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Selective Oxidation of Formaldehyde to Carbon Dioxide From High Ionic Strength Solution for Carbon-13 Analysis by Mass Spectrometry

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A method for determining the carbon isotopic composition of sub-milligram to milligram amounts of formaldehyde in high ionic strength aqueous solution has been developed. The procedure involves a highly selective two-step oxidation to CO_2, followed by purging, drying and cryogenic trapping of the CO_2. The ^{13}C content of processed samples is determined by mass spectrometry. Calibration of the method with an isotopic standard gave yields in the range 85–92%, while isotopic reproducibility (compared with quantitative combustion) was excellent. It is anticipated that the oxidation procedure will prove useful for the analysis of atmospheric formaldehyde collected by sulphito-based substrates.

Keywords: *Carbon-13; formaldehyde; mass spectrometry; selective oxidation*

Formaldehyde is a ubiquitous component of the environment: it is found in the background atmosphere,[1] in rural and urban air,[2] in indoor environments[3] and in biological systems.[4] Elevated levels of formaldehyde are irritating to the mucous membranes,[5] and long-term exposure to airborne formaldehyde has been linked to a higher incidence of cancer (relative to control groups) in laboratory animals.[6] Methods to determine the concentration of airborne formaldehyde in both indoor[7,8] and atmospheric[9,10] environments have therefore been developed. While formaldehyde concentration data alone can sometimes supply all the necessary information (*e.g.*, reactivity or toxicity) in a given situation, there are other occasions when further information is required, for example, the sources of the observed formaldehyde and the relative source strengths. For such occasions carbon isotopic analysis can be of great value. In our laboratory, the isotopic composition of formaldehyde in the clean atmosphere has been used to deduce relative source strengths of methane and non-methane natural hydrocarbons. The technique is fairly general, however.

A successful procedure involves collection of the formaldehyde sample,[11] followed by conversion into CO_2 which must be: (*i*), quantitative or nearly so; (*ii*), isotopically reproducible; (*iii*), highly selective for formaldehyde; (*iv*), effective in a typical collection matrix of high ionic strength; and (*v*), able to produce about 1 mg of CO_2 for mass spectrometry. In this paper, the final technique developed for this conversion is presented.

Experimental

Materials

All inorganic chemicals were of analytical-reagent grade without organic stabilisers; most of the reagents were pre-analysed to ensure a low organic carbon content. The isotopic standard used was the simple formaldehyde - hydrogen sulphite complex, sodium hydroxymethanesulphonate (HMSA). The salt was prepared by dissolving 3 g of $NaHSO_3$ in a minimum volume of H_2O, then adding a stoicheiometric excess (≈10%) of formaldehyde (as formalin, 37% m/m, Fisher Scientific). Crystals were formed in the solution on the addition of ethanol. These crystals were washed with ice-cold ethanol after recrystallisation and then dried at 110 °C for 2 h. Elemental analysis (Perkin-Elmer Model 240 C Elemental Analyser) showed a carbon composition of 7.79%, compared with the required 7.89% for the monohydrate $HOCH_2SO_3Na\cdot H_2O$. The crystalline product was also analysed by gas chromatography to ascertain whether any volatile impurities were present. One microlitre of an aqueous solution of the salt (≈3 M) was injected on to a PB-1 capillary column in a Hewlett-Packard Model 5880 gas chromatograph. The oven was temperature programmed from 35 to 235 °C at 2 °C min^{-1} intervals after an initial period of 10 min at 35 °C; the run was then continued isothermally at 235 °C for an additional 6 min. The flow-rate was 2.9 ml min^{-1}, the carrier gas N_2 and detection was by flame ionisation. Aside from a small signal, which was attributed to formaldehyde, no peaks were apparent. Conspicuously absent (*i.e.*, less than 30 ng of C) were the anticipated carbon contaminants, methanol (present at 10% levels in formalin as a stabiliser) and ethanol (the recrystallisation solvent).

Apparatus and Procedure

Atmospheric formaldehyde is usually collected in solution or on impregnated filters which are subsequently washed. As carbon isotopes are to be determined, only inorganic collection substrates are suitable; those found to be most useful have been sodium or potassium hydrogen sulphite (pyrosulphite) impregnated filters. Subsequent extraction gives about 15 ml of high ionic strength sulphite - sulphate solution.

All reactions are conducted under a positive pressure of helium and/or a helium purge. The solution is contained in a round-bottomed flask equipped with a purging frit and micro-electrode (introduced via an Ace-thread micro-electrode port). The purge gas exits through a small cooled condenser and then passes via high-vacuum fittings (Cajon) to a series of traps and valves which isolate the product, CO_2, from the H_2O and He purge gas.

Samples were treated according to the following procedure: (*i*), an aliquot of the solution is taken to be analysed for formaldehyde content. Helium purge gas is turned on at a low flow-rate; (*ii*), concentrated $HClO_4$ (72%, Mallinckrodt) is added dropwise through the micro-electrode port until the pH is ≈1. The helium purge is continued for 1 h to remove SO_2 and any volatile organic substances; (*iii*), aqueous NaOH (CO_3^{2-} free) is added via a syringe to bring the pH to ≈12, followed by a 0.2-ml aliquot of H_2O_2 solution (30% unstabilised, Ashland). The sample is heated and kept at boiling-point until gas evolution stops (about 20 min). The formaldehyde is converted to formate at this point, and excess of H_2O_2 destroyed; (*iv*), the solution is re-acidified (to pH ≤4) with concentrated $HClO_4$ and purged for 1 h to remove any

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contaminant CO_2. The traps and valves downstream of the reaction vessel are flushed with helium, each trap is then cooled to the appropriate temperature with a Dewar flask containing liquid N_2 or a mixture of dry ice and acetone; (*v*), 1 g of $HgCl_2$ (Mallinckrodt) and 1.0 g of $AgClO_4$ (Aldrich) are added through the port. This combination of reagents quantitatively and selectively oxidises HCOOH to CO_2. The reaction proceeds at boiling-point for 2 h with a constant helium purge. The CO_2 is collected at −196 °C; (*vi*), the heating and the helium flow are then discontinued. Helium is removed by vacuum from the CO_2 sample tube to a remaining pressure of <1 Torr. The contents of the sealed U-tube are submitted for determination of CO_2 yield and ^{13}C content. In experiments to date, both yield and ^{13}C content have been determined at the University of Arizona Laboratory of Isotope Geochemistry. Yield is determined by pressure, temperature and volume measurement; stable carbon isotope ratios are determined on a modified VG 602 mass spectrometer dedicated to CO_2 analysis. The McKinney - Nier experimental arrangement,[12] which compares the signal of a sample with that of a standard, is capable of 0.05‰ internal precision, with system analytical precision on most samples better than 0.1‰ (where the symbol "‰" denotes "per thousand").

Discussion

Effect of Chemical Parameters

The acidification, purging and H_2O_2 oxidation of formaldehyde to formate are based on well known reactions, and application of these reactions has been straightforward. The key reaction, *i.e.*, the one that provides the selectivity necessary to the method, is based on the oxidation of formic acid by $HgCl_2$:

$$HCOOH + 2HgCl_2 \rightarrow CO_2(g) + Hg_2Cl_2(s) + 2HCl \quad (1)$$

It has long been known that this is both a selective and quantitative conversion, but only at low ionic strength, with buffering[13] to pH 4 and, specifically, with low concentrations of chloride and sulphate.[14]

Unfortunately, the collection of atmospheric formaldehyde with sulphito surfaces produces solutions of high ionic strength, of which soluble sulphate is a major component. It is hypothesised that complexation of Hg^{2+} by these anions substantially affects the kinetics of the reaction. A series of modifications to the general procedure was therefore undertaken in an attempt to determine the effects of chemical complexation, not only on the kinetics, but also on the carbon isotopic composition. The ultimate goal was to find conditions where specificity of the reaction and measurable kinetics could be achieved despite the unfavourable matrix. Such a study has not previously been reported in the literature.

Samples prepared from 10% $NaHSO_3$ solution and weighed amounts of standard HMSA were run through the Hg_2Cl_2 oxidation procedure under the following conditions: sample 1, acidified with concentrated H_2SO_4 (98%, EM Science); sample 2, acidified with concentrated HCl (37% MCB Reagents) (in both of these samples the given acids replaced $HClO_4$ in the method described above, and no silver salts were added); sample 3, $HClO_4$ was used for acidification and $AgNO_3$ was added; sample 4, used the method above, *i.e.*, $HClO_4$ and $AgClO_4$. Table 1 shows both the yields and isotopic compositions of the CO_2 products (the yield was insufficient in sample 2 to allow isotopic analysis).

It is clear from the last column of Table 1 that the isotopic composition of the CO_2 product is strongly dependent on yield. However, it needs to be shown that under the reaction conditions proposed here (see sample 4), fractionation is negligible.

Table 1. Chemical enhancement and inhibition of $HgCl_2$ - HCOOH reaction

Sample No.	Yield, %	$\delta^{13}C$,* ‰
1	20	−53.7
2	1.6	—
3	85	−37.5
4	91	−36.6

* $\delta^{13}C$ = isotopic composition of samples, where ‰ is per thousand (per 10^3).

Silver Salts in the $HgCl_2$ - HCOOH Reaction

The chloride concentration in sample 2 was ≈1 M; the dominant Hg^{II} species under these conditions should be $HgCl_4^{2-}$. Mercury(II) sulphate decomposes in aqueous solution to form a sparingly soluble basic sulphate, $HgSO_4.2HgO$. The negative effects of sulphate and chloride on the yield are illustrated dramatically in Table 1. Because complexation of the mercury results in poor yields, two important chemical changes were implemented to address the situation. The first was use of the non-complexing perchloric acid for the acidification steps, and the second was the addition of silver salts. The original idea behind the addition of silver to the $HgCl_2$ - HCOOH system is conveyed by the following set of reactions

$$HgCl_2(aq) \rightleftharpoons HgCl^+(aq) + Cl^-(aq) \quad (2)$$

$$Ag^+(aq) + Cl^-(aq) \rightarrow AgCl(s) \quad (3)$$

$$HgCl_2(aq) + Ag^+(aq) \rightarrow HgCl^+(aq) + AgCl(s) \quad (4)$$

While this mechanism may be the correct one, other possibilities, such as a catalytic effect by the silver, cannot be ruled out. Dhar[14] observed the catalysis of the $HgCl_2$ oxidation of formate, when oxidants such as $KMnO_4$, $K_2Cr_2O_7$ or H_2O_2 were added in small amounts, and similar effects were noted for the Ag^+ - formate reaction; however, the two oxidants (*i.e.*, Hg^{2+} and Ag^+) were not tried together. It appears unlikely that silver ion is involved directly as an oxidising agent, as Ag(s) is not observed as a product in the reaction mixture, while AgCl(s) and $Hg_2Cl_2(s)$ are clearly distinguishable.

Isotopic Reproducibility

To evaluate the method for possible fractionation effects, the HMSA standard was run through the chemical oxidation procedure and the carbon isotopic values compared to those obtained from quantitative combustion. The ^{13}C content is expressed as $\delta^{13}C$:

$$\delta^{13}C\ (‰) = \frac{(^{13}C/^{12}C)\ \text{sample} - (^{13}C/^{12}C)\ \text{standard}}{(^{13}C/^{12}C)\ \text{standard}} \times 1000 \quad (5)$$

Table 2. Carbon-13 content of HMSA isotopic standard determined by combustion (CMBN)

Sample	Yield, %	$\delta^{13}C$, ‰
CMBN 1	100	−36.2
CMBN 2	100	−37.5
		Mean = −36.8 ± 0.9

Table 3. Carbon-13 content of HMSA isotopic standard (STND) determined by chemical oxidation

Sample	Yield, %	$\delta^{13}C$, ‰
STND 1	85	−37.5
STND 2	92	−34.6
STND 3	91	−36.6
STND 4	90	−35.1
		Mean = −36.0 ± 1.3

Table 4. Fractionation factors for the $HgCl_2$ - HCOOH reaction

Sample (from Table 1)	Yield (F), %	$\delta^{13}C$, ‰	k_1/k_2
1	20	−53.7	1.020
3	85	−37.5	1.002
4	92	−36.6	0.999

Table 5. Isotopic exchange (EXNG) between $HO^{12}CH_2SO_3^-$ (aq) and $H^{13}CO_3^-$ (aq)

Sample	Yield, %	HMSA/μmol	$Ba^{13}CO_3$/μmol	$\delta^{13}C$, ‰
STND 4 ..	90	33.4	—	−35.1
EXNG 1 ..	73.8	18.5	52.4	+504
EXNG 2 ..	68.5	23.9	38.8	+23.3

The PDB (from Peedee Belemnite, a South Carolina carbonate formation) standard, with a $^{12}C/^{13}C$ ratio[15] of 88.99, has been used for all data here.

Combustion of samples was performed at 900 °C in oxygen over CuO to assure complete conversion of the carbon to CO_2. For the chemical oxidation procedure, weighed amounts (*ca.* 5 mg) of the HMSA standard were dissolved in 10 ml of 10% $NaHSO_3$, and the solution was then subjected to the chemical oxidation procedure as described above. Isotopic results are given in Tables 2 and 3. Agreement between the two methods was good, but the spread of values was wider than expected. This may result from either inhomogeneities in the HMSA standard (*e.g.*, two different crystalline forms with different values of $\delta^{13}C$), or a slow loss of formaldehyde from the solid, resulting in fractionation over a period of time.

Overall, from the data in Table 1 (sample 4), and Tables 2 and 3, it was concluded that uncertainties owing to fractionation in the oxidation procedure were negligible compared to the magnitude of the effect being measured in the atmosphere.

Evidence for a Change in Mechanism

The kinetics of the original reaction between HCOOH and $HgCl_2$ were known to be pH dependent, reaching a maximum[13] at pH 4. While accurate reaction rate data as a function of pH have not been obtained for the current procedure, pH does not seem to be a critical factor when silver salts are added. In fact, the reaction is customarily run at pH 1 with a high yield in the above procedure, and samples which have been run at pH 2 do not yield measurably different results.

A change in the isotopic ratio of a reaction product relative to the starting material (isotopic fractionation) can occur by three main processes; the one of concern here is the kinetic isotope effect, characterised by the fractionation factor, k_1/k_2, where k_1 is the reaction-rate constant for the lighter isotopic molecule and k_2 that for the heavier isotopic molecule. The fractionation factor can be determined[16] from the fractional conversion of the reaction, F, as follows:

$$k_1/k_2 = \frac{\log(1-F)}{\log[1-(FR_p/R_o)]} \quad \ldots \quad (6)$$

where R_o is the isotopic ratio in the initial reactant molecule, and R_p the isotopic ratio in the product molecule. The fractionation factor is characteristic for a given reaction.

Table 4 gives the calculated fractionation factors for the reaction conditions of Table 1; R_o was calculated from the mean composition of Table 2. The addition of silver (sample 1 to sample 3) lowered the fractionation an order of magnitude from 2 to 0.2%; other samples have confirmed this general relationship. Smaller fractionation factors are generally indicative of lowered activation energies in a family of reactions, although other parameters (*e.g.*, steric hindrance or induction effects) must be considered.[17] It is apparent, however, that a dramatic change in the reactive species or the reaction mechanism occurs when silver is added to the system.

Method Selectivity

Blanks for the procedure were determined by following all the individual steps except for the addition of formaldehyde. The total amount of CO_2 produced was less than 50 μg, which represents less than 5% of a typical sample.

The atmosphere contains compounds with similar chemical properties to formaldehyde, and it was not known whether the collection procedure would be sufficiently discriminating against these species. Further, while the reactions incorporated into the procedure were designed to be selective, the effect of the modifications described above (addition of silver salts and $HClO_4$) was unknown. Therefore, the selectivity of the method was tested by spiking a standard formaldehyde solution with some model organic compounds and running it through the chemical oxidation procedure.

A solution containing 10 μl of CH_3COOH (Mallinckrodt), 10 μl of CH_3OH (EM Science), 10 μl of CH_3COCH_3 (Mallinckrodt), and 5 ml of CH_3CHO vapour (Aldrich) (all analytical-reagent grade) was made up to 10 ml in a calibrated flask with a 5% $NaHSO_3$ solution. The CH_3CHO vapour was bubbled slowly into the solution via a syringe. A 250-μl aliquot of this organic solution and a weighed standard addition of HMSA were then added to 10 ml of 10% $NaHSO_3$ solution in the reaction vessel; the complete oxidation procedure was then followed. The CO_2 produced during the 2 h reaction was collected in 3 M carbonate-free NaOH(aq), the collection solution was then heated under a helium purge to remove any volatile substances. The solution was finally acidified and purged with helium; drying and collecting of CO_2 were performed as in the standard procedure.

Results of the isotopic analysis showed a yield of 95.2% based on 3.6 mg of added HMSA and $\delta^{13}C$ of −35.0‰. This impressive isotopic reproducibility was achieved despite the very high levels of non-formaldehyde carbon. The original method[18] was known to be highly selective (used, for example, to determine formic acid in acetic acid); the modified method obviously retains excellent selectivity in the presence of compounds with similar chemical and physical properties. The only known interfering atmospheric compound is formic acid: for the results to be valid, samples must not contain formic acid in appreciable amounts.

Isotopic Exchange

The literature does not suggest that carbon exchange between formaldehyde and carbon dioxide is likely, as the former possesses essentially non-exchangeable protons.[19] However, because CO_2 is by far the most dominant carbon-containing gas in the atmosphere, it was considered necessary to demonstrate a complete selectivity of the method against CO_2 contamination at an isotopic level. The aqueous reaction examined was

$$HO^{12}CH_2SO_3^- + H^{13}CO_3^- \overset{K}{\rightleftharpoons} HO^{13}CH_2SO_3^- + H^{12}CO_3^- \quad (7)$$

Experimentally, sodium sulphite (0.8 g, Mallinckrodt), sodium hydrogen sulphite (0.2 g, Fisher), HMSA standard (mg levels) and $Ba^{13}CO_3$ (mg levels, 99 at.-% ^{13}C, Sigma) were added to the reaction chamber, followed by 10 ml of H_2O. The apparatus was sealed and left to stand at room temperature (25 °C) for 24 h. The standard oxidation procedure was then followed from step (*ii*) above, except that purging was extended to 2 h in step (*iv*). Under these conditions, the sulphate concentration (present due to oxidation) is sufficient to precipitate the barium and thereby release the carbonate (barium sulphite is also insoluble).

The sulphite to hydrogen sulphite ratio in solution gives a pH of about 7.7; at this buffered pH, the carbonate system will be largely HCO_3^- with some CO_2 formed {*i.e.*, $[HCO_3^-]/[CO_2](aq)$ = antilog (pH − pK) = 22.4}. Simultaneously

solving K_A, Henry's law ($K_H = 10^{-1.5}$ mol atm^{-1}) and the mass balance equations indicate that the equilibrium state of the CO_2 system is 86% aqueous, with 96% of the aqueous form present as HCO_3^-. Note that a 99 at.-% ^{13}C compound means a $\delta^{13}C$ of $\approx +10^7$‰, as mathematically defined in equation (5). As the isotopic analysis is capable of resolution to better than 1‰, this experiment is potentially extremely sensitive to any ^{13}C incorporated into the final sample from the original $Ba^{13}CO_3$ source. Because of the experimental design, carbon-13 can appear in the final sample either by incorporation into the HMSA [equation (5)] or due to incomplete purging of $^{13}CO_2$ (aq) in the initial acidification step. The data, therefore, give an upper limit on both processes. Data for two experiments are given in Table 5.

The theory of isotopic exchange chemistry[20] leads to the following expression

$$\mathrm{Ln}\frac{(X_\infty - X_o)}{(X_\infty - X)} = k(A + B)t \quad \ldots \ldots \quad (8)$$

where X_∞, is the $[HO^{13}CH_2SO_3^-]$ at equilibrium; X_o, the $[HO^{13}CH_2SO_3^-]$ at the start of the experiment; X, the $[HO^{13}CH_2SO_3^-]$ at the end of the experiment; A, is $[HO^{12}CH_2SO_3^-] + [HO^{13}CH_2SO_3^-]$; B, is $[H^{12}CO_3^-] + [H^{13}CO_3^-]$; t is time in min; and k, is the exchange rate constant.

For EXNG 1, $k = 9.6 \times 10^{-4}$ l mol^{-1} min^{-1} and for EXNG 2, $k = 7.7 \times 10^{-4}$ l mol^{-1} min^{-1}. Considering the errors involved in working with small amounts of exchanged isotopes, the results are in good agreement. Whether or not these small but positive results indicate actual isotopic exchange or are caused by the residual solubility of CO_2 is unknown, but the latter seems more likely. It can be calculated that the observed enrichment corresponds to a CO_2 purging efficiency of 99.998% for sample EXNG 1 and 99.9996% for sample EXNG 2. The rate of isotopic exchange (if indeed non-zero) is extremely small. When the $\delta^{13}C$ of any collected atmospheric CO_2 (−7‰) is compared[21] with the composition used in the laboratory simulation above (+10^7‰) it is clear that there should be no measurable isotopic exchange between formaldehyde and CO_2 collected from the atmosphere.

Conclusion

Formaldehyde can be oxidised to CO_2 selectively and quantitatively from dilute aqueous solution by a two-step process, involving conversion to formate by reaction with basic H_2O_2 and oxidation of HCOOH with $HgCl_2$. The latter reaction imparts remarkable selectivity to the method so that the oxidation can be performed in the presence of other organic compounds. The addition of silver salts to the reaction mixture increases the rate of reaction considerably; high concentrations of ionic compounds which constitute an interference otherwise can be readily tolerated. A change in the chemical mechanism is apparent when silver salts are used. Isotopic reproducibility of the method is excellent, and percentage yields are high (generally greater than 90%). The possibility of isotopic contamination by atmospheric CO_2 via isotopic exchange is non-existent. The method has been used to measure the ^{13}C content of formaldehyde in the clean atmosphere.[11]

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Determination of the Enantiomeric Composition of Cycloprofen by Proton Nuclear Magnetic Resonance Spectroscopy With a Europium(III) Shift Reagent

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The enantiomeric composition of cycloprofen was determined in a simple and reliable manner by proton nuclear magnetic resonance spectroscopy with a chiral europium(III) shift reagent. Effective complexation with the shift reagent took place in CCl_4 after conversion of the enantiomeric sample into a mixture of the methyl ester derivatives. Optimisation of the experimental conditions in terms of substrate concentration and shift reagent to substrate molar ratio led to two sets of enantiomeric resonance signals of utility for quantitative purposes. Analysis of synthetic enantiomeric mixtures by the proposed method demonstrated an excellent agreement between the assay results and the known masses of each enantiomer present in the mixture samples. The average ± standard deviation recovery values were 100.1 ± 0.4% ($n = 6$) and 99.9 ± 0.6% ($n = 6$) of (−)-cycloprofen depending on whether the quantification is based on the α-methyl protons or ester methyl protons, respectively.

Keywords: *Cycloprofen; enantiomeric composition; proton nuclear magnetic resonance spectroscopic analysis; lanthanide shift reagent*

Cycloprofen, α-methylfluorene-2-acetic acid, is a non-steroidal anti-inflammatory agent that belongs to the class of the 2-arylpropionic acids.[1] As with other compounds in this chemical group, the anti-inflammatory activity of cycloprofen resides only in the *S*(+)-enantiomer. In addition, the racemic modification is known to undergo stereospecific metabolic chiral inversion, a unique biotransformation whereby the inactive *R*(−)-enantiomer slowly converts into the pharmacologically active *S*(+)-antipode.[2–4]

Stereospecific assays are required to discern relationships between the concentration of a chiral drug and the pharmacological and toxicological effects it may elicit *in vivo*. From a regulatory viewpoint, it is essential to determine not only the amount of the biologically active enantiomer present but also to establish the extent of its contamination with the inactive enantiomer. Quantification of the enantiomers of ^{14}C-cycloprofen has been accomplished at the microgram level by a laborious procedure that entails conversion into a pair of L-leucine diastereoisomers which are separated by thin-layer chromatography and measured *in situ* by radiometry.[2] In this paper an alternative approach to the determination of the enantiomeric composition of cycloprofen is described, namely one that combines the use of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and a lanthanide shift reagent.

Experimental

Apparatus

Proton NMR spectra were recorded using a 90 MHz Varian (Sunnyvale, CA, USA) EM-390 spectrometer operating at an ambient probe temperature of 35 ± 1 °C.

Samples and Materials

Samples of (+)-, (−)- and (±)-cycloprofen were a gift from Squibb (Princeton, NJ, USA). The tetramethylsilane (TMS), carbon tetrachloride (CCl_4), tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$] and tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]praseodymium(III) [Pr(hfc)$_3$] were purchased from Aldrich (Milwaukee, WI, USA). Tetramethylsilane was made free of tetrahydrofuran by successive washes with sulphuric acid and saturated potassium carbonate solution. Carbon tetrachloride was distilled prior to use and stored over molecular sieves type 4A (Aldrich). Eu(hfc)$_3$ and Pr(hfc)$_3$ were stored over P_2O_5 *in vacuo* or under a dry N_2 atmosphere.

Preparation of Samples

An accurately weighed amount of cycloprofen (15–16 mg) was placed in a ground-glass, round-bottomed flask, mixed with a mixture of methanol (40 ml) and hydrochloric acid (concentrated reagent-grade, *ca.* 38%) (2 ml), and refluxed for 1 h. The reaction mixture was evaporated to a small volume, and extracted with diethyl ether (3 × 15 ml). The combined diethyl ether extracts were evaporated to dryness under a stream of dry N_2, and the residue was dried in a vacuum oven at 50 °C until a constant mass was attained. Alternatively, the sample was placed in a glass-stoppered flask, mixed with a 0.25 M solution of diazomethane in diethyl ether (3 ml), and allowed to stand at room temperature for 5 min. The solution was then evaporated to dryness under a stream of dry N_2, and the residue was dried to a constant mass at 50 °C in a vacuum oven.

Determination of the Enantiomeric Purity

The residue of the methyl ester derivatives dissolved readily in CCl_4 (0.5 ml) containing 1% v/v of TMS, and the solution was transferred into an NMR tube that contained Eu(hfc)$_3$ (22–23 mg). After mixing, the solution was allowed to stand at room temperature for 10 min, and then used to record the ^{1}H NMR spectrum. The relative intensities of the resonances (peak heights or peak areas) for the enantiomeric ester methyl protons at 6.68 p.p.m. [(+)-enantiomer] and 6.76 p.p.m. [(−)-enantiomer] or, alternatively, of the enantiomeric doublets for the α-methyl protons at 4.04 p.p.m. [(+)-enantiomer] and 3.94 p.p.m. [(−)-enantiomer] were measured. Measurement of peak heights was carried out manually using a ruler

and peak areas were measured using the electronic integrator that is attached to the NMR spectrometer. Both methods gave equivalent results with the accuracy being better than 99.8% of the known amounts of enantiomers present in the synthetic mixtures tested. The measurements were used to calculate the percentages of each enantiomer in the sample from the following equations: % (+)-enantiomer = $100 \times A_{(+)}/A_{(+)} + A_{(-)}$ and % (−)-enantiomer = $100 \times A_{(-)}/A_{(+)} + A_{(-)}$, where $A_{(+)}$ = peak area (or peak height) of the resonance signal for the (+)-enantiomer, and $A_{(-)}$ = peak area (or peak height) of the resonance signal for the (−)-enantiomer.

Results and Discussion

Equilibrium between a substrate and a lanthanide shift reagent is usually reached rapidly on the NMR time scale.[5] Consequently, only a single time-averaged spectrum results from the average of the complexed and uncomplexed substrate molecules.[6] A chiral lanthanide shift reagent co-ordinating to each of the two enantiomers can form rapidly equilibrating complexes that are diastereoisomeric and capable of generating different average chemical shifts. Differences in chemical shifts may arise from at least two sources: (*i*) differences in the equilibrium constants of each enantiomeric complex, thereby resulting in a larger shift for the complex having the larger binding constant; and (*ii*) differences in geometry between the diastereomeric complexes, which translates into differences in the induced shifts (Δδ) for corresponding signals in the two complexes.[7] When these conditions are met, NMR spectroscopy can serve as an

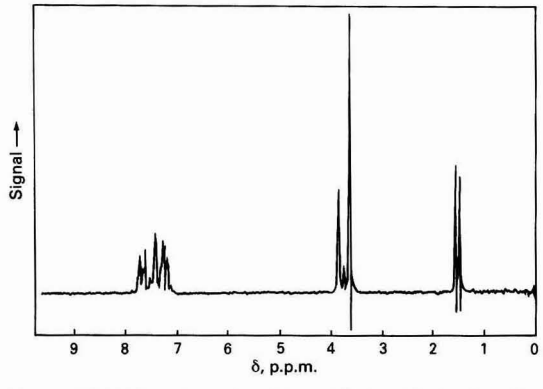

Fig. 1. ^{1}H NMR spectrum of a mixture of (+)- and (−)-cycloprofen methyl esters in CCl_4

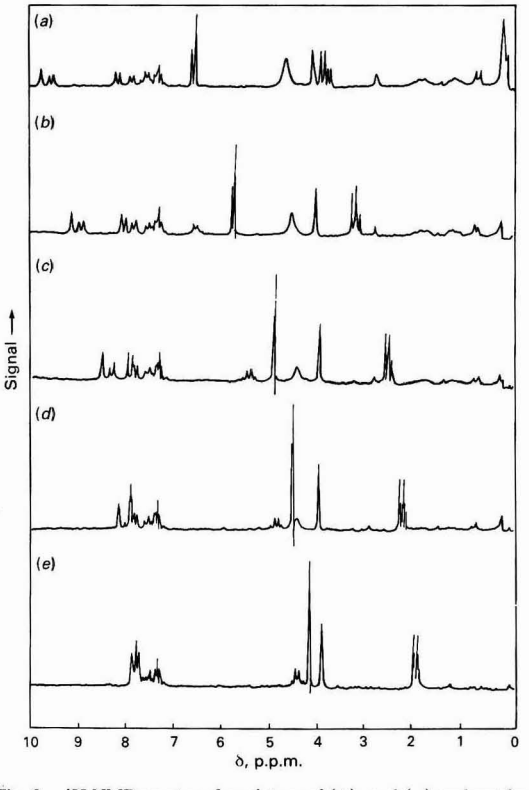

Fig. 2. ^{1}H NMR spectra of a mixture of (+)- and (−)-cycloprofen methyl esters, 0.127 M in CCl_4, after complexation with various molar equivalents of Eu(hfc)$_3$. Molar ratio: (*a*) 0.296; (*b*) 0.207; (*c*) 0.129; (*d*) 0.089; and (*e*) 0.045

Table 1. Shift data for a mixture of (+)- and (−)-cycloprofen methyl esters after complexation with various molar ratios of Eu(hfc)$_3$ to substrate. Total concentration of drug in CCl_4 was 0.127 M

	C–CH_3 signal					CO_2CH_3 signal				
	(+)-Enantiomer		(−)-Enantiomer			(+)-Enantiomer		(−)-Enantiomer		
Molar ratio	δ	Δδ	δ	Δδ	ΔΔδ	δ	Δδ	δ	Δδ	ΔΔδ
0.000	1.52	0.00	1.52	0.00	0.00	3.66	0.00	3.66	0.00	0.00
0.014	1.63	0.11	1.63	0.11	0.00	3.79	0.13	3.79	0.13	0.00
0.029	1.77	0.25	1.75	0.23	0.02	3.96	0.30	3.96	0.30	0.00
0.045	1.93	0.41	1.91	0.39	0.02	4.15	0.49	4.15	0.49	0.00
0.062	2.06	0.54	2.03	0.51	0.03	4.30	0.64	4.32	0.66	0.02
0.080	2.19	0.67	2.15	0.63	0.04	4.45	0.79	4.47	0.81	0.02
0.089	2.21	0.69	2.17	0.65	0.04	4.47	0.81	4.50	0.84	0.03
0.109	2.39	0.87	2.34	0.82	0.05	4.70	1.04	4.73	1.07	0.03
0.161	2.86	1.34	2.78	1.26	0.08	5.26	1.60	5.30	1.64	0.04
0.207	3.31	1.79	3.21	1.69	0.10	5.80	2.14	5.86	2.20	0.06
0.244	3.60	2.08	3.48	1.96	0.12	6.15	2.49	6.21	2.55	0.06
0.256	3.69	2.17	3.57	2.05	0.12	6.27	2.61	6.34	2.67	0.07
0.270	3.80	2.28	3.67	2.15	0.13	6.40	2.74	6.47	2.61	0.07
0.282	3.88	2.36	3.75	2.23	0.13	6.49	2.83	6.57	2.91	0.08
0.296	4.04	2.52	3.91	2.39	0.13	6.68	3.02	6.76	3.10	0.08
0.309	4.13	2.61	4.00	2.48	0.13	6.80	3.14	6.89	3.23	0.09

effective means of investigating the optical purity of organic molecules. In contrast to chromatographic techniques, NMR is free from restraints such as the need for samples of pure analytes for use as reference standards and of enantiomerically pure chiral shift reagents as they only affect the position but not the relative size of the bands stemming from each of the enantiomers in the mixture.

Cycloprofen was not amenable to direct enantiomeric analysis by ^{1}H NMR with lanthanide shift reagents because of poor co-ordination with the lanthanide ion. Conversion of the drug into the methyl ester derivative circumvented the problem because it is well known that esters can co-ordinate more effectively than their carboxyl-containing parent compounds.[8–15] Esterification with either methanolic hydrochloric acid or ethereal diazomethane was rapid and quantitative and afforded a product of such purity that it did not require further purification. Quantitative conversion of the acid into the methyl ester derivative was ascertained in two ways: (*i*) the ratio of the integrals for the protons of the α-methyl and ester methyl groups was monitored for different periods of time and no change was noticed after refluxing for 1 h; and (*ii*) it was observed that whereas the carboxylic acid form was only sparingly soluble in CCl_4, the residue of the methyl esters dissolved almost immediately. Fig. 1 shows the complete ^{1}H NMR spectrum of the uncomplexed enantiomeric methyl esters of cycloprofen in CCl_4. The α-methyl protons resonated as a doublet centred at 1.52 p.p.m., while the ester methyl protons gave rise to a singlet at 3.36 p.p.m. Additionally, the methylene protons of the fluorene moiety were detectable as a singlet at 3.85 p.p.m. and the aromatic protons of the same moiety resonated as multiplets in the 7.8–7.1 p.p.m. spectral region. The addition of $Eu(hfc)_3$ to the mixture of enantiomeric methyl esters caused all of the signals to shift to lower field, some of the signals to split into two, and a marked resolution of certain aromatic signals and of signals in the aliphatic region. Further, in the presence of the shift reagent the spectra of the methyl esters of cycloprofen gave evidence that the sense of non-equivalence was different for different sets of protons. For instance, while the resonance for the α-methyl protons of the (+)-enantiomer was shifted to a greater extent than that for the (−)-enantiomer the situation was reversed for the ester methyl signals. These differences in the sense of equivalence clearly demonstrate that the induced enantiomeric shift differences ($\Delta\Delta\delta$) between the signals for the two enantiomers are not simply the result of different equilibrium constants but also of differing structural features for each of the enantiomer - shift reagent co-ordination complexes.[7]

The magnitude of $\Delta\delta$ and $\Delta\Delta\delta$ in the presence of $Eu(hfc)_3$ was found to vary proportionally to the changes in shift reagent to substrate molar ratios. Optimum molar ratios and concentrations of substrate for use in the analysis were

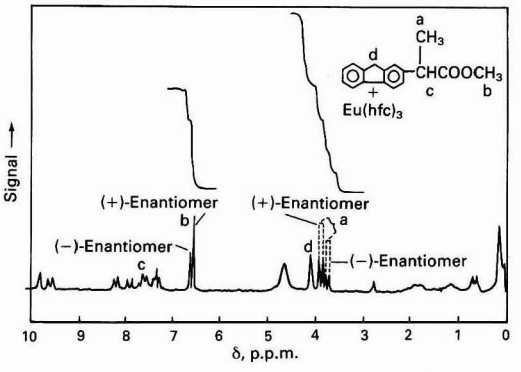

Fig. 4. ^{1}H NMR spectrum of a mixture of (+)- and (−)-cycloprofen methyl esters, 0.127 M in CCl_4, after complexation with 0.207 molar equivalents of $Eu(hfc)_3$

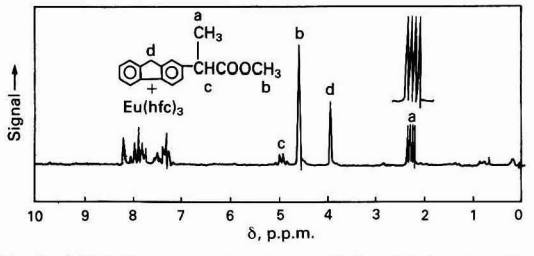

Fig. 3. ^{1}H NMR spectrum of a mixture of (+)- and (−)-cycloprofen methyl esters, 0.127 M in CCl_4, after complexation with 0.089 molar equivalents of $Eu(hfc)_3$

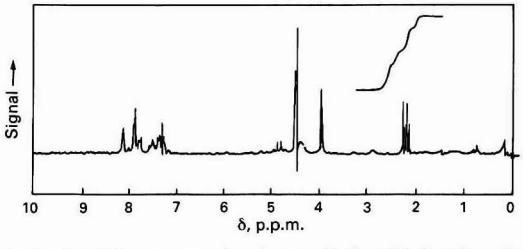

Fig. 5. ^{1}H NMR spectrum of a mixture of (+)- and (−)-cycloprofen methyl esters, 0.127 M in CCl_4, after complexation with 0.089 molar equivalents of $Eu(hfc)_3$ and enrichment with the (+)-enantiomer

Table 2. Determination of the enantiomeric composition of synthetic mixtures of (+)- and (−)-cycloprofen by ^{1}H NMR with a chiral lanthanide shift reagent

	Amount added*			Amount of (−)-enantiomer found					
				C–CH_3 signal			CO_2CH_3 signal		
Sample	(+)-Form/ mg	(−)-Form/ mg	(−)-Form, % of mixture	mg	% of mixture	Recovery,† %	mg	% of mixture	Recovery,† %
1	118.16	2.51	2.08	2.56	2.12	101.9	2.52	2.09	100.4
2	7.62	7.52	49.67	7.50	49.57	99.7	7.55	49.89	100.4
3	5.82	9.34	61.61	9.41	62.08	100.7	9.33	61.59	99.9
4	10.41	4.88	31.78	4.84	31.76	99.8	4.86	31.82	100.2
5	11.83	3.67	23.68	3.65	23.54	99.4	3.63	23.44	98.9
6	8.43	6.72	44.36	6.71	44.28	99.8	6.68	44.10	99.4
Average						100.2			99.9
SD						0.93			0.55

* Total concentration of drug (0.127 M) in CCl_4 and $Eu(hfc)_3$ to substrate molar ratio of 0.296.
† Recoveries were calculated from (amount found ×100)/amount added.

systematically investigated by using a constant concentration of substrate and varying concentrations of shift reagent. The desired change in molar ratio was attained by adding a known amount of shift reagent to an NMR tube followed by aliquots of a substrate stock solution in CCl_4. With $Eu(hfc)_3$ as the shift reagent, molar ratios in the range 0.000–0.309 produced the spectral changes shown in Fig. 2 without any evidence of line broadening. The $\Delta\delta$ values were found to be sensitive to both the distance and the orientation of the resonating nuclei with respect to the paramagnetic centre.[16–19] Table 1 summarises the shift data for (+)- and (−)-cycloprofen methyl esters in CCl_4 at various $Eu(hfc)_3$ to substrate molar ratios and a substrate concentration of 0.127 M.

