicely demonstrated by the observation that over 20% of the references are from papers delivered at conferences and that are likely to be overlooked by many of those not in the conference jet-set. However, this volume provides an even greater service at this particular time by its reporting of developments in inductively coupled plasmas and related techniques, which with the advent of commercial instruments are rapidly demonstrating their potential in the trace-element field. Personally, I appreciate this publication very much and I recommend this particular volume to all practising analysts who use atomic-spectroscopic methods.

JOHN AGGETT

ESTIMATING THE HAZARD OF CHEMICAL SUBSTANCES TO AQUATIC LIFE. Edited by JOHN CAIRNS, JR., K. L. DICKSON and A. W. MAKI. *ASTM Special Technical Publication* 657. Pp. viii + 278. Philadelphia, Pa.: American Society for Testing and Materials. 1978. Price $19.50.

A workshop was held in July 1977 to discuss the "Application of aquatic toxicity testing methods as predictive tools for aquatic hazard evaluation." Originally proposed by A. W. Maki it was called together as a result of the US Toxic Substances Control Act 1976. The workshop members included chemists, toxicologists, ecologists and aquatic biologists who were organised into specialist groups. Each group was assigned one of the following topics: (*a*) toxicological effects, (*b*) environmental concentration, (*c*) environmental fate, (*d*) hazard assessment and (*e*) procedures for estimating hazards to aquatic life, and these topics formed the basis of five one-day workshop sessions. Each session was opened with the reading of a plenary paper by a member of the specialist group. The workshop discussion that followed was summarised by the specialist group for that session and later approved by the other members of the workshop. This book is the publication of the plenary papers and the discussion reports for each of the workshop sessions.

It is inevitable, with a workshop of this type, that much overlap and repetition of ideas and subject matter occur between sessions. In fact, the plenary papers for sessions (*b*) and (*c*) cover much of the same ground and the two specialist session groups report a single discussion. Such repetition can be useful as it often provides an alternative viewpoint. However, stricter directives to authors concerning their subject matter could have been useful in providing more of the relevant subject material presented for some sessions. The plenary papers vary a great deal in their length, breadth of coverage and their citation of scientific literature, though the discussion reports do attempt to balance the individual papers. Nevertheless, the workshop acknowledges that many of its findings are related to organic chemicals but suggests that most aspects of hazard assessment apply equally well to the inorganics.

Much of the content of the book relates to two documents that are conveniently reproduced in the appendices. One is a "Proposed working document for the development of an ASTM draft standard on standard practice for a laboratory testing scheme to evaluate hazard to non-target aquatic organisms," by the ASTM sub-committee E35.21 on Safety to Man and Environment. The other is the "Criteria and rationale for decision making in aquatic hazard evaluation (third draft)," by the Aquatic hazards of pesticides task group of the American Institute of Biological Sciences. The applicability of these documents and a third alternative proposed by R. A. Kimerle, a member of the workshop, was discussed.

In its conclusion the workshop lists several areas of scientific research where future work would aid the assessment of the hazards of chemical substances to aquatic life.

In general, this book is well written and presented and would be a useful addition to the library of any scientist or administrator working in this field, particularly as it gives reasonably up to date information on hazard evaluation in aquatic systems as interpreted by a number of different individuals and committees.

K. R. BULL

LIQUID SCINTILLATION COUNTING. Volume 5. Edited by M. A. CROOK and P. JOHNSON. *Proceedings of a Symposium on Scintillation Counting organised by the Radiochemical Methods Group (Analytical Division, The Chemical Society), Bath, England, September* 13–16, 1977. Pp. x + 222. London, Philadelphia and Rheine: Heyden. 1978. Price $28; £14; DM64.50.

The change of title of the Symposium from Liquid Scintillation Counting was justified by the contents, which incorporated whole body counting and radioimmunoassay. There was little that was novel, either at the meeting or in this volume except that it is very useful to have the cross-fertilisation of ideas between disciplines. The quality of the writing is good, but the use of single spacing on a typewriter to ease reproduction causes references and superscripts to run into the previous line and results in some confusion in places.