Unambiguous identification of the enantiomeric signals was accomplished by comparing the intensities of the resonance signals before (Figs. 3 and 4) and after enrichment in one of the enantiomers (Fig. 5). The most useful signals for quantitative work were those of the enantiomeric α-methyl protons at 6.76 p.p.m. [(−)-enantiomer] and 6.68 p.p.m. [(+)-enantiomer] (the expansion is shown as an inset in Fig. 3), and ester methyl protons at 4.04 p.p.m. [(−)-enantiomer] and 3.91 p.p.m. [(+)-enantiomer]. Adding 0.089 molar equivalents of $Eu(hfc)_3$ to 0.127 M cycloprofen methyl esters in CCl_4 resulted in a useful 0.04 p.p.m. resolution of the enantiomeric α-methyl proton signals into two alternating equidistant doublets, and in the incomplete resolution of the ester methyl signals (Fig. 3). Raising the concentration of the shift reagent caused first an overlapping of the lines at the centre of the alternating doublets from the α-methyl protons and later a crossing over when the $\Delta\Delta\delta$ value became larger than the coupling constant for the doublet, this change occurring at a molar ratio of 0.207. As the molar ratio of shift reagent to substrate increased, the degree of non-equivalence of the enantiomeric ester methyl proton signals also increased in a proportional manner. Sufficiently large $\Delta\Delta\delta$ values for both the α-methyl and ester methyl signals were obtained at a molar ratio of 0.296 and a substrate concentration of 0.127 M in CCl_4, thus providing the analyst with two possible ways of measuring the enantiomeric composition of cycloprofen samples (Fig. 4). However, at higher molar ratios these signals started to overlap with those of both the aromatic protons and the shift reagent.

In comparison with $Eu(hfc)_3$, the use of $Pr(hfc)_3$ as the shift reagent caused severe line broadening even at low shift reagent to substrate molar ratios. As the principal purpose of a chiral shift reagent is to resolve a given pair of enantiomeric signals with the least evidence of line broadening, $Pr(hfc)_3$ was considered inadequate for the purpose on hand.

Several synthetic mixtures, made up by weighing each of the enantiomers of cycloprofen in the proportions shown in Table 2, were assayed by the proposed NMR method. The results of these assays agreed closely with the known masses of each enantiomer in the mixture tested, whether they were based on the integrals of the α-methyl or ester methyl proton resonances. The same data demonstrated the good accuracy of the NMR method. The average ± standard deviation (SD) recovery values were 100.1 ± 0.4% (n = 6) and 99.9 ± 0.6% (n = 6) of (−)-cycloprofen depending on whether the quantification is based on the α-methyl protons or ester methyl protons, respectively.

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Determination of Multi-pesticide Residues in Cereals, Cereal Products and Animal Feed Using Gel-permeation Chromatography

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Residues of organophosphorus, organochlorine and synthetic pyrethroid pesticides and insect growth regulators were determined in cereals and cereal products. Samples were extracted with acetone - methanol and the extracts cleaned-up by gel-permeation chromatography. The mean recoveries were: 99%, organophosphorus; 94%, organochlorine; 99%, synthetic pyrethroid; and 99%, insect growth regulators.

Keywords: *Multi-pesticide residue; gel-permeation chromatography; cereal; cereal product*

Gel-permeation chromatography (GPC) using cross-linked dextran gels has become an established and valuable technique in biochemistry. The technique is widely used for the separation of proteins and other biomolecules of high relative molecular mass in an aqueous or buffered solvent system.[1] Separation is achieved on the basis of molecular size, itself a function of the shape and volume of the molecule, but other mechanisms including partition and adsorption might also be involved. Gel-permeation chromatography was restricted to aqueous solutions until the introduction of an alkylated form of Sephadex gel, which allowed the separation of molecules in organic solvent systems.[2]

A review by Mulder and Buytenhuys[3] described the use of polydextran (Sephadex LH-20), polystyrene (Bio-Beads, Styragel) and polyacrylamide gels with organic solvents to separate a range of low relative molecular mass compounds of widely different physical and chemical properties.

The use of GPC as a clean-up procedure for pesticide residues was reported by Růžička *et al.*,[4] who used Sephadex LH-20 to separate a number of organophosphorus (OP) compounds obtained from plant extracts, although it was concluded that GPC was of limited value because much of the pigmented material extracted from plants such as cabbages co-eluted with the pesticides. Horler[5] investigated the use of Sephadex LH-20 in the determination of pesticides in grain and this gel was found suitable for their clean-up prior to thin-layer chromatography, but no indication was given of the effectiveness of clean-up when analysing the extracts by gas - liquid chromatography (GLC).

A gel (Bio-Beads) prepared from polystyrene has been more extensively evaluated as a clean-up procedure in pesticide residue analysis. Stalling *et al.*[6] reported the use of GPC with Bio-Beads SX-2 to separate organochlorine (OCl), OP and polychlorinated biphenyl (PCB) compounds from lipids contained in fish extracts. This procedure was later automated by Tindle and Stalling[7] and used to separate pesticides from other fat-containing samples. Griffitt and Craun[8] evaluated GPC as a method for separating pesticides from fats and oils. Bio-Beads SX-2 has also been used as a clean-up technique prior to the determination of octachlorostyrenes in fish extracts.[9]

The relative molecular mass exclusion of the Bio-Beads SX gels varies according to the type of gel used. Bio-Beads SX-2 has a nominal exclusion limit of 2700 while SX-3 has an exclusion of 2000. The use of SX-3 which has a lower exclusion limit, should prevent a greater proportion of the high relative molecular mass materials from gaining access to the pores of the gel and this should improve the separation between the lipid-like materials and the pesticides.

Johnson *et al.*[10] reported the use of Bio-Beads SX-3 as a clean-up procedure for animal and plant extracts prior to the determination of OCl and OP pesticides, herbicides and PCB compounds. The wide applicability of Bio-Beads SX-3 is indicated by its use in the identification of PCB compounds, polychlorinated terphenyls, organic pollutants in human adipose tissue,[11,12] in the determination of OP residues in fruit and vegetables,[13] the detection of synthetic pyrethroid (SPYR) residues in plant tissues and soils,[14,15] the determination of chlorpyrifos and its pyridinol metabolite in vines,[16] the analysis of OCl residues in vegetable oil refinery by-products[17] and the determination of OP, OCl, pyrethroid pesticides and fungicides in fruit and vegetables.[18]

A recent review by Shepherd[19] cites the acceptance of GPC for sample clean-up by the Association of Official Analytical Chemists, and that the IUPAC pesticide commission regards GPC as the most applicable clean-up stage for multi-residue procedures. Specht and Tillkes[20] have presented GPC elution data and mini silica gel chromatography for more than 400 pesticides and their metabolites and pollutants as part of a multi-residue method for food and feed of vegetable and animal origin.

One commodity which has received only limited investigation, since the initial work reported by Horler,[5] is wheat. Recent introduction of statutory regulation for maximum residues limits for pesticides in cereals,[21] and an increasing concern over pesticide residues in food, has required the determination of low levels of a wide range of compounds as part of a food surveillance programme. The cereal products that contain high levels of lipids such as wheat germ and bran are particular commodities which require improved clean-up procedures.

The objective of this work was to investigate the application of GPC to the determination of OP, OCl, insect growth regulators (IGRs) and SPYR residues in cereal products.

Experimental

Reagents

Hexane. Glass-distilled grade, from Rathburn Chemicals.

Dichloromethane. Glass-distilled grade, from Rathburn Chemicals.

Methanol. High-performance liquid chromatography (HPLC) grade, from Fisons Scientific Apparatus.

Acetone. Analytical-reagent grade, from BDH.

Acetonitrile. HPLC grade, Rathburn Chemicals.

Extraction solvent. Acetone - methanol (1 + 1, v/v).

Sodium sulphate. Anhydrous analytical-reagent grade, from BDH.

Sodium sulphate solution, 2% *m/v*.

Aluminium oxide. Merck neutral, used without pre-treatment.

Bio-Beads SX-3. 200–400 mesh, from Bio-Rad Laboratories.

Elution solvent. Dichloromethane - hexane (70 + 30, v/v) for GPC column. De-gassed before use by purging with helium.

Apparatus

Laboratory blender. Silverson Machines.
Rotary evaporator. Buchi.
Centrifuge. MSE GF4.
Low-speed grinder. Glen-Creston.

Gel-permeation Chromatography

Column. Anachem glass preparative chromatography column (500 × 25 mm i.d.) fitted with a 40–60 μm porous polytetrafluoroethylene (PTFE) bed support and adjustable plunger.

Sample introduction. Rheodyne PTFE rotary valve fitted with a 5-ml PTFE sample loop.

Solvent delivery. Waters Model M45 HPLC pump; flow-rate, 4 ml min^{-1}.

Fraction collector. LKB Helirack; collection time, 2.5 min per fraction.

Column Preparation

Fifty grams of the gel (Bio-Beads SX-3) were placed in a 500-ml conical flask, 100 ml of GPC elution solvent were added and the gel was left to stand for 24 h at 5 °C. The fully swollen gel was then de-gassed, by applying a vacuum to the conical flask, before slurry packing the column. After the gel had settled the plunger was depressed until a bed height of 30–35 mm was obtained. Elution solvent was pumped through the column at a flow-rate of 4 ml min^{-1} for 2 h prior to use.

Chromatographic Apparatus

Determination of organochlorine compounds

A Perkin-Elmer Model F17 instrument (or equivalent) equipped with a ^{63}Ni electron capture detector (ECD) was operated under the following conditions: detector and injector temperature, 250 °C; nitrogen carrier gas flow-rate, 30 ml min^{-1}; and detector purge gas flow-rate, 30 ml min^{-1}. A 2 m × 4 mm i.d. glass column packed with a mixed packing consisting of 3% OV-61, 7.5% QF1 and 3% XE60 (in the ratio of 2 + 2 + 1, m/m) on Chromosorb W AW DMCS (80–100 mesh) (all from Phase Separations, Clwyd, UK) was operated isothermally at 195 °C.

Determination of organophosphorus compounds

A Pye Model 204 instrument (or equivalent) equipped with a flame photometric detector (FPD) was operated under the following conditions: nitrogen carrier gas flow-rate, 30 ml min^{-1}; detector gas flow-rates, hydrogen, 30 ml min^{-1}; and air, 30 ml min^{-1}. A 1.52 m (5 ft) × 4 mm i.d. glass column packed with a mixed packing (as described above) was operated isothermally at 200 °C.

Determination of synthetic pyrethroids

A Pye Model PU 4550 instrument (or equivalent) equipped with a ^{63}Ni ECD was operated isothermally at 240 °C. A Mega-bore DB1 (Jones Chromatography, Mid-Glamorgan, UK) fused silica column (12 m) with a nitrogen carrier gas flow-rate of 12 ml min^{-1} and detector purge gas flow-rate of 40 ml min^{-1}. The injector and detector temperatures were set at 275 and 300 °C, respectively.

Determination of insect growth regulators

A Millipore - Waters HPLC system (or equivalent) consisting of a Model 6000 pump, WISP 101 auto-injector, a Model 450 variable-wavelength ultraviolet detector and Z-module C_{18} radial compression column was used. The mobile phase for methoprene and hydroprene was acetonitrile - water (80 + 20, v/v) at a flow-rate of 2 ml min^{-1} and for fenoxycarb, acetonitrile - water (60 + 40, v/v) at a flow-rate of 2 ml min^{-1} was used. The detector wavelength was set at 351 nm for hydroprene and methoprene or 238 nm for fenoxycarb.

Procedures

Extraction of samples

Approximately 100 g of the sample were ground to a coarse powder using the Glen-Creston mill. Twenty-five grams of the coarsely ground sample were accurately weighed into a 250-ml centrifuge bottle. Extraction solvent (100 ml of acetone - methanol) was added to the centrifuge bottle and the mixture blended at high speed for 2 min prior to centrifugation at 2500 rev min^{-1} for 5 min. The supernatant liquid was decanted off into a 1-l separating funnel. A further 80 ml of extraction solvent were added to the centrifuge bottle and the mixture was blended and centrifuged as before. The supernatant liquid was decanted off into the separating funnel as before.

Liquid - liquid partition

Sodium sulphate solution (400 ml) and 50 ml of dichloromethane were added to the separating funnel. The mixture was gently shaken for 1 min and the layers were allowed to separate. The lower, dichloromethane, layer was run off and passed through a glass column containing 10–15 g of anhydrous sodium sulphate. The dried dichloromethane extract was collected in a 500-ml round-bottomed flask. Partitioning was repeated with two further 50-ml aliquots of dichloromethane. The sodium sulphate was rinsed with two 5-ml aliquots of dichloromethane. The combined dichloromethane extracts were reduced to approximately 1 ml using a rotary evaporator ensuring that the temperature of the water-bath did not exceed 50 °C. The concentrated extract was transferred into a 25-ml calibrated flask and the round-bottomed flask rinsed with three 5-ml aliquots of GPC elution solvent. The washings were added to the calibrated flask and made up to volume with GPC elution solvent.

Gel-permeation chromatography

A 5-ml aliquot of the prepared extract was injected on to the GPC column and twenty 10-ml fractions were collected. The GPC column was washed for a further 10 min before the next sample was introduced. The total GPC run time was 60 min.

For the determination of OP pesticide residues, fractions 8–10 (71–100 ml) were collected and combined in a 100-ml round-bottomed flask and the solvent was reduced to a low volume (*ca.* 1 ml) by rotary evaporation. The concentrated extract was transferred into a 5-ml calibrated flask and the round-bottomed flask was rinsed with two 1-ml portions of acetone which were then added to the calibrated flask. The extract was made up to volume with acetone for subsequent GLC-FPD analysis.

For the determination of OCl pesticide residues, fractions 10–14 (91–140 ml) were collected and combined and the solvent was reduced to a low volume as above. The round-bottomed flask was rinsed with hexane and the extract was made up to volume with hexane.

Alumina mini-column clean-up for pyrethroids and insect growth regulators

Fractions 8–10 (71–100 ml) containing the SPYRs or fractions 7–11 (61–110 ml) containing the IGRs required further cleaning up. Although fractions 8–10 might contain OP

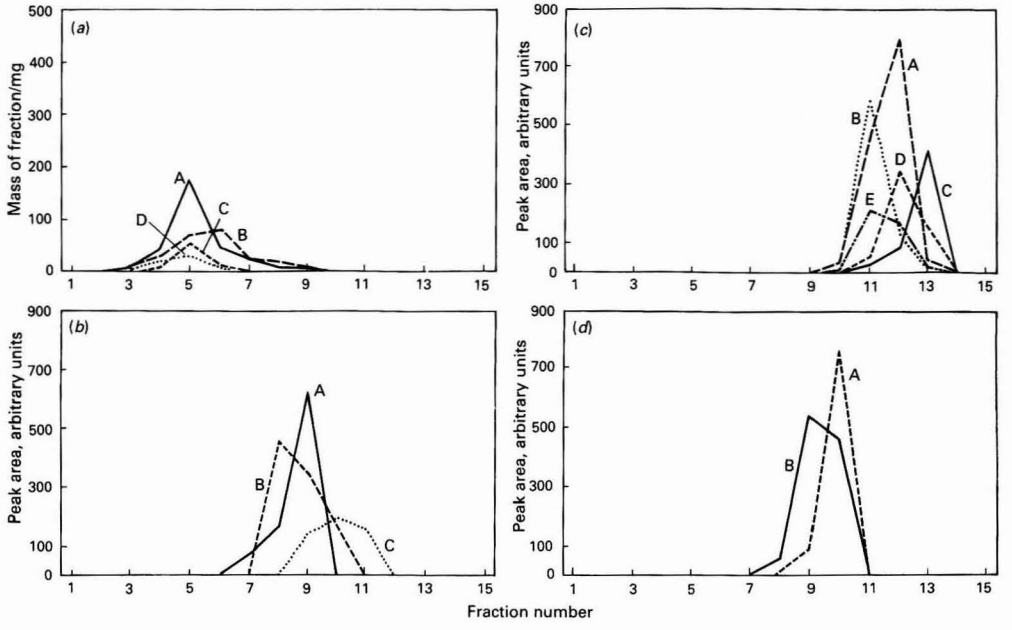

Fig. 1. Elution profiles from Bio-Beads SX-3 column: (*a*) wheat and wheat products; A, wheat germ; B, wheat; C, rice; and D, wheat bran; (*b*) insect growth regulators; A, methoprene; B, hydroprene; and C, fenoxycarb; (*c*) organochlorine pesticides; A, γ-HCH; B, α-HCH; C, PCB; D, *o,p'*-DDT; and E, *o,p'*-DDE; (*d*) organophosphorus pesticides; A, chlorpyrifos-methyl; and B, pirimiphos-methyl

pesticides, it is advisable not to quantify them after this clean-up step.

A clean-up column was prepared by adding 2 g of alumina to a 10 cm diameter powder funnel plugged with glass wool. The required fractions were combined and transferred into the funnel and the solvent was allowed to pass through the alumina into a 100-ml round-bottomed flask. The column was rinsed with three 5-ml portions of GPC elution solvent and the eluate was evaporated to a low volume (*ca.* 1 ml) by rotary evaporation. The final traces of solvent were removed under a stream of nitrogen. For the determination of pyrethroids, the residue was dissolved in hexane and transferred into a 5-ml calibrated flask for subsequent GLC analysis. For the IGRs the residue was dissolved in acetonitrile prior to HPLC analysis.

Pesticide Elution Profile

Compounds representative of four classes of pesticide were studied: (*i*) organophosphorus pesticides, etrimfos, chlorpyrifos-methyl, pirimiphos-methyl, malathion and fenitrothion; (*ii*) organochlorine pesticides, hexachlorobenzene (HCB), α-benzenehexachloride (HCH), γ-HCH, heptachlor, heptachlor epoxide, dieldrin, *p,p'*-dichlorodiphenyldichloroethylene (DDE), *p,p'*-dihydroxydichlorodiphenylmethane (DDD), *o,p'*-dichlorodiphenyltrichloroethane (DDT) and *p,p'*-DDT; (*iii*) synthetic pyrethroids, permethrin, deltamethrin and cypermethrin; (*iv*) insect growth regulators, hydroprene, methoprene and fenoxycarb.

Mixtures of the selected compounds from each class of pesticide were prepared in the elution solvent mixture. A 5-ml aliquot of the standard mixture solution was injected on to the GPC column and 10-ml fractions were collected. Each fraction was analysed using GLC or HPLC to determine the amount of pesticide present. The OP pesticides in the elution solvent were determined directly using GLC-FPD. For the OCl and SPYRs the elution solvent was removed from each fraction, by rotary evaporation, and the residue then made up to 10 ml with hexane prior to examination by GLC-ECD. A similar procedure was followed for the IGRs except that the fractions were re-dissolved in acetonitrile prior to HPLC examination. The fractions containing each pesticide are shown in Fig. 1.

Spiking of Commodity Extract Solutions

Each commodity was extracted with acetone - methanol and a mixed standard solution of each class of pesticide, prepared in dichloromethane - hexane (3 + 1, v/v), was added to the concentrated extract, previously prepared, prior to making up to the final 25 ml with the GPC elution solvent.

Extracted Solids Profile

Each commodity studied was extracted, the solvent partitioned and a 5-ml aliquot injected on to the GPC column. Fractions (10 ml) were collected over the total volume required to elute all the pesticides as determined above. Each fraction was transferred into a pre-weighed 25-ml beaker and excess of solvent evaporated off over a boiling water-bath. The beakers were placed in a desiccator until cool, re-weighed and the mass of the extracted solids was determined.

Results and Discussion

The elution volumes required from the Bio-Beads SX-3 column for the pesticides studied are given in Table 1. For each class of pesticide there was a range of fractions in which the compounds eluted from the GPC column. The separation between the classes of pesticides was, however, incomplete. The main overlap occurred between the OP, SPYR and IGRs. However, because of the different chromatographic techniques subsequently used to determine the compounds in each class of pesticide there would be little possibility of any of the compounds of one class interfering with the analysis of another class.

The majority of the co-extractives from the commodities

Table 1. Elution volumes of organophosphorus, organochlorine, synthetic pyrethroid and insect growth regulators from a Bio-Beads SX-3 GPC column

Pesticide	Elution volume/ml
Organochlorine—	
Heptachlor	91–120
α-HCH	91–120
Hexachlorobenzene (HCB)	91–130
γ-HCH	91–130
β-HCH	121–130
Dieldrin	91–130
o,p'-DDE	91–130
o,p'-DDT	91–130
o,p'-DDD	91–130
p,p'-DDT	91–130
PCB (Arochlor 1254)	91–140
Organophosphorus—	
Malathion	71–100
Pirimiphos-methyl	71–100
Fenitrothion	81–100
Chlorpyrifos-methyl	81–100
Etrimfos	81–100
Synthetic pyrethroids—	
Permethrin	71–100
Cypermethrin	71–100
Deltamethrin	71–100
Insect growth regulators—	
Methoprene	61–80
Hydroprene	61–90
Fenoxycarb	81–110

Table 2. Elution volumes for solids extracted from cereals and cereal products

Commodity	Elution volume/ml
Wheat	1–80
Wheat bran	21–80
Wheat germ	21–80
Animal feed	21–80
Rice	31–60

eluted from the GPC column in fractions 4–8, before the pesticides. The effect of injecting more concentrated grain extracts equivalent to 2.5 or 5 g ml^{-1} (instead of the 1 g ml^{-1}) was also investigated; for wheat, no broadening of the extracted solids profile occurred. However, when commodities with a high fat content (*e.g.*, wheat germ) were examined the solids eluted over a greater number of fractions, including some of those containing the pesticides. Stalling *et al.*[6] reported that when using Bio-Beads SX gels, the broadening of the lipid peak occurred when solutions of 500 mg ml^{-1} were injected, and concluded that the concentrations of lipid applied to the gel column should not exceed 100 mg ml^{-1}.

The solvent volumes in which the other substances extracted from each commodity eluted are presented in Table 2. To determine the efficiency of the GPC procedure for cleaning up samples, extracts were prepared from each commodity and subjected to GPC. The fractions for the two main regions of interest, fractions 10–14, *i.e.*, those which would contain any OCl and Arochlor 1254 were combined.

Similarly fractions 8–10, *i.e.*, those containing OP, and SPYRs were also combined. The solvent was removed and the mass of the co-extractives, if present, determined. The efficiency was calculated as the percentage of the extract mass removed from each of the two regions compared with the total co-extractive mass. The results obtained are presented in Table 3.

The efficiency of the GPC procedures for the clean-up of extracts containing OCl pesticides prior to residue determination (Table 3) based on the mass of the co-extractive material was 100%. However, for the other three classes of pesticides (OP, SPYR and IGR) the efficiency was lower. For analysis of OP pesticides by GLC-FPD this decreased efficiency of clean-up caused no problems as no interference was observed and a stable base line was maintained throughout the chromatographic run. However, the inclusion of the alumina mini-column was required for the SPYR and IGR classes as interferences were observed when analysing for these compounds. As the GPC procedure has previously been shown to eliminate up to 97% of the co-extracted solids, the mini-column was only required to remove a small amount of the residual material. Other workers[14,15] have reported the use of alumina or Florisil post-GPC clean-up columns when determining SPYR residues in high fat content matrices.

Table 3. Clean-up efficiency for cereal and cereal products

Commodity	Clean-up,%	
	OP and pyrethroid	OCl
Wheat	95	100
Wheat bran	90	100
Wheat germ	97	100
Animal feed	97	100
Rice	100	100

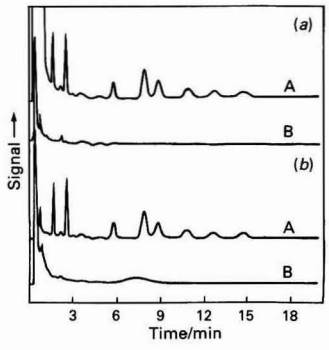

Fig. 2. Chromatograms using (*a*) GPC clean-up; and (*b*) alumina clean-up of A, spiked wheat; and B, control wheat

Although the clean-up achieved is better than 90%, on a mass basis, it is possible that very small amounts of components can be detected with the very high sensitivity achieved by some selective detectors. Such components may interfere with or co-elute with pesticides, and suitable confirmatory techniques should always be employed when the presence of pesticide residues is indicated.

The recovery of OP or OCl pesticides from each commodity examined is presented in Tables 4 and 5. Fig. 2 shows representative chromatograms of spiked (OCl pesticides at *ca.* 0.05 mg kg^{-1}) and control wheat samples after clean-up using conventional alumina column chromatography and GPC. Gel-permeation chromatography as a clean-up procedure is as effective as the alumina column, however, the advantage of a single technique for use with many sample types over a range of pesticides supports the use of GPC as a flexible clean-up procedure. Recoveries of the SPYRs and IGRs were only determined from spiked wheat and the data are given in Tables 6 and 7. The recovery experiments were restricted to spiked extracts as the objective of this work was to examine the effectiveness of the GPC procedure. The solvents used for extraction of pesticide residues from commodities are in common usage and data exist to show their effectiveness for extracting pesticide residues from treated commodities.[22]

Table 4. Percentage recovery of organophosphorus pesticides from spiked extracts. Results given are the means of three determinations (range found)

Commodity	Pesticide (spike concentration)				
	Etrimfos (0.97 mg kg^{-1})	Chlorpyrifos-methyl (1.4 mg kg^{-1})	Pirimiphos-methyl (1.15 mg kg^{-1})	Malathion (1.4 mg kg^{-1})	Fenitrothion (0.97 mg kg^{-1})
Wheat	96 (88–112)	99 (88–115)	92 (89–104)	100 (84–119)	103 (85–106)
Wheat bran	100 (100–101)	97 (93–100)	98 (92–104)	102 (100–107)	100 (100)
Wheat germ	103 (92–102)	101 (100–103)	99 (96–103)	103 (100–106)	101 (100–104)
Animal feed	97 (92–100)	91 (86–101)	97 (95–99)	103 (100–106)	95 (84–103)
Rice	100 (93–100)	91 (87–100)	98 (97–100)	100 (100)	101 (94–110)

Table 5. Percentage recovery of organochlorine pesticides from spiked extracts. Results given are the means of three determinations (range found)

Commodity	Pesticide (spike concentration)										
	Heptachlor epoxide (0.049 mg kg^{-1})	*p,p'*-DDE (0.089 mg kg^{-1})	Dieldrin (0.057 mg kg^{-1})	*o,p'*-DDT (0.048 mg kg^{-1})	*p,p'*-DDD (0.041 mg kg^{-1})	*p,p'*-DDT (0.046 mg kg^{-1})	HCB (0.027 mg kg^{-1})	α-HCH (0.026 mg kg^{-1})	γ-HCH (0.032 mg kg^{-1})	Heptachlor (0.020 mg kg^{-1})	β-HCH (0.033 mg kg^{-1})
Wheat	—	96 (83–108)	95 (85–106)	94 (77–116)	95 (80–114)	92 (81–107)	97 (99–107)	93 (88–103)	94 (89–103)	93 (86–102)	91 (80–102)
Wheat bran	94 (91–97)	95 (93–99)	—	92 (92–94)	—	99 (97–100)	95 (91–99)	—	98 (96–102)	—	92 (89–94)
Wheat germ	93 (91–95)	94 (93–98)	—	95 (92–96)	—	96 (93–97)	77 (73–79)	—	93 (92–93)	—	89 (87–90)
Animal feed	94 (93–95)	96 (94–96)	—	97 (84–99)	—	94 (89–97)	78 (77–79)	—	93 (92–93)	—	90 (89–90)
Rice	94 (92–96)	96 (94–99)	—	97 (90–100)	—	96 (93–97)	90 (88–94)	—	102 (101–104)	—	95 (93–96)

Table 6. Percentage recovery of synthetic pyrethroids from spiked extracts. Results given are the means of three determinations (range found)

Commodity	Pesticide (spike concentration)		
	Permethrin (1.6 mg kg^{-1})	Cypermethrin (2.1 mg kg^{-1})	Deltamethrin (2.0 mg kg^{-1})
Wheat	100 (95–105)	103 (101–103)	99 (98–100)
Wheat germ	101 (100–103)	98 (97–100)	98 (98–102)
Wheat bran	99 (94–103)	94 (90–97)	98 (95–102)

Table 7. Percentage recovery of insect growth regulators from spiked wheat extracts

IGR (spike concentration)	Recovery,*(range)
Methoprene (5.4 mg kg^{-1})	97 (95–99)
Hydroprene (5.1 mg kg^{-1})	107 (101–122)
Fenoxycarb (5.5 mg kg^{-1})	92 (82–101)

* Mean of six determinations.

The recoveries of the OP pesticides from spiked extracts (Tables 4–7) were greater than 90%, the over-all mean recovery being 99% (range 92–103%). Similarly a mean recovery of 94% (range 77–102%) was obtained for the OCl pesticides. The recoveries of the SPYRs and IGRs were determined in fewer commodities. However, the results obtained (Tables 6 and 7) showed that more than 90% of these pesticides could be recovered. For the SPYR pesticides the over-all mean recovery was 99% (range 94–103%) and for the IGR pesticides 99% (range 92–107%).

The results presented in this study show GPC to be a valuable procedure for the clean-up of cereals, cereal products and animal feeds prior to pesticide residue determination. Gel-permeation chromatography offers a number of advantages over other procedures. It can be automated, many procedures have been extensively evaluated and good clean-up of sample extracts, suitable for determination of residue content with specific GLC detectors can be achieved. Also, in the experience of this laboratory the GLC columns employed for the determination of pesticide residues show little deterioration in resolution after many samples thus considerably prolonging their usefulness. The GPC gel packing appears to be robust; after 2 years of constant use, with many different sample types, no appreciable deterioration in separation has been observed.

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Single-step Multi-cartridge Clean-up for Organophosphate Pesticide Residue Determination in Vegetable Oil Extracts by Gas Chromatography

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A multi-cartridge system has been developed which, in a single step, performs the extraction and clean-up of organophosphate (OP) pesticide residues from oils and fatty extracts. A solution in hexane containing up to 1.8 g of lipidic material is loaded on to an Extrelut-3 column to which a silica-gel cartridge and a C_{18} silica cartridge have been connected in series. The OP pesticide residues are eluted with 15 ml of acetonitrile. Carry-over of fatty material is in the range 2–5 mg per 1.8 g of different oils, which makes the final solution amenable to capillary gas chromatography. Recoveries of 23 OP pesticides were in the range 82–111%. The whole procedure takes *ca.* 20 min and compares favourably with current procedures.

Keywords: *Organophosphate pesticide residue; partition clean-up; vegetable oil; gas chromatography; flame photometric detector*

The performance of an on-column hexane - acetonitrile partition with respect to the clean-up of fatty extracts for the determination of organophosphate (OP) pesticide residues by gas chromatography (GC) with a flame-photometric detector operated in the phosphorus mode (FPD-P) has previously been reported.[1] Up to 1.8 g of olive oil and fatty extracts, such as those obtained from cereals, oil seeds and legumes by use of the current acetone extraction procedures,[2–12] were partitioned using disposable ready-to-use columns filled with Extrelut, a macroporous Kieselguhr-type material.

Good recoveries of nine OP pesticides with a wide range (0.05–0.43) of extraction p values (fraction of solute partitioning into the non-polar phase of an equi-volume two-phase system)[13,14] were obtained, together with a reduced carry-over of lipidic material in the eluate.

The reported clean-up has significant advantages over clean-up techniques in current use, *i.e.*, partitioning in a separating funnel,[2,15–17] gel-permeation chromatography[10–12,17–23] or sweep co-distillation.[2,17,24–28]

However, even a small amount of lipid present in the extract can be deleterious when capillary GC or capillary GC - mass spectrometry (GC - MS) is used for determination or confirmation purposes, especially at low levels. Further, the on-column injection mode in capillary GC is particularly sensitive to the bulk of co-injected lipidic material.

With this in mind we have developed a single-step clean-up in which silica gel plus C_{18} cartridges are used in series, downstream of an Extrelut column, to reduce further the co-extracted lipidic material in the final solution.

Following observations on the behaviour of olive oil components on thin-layer plates of silica gel and C_{18} silica, it was found that, with a C_{18} cartridge connected to the outlet of the Extrelut-3 partition column, it was possible to reduce significantly the amount of lipidic material in the final extract. A further reduction could be achieved by placing a silica-gel cartridge between the Extrelut-3 column and the C_{18} cartridge.

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Experimental

Apparatus

The GC analyses were carried out on a Perkin-Elmer Sigma 4B chromatograph equipped with a flame-photometric detector operated in the phosphorus mode (GC-FPD-P).

The column (1.8 m × 4 mm i.d.) used was of Pyrex glass, and was packed with 5% QF-1 on Chromosorb W-HP (100–120 mesh). The operating conditions were as follows: helium carrier gas, 60 ml min^{-1}; column oven, 190 °C; inlet and outlet blocks, 225 °C.

Materials

Ready-to-use Extrelut-3 columns (Merck, Darmstadt, FRG, Cat. No. 15327) were used as received for the on-column partition. Sep-Pak silica gel cartridges (Waters Associates, Milford, MA, USA, Part No. 51900) and Sep-Pak C_{18} cartridges (Waters Associates, Part No. 51910) were also used.

Reference standards. Compounds of analytical purity were from the collection of this laboratory. The pesticide names used here are those quoted in "The Pesticide Manual."[29]

Solvents. Hexane, acetonitrile (used after saturation with hexane), acetone and methanol (all re-distilled in glass).

Procedure

Prepare a solution in hexane containing up to 0.6 g ml^{-1} of olive oil or lipidic material (resulting from the extraction of oily crops by one of the current procedures).[2–6,17]

Pipette 3 ml of the above lipid solution on to the top of an Extrelut-3 column, at the Luer tip of which a silica-gel cartridge and a C_{18} cartridge (in descending order) have been connected (the two cartridges are linked together by means of a small glass connector). Wait 10 min to obtain an even distribution. Next, elute under gravity alone the assembled columns with three 5-ml portions of acetonitrile (saturated with hexane). Collect the eluate in a 50-ml Erlenmeyer flask and add 4 ml of methanol. Evaporate the eluate to dryness by means of a rotary evaporator (50–55 °C water-bath, reduced

pressure). Remove the last traces of solvent in a gentle stream of nitrogen. Add an appropriate volume of acetone (1–2 ml) and inject 1–5 μl of the solution into the GC-FPD-P.

Calculate the amount of OP pesticides present by comparing the peak heights for unknown samples with the corresponding peaks for standards, according to established procedures.

Results and Discussion

The performance of the on-column partition system has been studied with respect to its ability to remove lipidic material and to recover more OP compounds than those reported in the previous paper.[1]

In Table 1 are reported the amounts of lipidic material released in the acetonitrile eluate when 1.8 g of different commercial oils (including olive oil, peanut oil, maize oil, soya bean oil and mixed seed oil) are loaded on to either of the three systems investigated (*i.e.*, Extrelut-3 partition column alone, Extrelut-3 connected to a C_{18} cartridge and Extrelut-3 connected to a silica-gel and a C_{18} cartridge in series), elution being effected with 15 ml of acetonitrile. The results show that the greatest reduction, compared with Extrelut-3 alone, is achieved by the introduction of the C_{18} cartridge, which evidently removes highly non-polar components.

Table 1. Mean (n = 6) amounts of lipidic material released into the eluate with 1.8 g of different oils applied to the three systems investigated

	Lipid in the eluate/mg [mean (n = 6) ± SD]		
Oil type	Extrelut-3	Extrelut-3 plus C_{18} cartridge	Extrelut-3 plus silica and C_{18} cartridges
Olive oil	31.4 ± 3.60	6.1 ± 1.70	2.6 ± 0.69
Peanut oil	26.1 ± 0.30	6.6 ± 0.26	3.8 ± 0.15
Corn oil	34.8 ± 5.76	7.8 ± 1.21	4.9 ± 0.63
Soya bean oil	210.9 ± 9.80	7.0 ± 2.41	2.1 ± 0.76
Mixed seed oil	126.4 ± 9.80	5.6 ± 0.80	1.8 ± 0.30

In Table 2 are presented the results for the recovery experiments on 23 OP compounds obtained with the system described (consisting of an Extrelut-3 column plus silica gel and C_{18} cartridges in series). For the group containing the first 18 compounds in Table 2, the recovery of the different compounds was studied at spiking levels ranging from 0.1 to 1.4 mg kg^{-1} (level I) and at levels five times greater (level II). For the group containing the last five compounds, the recovery of the different compounds was studied at levels ranging from 0.5 to 2.0 mg kg^{-1} (level I) and at levels three times greater (level II). Data for the recovery experiments are the mean plus standard deviation for six replicates carried out with 1.8 g of spiked olive oil, which was chosen as the model lipid for the recovery experiments.

As can be seen, all the OP compounds investigated were recovered satisfactorily with values ranging between 82 and 111%. Similar recovery values were obtained when the Extrelut-3 alone or the Extrelut-3 plus C_{18} cartridge system was used during the development of the described procedure. The compounds studied were not separated in a single run under the GC conditions adopted, hence, the recovery experiments were carried out with mixtures of OP compounds that could be separated in a single run.

Compared with those cited in the previous paper,[1] the compounds studied here have a wider range of p values, *i.e.*, from <0.01 to 0.43. The p values listed in Table 2 were determined in our laboratory and they can be used to evaluate the applicability of the described procedure. The range of p values demonstrates the wide applicability of the Extrelut-3 partition step, covering a variety of compounds with different polarities and water solubilities, wider than those studied in the previous paper, for instance from dimethoate (polar) to bromophos-ethyl (non-polar). It is reasonable to assume, therefore, that other compounds having p values in this range could be recovered through the partition step.

The over-all procedure described consists essentially of an on-column partition step followed by two on-line steps in which some highly polar and highly non-polar components of the vegetable oils are removed by a chromatographic mechanism. The final eluate contains very small amounts of lipidic material and is amenable to both packed and on-column

Table 2. Mean (n = 6) recovery values of 23 organophosphate pesticides from 1.8 g of olive oil spiked at different levels

		Spiking level/ mg kg^{-1}		Recovery, % [mean (n = 6) ± SD]	
Pesticide	p value*	I	II	I	II
Diazinon	0.28	0.2	1.0	88 ± 2.9	93 ± 5.6
Etrimfos	0.20	0.4	2.0	91 ± 5.3	92 ± 4.1
Chlorpyrifos-methyl	0.18	0.4	2.0	96 ± 3.5	97 ± 5.8
Pirimiphos-methyl	0.21	0.3	1.5	90 ± 3.3	91 ± 3.4
Chlorpyrifos	0.27	0.5	2.5	92 ± 2.9	93 ± 2.8
Bromophos	0.26	0.6	3.0	95 ± 4.3	93 ± 4.7
Bromophos-ethyl	0.43	0.6	3.0	86 ± 2.9	82 ± 7.7
Malathion	0.05	1.0	5.0	100 ± 3.4	103 ± 2.0
Fenitrothion	0.06	0.6	3.0	95 ± 3.1	96 ± 7.1
Methacrifos	0.08	0.1	0.5	87 ± 6.7	88 ± 6.6
Fonofos	0.19	0.2	1.0	95 ± 5.5	86 ± 5.3
Fenchlorphos	0.25	0.4	2.0	91 ± 5.6	88 ± 6.0
Dimethoate	<0.01	0.6	3.0	108 ± 5.8	102 ± 7.0
Parathion-methyl	0.03	0.7	3.5	98 ± 5.9	89 ± 2.7
Parathion	0.05	1.0	5.0	91 ± 4.4	88 ± 3.8
Metidathion	0.03	1.2	6.0	102 ± 6.3	106 ± 7.0
Carbofenothion	0.19	1.4	7.0	92 ± 8.3	96 ± 6.2
Ethion	0.14	1.0	5.0	99 ± 6.2	96 ± 5.5
α-Phosphamidon	<0.01	2.0	6.0	95 ± 9.4	96 ± 8.7
β-Phosphamidon	<0.01	2.0	6.0	104 ± 9.5	91 ± 7.2
Fenthion	0.09	0.5	1.5	100 ± 2.9	83 ± 3.4
Isofenphos	0.11	0.8	2.4	105 ± 6.9	96 ± 4.5
Tetrachlorvinphos	0.04	2.0	6.0	111 ± 4.3	99 + 2.7

* p values between hexane and acetonitrile by GC-FPD-P after single distribution (extraction p-value is the fraction of solute partitioning into the non-polar phase of an equi-volume two-phase system).

capillary injection systems. The gas chromatograms are all free from interfering peaks and are indistinguishable from those obtained with the standard solution of pure pesticides.

The described procedure appears to be a very rapid way of preparing fatty sample extracts for the screening of OP compounds. It takes *ca.* 20 min and compares favourably with the current procedures cited earlier, and also from the point of view of the quality of the clean-up, the simplicity of the operations and the minimum requirement for glassware and reagents.

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Development of a Method for the Determination of Phthalate Esters in Sewage Sludge Including Chromatographic Separation From Polychlorinated Biphenyls, Pesticides and Polyaromatic Hydrocarbons

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A method was developed that allows phthalate esters (PEs) to be determined in sewage sludge samples by use of gas chromatography (GC) with electron-capture detection. Discrimination of the PEs in the split injector of the GC system was detected and the resulting problems are discussed. The ultrasonic technique was optimised for extraction time and number of extractions and compared with the Soxhlet extraction technique. It was shown that extraction efficiency is slightly better with ultrasonication than using Soxhlet extraction. The Soxhlet extraction also yielded much higher blank levels. The extract clean-up was carried out by dual column liquid chromatography with neutral alumina and Florisil. In this way separation of polychlorinated biphenyls, pesticides and polyaromatic hydrocarbons from the PEs was attained. This procedure was used successfully to determine PEs in several sewage sludge samples.

Keywords: *Phthalate ester; discrimination in split injector; sewage sludge; ultrasonic extraction; liquid chromatography clean-up*

The application of municipal sewage sludge in agriculture and forestry is a good way of fertilising crops and plants and it serves to reduce the amount of sewage sludge which has to be disposed of. However, it may also enrich the soil with a variety of inorganic, *i.e.*, heavy metals, and organic compounds that may be toxic to either plants or animals.

One group of organic compounds identified in sewage sludge samples are phthalate esters (PEs). These compounds are widely used as plasticisers with a total annual production of 20×10^9 kg.[1] Owing to their high rate of production and application PEs are ubiquitous contaminants in the biosphere and have been found in environmental samples.[2–5] Although PEs are considered to be substances of low toxicity their input into the soil and potential uptake into crop plants is of special interest as hepatotoxic,[6] mutagenic[7] and carcinogenic[8] effects have been observed. For this reason it is necessary to know the amount of PEs present in sewage sludges in order to evaluate their potential enrichment in the soil.[9] As there is evidence for the occurrence of polychlorinated biphenyls (PCBs), pesticides[10] and polyaromatic hydrocarbons (PAHs)[11] in sewage sludge the separation of PEs from these compounds and other interfering substances is necessary.

Several publications that deal with the presence of phthalate esters in sewage sludge exist.[11–14] These investigations however are either only semi-quantitative[14] or they are designed for the analysis of a wide spectrum of organic priority pollutants.[11–13] The objective of this research was to develop a method for the determination of PEs in sewage sludge including the separation of interfering compounds for reliable gas chromatography with electron-capture detection (GC-ECD). Five PEs included by the Environmental Protection Agency in a list of priority pollutants were selected for this research: dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butylbenzyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP).

Experimental

Reagents

Solvents. Acetone, hexane and methylene chloride (Merck); all solvents were doubly distilled.

Alumina. Neutral, Brockman activity I, 70–230 mesh (Merck); heated to 200 °C for several hours, cooled in a desiccator and de-activated with 15% (m/m) doubly distilled water.

Florisil. Activated, 60–100 mesh (Merck), heated to 300 °C for at least 8 h, then cooled in a desiccator.

Phthalate esters. DMP, DEP, DBP, BBP and DEHP (Fluka).

Pesticides. 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), α-benzenehexachloride, γ-benzenehexachloride, aldrine and hexachlorobenzene (Baker).

PCBs. PCB 60 (Baker).

PAHs. Naphthalene, pyrene, fluoranthene, phenanthrene, benzo[*a*]pyrene, anthracene and perylene (Polyscience Corporation).

Internal standards. 9-Bromophenanthrene (BrP) and dimethyl isophthalate (DMIP) (Fluka).

Apparatus

Glassware. All items were cleaned with Extran (Merck), rinsed with doubly distilled acetone, heated at 300 °C for at least 10 h and stored in clean aluminium foil. Before use they were rinsed with the solvent to be applied.

Liquid chromatography columns, 300 × 9 mm i.d. These were filled with either 15.0 g of alumina or 7.5 g of Florisil.

Ultra turrax homogeniser.

Freeze-drying apparatus.

Ultrasonic water-bath.

Centrifuge.

Mill. A Retsch mill was used to grind the freeze-dried sewage sludge (<0.2 mm).

Rotary evaporator.

Gas chromatograph. Varian Model 3700 instrument, equipped with a ^{63}Ni electron-capture detector and a split-injector. A DB-5 fused silica capillary column (30 m, 0.25 μm film thickness) was used for the separation of the PEs. The operating conditions were as follows: injection port, 250 °C; electron-capture detector, 300 °C, column temperature, 140 °C for 3 min, increased at 12 °C min^{-1} to 260 °C, then held for 10 min; carrier gas, helium; and make-up gas, nitrogen.

Integrator. A Spectra-Physics Model SP 4290 integrator was used to measure peak areas.

Procedure

Sampling

Samples of anaerobic digested sewage sludge were collected at different sewage treatment plants in and near the town of Bayreuth in the north-east of Bavaria, FRG. The sludge was collected in clean dark glass-stoppered bottles, immediately transported to the laboratory and stored at 4 °C for not more than 4 d.

Extraction of samples

Ultrasonic extraction. Homogenise the sewage sludge with the Ultra turrax homogeniser for about 5 min. Prepare a PE standard solution in methanol. For recovery and extraction efficiency studies spike the sewage sludge with the methanol standard solution. Homogenise the samples a second time. After storage for 24 h at 4 °C for equilibration take subsamples for freeze-drying. Grind the dried sewage sludge with a mill to obtain particles with a diameter of less than 0.2 mm. Place about 1.0 g of the dried and ground sludge into a centrifuge tube, add 40 ml of methylene chloride and place the sample in an ultrasonic water-bath for 30 min. Settle the suspended particles by centrifugation and filter the supernatant with a glass fibre filter-paper. Repeat the extraction four times with fresh solvent and concentrate the five combined extracts to *ca.* 1–2 ml.

Soxhlet extraction. Pre-extract the Soxhlet paper thimble with methylene chloride for 24 h. Extract about 1 g of the dried and ground sewage sludge for 48 h with 300 ml of methylene chloride. Evaporate the solvent extract to *ca.* 1–2 ml with a rotary evaporator.

Liquid chromatography (LC) clean-up procedure

Transfer the methylene chloride extract into hexane and concentrate the hexane phase to *ca.* 1–2 ml with a rotary evaporator. Fill a glass column with 15 g of de-activated alumina (15% water, m/m). Introduce the hexane concentrate on to the column and elute with three 30-ml fractions: (*i*) hexane, (*ii*) 10% methylene chloride in hexane and (*iii*) 50% methylene chloride in hexane. Fill a second glass column with 7.5 g of activated Florisil and pour the last fraction from the alumina column (which contains the PEs) through the Florisil column. Elute this column with 30 ml of methylene chloride followed by 30 ml of 5% acetone in methylene chloride. This last fraction contains all five of the PEs included in this study. Evaporate the last fraction with a rotary evaporator and finally under a stream of purified nitrogen to dryness. Add hexane with the internal standards DMIP and BrP and analyse by GC-ECD. Find the linear response range of the electron-capture detector and calibrate the measuring system by determining the response factors (R_F values) of the PEs with the internal standards DMIP and BrP.

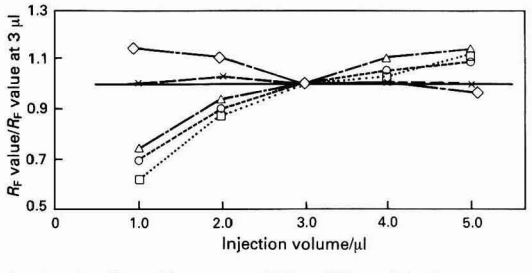

Fig. 1. R_F values with respect to BrP at different injection volumes. □, DMP; ○, DEP; △, DBP; ◇, BBP; and ×, DEHP

Results and Discussion

Discrimination in the Split-injector System

In modern GC analysis it is fairly common to use internal standards (IS) for the calibration of the GC system and for the evaluation of samples. For this purpose a response factor is calculated using the following equation:

$$\text{Response factor } (R_F) = \frac{\text{IS response}}{\text{IS concentration}} \times \frac{\text{analyte concentration}}{\text{analyte response}} \quad (1)$$

The advantage of using R_F values is that after the IS has been added to the sample extract, a knowledge of the solvent volume is no longer necessary; the injection volume should not affect the results of the quantification. To test for the latter assertion, different volumes (1–5 µl) of the same standard solution were injected several times and the R_F values with respect to BrP determined. In Fig. 1 the injection volumes are plotted against the R_F values which are normalised to the R_F value at 3 µl.

If the R_F value is independent of the injection volume, then the plots for all the PEs should give a straight line parallel to the *x*-axis. However, the R_F values, particularly those of DMP, DEP and DBP, show a different behaviour. These deviations are caused by the fact that discrimination effects due to the molecular size and polarity of the different PEs and the IS occurred in the injector port. This discrimination can arise when the more volatile sample components distil from the syringe needle at a greater rate than the less volatile

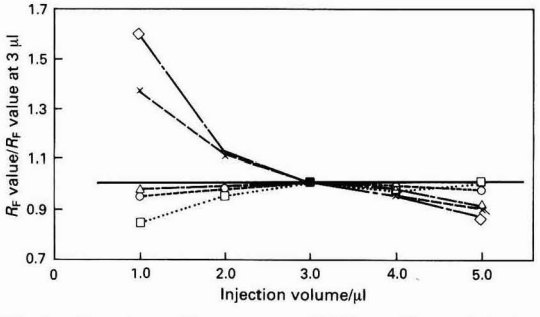

Fig. 2. R_F values with respect to DMIP at different injection volumes. □, DMP; ○, DEP; △, DBP; ◇, BBP; and ×, DEHP

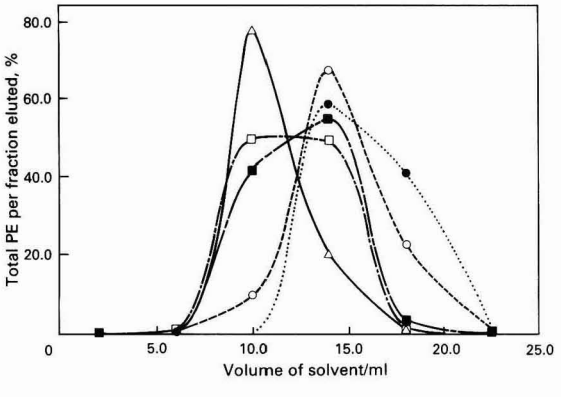

Fig. 3. Elution of PEs from alumina with 50% CH_2Cl_2 in hexane. ●, DMP; ○, DEP; □, DBP; ■, BBP; and △, DEHP

Table 1. Recovery of PEs from the alumina column (n = 5 in all instances)

PE	Recovery, %		
	x*	s†	s/x
DMP	98.0	2.4	2.5
DEP	99.5	1.7	1.7
DBP	98.5	2.7	2.8
BBP	99.0	2.4	2.4
DEHP	103.1	5.8	5.6

* Mean value.
† Standard deviation.

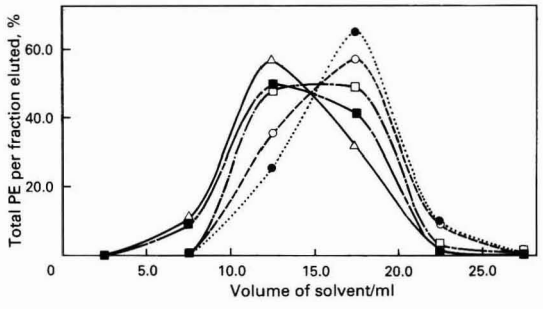

Fig. 4. Elution of PEs from Florisil with 5% acetone in CH_2Cl_2. ●, DMP; ○, DEP; □, DBP; ■, BBP; and △, DEHP

components.[15] Thus the response of the less volatile IS decreases due to discrimination and the R_F value of the more volatile PEs will decrease, as can be calculated using equation (1) and can be seen in Fig. 1. This influence of losses inside the syringe needle decreases as the size of the sample injected increases. It can also be seen that the R_F values of BBP and DEHP almost follow the ideal line. This leads to the assumption that the extent of the discrimination is very similar for BBP, DEHP and the IS BrP. For this reason BrP can be used for the quantification of BBP and DEHP but cannot be used as an IS for DMP, DEP and DBP.

An alternative IS was therefore required. As for the first IS the R_F values for the second IS, DMIP, were plotted against the injection volumes (see Fig. 2). In this instance, consequently the opposite effect is observed. The R_F values of DMP, DEP and DBP follow the ideal line fairly well, whereas those of BBP and DEHP show considerable deviation. Now the R_F values of the less volatile PEs increase with decreasing injection volumes, as the analyte responses decrease [see equation (1) and Fig. 2]. Because of the discrimination effects mentioned above DMIP was finally chosen for the quantification of DMP, DEP and DBP, whereas BBP and DEHP were determined with BrP as the IS.

Development of an LC Method for PE Clean-up

Generally sewage sludge contains large amounts of dissolved and suspended organic material. Strachan *et al.*[14] found that an average of 10.6% of sludge dry mass and 36% of the total organic carbon in sludge can be extracted by use of non-aqueous solvents. These high levels of co-extracted organic compounds, which lead to intensely coloured sample extracts, present a considerable challenge to precise and accurate determination of PEs in sludges. Also compounds interfering with the GC-ECD analysis, such as PCBs or pesticides, have to be separated from the PEs. Therefore a very selective and efficient extract clean-up procedure is necessary to produce final extracts of sufficient quality for reliable GC-ECD determination.

Table 2. Recovery of PEs from the Florisil column (n = 5 in all instances)

PE	Recovery, %		
	x	s	s/x
DMP	101.6	1.4	1.4
DEP	100.4	1.8	1.8
DBP	100.7	3.2	3.2
BBP	99.6	4.5	4.6
DEHP	103.1	5.8	5.6

Most of the methods described for the clean-up prior to GC analysis, however, were elaborated for sample matrices other than sewage sludge[16,17] or for techniques other than GC-ECD[12] and therefore are not suitable for sludge extract clean-up under the present conditions. Others require large volumes of eluents,[18] show only poor recovery[19] or use benzene,[20] which should be avoided if possible because of its carcinogenic effect. Several methods with only one LC column with different solid phases were tested in this study but none of them resulted in sufficiently clean extracts without undesirable interferences with the GC-ECD analysis. Therefore, following Russel and McDuffie[20] a modified dual LC clean-up procedure was developed. First 15% de-activated (water, m/m) alumina was used and various solvents or solvent mixtures were evaluated in order to establish a procedure for the elution of the PEs. Initially these experiments were carried out with standard solutions. After a suitable elution pattern had been found, it was then tested for clean-up efficiency and separation of PAHs, PCBs and pesticides.

To remove extremely non-polar compounds the column was flushed with 30 ml of hexane and 30 ml of 10% methylene chloride in hexane. It was established that all pesticides, PCBs and PAHs used in this study eluted in the first fraction with 30 ml of hexane. This first fraction can be further separated using a silica gel column chromatography procedure as described by McIntyre *et al.*[10] or Russel and McDuffie[20] to obtain one fraction containing the PCBs and another containing pesticides and PAHs. Another 30 ml of 50% methylene chloride in hexane eluted all of the PEs from the column. The elution patterns for the five PEs tested are shown in Fig. 3. Recovery studies were performed for the alumina column and the results are given in Table 1. Examination of the table reveals that the recoveries of the five PEs are quantitative with a small standard deviation. It was found, however, that the third fraction containing the PEs was still coloured and that interference with the GC analysis occurred. Hence the use of Florisil as a second LC column packing material was evaluated for further clean-up of the last alumina fraction. The experiments with the Florisil columns were carried out in the same manner as those with the alumina columns.

The Florisil column was eluted first with 30 ml of 50% methylene chloride in hexane and then with 30 ml of pure methylene chloride. No PEs were present in either fraction as GC-ECD revealed. For the third fraction several mixtures of acetone in methylene chloride were tested with regard to elution, recovery of the PEs and clean-up efficiency. The optimum result was found at 30 ml of 5% acetone in methylene chloride. The fractional elution of the different PEs from the Florisil column with 5% acetone in methylene chloride and the recoveries are shown in Fig. 4 and Table 2, respectively. In the final procedure the third fraction from the alumina column was introduced directly on to the Florisil column so that time could be saved by not evaporating this fraction prior to passing it through the Florisil column. This LC clean-up procedure has the following advantages compared with other methods: the solvent consumption can be

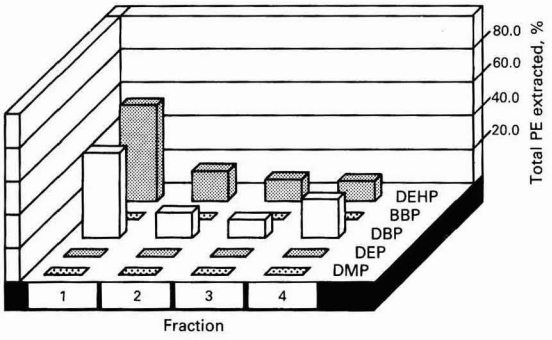

Fig. 5. Percentage recoveries of PEs from unspiked sewage sludge with successive extractions using ultrasonication

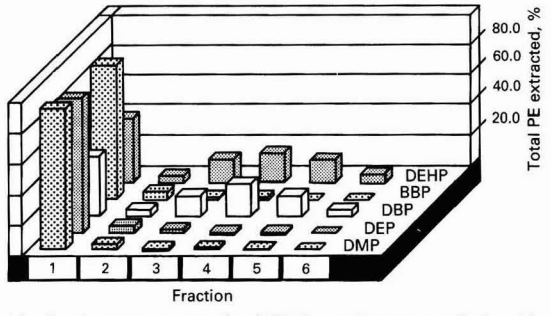

Fig. 6. Percentage recoveries of PEs from spiked sewage sludge with successive extractions using ultrasonication

kept fairly low if the complexity of the matrix is taken into account; the method allows five PEs to be determined in only one fraction for the GC analysis; it allows the separation of PEs from PCBs, pesticides and PAHs and, using the methods described above, separates PCBs from pesticides and PAHs; the recovery of the PEs is complete. Hence, a quantitative GC-ECD analysis of sewage sludge is possible without the interference of accompanying components owing to this rigorous clean-up procedure.

Optimisation of the Ultrasonic Extraction Method

Several methods are described for the extraction of sewage sludge or soil samples with organic solvents: (*i*) homogenisation and centrifugation of the liquid sludge[11,20]; (*ii*) Soxhlet extraction of liquid or dried sludge[10,21]; and (*iii*) ultrasonic extraction of dried sludge.[12,22] The first of these extraction methods was examined, but as it was hindered by the formation of resistant emulsions it was no longer taken into consideration. The ultrasonic extraction technique was then examined and optimised. Methylene chloride was chosen as the solvent because as has been previously described[11,15,22,23] it exhibits the best extraction efficiency for non-liquid matrices. To test the extraction efficiency of ultrasonication a sub-sample was extracted four times and the solvent extracts were collected and analysed separately. The percentage recoveries of the four extractions are presented in Fig. 5. Examination of the results indicates that a great proportion is recovered in the first extraction. However, a considerable amount is still recovered in the fourth extraction, which, with respect to DBP, is even greater than in the third extraction. Another sub-sample which was spiked before analysis was therefore treated in the same manner as the unspiked sub-sample (see Fig. 6).

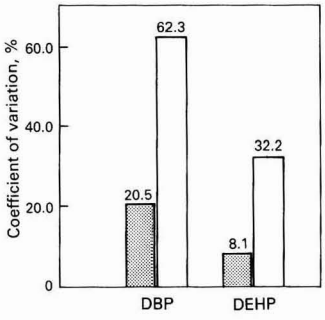

Fig. 7. Comparison of the coefficients of variation for the extraction of ground and unground sewage sludge. ▨, Ground sewage sludge (n = 5); and □, unground sewage sludge (n = 4)

Examination of these results indicates that those PEs which could not be detected in the unspiked samples, namely DMP, DEP and BBP, were detected mainly in the first two extractions with negligible amounts being recovered in the other fractions. The behaviour of DBP and DEHP is analogous to that of the unspiked sub-sample. Considerable amounts are detected in the fourth and even fifth extractions which are higher than the amounts in the second and third extractions.

The results lead to the conclusion that the spiked compounds are incorporated on to the sample matrix in a different way than the compounds already present in the sample and that both the spiked and the unspiked compounds cannot be extracted equally. Both DBP and DEHP are bound to the sludge matrix during the treatment of wastewater and, therefore, are more strongly adsorbed on to the different non-extractable organic compounds in the sludge than are the spiked PEs. These sorptive forces have different intensities. A large proportion (40–50%) can be extracted in the first extraction as can be seen in Fig. 6 and therefore behaves similarly to the spiked PEs. A more intensive or longer ultrasonication is however needed to extract the other 50–60% of the PEs. Even though only negligible and non-detectable amounts of DBP and DEHP could be extracted in the sixth and seventh extractions, respectively, the possibility that a portion of the PEs may still remain in the sludge after such an exhaustive extraction cannot be excluded. Ogner and Schnitzer[24] have found that fulvic acids can complex phthalates and that these PEs could not be re-extracted with organic solvents. In general it is therefore not possible to perform a complete extraction or recovery of the PEs originally existing in the sewage sludge. On the other hand it can be expected that the relative percentage of the PEs extracted is fairly high because of the small amounts of fulvic acids present in anaerobically digested sludges.[25] Because the total recovery cannot be determined, as mentioned above, it is important that the reproducibility of the extraction and analysis procedure is ensured. To examine the reproducibility and the influence of sludge grinding, sub-samples of ground and unground sludge were analysed and the coefficients of variation of the results for DBP and DEHP were determined (Fig. 7).

It is evident that grinding the sludge strongly reduces the coefficient of variation. This is because the heterogeneity of the untreated dried sludge is offset by grinding. Comparison with other investigations shows that in this study the reproducibility of the analysis of ground sludge is equal to or better than that reported previously for DBP and DEHP.[10,11]

Comparison of Ultrasonic and Soxhlet Extraction Techniques

In Table 3 the two extraction techniques are compared in terms of extraction efficiency, blank levels and extraction

Table 3. Comparison of Soxhlet and ultrasonic extraction of sludge samples

PE	Blank level/μg g^{-1} Soxhlet extraction	Ultrasonic extraction
DBP	50.3	1.6
DEHP	3.3	2.9
	Concentration/μg g^{-1} (standard deviation)	
	Soxhlet extraction	Ultrasonic extraction
DEHP	92.5 (6.4)	109.4 (8.1)
Extraction time/h	72	5

Table 4. Concentrations of PEs in sewage sludge samples

Location	Concentration/μg g^{-1} (dry mass) DMP	DEP	DBP	BBP	DEHP
Sonnefeld	ND*	ND	2.8	0.6	224.1
Coburg	ND	ND	2.6	0.3	73.5
Naila	ND	ND	3.1	ND	229.8
Weidhausen	ND	ND	2.3	0.5	65.8
Mitwitz	ND	ND	17.4	0.7	149.5
Rehau	ND	ND	24.1	ND	74.7
Rodach	ND	ND	236.0	0.3	133.9
Hof	ND	ND	4.1	0.2	480.6
Bayreuth	ND	ND	25.7	ND	180.7

* ND: Not detected.

time. The blank levels especially for DBP are very high for Soxhlet extraction. This is probably due to gross contamination of the extraction thimbles although they were pre-extracted for 24 h. Similar blank level problems with Soxhlet extraction have been reported by Peterson and Freeman.[22] The comparison of the extraction efficiency is therefore limited to DEHP only. It can be seen that ultrasonication was slightly better than Soxhlet extraction with almost the same standard deviations. Considering the laborious preparation time and larger solvent volumes required for Soxhlet extraction it is obvious that the Soxhlet extraction technique is not useful for the determination of PEs in sewage sludge samples.

Results for Several Sewage Sludge Samples

The analytical method described has been used to determine the amounts of PEs in several sewage sludge samples of wastewater treatment plants near the town of Bayreuth in the north-east of Bavaria, FRG. The results are given in Table 4. It can be seen that only DBP and DEHP and trace amounts of BBP are present in these sludges. These results are in agreement with those of other workers. Strachan *et al.*[14] reported concentrations of DBP and DEHP from 120 to 600 μg g^{-1}. In addition Petrasek *et al.*[27] demonstrated that DMP and DEP are almost completely degraded during the wastewater treatment process whereas only 44% of DBP and none of the DEHP are degraded. The high levels of DBP and particularly DEHP are explicable if it is taken into account that these two compounds are the most commonly used and therefore are the most widely distributed PEs in the environment.

Conclusion

The method of analysis discussed here combines a rapid and reproducible ultrasonic extraction with a rigorous clean-up procedure for the determination of PEs in complex matrices such as sewage sludge. The precision and accuracy of the method were verified and the problems with discrimination in the injector of the GC-ECD system were solved. This method is economical and has been used successfully to determine the amounts of PEs in several sewage sludge samples.

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Characterisation of a Partially Neutralised Aluminium Solution Using Gel-filtration Chromatography

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An aluminium solution (0.10 M) was hydrolysed such that the [OH]: [Al] ratio = 2, at 25 °C by a mild, slow procedure involving the addition of NaOH to an aluminium salt. In order to separate and characterise the various forms of aluminium present in solution a gel-filtration chromatographic technique was used. The collected effluents were monitored by the usual chemical methods (Ferron assay and atomic absorption spectrometry) and by ^{27}Al nuclear magnetic resonance spectroscopy. The results show that size exclusion is not the only parameter to be considered, the chromatograms being perturbed by charge effects. By varying the experimental conditions, collected samples containing only Al monomers and polymers could be obtained. The polymeric species are mainly represented by the Al_{13} form.

Keywords: *Gel-filtration chromatography; partially neutralised aluminium solution; aluminium-27 nuclear magnetic resonance spectroscopy*

In a recent study,[1] partially neutralised aluminium solutions (0.1 M) hydrolysed at 25 °C by a mild, slow procedure involving the addition of NaOH to an aluminium salt were analysed. It was shown that in the range where the ratio of [OH]:[Al] = 0–2, the number of Al species is minimal, the dominant polymer being that of the Al_{13} form. A small amount (≈10%) of polymeric species remained unidentified although limited nuclear magnetic resonance (NMR) results seemed to indicate that tetrahedrally co-ordinated Al is also present in these latter species. In an effort to characterise the various forms of Al present in solutions, an attempt was made to separate them using a gel-chromatographic technique, which in principle should physically separate a mixture of molecules according to their size. This is true provided the gel matrix has an adequate pore size, is completely inert and that the various species to be separated are not in fast equilibrium with each other (*i.e.*, each species has a sufficiently long lifetime). This technique has been used in previous studies[2,3] conducted on hydrolysed aluminium solutions but prepared in a manner different from that used in this work. Schönherr and Frey[2] examined Al solutions (2 M) hydrolysed from metallic aluminium. The Sephadex G-10 chromatographic gel used was eluted with pure water. The two overlapping conductivity peaks found were analysed using a method based on the Ferron assay but all fractions showed a mixture of monomers and polymers. Akitt and Farthing[3] analysed two types of hydrolysed Al solutions, one starting from Al metal and the other hydrolysed by Na_2CO_3. The fractions were monitored by conductivity, pH, pCl and ^{27}Al NMR. The results for the first type of solution confirmed in more detail the previous study.[2] The second type of solution consisted of 75% Al as Al_{13} the rest being species that could not be observed using NMR. The chromatographic results showed two peaks consistent with the original content of the solutions examined. The gel in that instance was Sephadex G-25 eluted with 1×10^{-3} mol dm^{-3} KNO_3. A recent study[4] reported results obtained using a pre-swollen Sephadex G-25 gel eluted with 0.1 M KCl. The aluminium chlorohydrate solutions (0.2–2.5 M Al) used were prepared at a ratio of [OH]:[Al] = 2.5 from a spray-dried commercial product. From ^{27}Al NMR analysis, it was concluded that these solutions contain a range of hydrolysis complexes of high and intermediate relative molecular mass (with octahedral and tetrahedral Al) in addition to a limited amount of Al_{13} and monomeric species. The gel filtration chromatographic profiles given as relative refractive index *versus* elution volume show essentially two peaks, attributed to high relative molecular mass and intermediate relative molecular mass Al polymers (both being larger than the Al_{13} species), respectively.

In this work, previously studied hydrolysed solutions[1] were examined in detail; the collected effluents from Sephadex G-10 gel were monitored by conductivity, pH, total Al concentrations, monomer Ferron assay and ^{27}Al NMR.

Experimental

Partially Neutralised Al Solutions

Starting from the Al salt, $Al(NO_3)_3.9H_2O$(Merck), a hydrolysed Al solution was prepared, as described previously,[1] at 25 °C up to a level of [OH]:[Al] = 2 and then aged for 24 h. This solution with a total Al concentration of 0.1 M contains 15.7% monomeric species, 74.7% Al_{13} polymers and therefore approximately 10% of unidentified polymeric species.