The twenty-six chapters are divided into five parts and I have chosen for comment what I considered to be outstanding in each section. The plenary lecture on sample preparation by Bransome gives timely warning and advice, which is of value to us all. Immunoassay is a powerful method, in that it can identify several hundred constituents in a biological fluid, but unfortunately the plenary lecturer does not include any references with which to follow up the technique. I would like to see the technique of Ayrey, for using heavy metals loaded into scintillators, developed further as it seems to be a powerful method for counting electron-capture nuclides by measuring a higher proportion of K X-rays. Whole body monitoring for clinical applications is well served by two chapters by Burkinshaw and his colleagues from Leeds, with Boddy providing the cost effectiveness to persuade the clinician. The advances in instrumentation and technique were in no way new but the chapter by Horrocks is masterly in its presentation, if over simplistic in representing the liquid scintillation detector as linear down to energies below 100 keV. The criticisms by Bransome of the misuse of automatic counters is answered by Johnson in chapter 20 in a well written chapter on computing methods which warn you when your data are meaningless or of little value. The final two papers on measuring small amounts of radioactivity in minute precious samples by Burleigh and Otlet demonstrate what meticulous attention to detail can produce when using carbon-14 dating techniques.

This is the last symposium on liquid scintillation counting to be organised by the Radiochemical Methods Group and in the future the emphasis should be upon practical workshops to improve the use of the technique. This opinion reflects the well developed state of the method of liquid scintillation counting for which this five-volume series will provide a convenient reference library for all users.

J. A. B. GIBSON

THIN-LAYER CHROMATOGRAPHY. Second Edition. By JUSTUS G. KIRCHNER. *Techniques of Chemistry, Volume XIV*. Pp. xxvii + 1137. New York, Chichester, Brisbane and Toronto: John Wiley. 1978. Price £42.50; $84.

This is Volume XIV in the Techniques of Chemistry Series, and may be considered to be one of the standard authoritative handbooks available covering thin-layer chromatography. It is a weighty tome of over 1100 pages with over 6000 references. Since the first edition, several of the chapters have been revised completely and all have been modified and brought up to date. The volume retains the general format of the first edition and it is divided into two parts, the first part dealing with various techniques of thin-layer chromatography, and the second, much to the satisfaction of the practical reader, with applications. The latter includes acids, alcohols, alkaloids, amines, antibiotics, carbohydrates, dyes, hydrocarbons, lipids, nucleic acids, pesticides, pharmaceutical products, phenols, natural pigments, steroids, essential oils, vitamins and many others.

In addition, there is an Appendix listing compounds and types of compounds to be found in the book together with the test number of the reagent(s) to be used for their detection. There is both a subject and a compound index, which have been included in preference to an author index. To complete the various lists the addresses are given (mainly in the USA) of commercial firms mentioned in the text.

There is always something to criticise in any book if one is determined to do so and this is no exception; for example, plastics analysts might wish for more details of separations of additives other than plasticisers/stabilisers (perhaps more in the ultraviolet absorber range), and specialists

in other fields might also feel deprived, but this is a minor carp for no single movable volume can be exhaustive when covering so many fields. My advice to the chromatographer who has not got a reference volume and can afford this one is to buy it. D. SIMPSON

INSTRUMENTATION FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. Edited by J. F. K. HUBER. *Journal of Chromatography Library, Volume* 13. Pp. xvi + 204. Amsterdam, Oxford and New York: Elsevier. 1978. Price $34.75; Dfl180.

Any chemist who has been an instructor in one of the many high-performance liquid chromatography (HPLC) training courses of recent years is well aware of the need for a good, comprehensive book to help in the selection of equipment for specific analytical requirements. Unfortunately, despite the impeccable qualifications of each of the authors, this volume does not provide a satisfactory outline of the state of the art in instrumentation for liquid chromatography.