Chromatographic Technique

The gel chosen was Sephadex G-10 (Pharmacia) which has the smallest exclusion limit of that series, *i.e.*, a relative molecular mass for globular proteins of 700. This value corresponds to a Stokes radius of about 0.7 nm which is in the range of values proposed in the literature for the Al_{13} polymer, *i.e.*, 0.56–1.26 nm.[5,6] A weighed amount (103 g) of the gel was swollen overnight in 500 ml of 0.05 M $NaNO_3$ acidified with 0.1 M HNO_3 to the pH corresponding to the analysed Al solution (ratio of [OH]:[Al] = 2, pH = 4.2). After de-gassing, the gel was packed in an LKB2137 column having an internal diameter of 26 mm and a gel length of 500 mm. Before each experiment, the gel was conditioned by passing at least 300 ml of the chosen eluent solution through it. The volume, V_0, (corresponding to the volume of liquid found in the interstitial space between the gel beads) was determined with Dextran Blue 2000 (Pharmacia) and found to be 170 ml. A 5-ml volume of the studied Al solution was injected and eluted at a rate of 1 ml min^{-1} using a peristaltic pump. At the bottom of the column the pH and conductivity of the liquid being eluted

were continuously monitored by an LKB 2185 detector. Eluted fractions (5 ml) were automatically recovered in polypropylene tubes and assayed at appropriate points by ^{27}Al NMR and chemical methods (Ferron assay and atomic absorption spectrometry). In order to study the effect of the ionic strength on the separation properties of the gel, different eluent solutions were tested, *i.e.*, 0.05, 0.1 and 0.5 M $NaNO_3$. Also in one instance, before elution, the column was pre-treated by passing 20 ml of the studied Al solution through it after which the column was washed with acidified (pH 4.2) distilled water until no more Al was detected in the fractions being eluted.

Ferron Assay

The Ferron reagent was prepared following the procedure proposed by Bersillon *et al.*[7] Ferron forms a coloured complex with Al and the absorbance is followed[1] at 370 nm.

^{27}Al Nuclear Magnetic Resonance Spectroscopy

Spectra were obtained at 65.2 MHz on a Bruker WM 250 Fourier transform NMR spectrometer. Samples were placed in 10-mm tubes containing, at their centre, a capillary tube with an $[Al(OH)_4]^-$ reference solution of appropriate concentration for quantitative determinations. Calibrations were run with $Al(NO_3)_3.9H_2O$ standards.

Results and Discussion

Column Without Pre-treatment, Eluted With 0.1 M $NaNO_3$

The results as a function of the eluted volume (V_e) are given in Fig. 1 where the monomeric content is determined with Ferron and the polymeric content by subtracting from the total aluminium content (as obtained by atomic absorption spectrometry after acidification with an excess of HNO_3) the corresponding monomeric amount. As observed, the separation is far from satisfactory. Both peaks are wide and asymmetric. The polymeric peak passes through a maximum at $V_e \approx 215$ ml, *i.e.*, 1.3 V_0 and is accompanied by a sharp increase of conductivity and a decrease of pH to a value of 4.0. Certain fractions were analysed by ^{27}Al NMR. The results are given in Fig. 2 where the spectra, obtained at ambient temperature, are identified by their respective V_e values. It should be noted that these NMR spectra were taken $\approx$ 12 h after the fractions had been recovered from the Sephadex column. In the V_e range 168–188 ml only one signal located at 63 p.p.m. (with respect to the octahedral value fixed at 0 p.p.m.) is observed. This signal is attributed to the tetrahedral aluminium of the Al_{13} polymer. In this range of V_e, its intensity increased with V_e and is given in Fig. 1 in arbitrary units. For fractions >188 ml, two signals are observed corresponding to the tetrahedral and octahedral (monomers) aluminium species, respectively. The NMR results, therefore, confirm qualitatively the chemical analysis performed on the eluted fractions. The important superposition and asymmetric character of the two elution curves is a clear indication that apart from dimensional effects other uncontrolled mechanisms are perturbing the expected separations of the different Al species.

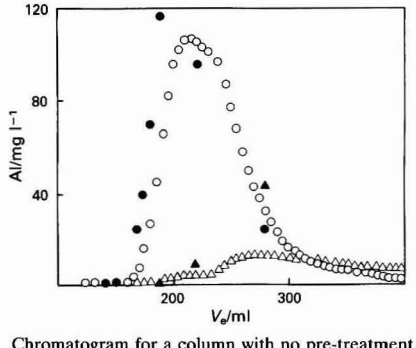

Fig. 1. Chromatogram for a column with no pre-treatment, eluted with 0.1 M $NaNO_3$. ○, Polymeric Al; △, monomeric Al; ●, intensity of NMR tetrahedral line in arbitrary units; and ▲, intensity of NMR octahedral line in arbitrary units

Column Without Pre-treatment, Eluted With 0.5 M $NaNO_3$

When the ionic strength of the eluent solution is increased to 0.5 M, the elution chromatogram given in Fig. 3 is noticeably improved when compared with the previous results (see Fig. 1). The polymeric peak is much more symmetrical and sharper. Its maximum is now located at $V_e \approx 200$ ml and its intensity has increased by a third, relative to the previously observed peak. The superposition with the monomeric peak is less pronounced although the latter still trails on to very large values of V_e.

Pre-treated Column, Eluted With Acidified Water of pH 4.2

In this instance, the chromatogram obtained (see Fig. 4) is improved, essentially with respect to the symmetry of both

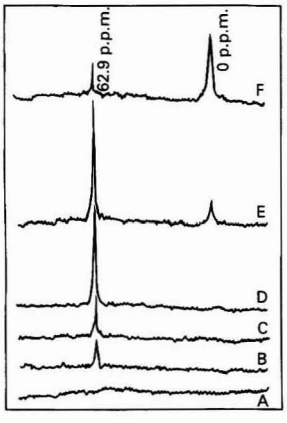

Fig. 2. ^{27}Al NMR spectra, obtained at ambient temperature, for different fractions recovered from the chromatogram shown in Fig. 1. V_e: A, 160; B, 168; C, 172; D, 188; E, 220; and F, 280 ml

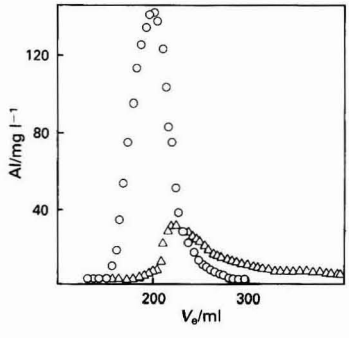

Fig. 3. Chromatogram for a column with no pre-treatment, eluted with 0.5 M $NaNO_3$. Symbols as in Fig. 1

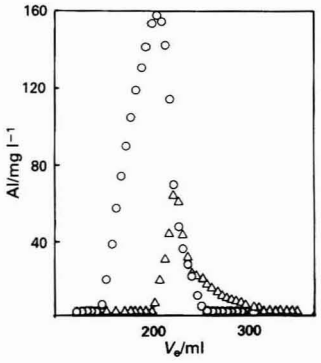

Fig. 4. Al pre-treated column, eluted with acidified water, pH = 4.2. Symbols as in Fig. 1

Table 1. Results for various experimental conditions

Experimental conditions	Monomeric Al species, %	Polymeric Al species, %	Al trapped in gel, %
$NaNO_3$, 0.05 M, 1 ml min^{-1} . .	18	54	28
$NaNO_3$, 0.1 M, 1 ml min^{-1} . .	14	70	16
$NaNO_3$, 0.1 M, 0.5 ml min^{-1} . .	13	70	17
$NaNO_3$, 0.5 M, 1 ml min^{-1} . .	18	62	20
Al pre-treated, acidified H_2O, 1 ml min^{-1}	17	64	19

Table 2. Quantitative comparison between ^{27}Al NMR and results obtained for various points of the "pure" polymer region of the Al pre-treated column

V_e/ml	Polymeric Al or total Al/mM	Tetrahedral Al/mM	Al_{13}/mM	Total Al : tetrahedral Al
160	2.15	0.15	1.95	14
170	3.35	0.25	3.25	13
180	4.40	0.25	3.25	18
190	5.25	0.30	3.90	17

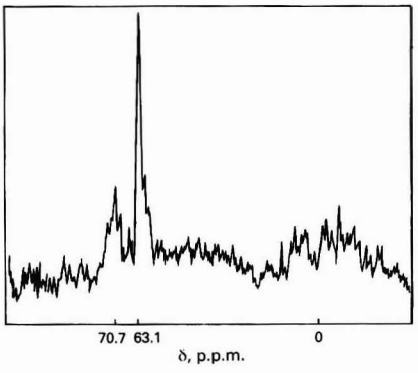

Fig. 5. ^{27}Al NMR spectrum, obtained at 87 °C, of the fraction with V_e = 185 ml, from the chromatogram shown in Fig. 4

curves. This results in higher recovered concentrations at the respective maxima; no more aluminium can be detected in fractions larger than 320 ml. In this chromatogram, two supposedly "pure" regions can be defined: pure polymer between V_e 140 and 200 ml and pure monomers between V_e 260 and 320 ml.

Table 1 summarises the results obtained for the different experimental conditions used. Reducing the elution rate by a factor of two does not affect either the results or the resolution of the chromatogram. In all instances, a certain percentage of aluminium injected is not recovered and remains trapped in the gel. The total amount of monomeric species initially introduced appears to be recovered.

As the initial injected aluminium solution contained approximately 84% of polymeric aluminium sub-divided as ≈74% Al_{13} and ≈10% unidentified polymers, some quantitative ^{27}Al NMR measurements were carried out in the "pure" polymer region identified previously for the Al pre-treated column. As discussed above, in this region, at room temperature, only a line at 63 p.p.m. which is identified with the tetrahedral aluminium of the Al_{13} polymer is observed. This NMR detectable Al_{13} polymer can then be compared with the amount of aluminium polymer determined from the chromatogram for different values of V_e. If the only polymer present in this region is the Al_{13} type then by taking the ratio of total Al: tetrahedral Al content a value close to 13 should be obtained. The results are given in Table 2.

The agreement between values in columns 2 and 4 of Table 2 are good for the first part of the "pure" polymer region with values close to 13 in column 5, however, the discrepancy increases for higher values of V_e, *i.e.*, when approaching the maximum of the polymer elution curve. Some indication of the presence of other types of polymers in these recovered fractions can be obtained by using ^{27}Al NMR conducted on solutions heated well above ambient temperature. The spectrum obtained for a solution recovered from the Al pre-treated column at V_e = 185 ml and heated to 87 °C is given in Fig. 5. A second line at 70.7 p.p.m. clearly appears in the tetrahedral region. Such a line can also be observed when the original aluminium solution is heated to similar temperatures.[1] This line reflects the presence of other species constituting the pool of unidentified polymers. Following an argument based solely on a size-exclusion criterion, the above data appear to indicate that the unidentified species have a molecular size which is not very different from that of Al_{13}. However, as indicated by the experiments conducted at different ionic strengths, other parameters such as charge can be involved. Sephadex G-10 is a highly cross-linked dextran gel, very hydrophilic with, in principle, no charged sites. Several workers[8–10] have indicated however that these gels do possess a small but variable number (1–60 μequiv. g^{-1}) of carboxyl groups.

The expected size separation will therefore be perturbed by ion-exchange effects which will depend on a large number of matrix and solution properties. For this gel, a detailed study of these effects together with selectivity coefficients has yet to be carried out. If the recognition of these perturbing effects is now well established their inclusion in a rigorous treatment of gel-filtration chromatography is still to be performed. A recent review[11] on the subject highlights the complexity of the problem. Finally, "pure" fractions containing only one NMR signal (either monomeric or polymeric) were examined as a function of time after elution. In both instances, the signals remained constant for up to 2 d (longer periods were not examined).

Conclusion

With regard to separation of the various aluminium species present in a solution where the ratio of [OH] : [Al] = 2 using a Sephadex G-10 gel, this work has clearly shown the perturbation brought about by charge effects. Under the experimental conditions used, a 10-fold increase in the ionic strength of the eluent solution does not lead to elution curves with good

resolution. By trying to block the exchangeable sites with aluminium during a pre-treatment stage of the column, a chromatogram showing "pure" polymer and monomer areas can be obtained. By examining the "pure" polymer area it can be stated that apart from the established Al_{13} polymer, a small amount of other polymers exist some of which also possess tetrahedral aluminium. It is also clear from these experiments that in solution, equilibrium between monomeric and polymeric species is reached very slowly; a separated Al_{13} polymer remains stable and does not revert back to the monomeric form for at least 2 d.

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Determination of the Components of Analgesic Mixtures Using High-performance Thin-layer Chromatography

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A spectrodensitometric method is described for the determination of the components of two analgesic mixtures. For the first mixture (paracetamol - ascorbic acid - caffeine - phenylephrine), the pharmaceutically active components were separated from each other and closely related degradation products and impurities on high-performance thin-layer chromatography (F_{254}) plates using methylene chloride - ethyl acetate - ethanol - formic acid (3.5 + 2 + 4 + 0.5) and methylene chloride - ethyl acetate - ethanol (5 + 5 + 1) as the developing systems. The other mixture (phenazone - phenacetin - caffeine) was separated efficiently from the degradation products using the same plates and acetonitrile - chloroform (1 + 1) as the mobile phase. The proposed method was used to determine these mixtures in commercial tablets.

Keywords: *Analgesic mixture; spectrodensitometry; high-performance thin-layer chromatography*

The analysis of paracetamol - ascorbic acid - caffeine - phenylephrine (mixture 1) and phenazone - phenacetin - caffeine (mixture 2) is required for the purpose of quality control of these drugs in their different dosage forms and for drug stability studies. However, most measurement techniques are not selective enough to determine the individual components both in a mixture and in the presence of their degradation products. Many methods have been reported for the determination of the components of these two mixtures either singly or as binary mixtures including spectrophotometry,[1–3] titrimetry,[4–6] thin-layer chromatography (TLC)[7–9] and high-performance liquid chromatography (HPLC).[10–13]

In this paper a selective method is described to separate and determine the components of mixtures 1 and 2 using high-performance thin-layer chromatography (HPTLC).

Experimental

Apparatus

A Camag TLC scanner and a Camag Nanomat sample applicator with a glass capillary were used (Camag, Muttenz, Switzerland).

Materials

Caffeine and paracetamol. Merck (Darmstadt, FRG).
Phenazone. Aldrich (Beerse, Belgium).
Phenacetin. Riedel de Häen (Seelze-Hannover, FRG).
Ascorbic acid. Kraemer and Martin (FRG).
Phenylephrine. Pharmachie Hahn (Hahn, FRG).
Acetanilide. Hoechst (Frankfurt, FRG).
p-*Phenatidine*. Merck.
p-*Chloroacetanilide*. Synthesised by acetylation of *p*-chloroaniline and characterised using spectroscopic and chromatographic methods to confirm the purity.[14]
Coffemed tablets. Chem. Pharm. Fabrik (Herbert Passauer, FRG). Labelled to contain 50 mg of caffeine, 200 mg of phenacetin and 250 mg of phenazone per tablet.
Rhino C tablets. CID (Giza, Egypt). Labelled to contain 5 mg of phenylephrine hydrochloride, 60 mg of caffeine and 400 mg of paracetamol per tablet.

Stock solutions of single components

Paracetamol - ascorbic acid - caffeine - phenylephrine (*mixture* 1). Stock solutions of each component were prepared by dissolving paracetamol (100 mg), ascorbic acid (200 mg), caffeine (100 mg) or phenylephrine (200 mg) in 50 ml of 80% ethanol.

Phenazone - phenacetin - caffeine (*mixture* 2). Stock solutions of each component were prepared by dissolving phenazone (100 mg), phenacetin (100 mg) or caffeine (100 mg) in 50 ml of acetonitrile.

Authentic mixtures

Authentic mixtures were prepared in ratios similar to those labelled to be present in the commercial tablets.

Authentic "Rhino C" mixture. Paracetamol (400 mg), ascorbic acid (60 mg), caffeine (35 mg) and phenylephrine (5 mg) were dissolved in 80% ethanol and made up either to 100 ml for the determination of paracetamol and caffeine or to 25 ml for the determination of ascorbic acid and phenylephrine hydrochloride.

Authentic "Coffemed" mixture. Phenazone (50 mg), phenacetin (40 mg) and caffeine (10 mg) were dissolved in acetonitrile and made up to 25 ml with the same solvent.

Chromatographic Conditions

The HPTLC plates were obtained from Merck (HPTLC silica gel F_{254}, pre-coated plates, 10 × 20 cm). The mobile phases methylene chloride - ethyl acetate - ethanol - formic acid (3.5 + 2 + 4 + 0.5) (I) and methylene chloride - ethyl acetate - ethanol (5 + 5 + 1) (II) were used for mixture 1 and acetonitrile - chloroform (1 + 1) (III) for mixture 2. The distance developed was *ca*. 5 cm.

Spectrophotometric Conditions

The plate was scanned at a speed of 1 mm s^{-1} and the recorder operated at a chart speed of 5 cm min^{-1}.

The sensitivity was 5 mV for ascorbic acid and phenylephrine, and 10 mV for the other components. The dimensions of the slit were 0.3 × 6 mm. The wavelengths used were 264 nm for ascorbic acid, 258 nm for phenazone, 274 nm for phenylephrine and caffeine, and 254 nm for phenacetin and paracetamol.

Procedures

For pure components

Different aliquots from each stock solution were transferred into 10-ml calibrated flasks and made up to volume with the

appropriate solvent to form the working solutions. The working solutions contained 0.2–2 mg ml^{-1} of paracetamol, caffeine, phenazone or phenacetin and 0.2–4 mg ml^{-1} of ascorbic acid or phenylephrine.

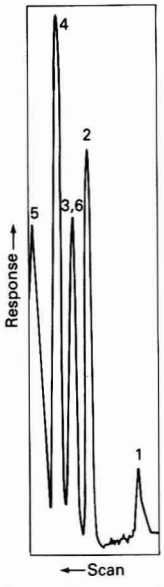

Fig. 1. HPTLC trace of authentic mixture 1, Rhino C. Mobile phase, I. 1, Phenylephrine hydrochloride; 2, ascorbic acid; 3, caffeine; 4, paracetamol; 5, *p*-aminophenol; and 6, colouring matter

Table 1. R_F values of a mixture of ascorbic acid, phenylephrine hydrochloride, paracetamol, caffeine and their degradation products

Component	R_F value*	
	Mobile phase I	Mobile phase II
Ascorbic acid	0.53	0.00
Phenylephrine hydrochloride	0.22	0.00
Paracetamol	0.87	0.67
Caffeine	0.69	0.52
p-Aminophenol	0.96	0.80
Colouring matter in Rhino C tablets	0.69	0.06

* Measured using a fluorescence quenching technique.

A 0.5-µl aliquot of the working solution of each component was spotted on an HPTLC plate using the glass capillary of the Camag Nanomat applicator. Alternate lanes were spotted, permitting the use of reference lanes for each sample lane.

The plate was developed horizontally in a developing chamber with the corresponding mobile phase for a distance of *ca.* 5 cm from the base line. The plate was air-dried and the spots were scanned using the Camag TLC scanner.

The peak height was measured and plotted against concentration (µg per spot).

For pharmaceutical preparations

Eight tablets of each preparation were powdered individually and then extracted with the appropriate solvent using an ultrasonic bath to give the corresponding sample solutions with the following concentrations: 122 mg per 25 ml for Coffemed tablets, one tablet per 25 ml for the determination of ascorbic acid and phenylephrine and one tablet per 100 ml for the determination of paracetamol and caffeine in Rhino C tablets.

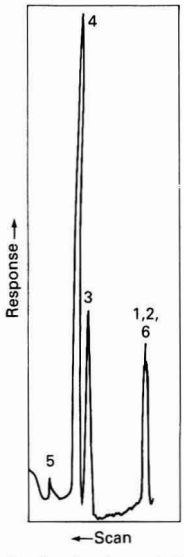

Fig. 2. HPTLC trace of authentic mixture 1, Rhino C. Mobile phase, II. 1, Phenylephrine hydrochloride; 2, ascorbic acid; 3, caffeine; 4, paracetamol; 5, *p*-aminophenol; and 6, colouring matter

Table 2. Analysis of Rhino C tablets using the proposed HPTLC method

Paracetamol		Caffeine		Ascorbic acid		Phenylephrine hydrochloride	
Claimed/µg µl^{-1}	Recovery,* %	Claimed/µg µl^{-1}	Recovery,* %	Claimed/µg µl^{-1}	Recovery,* %	Claimed/µg µl^{-1}	Recovery,* %
2	105.00	0.2	102.97	2.4	102.69	0.2	100.33
2	101.70	0.2	102.78	2.4	98.26	0.2	102.50
2	105.22	0.2	102.23	2.4	97.60	0.2	96.00
2	101.70	0.2	96.19	2.4	96.80	0.2	96.00
2	101.85	0.2	97.26	2.4	97.55	0.2	97.06
2	104.33	0.2	104.96	2.4	98.36	0.2	100.14
2	97.30	0.2	96.00	2.4	100.00	0.2	109.30†
2	105.78	0.2	101.40	2.4	107.27†	0.2	101.65
Mean	102.83		100.47		98.75		99.09
SD‡	2.78		3.47		2		2.7
RSD§	2.7		3.45		2.02		2.72

* Mean of six experiments.
† The result must be rejected according to the rejection rule (the rule for rejection of high or low values).[15]
‡ SD = standard deviation.
§ RSD = relative standard deviation.

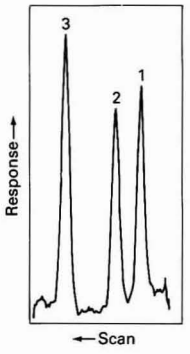

Fig. 3. HPTLC trace of Coffemed tablets. Mobile phase, III. Detection, 258 nm. 1, 0.5 μg of phenazone; 2, 0.1 μg of caffeine; and 3, 0.4 μg of phenacetin

Table 3. R_F values of a mxiture of phenacetin, phenazone, caffeine and their degradation products using mobile phase III

Component	R_F value*
Phenacetin	0.74
Phenazone	0.22
Caffeine	0.44
Acetanilide	0.79
p-Chloroacetanilide	0.79
p-Phenatidine	0.69

* Measured using a fluorescence quenching technique.

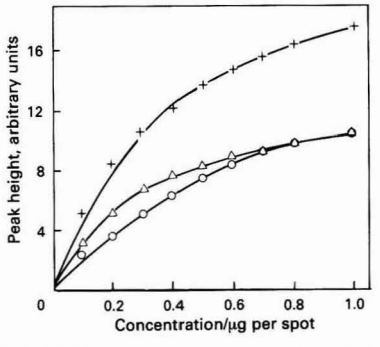

Fig. 4. Peak height - concentration relationship of phenacetin, phenazone and caffeine using HPTLC. ○, Phenacetin; △, phenazone; and +, caffeine. Mobile phase, III

For a 10 × 20 cm plate, 19 lanes were spotted. The working solutions and sample solutions of the authentic mixture were spotted alternately using the same glass capillary to avoid volume error (*i.e.*, the error during spotting). The glass capillary must be washed three times with the appropriate solvent after each spotting. The plate was then developed and scanned as described in the above procedure.

Discussion

The proposed method was used to separate and determine paracetamol, ascorbic acid, caffeine and phenylephrine in the presence of their degradation products dehydroascorbic acid and *p*-aminophenol (mixture 1). It was difficult to separate the four components, their degradation products (if present) and the colouring matter of the tablet coating by using one mobile

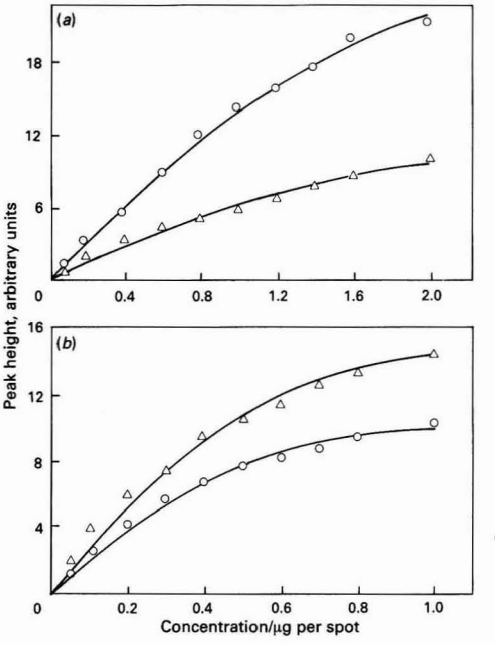

Fig. 5. Peak height - concentration relationship of ascorbic acid, phenylephrine hydrochloride, caffeine and paracetamol using HPTLC. (*a*) ○, Ascorbic acid; and △, phenylephrine hydrochloride. Mobile phase, I. (*b*) ○, Paracetamol; and △, caffeine. Mobile phase, II

Table 4. Analysis of Coffemed tablets using the proposed HPTLC method

Phenacetin		Phenazone		Caffeine	
Claimed/ μg μl^{-1}	Recovery,* %	Claimed/ μg μl^{-1}	Recovery,* %	Claimed/ μg μl^{-1}	Recovery,* %
1.2	102.79	1.5	104.32	0.3	104.87
1.2	98.14	1.5	100.72	0.3	100.00
1.2	99.44	1.5	99.86	0.3	103.66
1.2	100.00	1.5	99.64	0.3	103.66
1.2	96.53	1.5	96.70	0.3	101.36
1.2	110.20†	1.5	102.20	0.3	97.47
1.2	99.25	1.5	100.20	0.3	101.96
1.2	96.52	1.5	101.26	0.3	99.78
Mean	98.95		100.6		101.6
SD‡	2.18		2.19		2.46
RSD§	2.2		2.18		2.43

* Mean of six experiments.
† The result must be rejected according to the rejection rule.[15]
‡ SD = standard deviation.
§ RSD = relative standard deviation.

phase. For ascorbic acid and phenylephrine the acidic mobile phase methylene chloride - ethyl acetate - ethanol - formic acid (3.5 + 2 + 4 + 0.5) (I) was used, which avoided interference from other components, degradation products and colouring matter. Colouring matter interfered with the determination of caffeine however (both have the same R_F value of 0.69), but this interference was overcome by using the mobile phase methylene chloride - ethyl acetate - ethanol (5 + 5 + 1) (II). Paracetamol and caffeine were completely separated without interference from other constituents using mobile phase II (Tables 1 and 2, Figs. 1 and 2).

Phenazone, phenacetin and caffeine in Coffemed tablets (mixture 2) were separated effectively using the mobile phase acetonitrile - chloroform (1 + 1) (III) (Fig. 3). Their degradation products, acetanilide, *p*-chloroacetanilide and *p*-phenatidine did not interfere owing to the differences in R_F values between the active constituents and the degradation products (Table 3). Although acetanilide and *p*-chloroacetanilide have the same R_F value of 0.79, this has no significance for quality control purposes as both are degradation products.

Emission spectra for each component were recorded which showed absorption maxima at 274 nm for caffeine and phenylephrine, 264 nm for ascorbic acid, 254 nm for paracetamol and phenacetin and 258 nm for phenazone.

A calibration graph (peak height *versus* concentration) for each plate was constructed which was linear at low concentrations and non-linear at high concentrations. Determination using a second-order equation, rather than a linear regression equation, is recommended. This was valid up to 4 μg ml^{-1} of phenylephrine or ascorbic acid and 2 μg ml^{-1} of caffeine, phenazone, phenacetin or paracetamol. At concentrations greater than these no increase in peak height was observed.

Amounts of 100 ng per spot for phenylephrine and ascorbic acid, 25 ng per spot for caffeine and 50 ng per spot for paracetamol, phenacetin and phenazone are considered to be the lowest limits that can be applied (Figs. 4 and 5).

The second-order equations relating peak height to concentration are as follows. For mixture 1: ascorbic acid, $y = 0.0 + 0.1634x - 2.836x^2$, $r = 0.998$; phenylephrine, $y = 0.0 + 7.509x - 1.457x^2$, $r = 0.997$; paracetamol, $y = 0.0 + 0.211x - 0.116x^2$, $r = 0.998$; caffeine, $y = 0.0 + 0.089x - 0.150x^2$, $r = 0.997$; for mixture 2: phenacetin, $y = 0.0 + 9.988x - 2.439x^2$, $r = 0.999$; phenazone, $y = 0.0 + 0.122x - 3.656x^2$, $r = 0.997$; caffeine, $y = 0.0 + 0.195x - 5.620x^2$, $r = 0.997$ where y = peak height, x = concentration in μg per spot and r = correlation coefficient.

The sample solution was compared with a standard solution containing similar components and separated under the same conditions. Volume error was overcome by using the same capillary for application.

The proposed method was used to determine paracetamol, ascorbic acid, caffeine and phenylephrine in Rhino C tablets and phenazone, phenacetin and caffeine in Coffemed tablets. Statistical analysis of the results shows that the proposed method is accurate and precise (Tables 2 and 4). Further, the determination depends on the measurement of intact molecules of active components and not their hydrolytic products; hence the proposed method is selective and can be used for a stability-indicating assay. In addition the method is rapid and less expensive than high-performance liquid chromatography.

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Determination of Trace Impurities in High-purity Niobium by Inductively Coupled Plasma Atomic Emission Spectrometry After Anion-exchange Separation

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Inductively coupled plasma atomic emission spectrometry (ICP-AES) in combination with anion exchange with hydrochloric and hydrofluoric acid media was applied to the determination of trace-metal impurities in high-purity niobium samples. The column operating conditions of the anion-exchange method were selected on the basis of the mass distribution coefficients of the analytes. Molybdenum, tungsten and titanium having similar chemical properties to niobium were eluted simultaneously with magnesium, manganese and chromium. Iron and tantalum were eluted after niobium had been removed. These trace impurities were determined by ICP-AES using analysis lines that were free from spectral interference from the small amount of niobium which was present in the effluents. The results obtained for high-purity niobium oxide and niobium metal samples using ICP-AES combined with an anion-exchange method, agreed well with those obtained by electrothermal atomic absorption spectrometry without the separation of niobium and ICP-AES combined with a solvent extraction method .

Keywords: *Trace impurity; high-purity niobium; anion-exchange separation; inductively coupled plasma atomic emission spectrometry*

In recent years niobium has become a material of great scientific and technological significance. The determination of this element has been the subject of numerous studies using such techniques as emission spectrography,[1,2] radiometry,[3,4] spectrophotometry[5,6] and atomic absorption spectrometry.[7,8] Inductively coupled plasma atomic emission spectrometry (ICP-AES) offers several advantages over these methods; low detection limits, wide linear ranges in calibration graphs and the capability of simultaneous multi-element determinations. Despite these advantages, no report on the analysis of high-purity niobium by ICP-AES has been published.

The direct determination of trace impurities in the presence of a niobium matrix by ICP-AES is not possible owing to serious spectral interferences found in the complex atomic emission spectra. Separation procedures for niobium are essential in order to eliminate such interferences and improve signal to background ratios of impurity elements. Separation of trace impurities from niobium has been achieved by extraction - spectrophotometric methods for Ta[5] and stepwise extraction for Mo and W.[6] Basic studies on the anion exchange behaviour of these elements have been carried out with HF,[9] HCl and HF,[10–12] HCl and HNO_3[13,14] and HCl and oxalic acid,[15,16] and the techniques have been applied to the analysis of steels.[17–19] In these situations, Mo, W, Ti and other metals were separated sequentially and determined by spectrophotometry or volumetric analysis.

In this study, the anion-exchange technique was used for separation of impurities from niobium prior to ICP-AES measurements. The technique in hydrochloric and hydrofluoric acids was further investigated in order to obtain Mo, W and Ti together with Mg, Cr and Mn in the first effluent. In the separation procedure, Ta, which is the main impurity of niobium was co-eluted with Fe after the elution of niobium. In order to confirm the accuracy of the proposed method, a direct method using electrothermal atomic absorption spectrometry (ETAAS) and a solvent extraction method followed by ICP-AES were also investigated.

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Experimental

Chemicals

All chemicals used were of analytical-reagent grade. Ultrapur hydrochloric acid, nitric acid and hydrofluoric acid were obtained from Kanto Chemicals (Tokyo, Japan). Instrument calibration standards were prepared from commercially available 1 mg ml^{-1} standard solutions and/or high-purity materials. Distilled, de-ionised water, prepared using a sub-boiling distillation apparatus, was used throughout.

The resin used was a strongly basic anion-exchange resin, type AG 1-X8(100–200 mesh), supplied by Muromachi Kagaku Kogyo, (Tokyo, Japan). The anion-exchange column was prepared to a 20-cm bed height with a 10-ml bed volume, by using PTFE tubing with 0.8 cm i.d. For measurement of the mass distribution coefficients for the metals of interest, the air-dried resin in its chloride form was used.

High-purity niobium oxide (99.999%) purchased from D. F. Goldsmith Chemical & Metal, (Evanston, Il, USA), was used as a base matrix for the study of the elution behaviours and the recovery tests. Samples of high-purity niobium metal were obtained from the same manufacturer and from Soekawa Chemicals (Tokyo, Japan) and were of a nominal purity of 99.8 and 99.9%, respectively.

Apparatus

The sequential ICP-AES instrument used and its operating conditions are listed in Table 1.

For ETAAS, an SAS-760 atomic absorption spectrometer (Seiko Instrument & Electronics, Tokyo, Japan), equipped with a tungsten strip heater and a simultaneous deuterium background correction system was employed.[20,21] The strip heater was 7 mm wide and 55 mm long. An SAS-705V unit (Seiko Instrument & Electronics) was used as a power supply for the heater. Absorbances of Mg, Cr, Mn and Fe were measured under the following conditions: drying at 120 °C for 30 s, then heating to 800 °C in 10 s and holding at 800 °C for 10 s; atomisation at 2400 °C for 4 s; wavelengths, 285.2 nm for Mg, 357.9 nm for Cr, 279.5 nm for Mn, 248.3 nm for Fe; and sheath gas, 5.0 l min^{-1} of argon with 0.8 l min^{-1} of hydrogen.

Table 1. Instrumentation and operating conditions for ICP-AES

Instruments—	
Generator	Shimadzu ICPS-2H (Tokyo, Japan)
Spectrometer	Shimadzu CTM-100, 1-m Czerny - Turner with a 3600 grooves mm^{-1} grating
Operating conditions—	
ICP radiofrequency power/kW	1.2
Outer gas flow-rate/l min^{-1}	14
Inner gas flow-rate/l min^{-1}	1.0
Central gas flow-rate/l min^{-1}	1.0
Observation height/mm	15
Spectrometer slit-width/μm	10

Procedures

Determination of mass distribution coefficients

Air-dried resin (1 g) was placed in a 50-ml polyethylene bottle, followed by 0.1 ml of metal solution (1 mg ml^{-1}), added using a pipette. Appropriate concentrations of dilute acids were added to give a final volume of 10 ml. The bottle was shaken for 3 h and the remaining metal concentration in the solution was determined by ICP-AES.

Proposed method by anion exchange ICP-AES

A half gram of niobium and/or 0.715 g of niobium oxide were weighed and dissolved with 5 ml of HF followed by 1 ml of HNO_3. The solution was evaporated to dryness and the residue dissolved in 10 ml of 5 M HCl - 7 M HF. The solution was passed through a column, which had been washed previously with 20 ml of 1 M HCl, 20 ml of water, 20 ml of 1 M HF and 20 ml of 5 M HCl - 7 M HF in sequence (although there was a possibility of resin degradation in the high acidity media, it proved efficient for several separations). Niobium was adsorbed as a yellowish brown band 10 cm in length and impurities such as Ti, W and Mo were eluted with 50 ml of 5 M HCl - 7 M HF. The effluent for the first 10 ml and another 50 ml was collected in a PTFE beaker and 1 ml of H_2SO_4 and 1 ml of HNO_3 were added. After evaporation until fumes of sulphur trioxide were evolved, the solution was diluted to 25 ml with water. Niobium was eluted with 60 ml of 7 M HCl - 0.2 M HF. Residual impurities of Ta and Fe were eluted with 50 ml of 3 M H_2SO_4 - 0.1% H_2O_2. The effluent was treated in the same way as the first effluent and diluted to 50 ml with water. The concentrations of these elements were determined by ICP-AES using the lines listed in Table 2, which were selected with consideration to the sensitivity and spectral interferences from the small amount of niobium present in the effluents. Calibration was carried out using acid-matched standard solutions.

ETAAS analysis

An accurately weighed 1-g amount of niobium and/or 1.43 g of niobium oxide were dissolved with 10 ml of HF followed by 1 ml of HNO_3. Before AAS measurements, it was necessary to expel HNO_3 in order to avoid erosion of the tungsten strip heater. The solution was then evaporated to dryness and the residue dissolved in 10 ml of HF and diluted to 100 ml with water. The temperature programme of the ETAAS instrument was optimised using the 1% niobium solution prepared from niobium oxide. A test solution (10 μl) was pipetted on to the tungsten heater. The concentrations of Mg, Cr, Mn and Fe were determined by the standard additions method.

Solvent extraction ICP-AES

Tantalum can be separated from niobium by extraction from 0.6 M HF with 4-methylpentan-2-one [isobutyl methyl ketone (IBMK)]. The extraction was carried out using the method described by Grossman.[6] Tantalum, separated from 0.5 g of niobium into IBMK, was evaporated to dryness. The residue was dissolved with 0.5 ml of HF, and 0.5 ml of HNO_3 and 1 ml of H_2SO_4 were added. The solution was then evaporated until the evolution of sulphur trioxide fumes and diluted to 25 ml. The concentration of Ta in the solution was determined by ICP-AES.

According to the literature,[22] from 6 M H_2SO_4 - 10 M HF - 2.2 M NH_4F, Mo, W and Ti are less easily extracted into IBMK, although niobium fluoride can be extracted. Thus the following procedure was used for the determination of these elements. A 0.5-g amount of niobium was dissolved and treated so as to be matched, with respect to acid content, with 12.5 ml of 6 M H_2SO_4 - 10 M HF - 2.2 M NH_4F. Niobium was extracted into 12.5 ml of IBMK by shaking in a separating funnel for 5 min. The aqueous layer was transferred into a PTFE beaker and heated until sulphur trioxide fumes were evolved, and then diluted to 25 ml. Molybdenum, W and Ti in the solution were determined by ICP-AES.

Table 2. Line wavelengths for ICP-AES

Element	Wavelength/nm
Mg^{II}	279.553
Mn^{II}	257.610
Cr^{II}	205.552
Ti^{II}	323.904
W^{II}	207.911
Mo^{II}	202.030
Fe^{II}	238.204
Ta^{II}	240.063
Nb^{II}	309.418

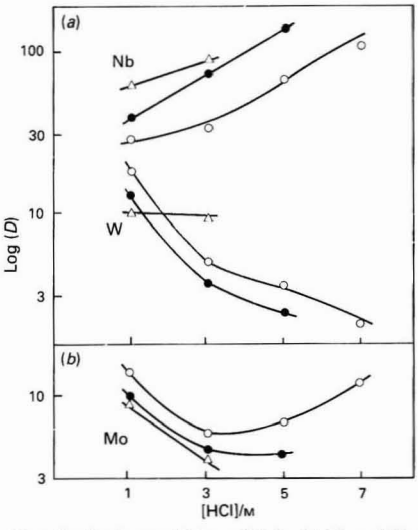

Fig. 1. Mass distribution coefficients (D) for (*a*) Nb and W; and (*b*) Mo between the anion-exchange resin AG 1-X8 and solutions containing various concentrations of hydrochloric and hydrofluoric acid. Hydrofluoric acid concentration: ○, 4; ●, 7; and △, 10 M

Results and Discussion

Anion exchange ICP-AES

For the simultaneous elution of Mo, W and Ti, mass distribution coefficients (D) in HCl - HF solutions of different compositions were determined. The results for Mo, W and Nb are shown in Fig. 1. Titanium showed no adsorption in the

Table 3. Mass distribution coefficients of analytes with different acid solutions

Solution	Element								
	Mg	Mn	Cr	Ti	Mo	W	Nb	Fe	Ta
5 M HCl - 7 M HF	<1	<1	<1	1	4	2	123	72	144
7 M HCl - 0.2 M HF	—	—	—	—	—	—	3	>500	>300
6 M H_2SO_4 - 0.1% H_2O_2	—	—	—	—	—	—	—	<1	6

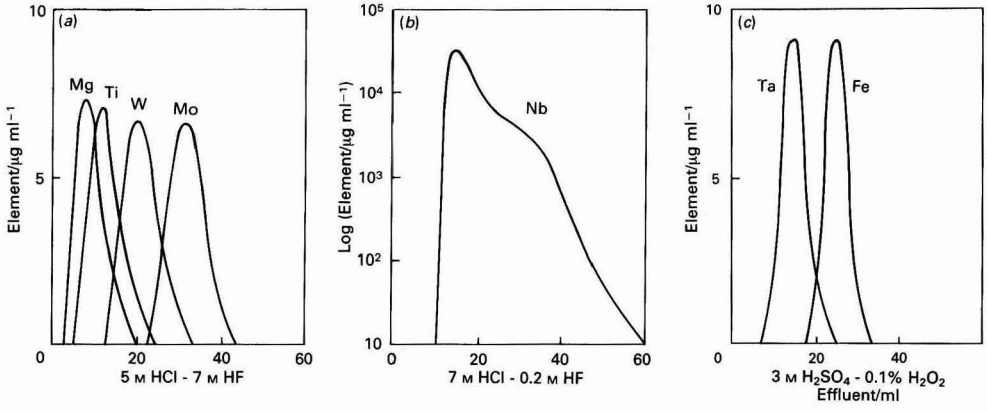

Fig. 2. Typical elution curves for (*a*) Mg, Ti, W and Mo; (*b*) Nb; and (*c*) Ta and Fe. The first 10 ml of (*a*) corresponds to the sample solution

solutions investigated. A 5 M HCl - 7 M HF mixture was suitable for the separation of Mo and W from niobium, because the *D* values of Mo and W were less than 5 and the *D* value of niobium was the highest among these solutions. The *D* values of other elements for 5 M HCl - 7 M HF are shown in the first row of Table 3. Magnesium, Mn and Cr could be eluted easily without any adsorption, but Fe and Ta were strongly adsorbed in that acid medium.

The 10 ml of bed volume could adsorb about 1 g of niobium in 5 M HCl - 7 M HF. In general, the eluent required two or three times the bed volume before the elements showing no adsorption were eluted. The volume eluted when the concentration of an element in the effluent reaches the maximum, $v_{max.}$, is given as $\omega D+i$,[19] where ω is the mass of a dried resin and i is the volume of a solution in the column. When ω and i are 5 g and 5 ml, respectively, $v_{max.}$ becomes 10 ml for $D = 1$ and 25 ml for $D = 4$ (Mo). So the eluent necessary for elution of Mo would be more than 30–40 ml. When 10 ml of 5 M HCl - 7 M HF solution containing 1 g of niobium passed through the column and a further 5 M HCl - 7 M HF, with a total volume of 120 ml in 10-ml increments, passed, niobium was eluted at an effluent volume of 30 ml and the niobium concentration reached 0.1 mg ml^{-1} at an effluent volume of 40 ml. The same concentration of niobium was obtained at a volume of 65 ml when 0.7 g of niobium was used and at a volume of 110 ml when 0.5 g of niobium was used. Based on these results, 0.5 g (as metal) was chosen as the appropriate amount of niobium sample.

A 0.5-g amount of niobium in 10 ml of 5 M HCl - 7M HF was passed through the column and 90 ml of 5 M HCl - 7 M HF was passed through in 10 ml portions. After each effluent was evaporated to produce 25 ml solutions containing 5% H_2SO_4, the concentrations of Mg, Cr, Mn, Mo, W and Ti were determined by ICP-AES. The results when niobium oxide was used, and 0.1 mg of each element added, are shown in Fig. 2(*a*). Chromium and Mn showed the same elution curve as Mg. Sixty millilitres of 5 M HCl - 7 M HF were sufficient to elute these elements completely and niobium, Fe and Ta were not detected in the 60 ml effluent. The elution behaviours of these six elements (Mg, Cr, Mn, Mo, W and Ti) added to the blank solution were almost the same as those shown in Fig. 2(*a*) but with slightly higher values of $v_{max.}$.

Tantalum is one of the elements that is most strongly adsorbed on to the anion-exchange resin in an HF medium; it can be eluted with high concentrations of HF solution, but a large amount of eluent is required.[9] On the other hand, niobium can be eluted easily with 7 M HCl - 0.2 M HF.[19] The *D* values of Ta and Fe with 7 M HCl - 0.2 M HF are shown in the second row of Table 3. Then prior to the elution of Ta and Fe, niobium was eluted with 60 ml of 7 M HCl - 0.2 M HF as shown in Fig. 2 (*b*). The concentration of niobium in the last portion of the effluent (50–60 ml) was 20 μg ml^{-1}. Although Ta can be eluted with NH_4Cl - NH_4F,[19] the solution contains a large amount of salts, therefore dilute sulphuric acid[23] was used. When a dilute sulphuric acid medium, to which 0.1% H_2O_2 was added, was used, both Fe and Ta had low *D* values shown in the third row of Table 3 and provided the elution curves shown in Fig. 2(*c*). Fifty millilitres of 3 M H_2SO_4 - 0.1% H_2O_2 was sufficient to elute the Fe and Ta completely. The effluent contained only 13–27 μg ($n = 4$) of niobium; this means that more than 99.99% of niobium was removed by this procedure.

On the basis of these results, the sequence of procedures for anion exchange, summarised under Procedures, was used in this work. The time required for the elution was about 7 h at a flow-rate of *ca.* 0.4 ml min^{-1}.

Table 4 shows the results of recovery tests. Each element was added to a solution prepared from niobium oxide and this was treated by the proposed procedure. The concentration of each element was determined by ICP-AES. The recovery of each element was 96.5–100.5% even at the μg level. Similar recoveries were obtained for the impurities added to the blank solution.

Other Methods

ETAAS

As can be seen in Fig. 3(*a*) the signal (absorbance) of Fe measured, with the reagent blank solution added, 0.1 μg ml^{-1}

of Fe shows a simple and sharp peak, while a sharp background peak and a low background peak are shown in Fig. 3(*b*) with the 1% niobium solution to which 0.1 μg ml^{-1} of Fe was added. This sharp background peak which might cause an incomplete background correction was significantly reduced by raising the ashing temperature from 400 to 800 °C as shown in Fig. 3(*c*). The effect of ashing temperature varying from 400 to 1000 °C on the signal was investigated and no change in the peak area or the peak height of the signal was found. The lowest ashing temperature necessary for the reduction of the background and the complete recovery of Fe was 800 °C.

In order to achieve a high accuracy in trace metal analysis, the behaviour of each metal should be similar in the sample solutions and in the calibration solutions. As shown in Fig. 3 by comparison of (*a*) and (*c*), the presence of niobium reduced the peak height of the Fe signal to about 50%. On the contrary, little difference in the peak area was observed between the solutions with and without niobium. Similar results were obtained for Mg, Cr and Mn.

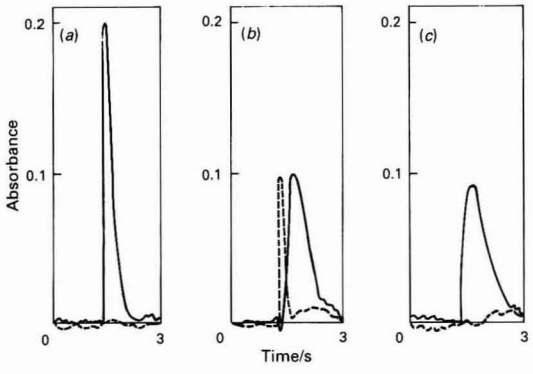

Fig. 3. Typical ETAAS signals for Fe(solid line) and background (broken line). (*a*) 0.1 μg ml^{-1} of Fe in 10% hydrofluoric acid, ashing at 800 °C; (*b*) solution containing 1% Nb and 0.1 μg ml^{-1} of Fe, ashing at 400 °C; and (*c*) same solution as in (*b*), at 800 °C. The ashing time was 20 s and the atomisation temperature was 2400 °C in all instances

Table 4. Recovery of impurities added to niobium solution by the proposed method

	Found/μg							
Added/μg	Mg	Mn	Cr	Ti	W	Mo	Fe	Ta
1.0 . .	1.02	1.00	1.0	1.0	1.1	1.1	—	—
10.0 . .	10.1	10.0	9.9	9.9	10.7	10.2	10.2	10.4
100	98.0	98.3	96.5	98.3	100.5	100.5	98.9	98.7

Solvent extraction ICP-AES

There was little possibility of determining non-volatile elements such as Ta, Mo, W and Ti by ETAAS, therefore ICP-AES after separation of niobium with a solvent extraction technique was investigated.

Tantalum could be extracted into IBMK from 0.6 M HF, a small amount of niobium being co-extracted. According to the extraction method described under Procedures, the recovery of Ta was 79 ± 3% (n = 3) and the quantity of niobium co-extracted was 0.087 ± 0.025 mg (n = 8). This concentration of niobium did not affect the measurement of the emission intensity of Ta.

From 6 M H_2SO_4 - 10 M HF - 2.2 M NH_4F, 96.7 ± 0.3% of niobium was extracted into IBMK, while 84% of Mo and 100% of W and Ti remained in the aqueous layer. From a study of the extraction behaviour of these elements by Milner *et al.*,[22] it was expected that the extraction efficiency for Nb would be 96% and the recoveries of Mo, W and Ti in the aqueous layer 90, 74 and 99%, respectively. The recoveries of W and Ti were better than those expected but that of Mo was a little worse. The residual concentration of niobium in the solutions was 0.664 ± 0.056 mg ml^{-1}. The emission intensities of the lines selected for W (207.911 nm) and Ti (323.904 nm) were not affected by the residual niobium. However, Mo (202.030 nm) could not be determined because of the spectral interference from niobium (201.980 nm). A concentration of 0.1 mg ml^{-1} of niobium caused a pseudo-signal equivalent to 0.2 μg ml^{-1} of Mo; ICP mass spectrometry was used for the determination of Mo instead.

Analytical Applications

Impurities in niobium oxide and two niobium metal samples were determined by the three methods described above. The results are shown in Table 5. No significant difference was observed between the values obtained by the proposed method and those by ETAAS and solvent extraction ICP-AES.

The detection limit (DL) was calculated as the concentration giving three times the standard deviation (σ) of the blank solution and the lowest quantitatively determinable concentration (LQD) was calculated from 10 σ. The results are listed in Table 6. The LQDs of the proposed method were some 2–10 times better than those obtained by ETAAS and were similar to those given by the extraction method. The simultaneous multi-element ICP-AES instrument, if available, could reduce the LQDs of the proposed method by half by reducing the final volume from 25 to 10 ml.

The blank values obtained by the proposed method were

Table 5. Analytical results of high-purity niobium oxide and metals in μg g^{-1} (mean of three determinations)

	Niobium Oxide*				Niobium metal*				Niobium metal†		
Element	Proposed method	ETAAS	Extraction	Quoted‡	Proposed method	ETAAS	Extraction	Quoted‡	Proposed method	ETAAS	Extraction
Mg	0.06 ± 0.01	(0.1)§	—	0.1	0.11 ± 0.03	(0.1)	—	<10	0.18 ± 0.03	0.2	—
Cr	(0.1)	ND¶	—	<2	0.8 ± 0.1	(0.6)	—	<10	7.2 ± 0.3	7.7 ± 0.1	—
Mn	0.20 ± 0.02	0.1	—	<1	0.10 ± 0.02	ND	—	<10	0.50 ± 0.02	0.4 ± 0.1	—
Ti	0.66 ± 0.01	—	0.6 ± 0.1	<1	1.3 ± 0.1	—	1.2 ± 0.3	<10	1.2 ± 0.2	—	1.7 ± 0.3
W	1.8 ± 0.2	—	1.7 ± 0.2	—	2.5 ± 0.3	—	(2.3)	<100	5.7 ± 0.3	—	5.7 ± 0.2
Mo	1.3 ± 0.1	—	1.3 ± 0.1‖	<1	2.0 ± 0.2	—	2.0 ± 0.5‖	<100	2.1 ± 0.2	—	3.0 ± 0.2‖
Fe	0.7	ND	—	<1	17 ± 1	16 ± 1	—	10	18 ± 1	—	15 ± 1
Ta	12 ± 1	—	12 ± 2	<50	242 ± 6	—	242 ± 10	240	349 ± 5	—	345 ± 18

* Niobium oxide and metal obtained from D. F. Goldsmith Chemicals & Metals.
† Niobium metal obtained from Soekawa Chemicals.
‡ Presented by manufacturer.
§ Values in parentheses are below the LQD (see Table 6).
¶ ND = Not determined.
‖ Determined by inductively coupled plasma mass spectrometry.

Table 6. Detection limits and lowest quantitatively determinable concentration of impurities in niobium

	DL/ng ml^{-1} (3σ)			LQD*/μg g^{-1} (10 σ)		
Element	Proposed method	ETAAS	Extraction	Proposed method	ETAAS	Extraction
Mg	0.12	1.2	—	0.02	0.2	—
Cr	5.1	12	—	0.8	2	—
Mn	0.3	1.2	—	0.05	0.2	—
Ti	0.9	—	0.9	0.1	—	0.1
W	15	—	15	2.5	—	2.5
Mo	10	—	100	1.8	—	16
Fe	2.7	39	—	0.9	6	—
Ta	13	—	13	4.4	—	2.2

* Calculated for 0.5 g of sample as niobium metal.

equivalent to 0.12 ± 0.02 μg g^{-1} for Mg and 0.9 ± 0.2 μg g^{-1} for Fe, and were negligibly small for the other six elements studied. To improve the DL and LQD values, it is important that the blank values be reduced. The blank value of Fe might arise mainly from the use of analytical-reagent grade HCl in the preparation of the 5 M HCl - 7 M HF and 7 M HCl - 0.2 M HF solutions. Forty millilitres of the HCl were used to prepare 80 ml of 5 M HCl - 7 M HF and 43 ml for 60 ml of 7 M HCl - 0.2 M HF. The use of a more highly purified acid to prepare these solutions would reduce the blank value of Fe.

Conclusions

The anion-exchange separation procedure combined with ICP-AES has been effectively applied to the determination of trace-metal impurities in high-purity niobium oxide and metals. The direct ETAAS method studied for the comparison was a rapid and simple technique, but although it provided a lower sensitivity and its application was limited to Mg, Cr, Mn and Fe. The solvent extraction ICP-AES technique had the advantage of being a simple procedure, however, the determination of Mo at trace levels was impossible owing to the residual niobium in the test solutions. The proposed method proved to have a satisfactory detection capability for the elements tested, providing sufficient accuracy and precision.

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Column Chromatographic Pre-concentration of Iron(III) in Alloys and Biological Samples With 1-Nitroso-2-naphthol-3,6-disulphonate and Benzyldimethyltetradecylammonium - Perchlorate Adsorbent Supported on Naphthalene Using Atomic Absorption Spectrometry

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The solid ion-pair material produced from the reaction between benzyldimethyltetradecylammonium chloride (BDTA) and sodium perchlorate on naphthalene provides the basis for a simple, rapid and selective technique for pre-concentrating iron from up to 500 ml of aqueous solution. Iron reacts with disodium 1-nitroso-2-naphthol-3,6-disulphonate (Nitroso-R salt) to form a water-soluble coloured chelate anion. The iron chelate anion forms a water-insoluble, stable iron - Nitroso-R - BDTA complex on naphthalene packed in a column. Trace amounts of iron are quantitatively retained on naphthalene in the pH range 3.5–7.5 and at a flow-rate of 1–2 ml min^{-1}. The solid mass is dissolved out from the column with 5 ml of *N,N*-dimethylformamide and iron is determined by means of an atomic absorption spectrometer at 248 nm. The calibration graph is linear for concentrations of iron over the range 0.5–20 μg in 5 ml of final solution. The standard deviation and relative standard deviation were calculated. The detection limit of the method was 0.0196 μg ml^{-1} of iron. The sensitivity for 1% absorption was 0.072 μg ml^{-1} (0.165 μg ml^{-1} by direct atomic absorption spectrometry of aqueous solution). The proposed method was applied to the determination of iron in standard alloys and biological samples.

Keywords: *Iron determination; atomic absorption spectrometry; naphthalene column retention; disodium 1-nitroso-2-naphthol-3,6-disulphonate; benzyldimethyltetradecylammonium - perchlorate adsorbent*

Disodium 1-nitroso-2-naphthol-3,6-disulphonate (Nitroso-R salt) has been used extensively as a reagent for the determination of metals since it was introduced in 1921 by van Klooster for the detection of cobalt.[1] A number of papers have been published on the Nitroso-R salt method for the determination of cobalt.[2–5] As the sensitivity of the cobalt - Nitroso-R salt reaction is very high, considerable interest has arisen in the spectrophotometric determination of cobalt. However, there have been differences in the choice of wavelength for the absorption measurement of the cobalt - Nitroso-R salt chelate anion, and in the effects of the amount of reagent and the elimination of interference arising from foreign ions. Confusing analysis data have been reported on the interference of other metal ions (iron, nickel, copper, etc.) forming metal chelates with this reagent. Adam and Pribil[5] have reported the extraction of the cobalt - Nitroso-R - methyltrioctylammonium complex in chloroform. This extraction made the method more sensitive and diminished the interference arising from the foreign metal ions present in the samples. In this instance, larger amounts of sodium chloride are used as a salting-out agent because of the incomplete interfacial separation of the two phases. Iron(II) and iron(III) also react with the Nitroso-R salt to form water-soluble, stable chelate anions. Preliminary experiments showed that both iron - Nitroso-R chelate anions form water-insoluble stable complexes in the presence of quaternary ammonium salts [*e.g.*, benzyldimethyltetradecylammonium chloride (BDTA), cetyltrimethylammonium chloride and methyltrioctylammonium chloride] in the aqueous solution.

Solvent extraction is a simple and convenient technique for the separation and concentration of metal ions, but it cannot be applied directly to the extraction of those metal ions that have poor solubility in conventional organic solvents. This technique is also inconvenient if an emulsion forms between the aqueous and organic phases on shaking. These difficulties can be easily overcome by using molten naphthalene as an extractant,[6–8] but the method cannot be applied to the extraction of thermally unstable complexes.[9,10] Solid - liquid separation, after adsorption of metal chelates on microcrystalline naphthalene, is more convenient, rapid and economical than the molten naphthalene method and can be applied to many types of metal chelates, *e.g.*, the hydrated 8-hydroxyquinoline complexes of magnesium[11] and cadmium,[12] the low-solubility 1,10-phenanthroline - tetraphenylborate complex of iron,[13] the slowly extractable 2-thenoyltrifluoroacetone complex of iron[14] and the unstable 2-mercaptobenzimidazole complex of palladium at high temperature.[9] The only inconvenience of the method is in the filtration, which increases the number of steps in the procedure and hence makes the method more time consuming.

A column chromatographic method has been developed in this laboratory for the separation and pre-concentration of metals by using various types of chelating agents and ion-pair adsorbents supported on naphthalene.[15–17]

Some materials such as green tea leaves,[18] thiol cotton,[19] polythioether foam,[20] C_{18}-bonded glass beads,[21] chelating resin,[22] XAD resins[23–26] and various metal oxides and hydroxides[27] have been used for the separation and concentration of metal ions. Although some of these are fairly efficient, their preparation is sometimes lengthy and rigid control of conditions is required. Further, the desorption of the metal chelate is carried out by the slow process of elution. Hence, the method is tedious.

This paper describes an efficient method of chromatographic pre-concentration and separation of iron with Nitroso-R salt and BDTA - ClO_4 adsorbed on naphthalene. Preliminary observations have revealed that the iron(III) ion is quantitatively retained by BDTA - ClO_4 supported on naphthalene in the presence of Nitroso-R salt over the pH range 3.5–7.5. The iron - Nitroso-R - BDTA complex cannot be applied to solvent extraction - atomic absorption spectrometry (AAS) because it has low solubility in non-aqueous organic solvents. Hence, the proposed method is simple, economical (it is not necessary to use much glassware), rapid (the solid mass is dissolved instead of being eluted) and sensitive (the adsorbent is dissolved in a small volume of organic solvent and the whole solution can be used for the

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absorption measurement). The metal retained on the column can be dissolved in a suitable solvent, together with the naphthalene, and determined directly by AAS. The conditions have been optimised and the method has been used for the determination of iron in standard alloys and biological samples.

Experimental

Apparatus

A Perkin-Elmer Model 403 atomic absorption spectrometer and a Toa-Dempa HM-5A pH meter were used. The hollow-cathode lamp for iron was obtained from Hamamatsu Photonics (Hamamatsu, Japan). All absorption measurements were performed under the following operating conditions: wavelength, 248 nm; slit setting, 0.3 mm; current, 10 mA; acetylene flow setting, 1.5 (pressure, 0.3 kg cm^{-2}); and air flow setting, 55 (pressure, 2.1 kg cm^{-2}). A funnel-tipped glass tube (60 × 6 mm i.d.) was used as the chromatographic column. All glassware and columns were washed with concentrated nitric acid - concentrated sulphuric acid (1 + 1) prior to use. The column was plugged with rough chemical fibre and then slurry-packed with the naphthalene material to a height of 1.3–1.7 cm.

Reagents

Standard iron solution (3 p.p.m.) was prepared by diluting 1000 p.p.m. AAS standard iron(III) chloride solution with doubly distilled water. Buffer solutions were prepared by mixing an appropriate ratio of 0.5 M ammonia and 0.5 M ammonium acetate, and 0.5 M acetic acid and 0.5 M ammonium acetate. Nitroso-R salt solution (0.5%) was prepared by dissolving 0.5 g of the reagent in 100 ml of doubly distilled water. Benzyldimethyltetradecylammonium chloride, naphthalene, *N*,*N*-dimethylformamide (DMF), Nitroso-R salt and all other reagents were of analytical-reagent grade unless specified otherwise. Doubly distilled water was used throughout.

Preparation of BDTA - ClO_4 Adsorbent

A solution of naphthalene was prepared by dissolving 20 g of naphthalene in 40 ml of acetone on a magnetic stirrer equipped with a heater. This solution was added to 1400 ml of doubly distilled water, containing 4 g of sodium perchlorate, with continuous stirring at room temperature. Next, a 500-ml solution of BDTA (2.0 g) in doubly distilled water, prepared on the magnetic stirrer/heater, was added gradually to the naphthalene solution in a fast stream with a syringe. The naphthalene mixture, co-precipitated with BDTA and sodium perchlorate, was stirred for *ca.* 3 h to form a uniform adsorbent and was then allowed to stand for 1–2 h. The supernatant solution was drained off by decanting and then the mixture was washed once with doubly distilled water. The aqueous naphthalene slurry was stored in a bottle.

General Procedure

An aliquot of the solution containing 0.5–20 μg of iron was placed in a 20-ml beaker and to this were added 14 ml of doubly distilled water and 1.0 ml of 0.5% Nitroso-R salt solution; the pH was then adjusted to *ca.* 5.5 with 0.5 ml of the buffer solution. The column loaded with naphthalene - BDTA - ClO_4 was conditioned at pH 5.5 by passage of 3–4 ml of the buffer solution at a flow-rate of 2 ml min^{-1} and then the sample solution was passed through the column. The packing was washed once with 3–5 ml of doubly distilled water, and the metal complex, together with the naphthalene, was dissolved out by passage of 5 ml of DMF through the column. This solution was aspirated into the air - acetylene flame of the AAS instrument, and the absorption was measured at 248 nm. The absorption values for various amounts of iron were measured and referred to a calibration graph established with a reagent blank prepared in a similar manner to the test solution.

Results and Discussion

Retention Characteristics of BDTA - ClO_4

Benzyldimethyltetradecylammonium chloride is a quaternary ammonium salt and has been used as a counter cation for the spectrophotometric determination of some metals. Preliminary studies showed that, in aqueous solution, the BDTA cation co-precipitates with microcrystalline naphthalene as ion pairs with various inorganic anions such as SCN^-, ClO_4^- and I^-. The co-precipitated naphthalene mixture showed strong adsorptive characteristics for the metal chelate anions. Therefore, different quaternary ammonium salts such as tetramethylammonium bromide, tetraethylammonium bromide (TEA), tetrabutylammonium bromide (TBA), benzyldimethylphenylammonium chloride, benzyltrimethylammonium chloride, benzyltriethylammonium chloride, cetyltrimethylammonium bromide (CTMA), cetylpyridinium chloride (CPC), methyltrioctylammonium chloride (MTOA) and BDTA were studied for the development of adsorbents.

Table 1. Precision and detection limits for iron

	Iron taken*/μg					
	4.5		9.0		12.0	
Chelating agent	Standard deviation of absorbance	Relative standard deviation, %	Standard deviation of absorbance	Relative standard deviation, %	Standard deviation of absorbance	Relative standard deviation, %
Nitroso-R - Zephiramine ..	7.5×10^{-4}	1.12	13.4×10^{-4}	1.00	14.7×10^{-4}	0.8

Chelating agent	Sensitivity for 1% absorption/ μg ml^{-1}	Mean absorbance of the reagent blank (x_B)	Standard deviation of reagent blank (s_B)	Analytical sensitivity (S)†/ ml μg^{-1}	Detection limit (c_L)‡/ μg ml^{-1}
Nitroso-R - Zephiramine ..	0.072	0.0018	0.0004	0.0611	0.0196

* $n = 5$.
† S, obtained from the slope of the calibration graph.
‡ $c_L = ks_B/S$; $k = 3$.[17,28,29]

Of these salts, only CTMA, CPC, MTOA and BDTA produced ion pairs with inorganic anions (SCN^-, ClO_4^- and I^-) as described above and co-precipitated with naphthalene. Tetraethylammonium bromide and TBA also produced ion pairs with ClO_4^- and co-precipitated with naphthalene. In the present study, the ion pair of the BDTA cation and ClO_4^- was used as an adsorbent. This adsorbent also showed strong adsorptive characteristics towards the various metal chelate anions.

Reaction Conditions

The retention of iron on naphthalene - BDTA - ClO_4 began to occur at pH 0.5, increased with an increase in pH, reached a maximum at pH 3.5 and remained constant in the pH range 3.5–7.5. The addition of 0.3–3.0 ml of the buffer solution did not affect the retention of iron; the use of 0.5 ml of the buffer solution is recommended. The flow-rate was varied from 0.5 to 10 ml min^{-1}. Over this range, the retention of iron was not affected. A flow-rate of 1–2 ml min^{-1} was used.

The retention capacity of the loaded naphthalene was evaluated by use of a column. A solution containing 300 μg of iron and 1.0 ml of the buffer solution was diluted to 20 ml with doubly distilled water in a beaker and passed through the column. The maximum concentration of iron retained on the adsorbent was 0.54 mg g^{-1}. It was also found that the retention capacity for iron depended on the amounts of BDTA and sodium perchlorate supported on the naphthalene. From 1.2 to 2.0-g amounts of BDTA and 2.0- to 4.0-g amounts of sodium perchlorate were shown to be sufficient for the retention of iron by the appearance of a clear-coloured adsorptive phase of the iron complex on the column. In this experiment, 2.0 g of BDTA and 4.0 g of sodium perchlorate were used for convenience.

Various volumes (0.1–3.0 ml) of 0.5% Nitroso-R salt solution were added to a sample solution containing 9 μg of iron. The retention of iron remained constant with the addition of >0.5 ml of Nitroso-R salt solution. In the present work, 1.0 ml of 0.5% solution was used for convenience.

The effect of the volume of the aqueous phase on the retention of iron on the column was investigated using the general procedure. The retention remained constant and maximum when the volume of the aqueous phase did not exceed 500 ml in the presence of 3.0 ml of 0.5% Nitroso-R salt solution. In subsequent work, 15 ml of the aqueous phase were used for convenience.

An attempt was made to dissolve out the iron - Nitroso-R - BDTA complex, together with naphthalene, from the column. As the solid mass of the iron complex and naphthalene is dissolved out in a small volume (3–5 ml) of solvent from the column, it is essential to select a solvent in which the chelate is highly soluble and which is flammable for AAS measurements. The solid mass is insoluble in conventional organic solvents such as benzene, toluene, xylene, 1,2-dichloroethane, hexane, nitrobenzene, chloroform, isoamyl alcohol, amyl alcohol, isoamyl acetate and 4-methylpentan-2-one, and in water-miscible solvents such as acetonitrile, propylene

Table 2. Effect of foreign salts and metal ions

Salt or ion	Tolerance limit
NaCl, $CH_3COONa.3H_2O$, NH_4Cl, Na_2SO_4	1 g*
KNO_3, L-ascorbic acid	500 mg
Thiourea	300 mg
KI, KSCN	50 mg
KH_2PO_4, $Na_2S_2O_3$	20 mg
NH_4F	10 mg
Potassium sodium tartrate	5 mg
$Na_2C_2O_4$, KCN	100 μg
Na_2EDTA, ammonium citrate	10 μg
Al^{III}, Zn, Ca, Mg	10 mg*
Mo^{VI}, Mn^{II}, Pb^{II}, Hg^{II}, Bi^{III}, Cd, Au^{III}, Ir^{III}, Os^{VIII}, Cr^{III}, Cr^{VI},† Ag^{I}‡	1 mg*
Ni^{II}	500 μg
Rh^{III}	300 μg
Cu^{II}	200 μg, 700 μg§
V^{V}, W^{VI}	100 μg
Pt^{IV}	70 μg
Pd^{II}, Ru^{III}	30 μg
Co^{II}	10 μg

* Maximum value tested; Fe^{III}, 9 μg, pH, 5.5; wavelength, 248 nm; 0.5% Nitroso-R salt solution, 1.0 ml; adsorbent, BDTA - ClO_4; solvent, DMF.

† Chromium(VI) was reduced to Cr^{III} by 1 ml of 1% ascorbic acid solution.

‡ One millilitre of 1% $Na_2S_2O_3$ solution was added as a masking agent.

§ Copper(II) was masked with 200 mg of thiourea.

Table 3. Analysis of samples for iron. Values in parentheses are approximate and not certified. (For NIES No. 3 and No. 6, the concentrations of the metal ions are given in μg g^{-1} except those for Na, K, Mg, Ca and P)

Sample	Composition, %	Iron, % Certified value	Iron, % Found*
NKK† 916 Aluminium Alloy	Si: 0.41; Cu: 0.27; Mn: 0.11; Mg: 0.10, Cr: 0.05; Ni: 0.06; Zn: 0.30; Ti: 0.10; Sn: 0.05; Pb: 0.04; V: 0.02; Zr: 0.05; Bi: 0.03; Ga: 0.03; Co: 0.03; Sb: 0.01; Ca: 0.03; B: 0.0006	0.54	0.56 ± 0.05
NKK 920 Aluminium Alloy	Si: 0.78; Cu: 0.71; Mn: 0.20; Mg: 0.46; Cr: 0.27; Ni: 0.29; Zn: 0.80; Ti: 0.15; Sn: 0.20; Pb: 0.10; V: 0.15; Bi: 0.06; Ga: 0.05; Co: 0.10; Sb: 0.01; Ca: 0.03	0.72	0.74 ± 0.04
NIST SRM‡ 94C Zinc Alloy	Mn: 0.014; Cu: 1.01; Ni: 0.006; Sn: 0.006; Al: 4.13; Cd: 0.002; Pb: 0.006; Mg: 0.042	0.018	0.0175 ± 0.0005
NIST SRM 629 Zinc Alloy	Cu: 1.50; Al: 5.15; Mg: 0.094; Pb: 0.0135; Cd: 0.0155; Sn: 0.012; Cr: 0.0008; Mn: 0.0017; Ni: 0.0075; Si: 0.078	0.017	0.0174 ± 0.0006
NIES§ No. 3 Chlorella	K: 1.24 ± 0.06; Ca: 0.49 ± 0.03; Mg: 0.33 ± 0.02; P: (1.7); and Mn: 69 ± 5; Sr: 40 ± 3; Zn: 20.5 ± 1.0; Cu: 3.5 ± 0.3; Co: 0.87 ± 0.05; Pb: (0.60); Cd: (0.026); Sc: (0.013)	0.185 ± 0.010	0.190 ± 0.011
NIES No. 6 Mussel	Na: 1.00 ± 0.03; K: 0.54 ± 0.02; Mg: 0.21 ± 0.01; Ca: 0.13 ± 0.01; P: (0.77); and Zn: 106 ± 6; Mn: 16.3 ± 1.2; As: 9.2 ± 0.5; Cu: 4.9 ± 0.3; Ni: 0.93 ± 0.06; Pb: 0.91 ± 0.04; Cd: 0.82 ± 0.03; Cr: 0.63 ± 0.07; Ag: 0.027 ± 0.003; Al: (220); Sr: (17); Se: (1.5); Co: (0.37); Hg: (0.05)	158 ± 8 (μg g^{-1})	163 ± 9 (μg g^{-1})

* Mean of four determinations; 1 ml of 10% thiourea was added as masking agent.

† NKK = Nippon Keikinzoku Kogyo.

‡ NIST SRM = National Institute of Standards and Technology (formerly National Bureau of Standards) Standard Reference Material.