The systems engineering concepts outlined in the Editor's introduction must be accepted as the methodology used by those engineers who actually design liquid chromatography apparatus for manufacture, but correlation of this mathematical approach with real performance from real hardware is not uniform throughout the book. However, individual chapters such as that on Electrochemical Detectors (H. Poppe), Sample Introduction Systems (J. C. Kraak) and Column Design (J. C. Kraak) stand out as examples of clear exposition, with good coverage of the subject and a good selection of easily understood line drawings of a range of individual components. Surprisingly, Professor Poppe's second chapter on other detectors is much less satisfactory, as it lacks the line drawings of flow cells, etc., which are so essential to an understanding of the sources of temperature and flow sensitivity in optical detectors in particular. However, this section is better in this respect than that discussing Radiometric Detectors (P. Markl), which relies solely on the mathematical approach. The remaining chapters on Pumps and Gradient Systems (M. Martin and G. Guiochon), Preparative Liquid Chromatography (A. Wehrli) and Mass Spectrometry (E. Kenndler and E. R. Schmid) do not reach the standard of the three best sections, but no part of the book is less than a useful guide to an understanding of HPLC instrument components.

The literature coverage of individual sections varies as much as the subject matter, but on the whole coverage is reasonably complete up to 1977, with one or two 1978 references. Within these limits, most of the major papers on each of these equipment topics do appear in the reference lists at the ends of separate sections.

It is a considerable disappointment that the standard of presentation of individual chapters is so variable. The cost of the book is not unreasonable for what is essentially a reference volume on apparatus, but is too high for recommendation for individual purchase. However, until a better balanced competitor appears this volume must be a good choice for the shelves of the larger chemical libraries. R. A. WALL

ENVIRONMENTAL POLLUTANTS: DETECTION AND MEASUREMENT. Edited by TAFT Y. TORIBARA, JAMES R. COLEMAN, BARTON E. DAHNEKE and ISAAC FELDMAN. *Environmental Science Research, Volume* 13. Pp. xii + 500. New York and London: Plenum. 1978. Price $51.

This is the 13th volume in the series Environmental Science Research and it records the proceedings of the 10th International Conference on Environmental Toxicity held at Rochester, New York, in May 1977. This conference, with the detection and measurement of environmental pollutants as its theme, was unusual in that there was a limited attendance of around only 30, of whom 75% were presenters of papers. The reason given in the Preface that this small gathering "should provide more meaningful discussion" is hardly convincing, nor is the international designation given to this conference, only two participants apparently being from an organisation outside the USA.

The book follows the pattern of the conference in that the 23 papers with their associated discussions, presented in five sessions, are published as chapters within five titled sections. The first section of four chapters deals with general aspects of the specification of analytical problems in the detection and measurement of pollutants in the environment. This is followed by a section with the somewhat misleading title of "More Familiar Principles" and containing a miscellancy of five singularly interesting but unrelated topics, as diverse as ion-selective electrodes and the

identification of the source and the fate of petroleum from oil spills at sea. The four chapters on methods for field use that follow strikingly illustrate the advances made in the sampling and detection of gaseous pollutants, ranging from the use of passive samplers involving gas permeation to that of lasers.

The true value of this volume is probably to be found in the last two sections where some of the latest and most powerful analytical techniques for use in pollution studies are described. The first five chapters concern the application of various microprobe methods, such as transmission electron microscopy, secondary ion mass spectrometry, auger electron spectroscopy and X-ray photoelectron spectroscopy to the analysis of airborne particulates arising from or associated with pollution. Also included is an account of the development and an assessment of the potential of one of the newest microanalytical techniques, that of electron energy loss spectroscopy. In the final section, devoted to a variety of physical analytical methods, the applications of the two well established techniques of X-ray and nuclear activation analysis are reviewed. Also, a valuable re-evaluation is made of the potential use of Raman spectroscopy in the chemical identification of airborne particulates. Other topics described are the use of time-of-flight spectroscopy for measuring the size distribution of airborne particulates and of surface ionisation mass spectrometry for the continuous analysis of both inorganic and organic pollutants in air or water.

This is a generally well produced volume, most chapters being of considerable individual merit. However, the reviewer finds difficulty in recommending it either to the general reader wishing for an overview of the present state of the art of detecting and measuring environmental pollutants, or to the instrumental specialist who has an interest in developing new and better methods of analysis. The book has something for each class of readership but possibly not sufficient to justify the high cost of around £25. Rather, this is seen as a reference book for the library shelf where all can have access.

R. Wood

Studies in Gas Chromatography - Chemical Ionisation Mass Spectrometry of Some Silicate Anions

Chemical ionisation mass spectrometry of trimethylsilyl derivatives of the anions SiO_4^{4-}, $Si_2O_7^{6-}$, $Si_3O_9^{6-}$ and $Si_3O_{10}^{8-}$ has been studied using isobutane as the reagent gas. The protonated molecular ion was observed in all recorded spectra. Also, the chemical ionisation spectra showed much fewer fragment peaks when compared with the corresponding electron impact spectra.

Keywords: *Chemical ionisation mass spectrometry*; *silicate anions*

J. B. ADDISON

Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada, B3H 3Z1.

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Internal Standardisation and its Value in the Assessment of the Suitability of the Column for Quantitative High-performance Liquid Chromatography

The requirement that the best precision be obtained for a pharmaceutical raw material assay using automated high-performance liquid chromatography, with valve injection, over long runs, suggested that the inclusion of an internal standard would be beneficial in allowing an additional method for the calculation of results. The accurate volume injection of the valve and loop system thus permitted standard calibration and subsequent sample evaluation by the peak-height ratio method as well as from absolute peak heights alone. After a number of experiments, column deterioration rendered the assays invalid, and retrospective analysis of the data demonstrated the merits of internal standard related parameters in showing the decline in performance of the column.

The major benefits derived from the approach adopted were criteria for judging the acceptability of the assay results, without excessive replication for a particular sample, and prior warning of the need to re-pack the column.

Keywords: *Quantitative high-performance liquid chromatography*; *internal standardisation*; *column assessment*; *pharmaceutical analysis*; *automatic injection*

R. A. MOORE

Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey, GU20 6PH.

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Determination of Small Amounts of Selenium in Organic Matter

Report prepared by the Metallic Impurities in Organic Matter Sub-Committee

Methods for determining small amounts of selenium in organic matter have been examined. Determination first requires oxidative destruction of organic matter. The usual oxidising systems employing sulphuric acid initially, with hydrogen peroxide, nitric and/or perchloric acids, gave very low recoveries of selenium when high proportions of fats were present in the samples. Continuous combustion and oxygen flask methods were also unsatisfactory. A wet-oxidation procedure in which nitric and perchloric acids (5 + 1) were followed by nitric and sulphuric acids gave satisfactory results. It was important to ensure that selenium was present as selenium(IV) by boiling the solution with hydrochloric acid after the oxidation. Colorimetric, gas - liquid chromatographic, fluorimetric and atomic spectroscopic methods were considered for the selenium determination; of these, the last two were selected for collaborative trials. For the fluorimetric finish, the solution, after oxidation, was treated with 2,3-diaminonaphthalene reagent, the complex was extracted with cyclohexane or dekalin and the fluorescence in the organic phase was measured in a spectrofluorimeter with excitation at 369 nm and emission reading at 525 nm. Atomic-absorption or fluorescence measurements were made after hydrogen selenide generation and atomisation. The precisions of analyses for samples with selenium contents from less than 0.1 to 10 $\mu g\ g^{-1}$ by the fluorimetric and hydride generation methods are illustrated.

Keywords: *Selenium determination*; *wet oxidation*; *fluorimetry*; *atomic spectroscopy*

ANALYTICAL METHODS COMMITTEE
The Chemical Society, Burlington House, London, W1V 0BN.

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Hydrocarbon Analysis of Naphtha Using Capillary-column Gas Chromatography Under Isothermal Conditions

Short Paper

Keywords: *Hydrocarbon analysis*; *naphtha*; *capillary-column gas chromatography*; *Kovàts retention indices*

PRADEEP KUMAR, S. L. S. SAROWHA and P. L. GUPTA
Indian Institute of Petroleum, Dehra Dun-248005, India.

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The Impact Cup: A Simple Aid in Flame Spectrometric Analysis at High Analyte Concentrations

Short Paper

Keywords: *Impact cup*; *flame spectrometry*; *high analyte concentrations*; *pneumatic nebuliser*

MALCOLM S. CRESSER
Department of Soil Science, University of Aberdeen, Meston Walk, Aberdeen, AB9 2UE.

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