§ NIES = National Institute for Environmental Studies.

carbonate and 1,4-dioxane, but is soluble in methanol, ethanol and DMF. Methanol and ethanol are better and more economical solvents, but are volatile and too unstable for absorption measurements. Hence, these solvents are not favourable, whereas DMF has high solubility, a low specific gravity and is stable and sensitive. In the present study, therefore, DMF was selected as the preferable solvent.

Small volumes (2–3 ml) of DMF were sufficient to dissolve the complex - naphthalene mixture, enhancing the sensitivity of the proposed method 2-fold. As only a small volume (3–5 ml) of this solvent is required, it was necessary to study the effect of the surplus water adsorbed on the naphthalene. It was found that the surplus water decreased the absorption signal by *ca.* 6% and led to errors in the determination. Therefore, it was necessary to eliminate the adsorbed water completely by aspiration.

Based on the optimum conditions described above, the calibration graph was linear over the concentration range 0.5–20 μg of iron in 5 ml of DMF solution. The precision of the method for the determination of various amounts of iron was studied, together with the detection limit. The results are given in Table 1. The sensitivity for 1% absorption was 0.072 μg ml^{-1} (0.165 μg ml^{-1} for direct AAS measurement of the aqueous solution). The pre-concentration factor was 100.

Effect of Foreign Ions

Various salts and metal ions were added individually to a solution containing 9 μg of iron and the general procedure was applied. The tolerance limits (error <3%) are given in Table 2. Of the salts examined, most could be tolerated in milligram to gram amounts. Ammonium citrate and disodium ethylenediaminetetraacetate (Na_2EDTA) interfered seriously. Of the metal ions studied, most did not interfere, even in the 1–10 mg range. Vanadium(V), W^{VI} and Pt^{IV} probably form ion pairs ($BDTA^+$ - VO_3^-, $BDTA_2^+$ - WO_4^{2-} and $BDTA_2^+$ - $PtCl_6^{2-}$) on the surface of the naphthalene - BDTA - ClO_4 adsorbent in the column. Cobalt, Cu^{II}, Ni, Pd and Ru formed water-insoluble complexes with this adsorbent in the column, and Pd^{II}, Ru^{III} and Co^{II} interfered in the determination. These metals, therefore, must be absent. Larger amounts (10 mg) of Al^{III}, Zn, Ca and Mg did not interfere. Silver(I) precipitated silver chloride in the presence of Cl^-, but it could be masked by $Na_2S_2O_3$. Interference from Cr^{VI} was suppressed considerably by reduction to Cr^{III} with ascorbic acid. Iron(III) and Fe^{II} formed similar green complex cations and were retained on the column.

The proposed method is highly selective hence rigid control of the conditions is unnecessary. From observations on authentic samples, it was found that the proposed procedure can be applied to the determination of iron in standard aluminium and zinc-based alloys and in biological samples, without prior separation.

Determination of Iron in Standard Aluminium and Zinc Alloys

A 0.1-g sample of the standard alloy was completely dissolved in 4–5 ml of hydrochloric acid (1 + 1) by heating on a water-bath and then 0.5–1.0 ml of 30% hydrogen peroxide was added to the solution. The excess of peroxide was decomposed by heating the solution on a water-bath. The solution was cooled and filtered, and the filtrate was diluted to 200 ml with doubly distilled water in a calibrated flask. An aliquot (2–3 ml) of this solution was taken, 1 ml of 10% thiourea solution was added and the general procedure was followed. The results are given in Table 3.

Determination of Iron in Chlorella and Mussels

Chlorella (0.1 g) was placed in a beaker and dissolved in 10 ml of aqua regia by heating on a hot-plate. The solution was cooled and filtered, and the filtrate was diluted to 200 ml with distilled water in a calibrated flask.

For mussels, a 0.5-g sample was dissolved in 7 ml of concentrated nitric acid. The solution was cooled and filtered, and the filtrate diluted to 100 ml with doubly distilled water.

In each instance, an aliquot (1–2 ml) of the final solution was taken, 1 ml of 10% thiourea solution was added and the general procedure followed. The results presented in Table 3 are in reasonable agreement with the certified values.

Conclusions

The adsorbent naphthalene - BDTA - ClO_4 forms complexes with various metal ions, such as Co, Ni, Ru and Pd, in the presence of Nitroso-R salt; hence, a similar method could be developed for their multi-element determination. The proposed method is the first attempt at separating and concentrating iron by using the reaction of the iron - Nitroso-R complex anion with the ion pair produced from a quaternary ammonium cation and an inorganic anion supported on naphthalene.

Solvent extraction provides an efficient separation technique, but, in general, it is difficult to extract some metals in the presence of cationic surfactants because of the incomplete interfacial separation of the two phases. In such instances, either large amounts of salting-out agents must be added to the solution for the complete extraction of the complex or the solution must be allowed to stand for long periods of time. The addition of these reagents could cause contamination of the sample in ultra-trace analysis (below the p.p.b. level), thereby leading to errors in the determination. This is a drawback to the method.

Advantages of the method are that it allows the use of simple glassware such as funnel-tipped columns and beakers and of small volumes of organic solvent for dissolution of the complex. The method also proved to be very efficient for the determination of trace amounts of iron in aluminium and zinc-based alloys and in biological samples. The sensitivity of the method could probably be improved further by use of other optical analytical techniques such as electrothermal AAS or high-performance liquid chromatography with ultraviolet - visible detection.

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Microwave Digestion of Carbonate Rock Samples for Chemical Analysis

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The use of a microwave oven has permitted rapid and complete digestion of powdered carbonate rock samples, replacing the classical methods of dissolution which involved lengthy multi-step processes. The procedure includes the initial open heating of the powdered rock samples with 0.5 M ethanoic acid to inhibit the subsequent formation of calcium fluoride, and a two-acid closed system, heated in the microwave oven. Boric acid is then added before further heating of the closed system. This ensures that the silica remains in solution, thereby allowing complete analysis of the major components, by atomic absorption spectrometry, to be carried out on a single solution. The results compare favourably with three previous analyses obtained by a variety of lengthy, classical methods. The advantages of the microwave digestion of carbonate rock include its accuracy, safety and rapidity, permitting the digestion and analysis of 12 rock samples within a working day. The detection limit of SiO_2 with this method was 0.20%.

Keywords: *Microwave digestion; carbonate rock; calcium determination; atomic absorption spectrometry*

Abu-Samra *et al.*[1] described one of the earliest uses of a microwave oven for wet digestion, they successfully completed the wet ashing of organic material. The main benefit of the technique is that it provides rapid, safe and efficient digestion, thereby allowing greater contamination control, eliminating the need for constant attention and reducing the risk of explosion.

The efficiency of microwave ovens has been greatly improved over the past decade, advancing from the use of domestic microwave ovens for open-vessel digestion to analytical laboratory instruments equipped for closed-vessel digestion. The need for the safe removal and treatment of acid fumes from the system has also been emphasised.[2] Nadkarni[3] drew attention to the fact that while the dissolution of geological materials in acids might take several hours of heating and require close supervision, the same operation can be undertaken in a microwave oven in 3–4 min. In the 1980s the use of microwave ovens has dramatically increased and a review by Matusiewicz and Sturgeon[4] chronicles the wider application of microwave digestion to the analysis of mineral, environmental and metallurgical samples.

Heating Process

As microwave heating is internal as well as external, the heat conduction stage is avoided because energy is instantly transferred to the sample by absorptive polarisation rather than by molecular collisions. The internal heating mechanically agitates and ruptures the surface layers of the sample, thereby providing a better contact between the acids and the sample.[5] The use of a closed pressurised vessel also aids digestion, by increasing the temperature of the sample solution, hence the required boiling-point is more rapidly achieved.

The Problem in Hand

Laboratory work was required to digest a variety of carbonate rocks and to carry out major component analysis, on a single solution, using atomic absorption spectrometry. The samples ranged from limestones containing organic material, to blocks taken from the extensive, disused stone mines at Combe Down, located south of Bath, in the UK. The Combe Down Oolite is a non-organic rock, consisting mainly of ooliths and detrital material of well worn shelly debris, in a mosaic matrix of coarse calcite.[6]

Previous attempts to digest carbonate rocks have proved complicated and time consuming. Barber[7] introduced a multi-step chemical separation and analysis of carbonate rocks. In this method, samples were digested in ethanoic acid, at 60 °C, for two periods of 12 h and then CaO, MgO, MnO and Fe_2O_3 were determined using atomic absorption spectrometry. Further separation with hydrogen peroxide was required before the analysis of trace elements. Robinson[8] developed a rapid method for the analysis of the carbonate fraction of limestones and dolomites, which involved dissolving the samples in cold 1 M hydrochloric acid. However, he discarded the non-carbonate fraction of the rock which might include clay, silica, feldspar and occasional sulphides and phosphates. In addition, the British Standard 2455[9] requires the lengthy preparation of two different solutions before analysis is possible.

A technique which would allow the rapid and complete digestion of powdered whole rock samples would be very time saving for the analytical geochemist. Despite the increasing popularity of the microwave digestion technique, a literature search failed to find an accepted method of digestion applicable to carbonate samples. Further, the CEM microwave manual supplied did not suggest a method, the most relevant application being the digestion of material from the sides of a cooling tower in Oklahoma.

Experimental

Instruments

The laboratory oven used was a CEM MDS-81D microwave digestion system. The instrument power was adjustable up to a maximum of 630 W. The pressure vessels supplied with the instrument were microwave-transparent, 120-ml Teflon perfluoroalkoxy resin (PFA) containers with detachable screw-top caps. Up to 12 vessels can be placed in the carousel which is rotated during the microwave process. An essential feature of the safety vent is the cap; this consists of a safety pressure relief valve, venting nut and vent tubing (Fig. 1). The design allows the safety valve to open when the internal pressure reaches 830 kPa, venting the system into a collection chamber. The valve then reseals allowing the pressure to increase again.

Reagents

All the chemicals and reagents used were of analytical-reagent grade. The following reagents are required: 42% v/v hydrofluoric acid; concentrated nitric acid; 0.5 M ethanoic acid; 4% m/v boric acid; and 10% m/v lanthanum chloride.

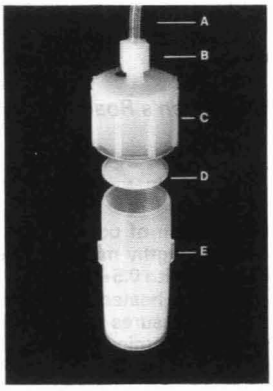

Fig. 1. Components of a microwave pressure vessel: A, venting tube; B, venting nut; C, vessel cap; D, safety valve; and E, vessel body

Procedure

A sequence of tests was undertaken in order to establish the most acceptable procedure. This was as follows: 200-mg portions of cleaned, dried and powdered (to pass through a 4 μm sieve) whole rock samples are weighed into 120 ml Teflon PFA vessels. A 20-ml volume of 0.5 M ethanoic acid is added to each and the open vessels are placed on a hot-plate at 150 °C until the evolution of carbon dioxide ceases. When cool, 1 ml of 42% HF and 3 ml of concentrated HNO_3 are added to each vessel. The vessels are sealed using a CEM capping station and placed in the carousel with the vent tubing attached and the venting nut tight. The carousel is placed in the microwave oven, the turntable switched on and the blower turned to full speed. Two programmes are set, 3 min at 100% power followed by 30 min at 90% power (a lower power setting is required if fewer than 12 vessels are used). The clear material of the oven door allows the progress of the digestion to be observed. The vessels are left to cool to room temperature, vented by loosening the venting nut and uncapped using the CEM capping station, then 10 ml of 4% boric acid are added to each. The vessels are replaced in the carousel following the same procedure and placed in the microwave for 10 min at 60% power. After cooling to room temperature the vessels are vented, uncapped and the solution made up to 100 ml with distilled, de-ionised water in preparation for the atomic absorption spectrometric analysis.

Problems Encountered

One problem, encountered at an early stage in the development of the technique, was that pressure dissolution alone failed to digest the sample completely. After treatment with HF and HNO_3 and closed heating in the microwave, in almost all instances a residue remained. The precipitate was always white in colour, even from the most organic-rich carbonate rock samples. Similar difficulties were encountered[10] when using the closed pressure dissolution method suggested by Xiao-quan *et al.*[11] for the determination of gallium.

The white residue remaining after pressure dissolution was analysed using X-ray diffraction and subsequently identified as calcium fluoride. It appeared that a pressure equilibrium in the sealed system had prevented the complete dissolution of the carbonate material. Subsequently, calcium fluoride formed by the reaction of the remaining carbonate with hydrofluoric acid. Hence, it was decided to introduce an earlier stage into the procedure, a reaction with 0.5 M ethanoic acid in an open system to allow the evolution of the carbon dioxide, thereby preventing a pressure equilibrium in the subsequent sealed system.

Table 1. Results of the microwave analyses (in percentage mass values) compared with those certified by the GFS and NIST and those of Galle[13] and Ingamells and Suhr[12] for the five standards analysed

			Result obtained		
Standard	Analyte	Certified value	Galle	Ingamells and Suhr	This work
GFS 400 ..	SiO_2	0.07	0.06	0.075	<0.20
	Al_2O_3	0.03	0.04	0.029	0.04
	Fe_2O_3	0.06	0.08	0.053	0.04
	CaO	30.49	30.45	30.51	30.41
	MgO	21.52	21.59	21.50	21.65
	Na_2O	—	0.01	0.04	<0.01
	K_2O	—	0.02	0.01	0.02
GFS 401 ..	SiO_2	2.03	1.98	2.09	1.99
	Al_2O_3	0.22	0.25	0.22	0.18
	Fe_2O_3	0.19	0.25	0.199	0.17
	CaO	50.25	50.41	50.07	50.00
	MgO	3.52	3.60	3.60	3.56
	Na_2O	—	0.01	0.02	<0.01
	K_2O	—	0.04	0.06	0.07
GFS 402 ..	SiO_2	2.45	2.59	2.63	2.41
	Al_2O_3	0.53	0.59	0.53	0.47
	Fe_2O_3	0.35	0.40	0.370	0.36
	CaO	46.82	46.78	46.65	47.02
	MgO	5.63	5.81	5.74	5.54
	Na_2O	—	0.01	0.02	<0.01
	K_2O	—	0.07	0.16	0.16
GFS 403 ..	SiO_2	1.76	1.82	1.81	1.81
	Al_2O_3	0.43	0.45	0.43	0.41
	Fe_2O_3	0.30	0.31	0.308	0.29
	CaO	38.13	37.94	37.99	38.57
	MgO	13.66	13.87	13.78	13.82
	Na_2O	—	0.01	0.03	<0.01
	K_2O	—	0.05	0.14	0.13
NIST SRM 88a ..	SiO_2	1.20	—	—	1.10
	Al_2O_3	0.19	—	—	0.18
	Fe_2O_3	0.28	—	—	0.26
	CaO	30.17	—	—	30.27
	MgO	21.29	—	—	21.26
	Na_2O	0.01	—	—	<0.01
	K_2O	0.12	—	—	0.12

Analysis

The concentrations of seven elements were determined using a Philips PU9000 atomic absorption spectrometer, calibrated using "cocktail" solutions prepared from 1000 p.p.m. standard solutions.

As a check on the analytical technique, tests were run on a set of standards of known composition. During the 1960s, the G. Frederick Smith Chemical Company (GFS) made available a series of 20 limestone and dolomite standards. Using classical methods of digestion, Ingamells and Suhr[12] produced chemical analyses of four of the samples; a dolomite (No. 400); two limestones (Nos. 401 and 402) and a limestone - dolomite blend (No. 403). Galle[13] performed similar analyses for the entire series of 20 samples.

For the present analysis the GFS standards 400, 401, 402, 403 and the National Institute of Standards and Technology (NIST) (formerly known as the National Bureau of Standards) Standard Reference Material (SRM) 88a Dolomite were digested using the above procedure and run on the atomic absorption spectrometer.

Concentrated solutions were analysed for SiO_2 and Al_2O_3 using a dinitrogen oxide - acetylene flame, while iron was determined as total iron oxide using an air - acetylene flame. The flame emission mode of the spectrometer was used to determine Na_2O and K_2O. For the determination of CaO and MgO, the solutions and cocktail calibration standards were diluted as follows: 200 μl of the concentrated solution and

500 µl of 10% lanthanum chloride made up to 10 ml with distilled, de-ionised water. The air - acetylene flame was used and less sensitive wavelengths selected, with burner rotation applied as required.

Results and Discussion

The results are summarised in Table 1, listed as percentage mass values. Each value represents an average of four separate analyses performed on each sample and is compared with the results of the GFS, of Ingamells and Suhr[12] and of Galle,[13] all of which were obtained by classical methods. The results compare favourably and are within the required accuracy for analysis of large scale chemical trends in a lithology. In addition, the four separate analyses showed excellent precision, emphasising the reproducibility of the sample digestion conditions in a closed pressurised vessel. As concluded by Marshall,[14] the great advantage of the microwave method is its speed. For the seven oxides mentioned, provided the cocktail solutions for calibration are already prepared, it is possible to digest and analyse accurately 12 samples within a working day.

As the detection limit of SiO_2 in the method described was about 0.20%, an accurate analysis of GFS 400 was not possible. This was not significant for the present research as none of the carbonate samples of interest contained such low levels of silica. Also, in all instances Na_2O was found to be below the detection limit of 0.01%.

The method of microwave digestion of carbonate rocks has been found to be rapid, accurate, safe and convenient.

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Multi-elemental Separation of Copper, Cobalt, Cadmium and Nickel in Natural Waters by Means of Colloidal Gas Aphron Co-flotation

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The separation, pre-concentration and multi-elemental determination of Cu, Co, Cd and Ni by co-flotation on $Fe(OH)_3$ in natural waters and synthetic media, of average and low salinity, using a colloidal gas aphron of sodium lauryl sulphate as an alternative to the conventional flotation on bubbling air or gas, has been studied. The separation process has been optimised by means of both univariate and simplex methods (COFLOT computer program). The results obtained after 5 min of flotation, show recoveries of greater than 90% for each of the elements, at concentrations of *ca.* 10 p.p.b. The concentration measurements during the optimisation process and those corresponding to the sublates were carried out by means of atomic absorption spectrometry.

Keywords: *Pre-concentration; multi-elemental; co-flotation; colloidal gas aphron*

The colloidal gas aphron (CGA) consists of small gas bubbles encapsulated in an aqueous, soapy film, the structure and properties of which have been studied by Sebba.[1] The CGA can be obtained from solutions of a great variety of surfactant agents. For the analytical chemist the CGA presents two fundamental characteristics, its small size and the existence of a double film encapsulating the gas (normally air). As a consequence of these properties, it is thought to have great potential as a pre-concentration and/or separation technique.[2] The methods of handling and the stability are now fairly well known.[3,4]

In the last decade some promising results have been published[2, 5–7] using CGA in connection with metals, dyes, sea water, etc. Woodburn *et al.*[8] have published results on the flotation of coal particles with CGA. However, despite the analytical potential which can be expected from them, they have been scarcely used as an alternative to the bubbling of gas in conventional flotation processes.

In this work the results of the optimisation process as applied to multi-elemental co-flotation of Cu, Co, Cd and Ni with $Fe(OH)_3$ and the CGA of sodium lauryl sulphate (NaLS) in natural and synthetic media of low and high salinity (0–3.5% NaCl) are summarised. The results obtained in the optimisation process and subsequent analysis of the sublates show that using the CGA allows for a separation and determination of these species in natural and low-salinity media similar to sea water. As a rule, a 5-min measuring time was used. The separation factor is greater than 90% for concentrations of less than 10 p.p.b. However, magnesium hydroxide precipitation takes place at the pH required for the multi-elemental co-flotation, inhibiting the separation process considerably. For this reason the methodology described does not apply to the analysis of sea water.

Experimental

The experiments were performed in columns (Fig. 1) as described elsewhere.[9,10] The sintered glass plates were removed from the columns because, in the aphron experiments, the gas bubbles were pre-formed and, therefore, the plates were not necessary.

The CGA generator was a similar system to that described by Sebba.[3] The aphron was pumped by an Eyela MP-3 peristaltic pump.

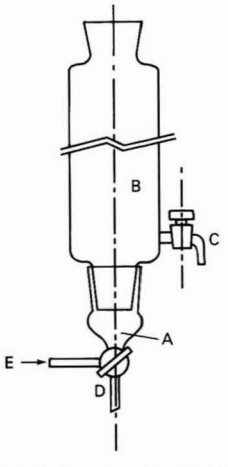

Fig. 1. Experimental device used for the flotation process. A, Detachable section; B, variable size column; C, stopcock; D, three-way stopcock; and E, CGA inlet

The determination of elements in the optimisation process and in the recovery of the sublates was performed using a Pye Unicam SP-9-800 atomic absorption spectrometer with an air - acetylene flame. Calibration was achieved using solutions of similar composition to the samples. The instrumental conditions chosen were as established by previous optimisation processes.[11]

Reagents

All of the reagents used were of analytical-reagent grade except the NaLS (Scharlau, pure) and were used without purification. Multi-standard Cu^{II}, Co^{II}, Cd^{II} and Ni^{II} (1000 p.p.m. each) stock solutions were used.

Optimisation Process of Co-flotation of Cu, Cd, Co and Ni

To 1 l of a 3.5% NaCl solution 2 ml of a multi-standard solution (1000 p.p.m. in all of the elements) were added.

Next, the amount of Fe^{III} (0.05 M $FeCl_3$ solution) that had been established in the optimisation process (either the univariate or the simplex method) was added. The pH was adjusted by the addition of ammonia solution and/or NaOH. The solution was gently shaken during the induction time, as indicated by the optimisation process, and then transferred into the flotation cell. The aphron flow of NaLS (which was produced a short while before) was introduced into the column through the lower tap by means of the peristaltic pump. Two 10-ml samples were collected, one after 2 min and another after 5 min of passing the aphron, taken from the lateral tap and transferred into screw-stoppered sample tubes containing 0.15 ml of concentrated HCl.

The response function of the optimisation methods (both univariate and simplex) is the sum of the percentages of the flotations of the four elements.

Procedure for the Recovery of Sublates in Low and High Salinity Media

Sample volumes of 0.5–5 l require the addition of 5 ml of 0.05 M Fe^{III}. The pH is adjusted to 11 ± 0.1 by the addition of NaOH and ammonia solution. The solution is shaken for 2–3 min and then passed to the flotation cell. The CGA flow is passed for 5 min by means of the peristaltic pump in position 7 (a flow-rate of 23 ml min^{-1}). The CGA is formed from a 2 g l^{-1} solution of NaLS.

After the CGA flow has stopped, the liquid is discharged through the lower tap in the column. The precipitate and the foam are treated with 10 ml of 6 M HCl and 1 ml of ethanol. The column is rinsed with a few millilitres of distilled water and the washings are collected with the precipitate and the foam, and made up to 25 ml.

Results and Discussion

Study of the Experimental Variables

The optimisation process was carried out by means of the univariate method, the simplex method and a factorial design. According to our previous knowledge of flotation processes with gas aphrons[12] the initial parameters to be optimised and controlled are: the pH of the medium; amounts of the co-precipitant species; CGA flow-rate at the entrance of the column; induction time; NaLS concentration; and flotation time.

Ionic strength is one of the factors with the greatest influence on this process; as a general rule, the greater the ionic strength the poorer the results. For this reason all optimisation experiments were carried out in 3.5% NaCl solutions.

pH of the medium

The pH of the medium is a highly significant factor in the flotation processes, therefore, it was the first variable to be optimised. Several experiments were carried out at different pH values, with the addition of 5 ml of the Fe^{III} solution. The generating solution (NaLS) of the aphron had a concentration of 2 g l^{-1} and the peristaltic pump was at position 10, equivalent to a flow-rate of 30 ml min^{-1}. All experiments were performed after shaking for 2 min, previously established by visual examination as the minimum time necessary to form the aphron. The results, after 5 min of passing the aphron flow, are plotted in Fig. 2. A pH of 11 was found to be the optimum for the flotation. It was not therefore necessary to add NaOH which could have caused difficulties in connection with the subsequent dissolution of the sublates being analysed.

Amount of co-precipitant species

Several experiments were carried out at pH 11, varying the amount of Fe^{III} and keeping the other variables constant. The

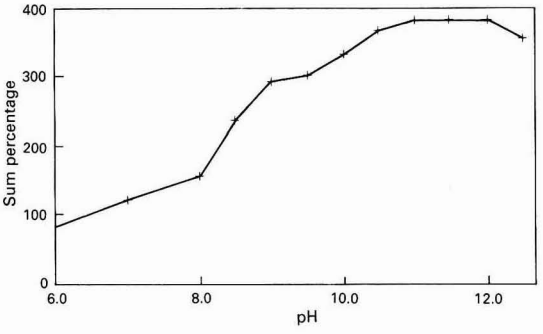

Fig. 2. Influence of the pH of the mother solution

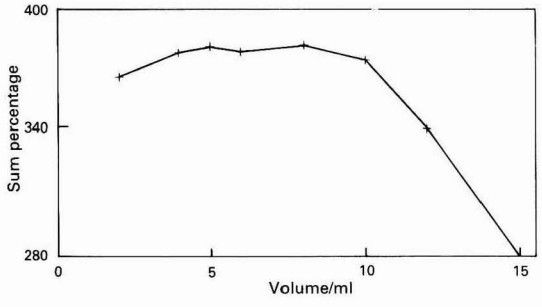

Fig. 3. Influence of the amount of Fe^{III} co-precipitant

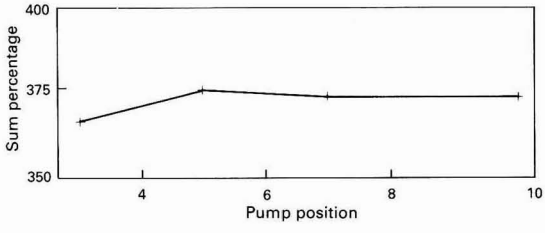

Fig. 4. Influence of the CGA flow-rate

results (Fig. 3) show that in the samples containing 2 and 10 ml of Fe^{III} similar recoveries were obtained. It was decided that 5 ml of the co-precipitating agent should be used. Thus, the co-precipitating agent is not in excess while ensuring that a noticeable amount of precipitate is obtained, facilitating the recovery of the sublates.

CGA flow-rate at the entrance of the flotation column

The flow of the CGA into the column might have some influence on the process, as it is a measure of the surfactant and of the CGA flow-rate through the column. Several experiments at pH 1, with 5 ml of Fe^{III}, were carried out in which the flow-rate was varied. The generating solution of the CGA was maintained at 2 g l^{-1}. The solution was shaken for 2 min before the passage of the CGA current. The results (see Fig. 4) show that a pump position of 5 (a flow-rate of 17 ml min^{-1}) gave the same results as at position 10 (30 ml min^{-1}). A position of 7 (23 ml min^{-1}) was chosen as the optimum. In this way a reasonable, not excessive, amount of foam was obtained. If the amount of foam is too great its subsequent destruction poses a number of problems in connection with the analysis of the sublates.

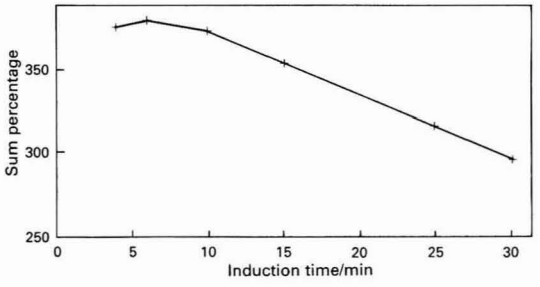

Fig. 5. Influence of the induction time

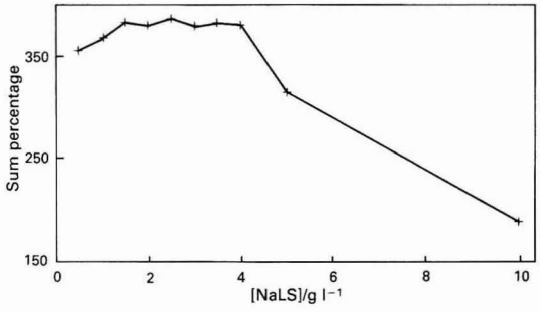

Fig. 6. Influence of the NaLS concentration of the initial solution on the formation of the CGA

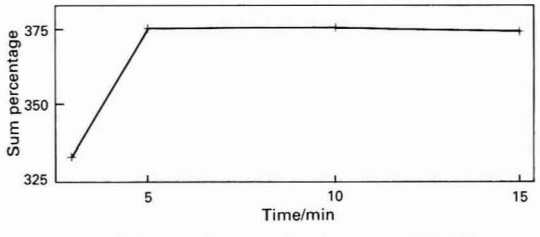

Fig. 7. Influence of the flotation time on the CGA flow

Table 1. Effect of sample volume

Column i.d./mm	Sample volume/ l	Fe^{III} added/ ml	Flotation, % Cu	Cd	Co	Ni	Total
60	0.5	2.5	92	96	96	96	376
	0.5	5.0	92	96	94	95	377
	1.0	5.0	92	96	95	95	382
80	0.5	2.5	94	98	95	96	376
	0.5	5.0	92	95	97	97	378
	1.0	5.0	92	99	97	98	375
	2.0	5.0	92	97	93	96	380
100	0.5	2.5	91	96	95	95	381
	0.5	5.0	91	96	96	93	386
	1.0	5.0	91	97	95	93	385
	2.0	5.0	92	97	96	97	378
	2.0	10.0	93	100	96	96	383
120	5.0	5.0	88	86	94	95	363*
	5.0	5.0	86	84	91	92	353
	5.0	25.0	95	97	94	97	383*
	5.0	25.0	92	93	90	91	366

* These values correspond to a flotation time of 8 min.

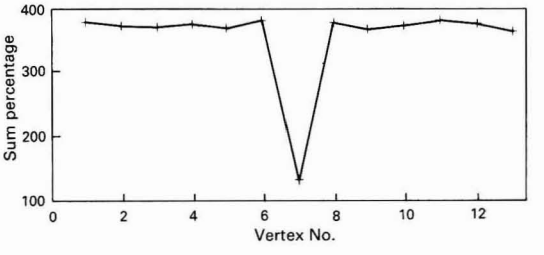

Fig. 8. Variation of response in simplex optimisation No. 1

Table 2. Factor definition and levels

Effect	Factor	Level Low(−)	High(+)
A	Co-precipitant/ml	3	10
B	Pump position	5	10
C	pH	9.5	12.5
D	Induction time/min	3	10

Induction time

The induction time can influence the conventional flotation processes in either a positive or a negative way. Several experiments were carried out under the stated conditions of pH, co-precipitant and flow-rate to determine the optimum induction time. The generating solution of the CGA was maintained at 2 g l^{-1} of NaLS. The experimental results shown in Fig. 5 indicate that a time of 10 min results in a deterioration in the separation of the species. Therefore a 5-min induction time was chosen as optimum; in this way, a time range for proper aphron formation was assured.

NaLS concentration in the CGA generating solution

Under the optimum conditions several experiments were performed while varying the NaLS concentration of the CGA generating solution. The surfactant added to the process will influence the stability and formation of the CGA. The results, presented in Fig. 6, show that concentrations of NaLS of between 1.5 and 4.0 g l^{-1} are the best. In practice a 2.0 g l^{-1} concentration was chosen for reasons of stability and the amount of foam produced, as discussed in the flow pumping experiments.

Flotation time

During the experiments, it was observed that after 3 min of flotation the solution became totally transparent, therefore, a short time in excess of this would be a suitable flotation time. An experiment in which the CGA flow was passed for 15 min, was carried out under the above optimum conditions. The results obtained (Fig. 7) indicate that a time of 5 min is sufficient to achieve optimum separation.

Sample volume

The sample volume and the size and shape of the column, as described in the literature, vary, giving rise to difficulties in the reproducibility of experiments with conventional flotation. There are relatively few devices available commercially, hence each worker has to design his own system; some modifications have therefore been made at the time of the study in order to reproduce some of the experiments. Nevertheless, experiments on CGA[12] show that very few modifications were necessary compared with those needed for traditional flotation processes. This was confirmed by substituting the upper part of the columns with others of different sizes, so that different columns, sample volumes and ge-

ometries could be studied. The results are presented in Table 1. They show that with sample volumes from 0.5 to 5 l it is unnecessary to change the experimental conditions as all of the separations are virtually identical. However, there was a slower separation for a 5-l sample because of the increased time needed for the CGA to reach the upper level of the column.

Optimisation by the Simplex Method

Optimisation by the simplex method using the COFLOT computer program[13] was used. Two simplex methods were employed: one started at a zone at which a good response was expected (simplex 1), the other from a less favourable zone (simplex 2). The variables considered in this instance were: the amount of the co-precipitant species; CGA flow-rate; pH; and induction time.

Other variables such as the concentration of the NaLS, which generates the aphron, were not considered because it would have been necessary to change the solution in every experiment, whilst not having much influence in the whole process. The flotation time was not evaluated, as all experiments previously performed have shown that 5 min is an adequate time in which to obtain a good separation.

The simplex evolution in both instances and the percentage of flotation are shown in Figs. 8 and 9. In both instances the search for the optimum value was complete when the sum of the percentages of the four elements was higher than 370 in all of the retained vertices. In both simplex methods 1 and 2, the optimum was reached after a relatively low number of experiments (14 and 17, respectively), despite simplex 2 being started at a theoretically unfavourable zone.

Table 3. Results for factorial design

		Flotation, %				
Experiment	Effect*	Cu	Cd	Co	Ni	Total
1	T†	90	64	92	93	339
2	A	92	73	92	84	341
3	B	89	67	92	92	340
4	AB	93	89	94	94	370
5	C	81	93	94	97	365
6	AC	89	95	95	99	378
7	BC	70	93	92	96	351
8	ABC	86	96	96	97	375
9	D	90	66	95	95	346
10	AD	74	68	69	77	288
11	BD	91	71	95	97	354
12	ABD	27	26	28	27	108
13	CD	55	88	91	97	331
14	ACD	73	90	88	90	341
15	BCD	53	87	90	94	324
16	ABCD	73	80	79	82	314

* For an explanation of Effect coding see Table 2.
† T is the estimate of the mean.

Table 4. Values of estimates

	Flotation, %				
Effect*	Cu	Cd	Co	Ni	Total
T†	76.62	77.88	86.38	88.19	329.06
A	0.75	77.88	6.25	6.94	14.68
B	3.88	1.75	3.12	3.31	12.06
AB	2.25	2.62	2.75	2.94	10.56
C	4.12	12.38	4.25	5.81	18.31
AC	8.50	0.75	5.12	4.94	19.31
BC	1.88	0.50	1.75	1.56	5.69
ABC	3.50	1.62	2.12	2.19	9.44
D	9.62	5.88	7.00	5.81	28.31
AD	4.50	5.25	7.12	6.44	23.31
BD	2.12	4.25	3.25	4.06	13.69
ABD	3.50	4.38	3.38	4.19	15.44
CD	0.62	1.88	3.38	2.56	8.44
ACD	6.25	4.00	4.75	3.69	18.69
BCD	3.62	2.75	2.12	3.06	11.56
ABCD	2.75	3.12	2.00	3.69	11.56

* For an explanation of the Effect coding see Table 2.
† T is the estimate of the mean.

Factorial Design

In order to obtain further information on the flotation process a factorial design at two levels for the same variables as in the simplex situation was carried out. The levels imposed and the results obtained are shown in Tables 2 and 3. The corresponding values for each element and for the sum of all four elements are presented in Table 4.

From the corresponding values, the induction time, followed by the pH, appears to be the most influential variable. With regard to the interactions involved, that which occurs between the amount of co-precipitant and induction time appears to be the most noticeable. The marked influence of the induction time can be explained as being due to the high value chosen (10 min) which corresponds to a time that starts to have a negative influence on the process (see Fig. 5). Such a fact was demonstrated in the optimisation of the univariate method.

The negative effect of the induction time is curious in that it is in contrast to all the available data for conventional processes,[9] where increasing the induction time generally favours the kinetics of the process and, therefore, the recovery factor is improved. As some of the fundamental differences between the conventional co-flotation processes and co-flota-

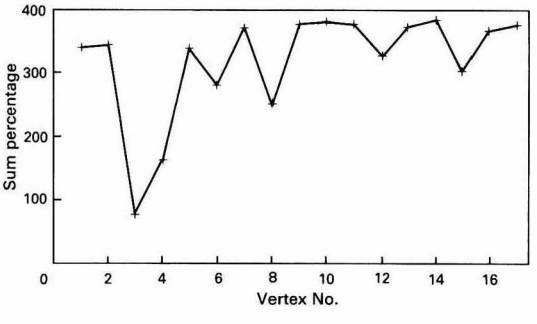

Fig. 9. Variation of response in simplex optimisation No. 2

Table 5. Optimum values reached in the optimisation and factorial design

	Pump flow-rate/ml min^{-1}	Fe^{III} added/ml	pH	Induction time	Flotation, %				
Method					Cu	Cd	Co	Ni	Total
Univariate	7.0	5.0	11.0	5.0	92	95	98	94	379
Simplex 1	6.1	7.1	11.9	1.0	89	96	95	94	374
Simplex 2	5.2	3.4	11.4	9.6	91	95	95	96	377
Factorial design	7.5	8.2	11.8	3.0	87	93	95	97	372

Table 6. Experiments on the recovery of sublates (values expressed in p.p.b.)

Element	3.5% NaCl* solution	Tap water*	Tap water†
Cu	9.8 ± 0.4	20.8 ± 1.4	11.5 ± 2.5
Cd	10.2 ± 0.8	10.8 ± 0.1	0.5 ± 0.1
Co	9.7 ± 0.9	10.0 ± 0.1	0.8 ± 0.1
Ni	9.7 ± 1.0	18.8 ± 1.3	6.8 ± 1.1

* Average values of five standard additions of 10 p.p.b.
† Direct determination.

tion based on the use of the CGA derive from the fact that the surfactant is present in the solution during the induction time and the size of the bubbles is much larger in the conventional processes, the evolution of the precipitate surface in the absence of the surfactant is very different compared with the situation where the surfactant is present in the solution.

The CGA mechanism must act by fixing the gas bubbles on the surface of the precipitate through a soapy shield, which itself becomes progressively destroyed as the bubbles ascend through the column. The results show that this mechanism works if the precipitate is not very old. Therefore, the induction time should be suppressed, or at least reduced to a minimum (1–2 min) to form the CGA.

The corresponding values for each separate element, however, show that the pH is the most important variable particularly for Cd (at low pH values the separation of Cd is diminished drastically). Table 5 gives all of the values obtained in the optimisation processes by both the univariate and the simplex methods; the best results of the factorial design are also shown. All of the results were very similar (11.0–11.9), showing that the optimum response had been reached, hence it can be assumed that pH is the most critical factor.

Sublate Recovery: Analysis of Real Samples

To study the analytical possibilities of the optimised co-flotation process, several experiments with 1 l of 3.5% NaCl solution containing 10 p.p.b. of each element were performed. The results obtained, after the corresponding blanks had been subtracted, are shown in Table 6. These results show recoveries of greater than 90% for all of the elements and a very acceptable degree of reproducibility at the concentrations used. In the same table a summary of the results for the analysis of tap water is presented, compared with a direct analytical determination, after the addition of 10 p.p.b. of each of the elements prior to analysis.

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Caesium-selective Poly(vinyl chloride) Membrane Electrodes Based on Calix[6]arene Esters

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Hexameric calix[6]arene ester compounds have shown an ability to extract large alkali metal ions such as caesium and rubidium in phase transfer studies. A potentiometric selectivity study has been made using two ester containing hexameric calix[6]arenes, *p-tert*-butyl hexaethyl ester (**1a**) and the unsubstituted hexaethyl ester (**1b**), as the ionophores for caesium ions. Six electrodes were prepared with 2-nitrophenyl octyl ether as the solvent mediator and various amounts of potassium tetrakis(*p*-chlorophenyl)borate as the ion exchanger in a poly(vinyl chloride) matrix. The ligands examined produced electrodes with near-Nernstian slopes; the selectivity coefficients for caesium with respect to rubidium, potassium, sodium, lithium, calcium, magnesium, hydrogen and ammonium were determined. Lifetimes of at least 1 month were estimated.

Keywords: *Caesium; ion-selective electrode; ionophore; calixarene*

There has been an increasing interest in analytical methods for determining the alkali metal ions, owing, in part, to the importance of lithium, potassium and sodium in biological processes, the susceptibility of rubidium and caesium to neutron activation, the production of radioactive caesium during nuclear fission processes and the possible use of lithium as a fusion fuel. Traditionally, caesium and the other alkali metals have been determined by flame photometry or atomic spectrometry (either emission or absorption). More recently, the technique of inductively coupled plasma mass spectrometry has been applied to the determination of caesium in serum[1] and the technique compared favourably with caesium determination by neutron activation analysis, the major trace analysis technique for caesium available to date.

Potentiometry involving ion-selective electrodes (ISEs) is now widely used, particularly in clinical assays for the determination of sodium and potassium.[2,3] The ISE response is generated by selective complexation of the target or primary ion by neutral carrier molecules dispersed in a poly(vinyl chloride) (PVC) matrix. However, relatively little attention has been paid to the development of similar caesium electrodes. One system, based on TMC-crown formazane, a dibenzo 14-membered ring cyclic compound, produced a good caesium ISE.[4] Some crown compounds with an 18–21 polyether ring size (*e.g.*, 21-crown-7) possess the optimum cavity size for caesium ions.[5] The potassium-selective crown ether, dibenzo-18-crown-6, has also been suggested for use in caesium electrodes in the absence of potassium ions and a range of caesium-selective ligands have been synthesised by Kimura *et al.*[6] The cryptahemispherand-3 of Cram and Ho[7] is capable of selective ion binding in the order $Cs^+ > Rb^+ \approx NH_4^+ > K^+ > Na^+ > Li^+$. Membranes based on valinomycin have been used in caesium and rubidium electrodes, provided the potassium activity is relatively low.[8]

The synthesis, structures and cation-binding properties of chemically modified calixarenes, a new class of macrocyclic molecular receptors, have been reviewed recently.[9,10] Such work has confirmed the ionophoric behaviour of many calixarene esters and ketones. The ability of some calix[4]arene structures to act as neutral carriers in sodium-selective PVC membrane systems has already been documented.[11–13] The performance of caesium-selective PVC membrane electrodes based on the *p-tert*-butyl hexaethyl ester, **1a**, and the unsubstituted hexaethyl ester, **1b**, calix[6]arene derivatives (Fig. 1) is now described.

Experimental

Materials

Details of the ligand synthesis have been published previously.[10] The membrane components: PVC, potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB), 2-nitrophenyl octyl ether (2-NPOE) and AnalaR grade tetrahydrofuran (THF) were obtained from Fluka; AnalaR grade chlorides of caesium, sodium, potassium, lithium, rubidium, magnesium, calcium and ammonium were supplied by Riedel-de-Häen and dissolved in distilled, de-ionised water.

Electrode Fabrication and Measurements

The general procedure for the preparation of the polymeric membrane was as follows: PVC, 180 mg; ligand, 2 mg; plasticiser 2-NPOE, 360 mg; and KT*p*ClPB, 0.5 mg if needed (Table 1) were dissolved in THF. The solution was then poured into a 30 mm square glass mould and covered to prevent particle contamination in a manner similar to that described by Moody and Thomas.[14] Gradual evaporation at room temperature gave a transparent flexible membrane of ≈0.1 mm in thickness. A 9-mm diameter disc was then removed with a cork borer and inserted into the cap of a Russell Model ISE 97-7809 gas sensing electrode (the diameter of the exposed membrane was 7 mm). The electrode design enables the PVC discs to be conveniently clipped on to the electrode tip, rather than being glued.[14] The internal reference electrolyte was 1×10^{-1} M CsCl. Fabricated electrodes were conditioned by immersion in a 1×10^{-1} M CsCl solution for at least 30 min prior to use. Electrochemical measurements were made relative to a saturated calomel reference electrode (SCE) using a Philips PM 9421 digital pH/millivoltmeter coupled to a Philips PM 8251 single pen recorder for the traces. The SCE was a Metrohm capillary tip

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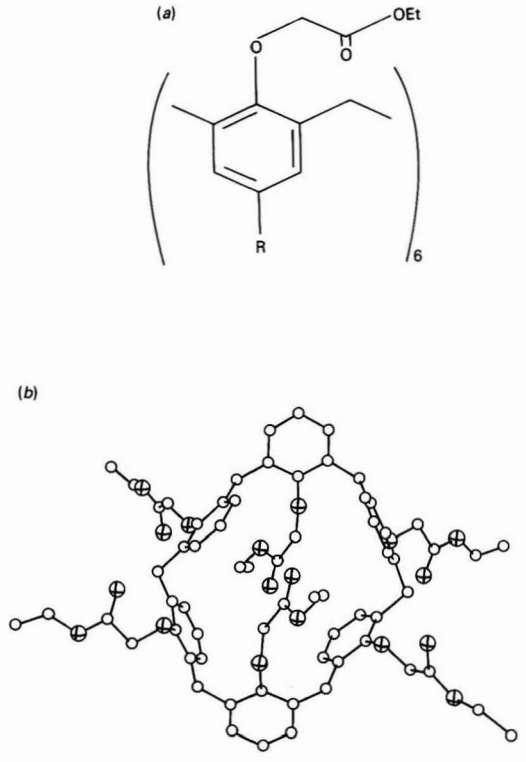

Fig. 1. (*a*) The calix[6]arene esters, **1a** and **1b**. In **1a**, R = Bu^t in **1b**, R = H. (*b*) A view of molecule **1b** almost normal to the plane of the six macrocyclic CH_2 carbon atoms. The molecule has a 0.404 and 0.47 nm separation between adjacent phenolic oxygen atoms. Carbon and oxygen atoms are shown as spheres of arbitrary radius, the oxygen atoms are larger and marked with a cross[10]

Table 1. Composition of the electrodes (all values in mg). Ligands as in Fig. 1.

	Electrode number					
Constituent	1	2	3	4	5	6
Ligand **1a** ..	0	1	1	0	0	0
Ligand **1b** ..	0	0	0	1	1	1
KT*p*ClPB ..	0.25	0	0.25	0	0.25	0.1
2-NPOE	180	180	180	180	180	180
PVC	90	90	90	90	90	90

model (Ref. 6.0750.000) chosen for its stability and low rate of KCl leakage. This arrangement ensured that any errors arising from variations in the junction potentials and possible potassium contamination of the sample were minimised. The selectivity coefficients ($k_{i,j}^{pot}$) were determined by the separate solution method using 1×10^{-1} M chloride salts of the cations involved and the Group II cation and hydrogen ion selectivities were confirmed by the mixed solution fixed interference method using 1×10^{-1} and 1×10^{-2} M solutions of the interfering ions, respectively. Injection experiments involved the addition of 0.180 ml of 1×10^{-1} M solutions of various cations into 20 ml of 1×10^{-4} M CsCl solution, which is equivalent to a 10-fold change in concentration, *i.e.*, 1×10^{-4}–1×10^{-3} M, for a caesium injection. Dynamic responses were measured by injection of 0.225 ml of a 1 M CsCl solution into 25 ml of a 1×10^{-3} M CsCl solution, on a fast chart speed (60 mm min^{-1}) thus producing a 1×10^{-3}–1×10^{-2} M change in CsCl concentration.

Table 2. Percentage extraction of alkali metal picrates into dichloromethane at 20 °C; values are ±5%. No picrate extraction in the absence of receptor

	Cation				
Ligand	Cs^+	Rb^+	K^+	Na^+	Li^+
1a	100	89	86	50	11
1b	95	94	51	10	5

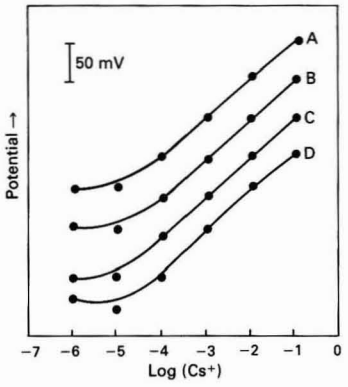

Fig. 2. Calibration graphs of electrodes incorporating the caesium-selective ligands. See Table 1 for membrane compositions. A, Electrode 2, ligand **1a**; B, Electrode 3, ligand **1a**; C, Electrode 4, ligand **1b**; and D, Electrode 5, ligand **1b**

Lifetime Studies

The lifetimes of the electrodes were studied by periodically re-calibrating them in standard solutions and calculating the response slope over the range 1×10^{-1}–1×10^{-4} M CsCl. During the observation period (4–6 weeks) the electrodes were used for a minimum of 2 h per week and stored in a 1×10^{-1} M CsCl solution when not in use.

Picrate Extraction Measurements

Metal picrates (2.5×10^{-4} M) were prepared *in situ* by dissolving the metal hydroxide (0.01 mol) in 2.5×10^{-4} M picric acid (100 ml). Solutions (2.5×10^{-4} M) of the calixarene derivative were prepared in dichloromethane. Equal volumes (5 ml) of receptor and picrate solutions were shaken (3 min) and the percentage extraction was measured from the absorbance of the resulting solution at *ca.* 378 nm.

Results and Discussion

Both the hexameric calixarene ester derivatives **1a** and **1b** exhibited good ion transport ability, with efficiency decreasing in the order $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ (Table 2). The phase transfer experiments represent a simple cation transfer system from an aqueous to an organic phase at a high pH and provide a rapid method of evaluating the selectivity behaviour of a new ligand. However, bearing in mind that the electrode membrane is a more complex ion-exchange system and that the conditions under which the selectivity of the ISE is examined are very different, the phase transfer results should be regarded only as a rough guide to the electrode selectivity.

Table 3. Response characteristics of the electrodes containing caesium-selective ligands. Membrane compositions as in Table 1

	Electrode number					
	1	2	3	4	5	6
	Ligand					
Parameter	None	**1a**	**1a**	**1b**	**1b**	**1b**
Slope/mV decade^{-1}	57.0	54.0	55.7	51.3	55.3	54.0
$-\text{Log}k^{\text{pot}}_{\text{Cs},j}$ $j = \text{Rb}^+$	0.52	1.24	0.99	1.85	1.52	1.78
$j = \text{K}^+$	1.21	0.66	0.79	2.68	2.53	2.49
$j = \text{Na}^+$	2.67	2.13	2.05	3.87	3.73	3.57
$j = \text{Li}^+$	3.36	3.29	3.25	4.20	4.45	3.92
$j = \text{Ca}^{2+}$	3.33	3.56	3.52	3.39	3.97	3.44
$j = \text{Mg}^{2+}$	3.02	2.80	3.02	4.04	3.92	3.85
$j = \text{H}^+$	1.82	1.95	3.04	3.71	2.70	3.02
$j = \text{NH}_4^+$	1.50	1.87	2.06	2.83	2.75	2.40

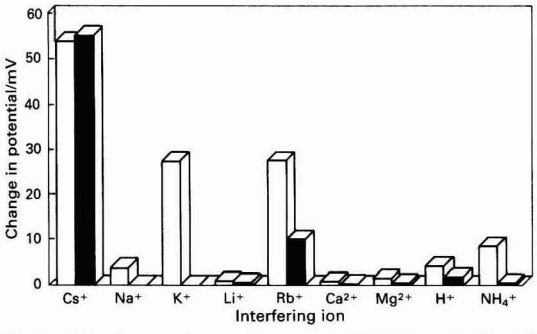

Fig. 3. Injection experiments. Response (mV) of Electrodes 3 and 5 to injections of 0.180 ml of 1×10^{-1} M cation solutions into 20 ml of 1×10^{-4} M CsCl

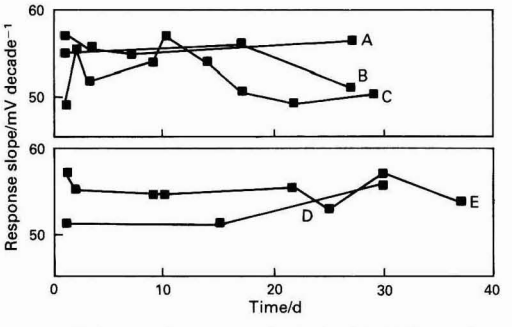

Fig. 4. Lifetime studies. Data obtained with PVC membrane electrodes showing average slopes over the range 1×10^{-1}–1×10^{-4} M CsCl. A, Electrode 1, no ligand; B, Electrode 2, ligand **1a**; C, Electrode 3, ligand **1a**; D, Electrode 4, ligand **1b**; and E, Electrode 5, ligand **1b**

Typical calibration graphs and response characteristics of the six electrodes are presented in Fig. 2 and Table 3, respectively. All five membranes containing the calixaryl ligands produced slopes in excess of 50 mV decade^{-1} change in caesium concentration over the approximate range $1 \times 10^{-4.5}$– 1×10^{-1} M for CsCl solutions (Fig. 2).

A selectivity pattern of $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ was obtained for Electrode 1 containing only KT*p*ClPB. The ion exchanger KT*p*ClPB used in these experiments is known to exhibit some caesium sensor activity in PVC membrane systems owing to an ion-exchange mechanism between the positive caesium ions and the large bulky negative anions. In the absence of a "best-fit" cavity mechanism, cations are drawn into the membrane in reverse order of their hydration enthalpies. With the alkali metals, this means that the preferred cation is that with the largest diameter and hence the lowest hydration enthalpy. The selectivity sequences obtained for Electrode 1 containing KT*p*ClPB only follow this predicted sequence[15] and the values are close to those previously published by other workers (see reference 16, p. 23, Fig. 5).

Electrodes 2 and 4 demonstrated the ability of the ligands to act as neutral carriers independent of the presence of the ion exchanger. Electrode 2, containing only ligand **1a**, displayed a near-Nernstian response to caesium ions in CsCl solutions over the range 1×10^{-4}–1×10^{-1} M. The potentiometric selectivity pattern against the Group I ions broadly followed the phase transfer trends, with reversal of the potassium and rubidium values and good preference for caesium over the Group II ions. The log $k^{\text{pot}}_{i,j}$ values for hydrogen and ammonium ions were reasonable but were improved by the addition of KT*p*ClPB in a 1:4 m/m ratio, equivalent to a 1.6:1, ligand:exchanger molar ratio (Electrode 3, Table 3).

The addition of the ion exchanger produced some stabilisation of the signal at low concentrations ($< 1 \times 10^{-4}$ M) but the selectivities against the Group I and Group II ions remained approximately the same.

Electrodes containing the ligand, **1b** also showed a near-Nernstian response to caesium ions (Fig. 2 and Table 3). The potentiometric selectivity pattern followed the Group I phase transfer trends with particular excitement for the selectivity data for caesium over potassium. Excellent log $k^{\text{pot}}_{i,j}$ values were obtained for the Group II, hydrogen and ammonium ions. The addition of KT*p*ClPB in the preparation reduced the selectivity against the hydrogen ion by an order of magnitude, but the other selectivity coefficients were largely unaffected (Electrode 5). An electrode containing a greater ligand:ion exchanger ratio of 10:1 m/m or 4:1 molar ratio, using ligand **1b**, was investigated. Although exhibiting good selectivity it still does not match the selectivity of ligand **1b** on its own (Table 3, Electrode 6).

Injection experiments were carried out to compare the responses of Electrodes 3 and 5 to additions of caesium and interfering ions (Fig. 3) and they confirm the selectivity data in Table 3. All the electrodes responded rapidly to changes in caesium concentration, with time constants of the order of a few seconds. The rate of response was only limited by the speed of stirring and the injection technique. Lifetime studies (Fig. 4) point to a stability of at least 1 month for both ligands.

In general ion exchangers such as KT*p*ClPB are incorporated into PVC membrane systems in small controlled amounts to reduce anion interference, lower the membrane resistance, improve electrical properties and enhance the selectivity characteristics by increasing the cation extraction efficiency

(see reference 16, p. 17). In this instance, the situation is further complicated by the caesium sensor abilities of the KT*p*ClPB, leading to the question of whether it is the exchanger or the ligand that is dominating the over-all performance of membranes incorporating both substances.

Examination of the selectivity data for the electrodes (Table 2) indicates that the dominant factor is the presence or absence of the calixarene ligand **1a** or **1b**. Both ligand-based systems perform considerably better as caesium sensors than the ion-exchange system in Electrode 1. The addition of the exchanger to membranes containing excess of ligand slightly reduced the selectivity against rubidium and caused some variation in hydrogen ion selectivity, but otherwise had no beneficial effect on selectivity.

Conclusion

Caesium ion-selective electrodes, incorporating calixarene based ionophores, have been fabricated and display excellent selectivity factors over other Group I and Group II ions and against hydrogen and ammonium ions. In all instances the electrodes incorporating the ligand **1b** had superior performances to those incorporating the ligand **1a**. The electrodes are robust, simple to prepare, have fast response times and lifetimes of at least 1 month.

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Poly(vinyl chloride) Membrane Electrode on a Graphite Substrate for the Potentiometric Titration of Tetramethylammonium Bromide With Sodium Tetraphenylborate

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A poly(vinyl chloride) membrane electrode, based on the ion-pair complex of tetramethylammonium bromide (TMAB) with sodium tetraphenylborate, is described. The electrode exhibits a linear response with a slope of 49 mV per decade of concentration at 20 °C within the range 1×10^{-4}–0.1 M TMAB, at an ionic strength of 0.1 M NaBr. The effect of the pH of the test solution and the interference of other cations and SiO_4^{4-} (or silicate ions of lower charge such as $HSiO_4^{3-}$ or $H_2SiO_4^{2-}$) were studied. Potentiometric titration was used to determine TMAB, in the presence of SiO_4^{4-}, with satisfactory results.

Keywords: *Tetramethylammonium bromide; potentiometric titration; poly(vinyl chloride) membrane on graphite*

Quaternary ammonium salts are important analytical, pharmaceutical and industrial chemicals, *e.g.*, in the synthesis of zeolites.[1] Many analytical methods, such as titrimetry,[2] gravimetry[3] and spectrophotometry,[4] are available for the determination of quaternary ammonium salts. Most of these methods are tedious, non-selective and difficult to apply to quaternary ammonium compounds with short hydrocarbon chains. Such compounds are normally used in low concentrations and are then difficult to determine with high precision and reproducibility.

In the present paper, a poly(vinyl chloride) (PVC) membrane supported on a graphite electrode, similar to those previously reported by Dowle *et al.*,[5] is described for the determination of tetramethylammonium bromide (TMAB). An electrode based on the ion pair of TMAB with sodium tetraphenylborate (TPB) in a PVC membrane was prepared.

We were particularly interested in the possible interference from SiO_4^{4-}, because the aim was to apply this electrode to the study of solutions used for the synthesis of zeolites, which contain silicate ions.

Experimental

Apparatus

For the addition of the titrant solution a Radiometer ABU 1[a] Auto-Burette was used (Radiometer, Copenhagen, Denmark). The pH and e.m.f. values were measured with an Orion (Cambridge, MA, USA) Model 701 pH meter and a Corning (Halstead, Essex, UK) 113 pH and millivoltmeter, respectively. The reference electrode was an Orion 90-02-00 double-junction electrode with an Orion 90-00-02 inner filling solution, the outer filling solution being 0.10 M NH_4F. Subsequently, the Orion 960 autochemistry system was used.

Reagents

Sodium tetraphenylborate solution. Analytical-reagent grade (Aldrich, Brussels, Belgium). The solution was standardised with 0.1 M $AgNO_3$.

Silver nitrate solution. Titrisol ampoule (Merck).

Tetramethylam:nonium bromide. Recrystallised from water shortly before use and dried over P_2O_5, pro analysi (Merck, Darmstadt, FRG).

Tetrahydrofuran. Pro analysi (Merck).

Chemicals for selectivity tests. Pro analysi (Merck).

Tetraethylammonium hydroxide (TEAOH), 20% solution (Janssen Chimica, Beerse, Belgium).

Sodium bromide. Pro analysi (Merck).

Buffer solutions. Titrisol ampoules (Merck).

Tritolyl phosphate. From UCB (Brussels, Belgium).

Poly(vinyl chloride) powder. From Solvic (Brussels, Belgium).

Hexadecyltrimethylammonium bromide [cetyltrimethylammonium bromide (CTAB)]. From Sigma (St Louis, MO, USA). Doubly distilled water was used to prepare all the solutions.

Sensing Material

The compound was prepared by reacting equivalent amounts of solutions of TMAB and TPB. The salt that precipitated was filtered on a glass filter and washed three times with water. The compound was then dispersed in water and dialysed with Dialysis tubing—Visking, 5–24/32 in (Medicell International, London, UK) for 48 h; it was then free of bromide (tested with $AgNO_3$ plus HNO_3). The material was air dried at 80 °C, ground in a glass pestle and stored in a dark-glass vessel.

Coating Solution

A mixture of 75 ml of tetrahydrofuran and 6.0 g of tritolyl phosphate was stirred in a closed dark-glass vessel, and 4 g of PVC powder were added slowly until complete dissolution was achieved.

A study of the membrane composition with various concentrations of sensing material showed no significant differences in the *E versus* log[TMAB] slopes from 0.4 to 20% m/m. Potential readings were carried out after 5 min in stirred solutions of TMAB [the ionic strength was not adjusted to a constant value (Fig. 1)]. The lowest concentration of active reagent, down to log[TMAB] = −5, containing 0.4% m/m of the sensing material, was chosen, because larger amounts are increasingly difficult to keep in suspension. In addition, economic reasons advocate the lowest concentration compatible with a reliable operation.

A similarly low concentration was reported by Dowle *et al.*[5] These workers also used tritolyl phosphate as a plasticiser. Previous investigations[6,7] had shown this to be an excellent compound for this application.

Additionally, 40 mg of the sensing material were added to the coating solution and stirred continuously until a finely dispersed suspension was obtained. This could take typically 1 h.

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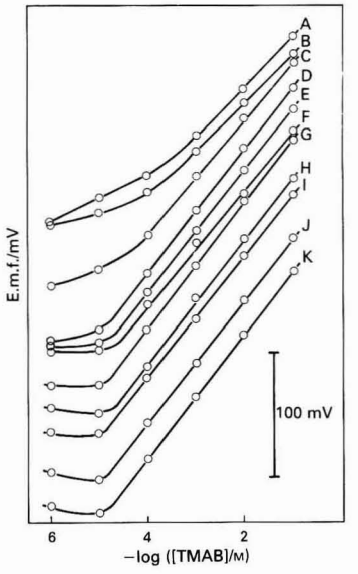

Fig. 1. Calibration graphs for TMAB electrodes. Sensing material concentration: A, 0.1; B,0.2; C, 0.3; D, 0.4; E, 0.7; F, 1.1; G, 1.6; H, 2.5; I, 5.0; J, 10; and K, 20% m/m

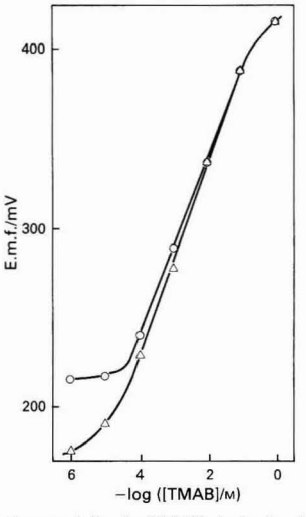

Fig. 2. Calibration graph for the TMAB electrode: △, solutions of TMAB without NaBr; and ○, solutions of TMAB with NaBr and total ionic strength 0.10 M

Electrode Preparation

As the substrate for the PVC membrane, a graphite rod (40 × 8 mm) was used, which was ignited in a colourless flame before use. A brass cap, to which a co-axial cable was soldered, was clamped to the rod. The rod was fixed in a PTFE tube (110 × 12 mm) and sealed with a silicone-rubber O-ring. About 30 mm of the rod protruded through the end of the Teflon tube. A co-axial cable was fixed on to the thicker end of the tube with a nylon screw. The electrode was immersed in the coating solution until 1 cm of the PTFE tube was wetted. The coated electrode was allowed to dry in air, and the coating procedure was repeated twice.

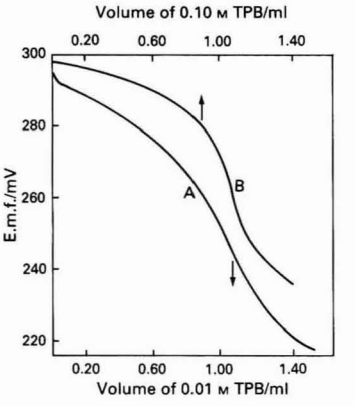

Fig. 3. Representative titration curves for the titration of A, 1.00 ml of 0.01 M TMAB; and B, 1.00 ml 0.10 M TMAB with 0.01 and 0.10 M TPB, respectively, in a total starting volume of 40.0 ml

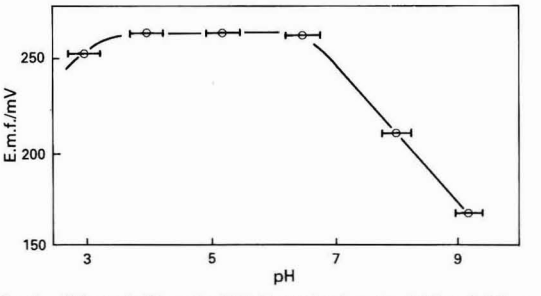

Fig. 4. Effect of pH on the TMAB electrode potential in a 0.001 M TMAB solution

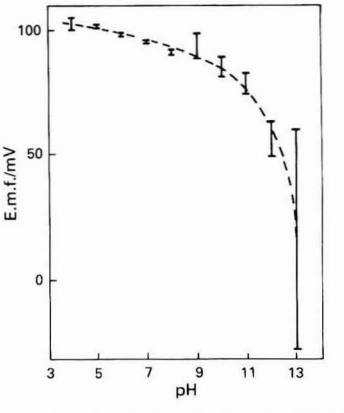

Fig. 5. Response (maximum time 5 min) of a TMAB electrode (0.4% m/m) in buffered 0.001 M TMAB solutions. The potential is drifting to more negative values

Preparation and Standardisation of a 0.01 M TPB Solution

A 3.42-g amount of TPB was weighed into a 1-l calibrated flask and the volume adjusted with distilled water. After 1 d, this solution was passed through a filter-paper (Schleicher & Schull No. 589/3). The concentration of this solution was determined with freshly prepared 0.1 M $AgNO_3$ by potentiometric titration, using the TMAB - TPB electrode and a double-junction electrode. A 25.00-ml portion of the filtered

Table 1. Selectivity coefficients for the TMA - TPB electrode

Interferent		
Cation	Anion	Log $k^{pot}_{TMA^+,j^{z+}}$
Li^+	Cl^-	−1.4
NH_4^+	Br^-	−1.1
Na^+	Cl^-	−1.3
K^+	Cl^-	−1.3
Ag^+	NO_3^-	4.5
Ba^{2+}	Cl^-	−1.4
Ca^{2+}	Cl^-	−1.3
Mg^{2+}	SO_4^{2-}	−1.4
Cu^{2+}	SO_4^{2-}	−1.2
Mn^{2+}	SO_4^{2-}	−1.3
Zn^{2+}	Cl^-	−1.3
Fe^{2+}	SO_4^{2-}	−1.5
Cd^{2+}	Cl^-	−0.9
Co^{2+}	Cl^-	−1.1
Al^{3+}	NO_3^-	−1.3
Fe^{3+}	Cl^-	0.5
CTA^+	Br^-	6.2

TPB solution and 15 ml of water were transferred into a 50-ml beaker. This sample was titrated with the standard $AgNO_3$ solution. The concentration was calculated by the second-derivative method as 0.00995 ± 0.00001 M (n = 4).

Preparation and Standardisation of a 0.01 M TMAB Solution

A 1.5983-g amount of dried and recrystallised TMAB was weighed into a 1-l calibrated flask and the volume adjusted with distilled water. A 1-ml aliquot of this solution and 39 ml of water were transferred into a 50-ml beaker and titrated potentiometrically with a 0.00995 M TPB solution by means of the TMAB electrode and a double-junction electrode. The titre of the TMAB solution was calculated as 0.01035 ± 0.00001 M (n = 4) corresponding to 1.5945 g of TMAB.

The potentiometric titration method of determining TMAB described here was not compared with the two-phase titration[2] or gravimetric[3] methods, because these last two methods were considered too inaccurate, particularly in the dilute solutions in which the TMAB - TPB electrode functions. It was not possible to use the spectrophotometric method[4] because no colour was developed in the TMAB solution on addition of the reagent dithiazone in a suitable pH range. Apparently, the chain length of TMAB is too short for this reaction, because the spectrophotometric method works well for quaternary ammonium salts such as TEAOH.

Results

Construction of the Calibration Graph

To construct the calibration graph (Fig. 2) suitable increments of standard 0.01 M TMAB were diluted in a 100-ml calibrated flask, sufficient to cover the concentration range 1×10^{-6}–1×10^{-3} M, for subsequent titration.

The total ionic strength of each solution was 0.1 M (except at the highest concentration) and NaBr was used as the supporting electrolyte. The highest concentrations were prepared from a standard solution of 1 M TMAB. A freshly prepared TMAB electrode and the reference electrode were immersed in these solutions, stirred at a constant rate and the e.m.f. values were recorded when stable readings were obtained, usually after 20 s, at ambient temperature (20 °C). These data were plotted *versus* −log[TMAB] (Fig. 2).

An electrode response of 49 mV per decade of concentration was determined over the linear range (7×10^{-5} < concentration < 1×10^{-1} M). The effect of the addition of NaBr to TMAB solutions, to equalise the ionic strength ($\mu = \frac{1}{2} \Sigma c_i z_i^2$, with c_i = concentration of ion type i, z_i = charge of the ion) at 0.1 M, is to cause a rise in electrode potentials below μ = 0.1 M. In a calibration graph for solutions without additional electrolyte the slope in the linear region is 53 mV per decade of concentration (Fig. 2). The response is sub-Nernstian.

Electrode Stability

Electrode potentials were stable to within 1 mV in 1 min at pH 5 and with 1×10^{-3} M TMAB. In addition, electrode stability is not a critical factor when the electrode is applied to potentiometric titrations.[8]

Standard Procedure for Potentiometric Titrations

An aliquot of a solution containing TMAB was transferred into a 50-ml beaker, water was added and the pH was fixed at a suitable value (see later) with very small volumes of HCl and/or NaOH; the total volume was adjusted to 40 ml with distilled water. The resulting solution was titrated with 0.01 M TPB, with constant magnetic stirring, using the TMAB electrode as the sensor and a double-junction electrode as the reference electrode.

In the titration curve for dilute TMAB (2.5×10^{-4} M) solution with 1×10^{-2} M TPB, the electrode potential dropped initially because the TPB added was not precipitated instantaneously. An example is given in Fig. 3, where two curves are shown for 2.5×10^{-4} and 2.5×10^{-3} M TMAB titrated with 0.01 and 0.10 M TPB, respectively.

Interference by Other Agents

Effect of pH

The object was to determine TMAB in solutions with pH values of *ca.* 13, in order to ascertain whether solutions at these pH levels interfered in the determination of TMAB. Therefore, the pH of a 1×10^{-3} M test solution was adjusted by the addition of very small volumes of NaOH and/or HCl. The respective electrode potential was monitored as a function of pH. From the graph (Fig. 4) obtained it is clear that pH has a negligible effect within the range 3.0–7.0, and in this range the electrode can be used for the determination of TMAB.

In a study of the influence of pH on the response of the electrode with buffered TMAB solutions, the potentials in stirred solutions (1×10^{-3} M TMAB) were monitored until the electrode potential was constant to within 1 mV in 5 min (maximum time: 5 min).

The total potential drop is shown in Fig. 5. Initially, the potential drifted to more negative values. It should be noted that a comparison of electrode responses to various buffer solutions can include the influence of ions other than H^+ or OH^-, as the buffers differ in chemical composition and ionic strength.

Effect of SiO_4^{4-} as an interfering agent on the volume of titrant at the equivalence point

For a cation-selective electrode positive ions, particularly, are suspected to interfere; interference by SiO_4^{4-} (or ions of lower charge such as $HSiO_4^{3-}$ or $H_2SiO_4^{2-}$) is not likely. Nevertheless, this was checked by adding 100 µl of 0.1 M Na_4SiO_4 to 1.00 ml of 0.01035 M TMAB and titrating with 0.00995 M TPB. In three experiments the following values for the volume of titrant at the equivalence point were obtained: 1.042, 1.039 and 1.040 ml. The mean value is 1.040 ml with a standard deviation of 0.0015 ml, which is in good agreement with results for experiments without SiO_4^{4-}.

Determination of selectivity coefficients

A method for the examination of interference is the determination of the selectivity of the electrode, obtained by using the separate solution method[9] and the equation:

$$\text{Log } k^{\text{pot}}_{\text{TMA}^+,J^{z+}} = (E_2 - E_1)/S + \log[\text{TMA}^+] - \log[J^{z+}]^{1/z}$$

where E_1 is the potential of the electrode in a TMA^+ solution of 0.01 M and $\mu = 0.1$ M (adjusted with KCl, except at Ag^+), and E_2 is the potential of the same electrode in a solution with $[TMA^+] = 0$ and $[J^{z+}] = 0.01$ M at the same ionic strength; j represents the interferent and S is the electrode slope (49 mV). Results are given in Table 1.

Conclusions

The electrode described here is easy to construct and was used successfully as a sensor in the potentiometric titration, with TPB of substances such as TMAB and TEAOH, even in the presence of SiO_4^{4-}, after adjusting to an appropriate pH. The low cost and ease of operation of potentiometric instrumentation make the potentiometric titration of TMAB a highly desirable alternative to many other methods.

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Adsorptive Stripping Voltammetric Determination of Pipemidic Acid in Human Urine

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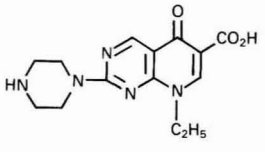
The electrochemical behaviour of pipemidic acid {8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylic acid}, a well known antimicrobial agent used for urinary infections, was investigated by linear-sweep, differential-pulse and square-wave voltammetry at a hanging mercury drop electrode. Two reduction processes were observed in Britton - Robinson buffers at acid pH, whereas only one or two processes were observed in alkaline solutions, dependent on the pH of the buffer employed. Adsorptive effects were used to accumulate the drug on to the electrode. The adsorbed species were measured voltammetrically by using a cathodic process appearing at −0.76 V in 0.1 M $HClO_4$. Linear calibration graphs were obtained in the range 2.5×10^{-9}–2.0×10^{-7} M. A simple procedure of extraction was employed for the determination of the drug in urine samples.

Keywords: *Adsorptive stripping voltammetry; pipemidic acid; urine analysis*

Pipemidic acid {8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylic acid} (**I**) has long been established as a useful therapeutic agent for urinary tract infections owing to its antibacterial spectrum against gram-negative bacteria.[1] Its effectiveness in this regard seems to be partly due to an efficient distribution to the urinary tract and its stability to metabolic inactivation. For instance, Shimizu *et al.*,[2] found that the concentrations of pipemidic acid in human urine were very high following a single dose. The highest levels in urine samples are obtained between 2 and 4 or 4 and 6 h, the concentrations of which, at therapeutic doses, are sufficient to meet the required minimum inhibitory concentration for antibacterial activity.



(I)

Increasingly, the monitoring of drugs is being considered a rational approach for the correct management of patient therapy. Regarding pipemidic acid, attention must be given in this respect, as the determination of the relationship between drug dose and drug level imparts an important aspect to the success of the treatment. Accordingly, chromatographic methods employing ultraviolet[3] and spectrofluorimetric[4] detection have been reported for the determination of the drug in biological fluids. The polarographic behaviour of pipemidic acid has been studied previously[5] and applied to the analysis of a pharmaceutical formulation. The aim of the present study was to investigate the adsorptive behaviour of this antimicrobial agent. Its application to the determination of the levels of pipemidic acid in the urine of patients undergoing therapy has been found feasible. Adsorptive voltammetry is a technique which has been proven suitable for the quantification of a large variety of biologically significant organic molecules.[6,7]

Experimental

Apparatus

Voltammetric experiments were carried out using either a computerised electrochemical measurement system (Inelecsa, Seville, Spain) or alternatively, a Metrohm E-611 potentiostat coupled to a Metrohm E-612 scanner was used. The linear sweep voltammograms generated by the latter instrument were recorded on a Graphtech WX-4421 *x* - *y* recorder. A Metrohm EA-410 hanging mercury drop electrode (HMDE) was used throughout as the working electrode. The drop size corresponded to three divisions of the micrometric dial of the electrode. A platinum wire was used for the auxiliary electrode with a saturated calomel reference electrode. The solutions were stirred using a 12-mm magnetic stirring bar (Metrohm, Herisau, Switzerland).

Materials

Pipemidic acid was obtained from Prodes Pharma Laboratories (Barcelona, Spain). Stock solutions (1.0×10^{-2} M) of pipemidic acid were prepared in 5.0×10^{-2} M NaOH each week. Solutions were stored at 4 °C and protected from the light. Britton - Robinson (B - R) buffers, of constant ionic strength and adjusted to 0.3 M with KCl, and standardised 0.1 M $HClO_4$ solutions were used as background electrolytes. All other reagents were of analytical-reagent grade (Merck, Darmstadt, FRG) and purified water was obtained by passing distilled water through a Milli-Q (Millipore-Waters, Milford, MA, USA) system.

Procedures

For voltammetric investigations, the test solution (20 ml) was de-aerated with oxygen-free argon for 15 min (and for a further 30 s before each new measurement). An equilibrium time of 10 s was allowed to elapse between the end of the stirring and the start of the potential scan.

Urine samples consisted of pooled human urine from at least ten healthy individuals, spiked with an adequate amount of the drug to give the desired final concentration.

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Sample purification was accomplished by solid - liquid extraction according to the following procedure. The sample (1 ml) was diluted to 10 ml with 0.025 M phosphate - acetate buffer, adjusted to pH 5 (with 0.8 M NaOH), and gently mixed. The resultant solution was passed through a reversed-phase C_{18} disposable Sep-Pak cartridge (Waters, Milford, MA, USA) previously activated by washing sequentially with 10 ml of water, methanol, and buffer solution. The eluate was discarded, the cartridge washed with 10 ml of water and the retained materials were eluted with 3 ml of methanol. The eluate was then collected in a 10-ml test-tube. The solvent was evaporated to dryness at 45 °C under an inert gas stream. The dried extract was re-dissolved in 5 ml of 0.1 M $HClO_4$ and transferred into the polarographic cell. Measurements were obtained in the linear-sweep voltammetric (LSV) mode under the following optimised conditions: accumulation potential, −0.5 V; scan rate, 1 V s^{-1}; and accumulation time, 20 s.

Quantification was achieved by the standard additions method.

Results and Discussion

Cyclic Voltammetry

Pipemidic acid undergoes two main reduction processes at the HMDE as seen from the cyclic voltammogram shown in Fig. 1, obtained for a 5.0×10^{-5} M solution of the drug in B - R buffer pH 3.04. The resultant peaks, namely 1 (−0.569 V) and 2 (−0.892 V), are symmetrical in shape with a width at their half-height of 30.3 and 63.4 mV, respectively, indicating adsorption-controlled processes.[8,9] At higher concentrations (2.0×10^{-4} M), the shape of the curves changed into waves which showed typical diffusion plateaux, the processes being controlled by diffusion under such conditions. When the concentration was lowered to 2.0×10^{-7} M, a negligible current was observed for the second peak, while the first peak was not visible at all. However, a well defined peak was again observed for the second reduction process if a 75-s time period preceeded the potential scan, showing the possibility for adsorptive accumulation of pipemidic acid at the HMDE.

Effect of pH

Linear sweep voltammograms obtained for 2.0×10^{-4} M solutions of pipemidic acid for pH values within the range 1–11, showed behaviour that resembled that of previous studies carried out using direct current (d.c.) polarography.[5] Peak 1 existed between pH 1 and 5, whereas peak 2 was clearly seen up to pH 7. Peak 2 split into two waves between pH 8 and 9, showed a single peak at pH 10, and at pH 11 split again; this was in good agreement with the d.c. polarographic study.[5] From this it was readily concluded that peak 2 was the most important for analytical purposes in the pH region from 1 to 7, where peak currents for this process were improved compared with those of peak 1 by a factor of about 3. Accordingly, the rest of this study was concentrated on peak 2. A linear potential scan in the negative direction following an accumulation time (t_{acc}) of 75 s, yielded well defined, symmetrical signals for peak 2 whose peak potential, E_p, varied linearly with pH in the range 1–5 according to the equation:

$$E_p\ (\text{mV}) = -66.7\ \text{pH} - 715.73$$

The maximum peak intensity was observed at pH 1, with a 0.1 M $HClO_4$ solution, which was consequently used as the background electrolyte for the remainder of the study.

Adsorptive Stripping Voltammetry

The variation of peak current (i_p) with accumulation potential, E_{acc}, from 0.0 to −0.600 V, did not produce any change in the value of i_p, therefore, an E_{acc} of −0.500 V was used in further studies. Adsorption of the drug on to the HMDE did not occur to any significant extent when the accumulation step was carried out under open circuit conditions.

The rate-determining step of the whole process was found to be dependent on the transport of the drug from the bulk of the solution to the electrode surface. Thus, the current recorded in a 2.0×10^{-7} M solution of pipemidic acid after 120 s of accumulation under unstirred conditions was 350 nA, whereas the use of a shorter t_{acc}, *i.e.*, 60 s, under forced mass convection yielded a value of 1450 nA. Adsorption - stripping cycles carried out for increasing values of the scan rate, v, under the above optimised conditions gave rise to reduction peaks with intensities that showed a linear increase with the scan rate between 0.1 and 1 V s^{-1}, according to the following relationship:

$$i_p\ (\text{nA}) = 1.09\ (v/\text{V s}^{-1}) - 1.06;\ r = 0.9997,\ n = 7$$

where r is the correlation coefficient and n is the number of cycles. This relationship was as expected for an adsorption-controlled process.

Different potential scan modes, *i.e.*, LSV, differential-pulse voltammetry (DPV), and square-wave voltammetry (SWV), were applied to the stripping analysis of the adsorbed pipemidic acid. It was found that the use of the pulse techniques did not improve the sensitivity attained by LSV. The limit of detection found using LSV was 2.5×10^{-9} M as against a limit of detection of 8.0×10^{-9} M obtained using either DPV or SWV. The dynamic linear range of calibration with LSV is extended from 7.5×10^{-9} up to 1.0×10^{-7} M, while the linear ranges obtained by DPV and SWV were restricted from 1.1×10^{-8} to 7.5×10^{-8} M. Further, the largest slope value in the plot of peak current *versus* concentration was obtained using LSV (5.1×10^6 μA l mol^{-1}) while values of 3.6×10^6 and 5.2×10^5 μA l mol^{-1} were achieved using SWV and DPV, respectively.

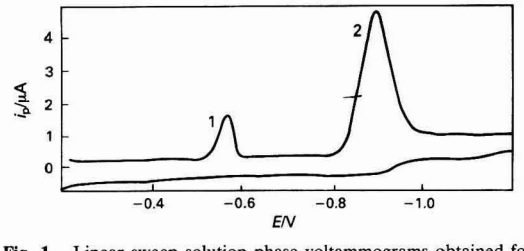

Fig. 1. Linear sweep solution phase voltammograms obtained for 5.0×10^{-5} M pipemidic acid in Britton - Robinson buffer pH 3.04 at a scan rate of 1.0 V s^{-1}. For details, see text

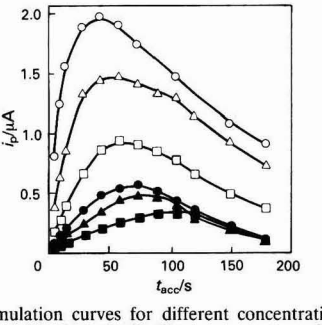

Fig. 2. Accumulation curves for different concentrations of pipemidic acid by LSV. E_p −0.500 V at a scan rate of 1.0 V s^{-1}. [Pipemidic acid]: ■, 4×10^{-8}; ▲, 7×10^{-8}; ●, 1×10^{-7}; □, 2×10^{-7}; △, 5×10^{-7}; and ○, 1×10^{-6} M

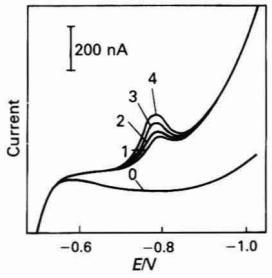

Fig. 3. Linear sweep voltammograms obtained for urine extracts of a urine sample, 0. a blank; and 1, containing 30.35 μg ml^{-1} of pipemidic acid. Curves 2–4 are for successive standard additions of 1.5 × 10^{-7} M of the drug, t_{acc} = 20 s, other conditions as in Fig. 2

Successive accumulation stripping cycles were carried out with increasing accumulation times for six different concentrations of the analyte. The resultant accumulation curves are shown in Fig. 2. As expected, the lower the bulk concentration of the drug, the lower the magnitude of the stripping current and the better the linear relationship between peak intensity and t_{acc}. In addition, a plot of the slope of the initial linear portions of the accumulation curves *versus* the corresponding concentrations gave rise to a linear graph with a slope of 9.04 × 10^7 nA s^{-1} mol^{-1}, indicating that the adsorption process follows a normal Langmuir-type isotherm in the initial region of the plot. As either the t_{acc} or the assayed concentration takes higher values, the peak current ceases to increase. In fact, it shows a trend to diminishing values that can even drop close to those achieved after a very short t_{acc}. This could be explained in terms of an adsorption equilibrium (not a saturation equilibrium) that is a function of the bulk concentration of the drug, which is reached as a compromise between the adsorption forces driving the molecules to the surface of the electrode and the possible repulsion interactions of the molecules in the adsorbed state, once a given coverage of the electrode has been reached.

Similar phenomena have been reported in the literature for molecules of biological importance.[10,11] As can be seen in Fig. 2, analytically useful relationships between peak intensity and concentration exist over the complete range of t_{acc}. However, it is better that quantification of this drug be performed by using those experimental conditions that fall within the initial zone of the accumulation curves where the maximum $i_p : t_{acc} : c$ ratios are attainable.

Analytical Application

Further study for the determination of the drug in human urine was performed, where determination would be useful for the screening of patients undergoing prophylactic or therapeutic treatment with pipemidic acid.

The above mentioned possibility to perform the pre-concentration step within a wide range of values for E_{acc} would provide the analyst with a useful approach to avoid interferences arising from the presence of naturally occurring components in urine which are able to form mercury salts (*e.g.*, cysteine). However, preliminary attempts to perform direct analyses in urine failed to give good blank measurements, and it was felt that a sample clean-up procedure was necessary prior to analysis. Accordingly, a solid - liquid extraction was used, because of the ease with which it can be carried out, the low cost and the good results obtained with other analytes.[12,13] The retention of the drug on the packing material of a C_{18} cartridge was studied in pH 2–9 buffered aqueous solutions. No significant differences were observed with variation in the pH, and recoveries in the range 98–99.5% were achieved in all instances. Optimum results were obtained at pH 5, and this was chosen for the extraction procedure as described under Experimental.

Accumulation curves obtained for urine extracts, showed that maximum adsorption was reached with a shorter t_{acc} than in aqueous solutions, probably owing to the presence of natural surfactants in the urine, which were retained in the C_{18} cartridge with the drug, and competed for the adsorption sites of the electrode. Therefore, a t_{acc} of 20 s was used throughout for real samples. A blank urine extract obtained by following this procedure is shown in Fig. 3 (line 0), together with a urine sample extract (line 1) containing 30.35 μg ml^{-1} of pipemidic acid. Quantification of the urine content of the drug was accomplished by standard additions (Fig. 3, lines 2–4). Five aliquots of urine were spiked with 30.35 μg ml^{-1} of the drug and processed according to the proposed procedure. At this concentration level the recovery attained was 82% and the assay precision expressed in terms of the relative standard deviation was 5.2%. Successive additions of pipemidic acid to a blank urine extract gave rise to a wide dynamic range of linearity covering almost two orders of magnitude from 2.0 × 10^{-7} to 1.0 × 10^{-5} M in the cell, with a slope of 6.3 × 10^4 μA l mol^{-1} and a correlation coefficient of 0.999 (n = 11).

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Polarographic Determination of Sorbic Acid in Fruit Juices and Soft Drinks

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A simple differential-pulse polarographic method using a laboratory-built hanging mercury drop electrode as the working electrode was developed for the determination of sorbic acid in fruit juices and soft drinks. Sorbic acid was extracted from the samples with diethyl ether. After reduction of the ethereal solution to a small volume by direct evaporation, the residual ether was dissolved in the supporting electrolyte (25 ml of acetonitrile + 1 ml of 0.06 M acetic acid + 0.8 g of tetraethylammonium bromide). Peak current was measured at −1.7 V. The working range of the method, without dilution or pre-concentration of the samples, was from 4 to 229 p.p.m. for the original juice and drink samples. The validity of the method was confirmed by parallel determinations using the method of the Association of Official Analytical Chemists and by recovery tests on a large variety of juice samples. Satisfactory recoveries and agreement in results from the two methods were obtained. The recovery and precision (relative standard deviation) of the method were 97 ± 4 and 100 ± 3%, respectively, for blackcurrant juice for five determinations.

Keywords: *Sorbic acid determination; soft drink; fruit juice; differential-pulse polarography*

Sorbic acid is a popular preservative used in many food products and is generally considered to be safe in application. Many assay methods have been reported for the determination of sorbic acid including visible spectrophotometry,[1] ultraviolet (UV) spectrophotometry,[2] gas - liquid chromatography,[3] high-performance liquid chromatography (HPLC)[4] and several titrimetric methods.[5,6] The UV method is sensitive and simple but is subject to interference by many substances which absorb in the UV region such as benzoic acid and saccharin. The visible spectrophotometric method[1] is time consuming because it uses steam distillation for separation prior to analysis, moreover, it is suspected that this method is interfered with by the ethanol present in many food products and beverages. However, no reports on the use of polarographic methods for the determination of sorbic acid in food have been found in the literature. A study of these methods might provide a solution to the interference problems caused by the presence of UV active compounds and ethanol in food.

The work reported in this paper describes an analytical procedure using the differential-pulse polarographic method for the determination of sorbic acid in an acetonitrile - acetic acid medium. This procedure provides a sensitive and selective method for sorbic acid determination in the presence of ethanol and other UV active compounds commonly used as food additives.

Experimental

Apparatus

A Princeton Applied Research (PAR) Model 174A polarographic analyser equipped with a Houston *x* - *y* recorder (RE-0089) was used to measure and record the differential-pulse polarograms. A hanging mercury drop electrode (HMDE) specially designed for use in an organic solvent was constructed in this laboratory using procedures described in the literature.[7] Mercury drops of the desired sizes were produced either from a PAR HMDE (Model 9323) or a PAR static mercury drop electrode (Model 303) and collected in a separate beaker. A laboratory-built HMDE equipped with a short platinum wire (1–2 mm), which projected out at the tip of a glass tube with a good platinum - glass seal, was used to pick up the mercury drop. The mercury drop wetted and completely covered the platinum wire which was used mainly as the current collector for the HMDE for electrochemical analysis in the organic solvent. A saturated calomel electrode was used as the reference electrode, with a saturated potassium nitrate solution as the liquid junction and acid-resistant Vycor as the restrictor to electrolyte flow. A coil of platinum wire was used as the counter electrode.

Reagents

All reagents used were of analytical-reagent grade except acetonitrile which was of HPLC grade. Standard sorbic acid solution, 1000 μg ml^{-1}, was prepared by dissolving 0.1000 g of sorbic acid in 100 ml of acetonitrile. Supporting electrolyte was prepared by dissolving 3.2 g of tetraethylammonium bromide in 100 ml of acetonitrile - acetic acid. The acetonitrile - acetic acid medium was prepared by mixing acetonitrile with 0.06 M acetic acid in the proportion 25 + 1. Other reagents used were diethyl ether, 10% m/m hydrochloric acid and 0.1% m/m hydrochloric acid.

Procedure

An appropriate amount of sample was diluted with distilled water to a concentration range of 25–60 p.p.m. of sorbic acid. A 4.5-ml volume of the diluted sample was pipetted into a separating flask and 0.5 ml of 10% m/m hydrochloric acid was added together with 25 ml of diethyl ether. The separating funnel was stoppered and shaken. The aqueous layer was discarded and the organic layer was washed twice with two 2-ml portions of 0.1% m/m hydrochloric acid. The purified layer was transferred into the electrochemical cell. The volume of the ether extract was reduced to *ca.* 1–2 ml by careful warming using a hot-plate for about 5 min. A 25-ml volume of the supporting electrolyte was then added.

The solution was flushed with nitrogen, pre-saturated with water - acetonitrile vapour, for 15 min. A mercury drop of suitable size was produced from a commercial HMDE, collected in a separate beaker and picked up by the laboratory-built HMDE with a short platinum wire, as described under Apparatus. The method of standard additions was used for sorbic acid determination in all of the samples studied.

Results and Discussion

Preliminary Investigation

An attempt was made to oxidise sorbic acid in an acetonitrile medium using a glassy carbon electrode, but no peak was found up to a potential of +2.0 V. As solvated protons of various acids can be reduced electrochemically, this might provide a suitable method of analysis. However, the difference in the half-wave potential between different acids in water is too small for the identification and quantification of acids.[8] Thus, organic solvents were chosen as the media for the reduction of the solvated proton of sorbic acid. Acetonitrile was used in the present work because of its wide working range, its commercial availability in a high-purity form and because it exhibits a large difference in half-wave potentials for different acids.[9]

As the presence of other acids as impurities in acetonitrile can affect the peak current of sorbic acid, 0.06 M acetic acid was added to acetonitrile in the proportion of 1 + 25 in order to mask the effect of this interference. The commercially available HMDE was not used directly in the organic medium, because of the uncontrolled and premature falling of the hanging mercury drop at high negative potentials, which occurs as a result of the dissolution of the silicone layer coated inside the capillary wall by the organic solvent.[10] These factors led to poor reproducibility of the drop surface and difficulty in holding the hanging mercury drop during the experimental period, hence the use of a laboratory-built HMDE in the present work.

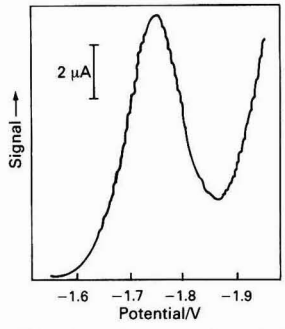

Fig. 1. Differential-pulse polarogram of a solution containing: sorbic acid, 1 mg; tetraethylammonium bromide, 3.2 g; and acetic acid, 0.014 g (operating parameters given in Table 1)

Table 1. Optimised parameters for differential-pulse polarographic determination of sorbic acid

Initial potential	−1.3 V
Modulation amplitude	50 mV
Scan rate	5 mV s^{-1}
Scan direction	Negative
Current range	10–50 μA
Mercury drop: volume	2.6×10^{-4} cm^3
mass	3.6×10^{-3} g
surface area	2.0×10^{-2} cm^2

Study of Analytical Parameters

The oxidation wave of sorbic acid was poorly defined. However, a symmetrical peak was obtained upon reduction of the solvated proton as shown in Fig. 1. Thus, the analytical use of the reduction peak was studied. The optimised parameters for using the differential-pulse polarographic method for sorbic acid determination are given in Table 1.

The calibration graph was found to be linear up to 40 μg ml^{-1} with a slope of 0.85 μA ml μg^{-1} and a correlation coefficient of 0.9994. The lowest detectable concentration was 0.7 μg ml^{-1} which is equivalent to 4 p.p.m. in the original sample. Thus, the working range of the method without dilution or pre-concentration of the samples is from 4 to 229 p.p.m.

The recovery and the precision of the method were studied by adding a known amount of analyte to an orange juice (177 p.p.m.) and a blackcurrant juice sample (111 p.p.m.). Using the proposed procedure, the recovery and precision (standard deviation) were 97 ± 4 and 100 ± 3%, respectively, for five determinations.

Interference Studies

Various food additives commonly found in fruit juices and soft drinks were investigated. These included ethanol, citric acid, stearic acid, tannic acid, malic acid, tartaric acid, ascorbic acid, succinic acid, benzoic acid, caffeine and saccharin. Their differential-pulse polarograms were obtained and no peak was found within the working potential range. Therefore, they are not expected to cause any significant interference in the determination of sorbic acid.

Food Sample Analysis

To test the validity of the method, two approaches were taken. Firstly, sorbic acid was added to various drinks and juices before the analysis was carried out. Secondly, parallel methods were used to analyse the same samples and the method of the Association of Official Analytical Chemists (AOAC)[1] was taken as the reference method. The results, summarised in Table 2, show that the method is specific, capable of working at low sorbic acid levels with a large variety of samples and capable of producing satisfactory accuracy. In addition, the working procedure is simple and does not

Table 2. Verification of the proposed method for the determination of sorbic acid in juice and drink samples

Sample	Sorbic acid added/μg ml^{-1}	Sorbic acid found/μg ml^{-1} AOAC method*	Sorbic acid found/μg ml^{-1} Proposed method	Difference, %
Apple drink	—	160	162	1.3
Garlic juice	—	874	840	−3.9
Lemonade	—	497	492	−1.0
Blackcurrant juice	111	—	111	0.0
Grapefruit juice	143	—	145	1.4
Lemon tea	145	—	147	1.4
Lemonade shandy	53	—	48	−7.5
Orange juice	177	—	174	−1.7
Sugar cane drink	66	—	61	−7.6
Wine	182	—	173	−4.9

* See reference 1.

require tedious clean-up steps as specified in the AOAC method.

Conclusion

A polarographic method employing differential-pulse voltammetry with the use of an HMDE was developed for the determination of sorbic acid in fruit juices and drinks. The procedure is simple and specific and is not subject to interference from many substances commonly found in soft drinks such as ethanol and benzoic acid. Moreover, it can be used for the analysis of intensely coloured juice samples. Thus, the scope of application of the proposed method for the determination of sorbic acid has been extended to cover juice and drink samples which cannot be analysed by the established AOAC procedure.

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Hydrogen Catalytic Waves for the Polarographic Determination of 2-Mercaptobenzothiazole in the Presence of Cobalt(II)*

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2-Mercaptobenzothiazole (MBT) is electrochemically inactive, but in complex combination with cobalt it forms a hydrogen catalytic wave, controlled by the kinetics of a protonation - deprotonation reaction. This wave has a surface character and can be used for analytical purposes in the determination of MBT with very high sensitivity (1×10^{-6}–1×10^{-5} M) in soluble samples or in solutions in a water - solvent mixture. The main parameters affecting this wave were studied, *i.e.*, cobalt, ammonium chloride, ammonia and acetic acid concentrations, ionic strength and solvent effect. A mechanism for the interpretation of both chemical and electron transfer reactions is proposed. Conditions suitable for analytical applications and real systems are described.

Keywords: *Polarography; hydrogen catalytic wave; 2-mercaptobenzothiazole determination; cobalt*

Some organic substances, which are polarographically inactive, produce characteristic waves when complexed with a series of metal ions, thus enabling a quantitative analytical determination. The ligand form of these substances becomes electroactive owing to the kinetic or catalytic processes occurring as a consequence of the structural changes, which, in turn, occur as a result of the coordinate binding with the complexing ions.

The hydrogen catalytic discharge produced by a series of compounds in the presence of Co^{2+} and Ni^{2+} ions is an important process that occurs in this class of reaction.[1] Several kinds of catalytic hydrogen waves produced by substances in ligand form are known: (1) catalytic hydrogen waves at half-wave potentials of about −1.04 V[2–4]; (2) catalytic hydrogen pre-waves at −1.1 V[5–10]; (3) maximum-shaped catalytic hydrogen waves at −1.47 V[11,12]; (4) maximum-shaped catalytic hydrogen waves at −1.69 V or Brdička-type waves[13,14] (see reference 1 and the complete list of the Brdička-type waves in the publications cited therein); (5) catalytic hydrogen double-waves of proteins, discovered also by Brdička[13,14] (literature also cited in reference 1). The discharge mechanism of type (3) only has been elucidated and quantitatively described.[1,11,12,15,16] In the other instances no direct experimental proof of the active species has been found, but it is generally assumed that the Brdička mechanism is valid. According to this mechanism a Co - O complex (formed by polarographic reduction of a Co^{II} complex) mediates the catalytic discharge on the basis of a kinetic mechanism represented by a protonation - deprotonation reaction. The catalytic wave in the Co - cycloserine system which can also be specified,[17] appears to be a Brdička-type system, but is actually formed by the superposition of two kinds of waves: the first is a diffusion-controlled wave and the second a surface-type discharge.

In this paper the polarographic determination of 2-mercaptobenzothiazole (MBT), which is inactive in simple form but produces a catalytic hydrogen wave of type (1) in the presence of Co^{II} ions is described. The Co^{III} - MBT system has been described previously in reference 4.

Experimental

Both direct current (d.c.) and alternating current (a.c.) polarograms were recorded with a Radelkis OH-105 type polarograph (Hungarian make). The polarographic cell was combined with a saturated calomel electrode as anode. The capillary constants were: t_1 (time of drop formation) = 5.72 s, m (rate of flow of Hg) = 1.14 mg s^{-1} and h (height of Hg reservoir) = 70 cm in 1 M NH_4Cl and 0.1 M NH_3 in an open-circuit system. The same instrument was also used to record the intensity - time (i - t) graphs at constant potential and displacement rate of the paper, 20 and 40 cm min^{-1}, respectively. The 0.05-s time response of the recorder was sufficiently short for this purpose.

For recording the ultraviolet spectra, a Specord M-40 spectrophotometer (Karl Zeiss, Jena, GDR) provided with microprocessor and data processing facilities was used.

All chemicals were of analytical-reagent grade. The solutions were prepared using a stock one-component solution at a suitable concentration. For example, the MBT stock solutions were prepared at 1×10^{-3} and 1×10^{-4} M concentrations; Co^{II} stock solutions at a 1×10^{-2} M concentration and NH_3 and NH_4Cl at 1 and 3 M concentrations, respectively. In this way, a few millilitres of these solutions were used and then diluted to 10 ml with distilled water as appropriate.

Results

2-Mercaptobenzothiazole has two forms: (1) >C–SH or thiole in alkaline medium and (2) >C=S or thione in acidic medium. Owing to this structural variation, the solubility and a series of other properties of the compound depend strongly on the pH of the solution. However, in the range of concentrations of 1×10^{-6}–1×10^{-4} M, where the polarograms can be recorded, MBT dissolves completely in water and in all other media investigated in this work.

D.c. and a.c. Polarographic Waves in the Co^{II} - MBT System

In the absence of complexing ions, MBT is polarographically inactive in both the thiole and thione forms. In the presence of Co^{2+} ions (1×10^{-3} M) and MBT (2×10^{-5} M) two processes can be observed on a d.c. polarogram (curve 1, Fig. 1): (1) the displacement of the cobalt wave from the half-wave potential of −1.11 V (not shown in the figure), in the absence of MBT, to −0.91 V, which is the region peculiar to catalytic cobalt pre-waves[1]; (2) the formation of a maximum-shaped wave at a half-wave potential ($E_{\frac{1}{2}}$) of −1.04 V. This last wave is not a polarographic maximum of the first-order type, but a catalytic hydrogen wave, as shown, and confirmed by other experimental data, using direct microscopical observation of the hydrogen bubbles on the mercury drop surface in the potential range where this process occurs.

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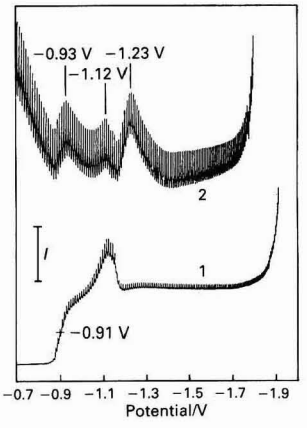

Fig. 1. 1, D.c. and 2, a.c. (60 Hz) polarograms of the solution: $[Co^{2+}]$, 1×10^{-3} M; [MBT], 2×10^{-5} M; $[NH_4Cl]$, 1 M; and $[NH_3]$, 0.1 M. Initial potential, −0.7 V; sensitivity: 1, $I = 3 \times 10^{-7}$ A; and 2, $I = 3 \times 10^{-8}$ A

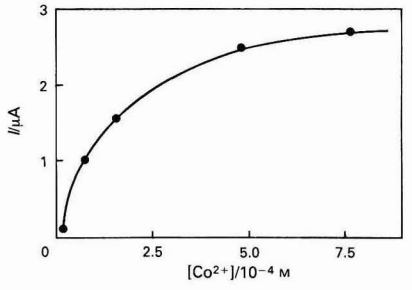

Fig. 2. Variation of the hydrogen catalytic wave as a function of Co^{2+} concentration. Electrolyte: 1.5×10^{-5} M MBT; 1 M NH_4Cl; and 1×10^{-1} M NH_3

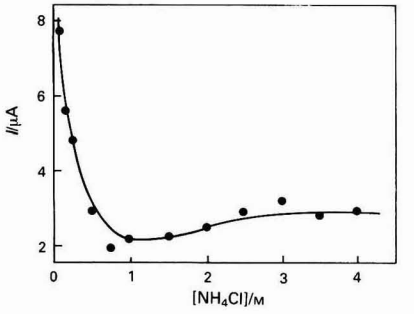

Fig. 3. Variation of the hydrogen catalytic wave as a function of NH_4Cl concentration. Electrolyte: 1.5×10^{-5} M MBT; 1×10^{-3} M Co^{2+}; and 1×10^{-1} M NH_3

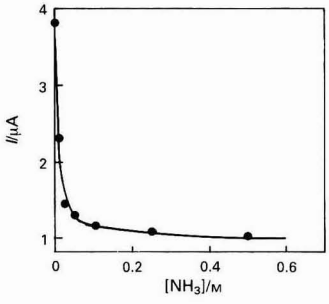

Fig. 4. Variation of the hydrogen catalytic wave as a function of NH_3 concentration. Electrolyte: 1.5×10^{-5} M MBT; 1×10^{-3} M Co^{2+}; and 1 M NH_4Cl

As shown, curve 1, Fig. 1, the limiting current of the diffusion controlled Co^{2+} wave is much higher than the limiting current of the catalytic cobalt pre-wave. However, only the two processes described are visible on the polarogram.

The three waves, occurring in this system, are clearly shown on the a.c. polarogram (curve 2, Fig. 1, 60 Hz): a cobalt catalytic pre-wave with a maximum at −0.93 V, a hydrogen catalytic wave at −1.12 V and the diffusion controlled cobalt wave at −1.23 V.

In the potential range of −0.9 to −1.2 V the current falls abruptly to the cobalt diffusion value, owing to the desorption of the polarographic active species.

Another tool for determining the nature of the polarographic processes is the effect of the mercury reservoir height. It has been found that while the cobalt pre-wave drops with a decrease of the mercury-column height, the hydrogen catalytic wave increases considerably under the same conditions. This is further proof of the surface character of the cobalt wave at −1.23 V mentioned above.[1,17]

Effect of Cobalt Concentration

The concentration of cobalt ions has a strong influence on the hydrogen catalytic wave. The wave height at cobalt concentrations in the range of 2×10^{-5}–7.5×10^{-4} M has been measured, as shown in Fig. 2. The wave increases gradually with cobalt concentration, tending to reach a pseudo-plateau for higher values. For analytical purposes a range of higher cobalt concentrations is therefore, more favourable, as the hydrogen catalytic wave is less influenced by the variations of medium composition.

Effect of Ammonium Chloride Concentration

In ammonia buffer the variation of NH_4Cl concentration simultaneously modified two solution parameters with opposite influences on the hydrogen wave: (*i*) it decreases the pH value, which contributes to an increase of the protonation source and (*ii*) it enhances the stability of the cobalt - amine complexes, hence contributing to a decrease of the concentration of active species. From the relationship between wave height and NH_4Cl concentration (Fig. 3) one can see the predominant effect among the two influences mentioned.

Thus, in the concentration range below 1 M NH_4Cl, the wave decreases rapidly, which shows that the displacement of the equilibrium between the complexes is in the direction of the cobalt - amine species. At higher concentrations the hydrogen catalytic wave increases slightly, probably as a consequence of the increase of the buffer capacity and the diminution of the pH value. At a concentration of NH_4Cl that is greater than 1 M the variation of the wave height is small.

Effect of Ammonia Concentration

The variation of the wave height as a function of NH_3 concentration, represented in Fig. 4, shows that a strong decrease in wave height occurs at concentrations of NH_3 below 0.1 M. For a further increase of the ammonia concentration a slight decrease of the catalytic current only is noticed. In the range 0.1–0.5 M NH_3, the wave height is almost stabilised and again in this instance the conditions are suitable for analytical determination.

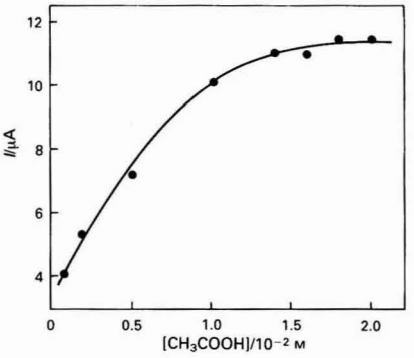

Fig. 5. Variation of the hydrogen catalytic wave as a function of CH_3COOH concentration. Electrolyte: 1.5×10^{-5} M MBT; and 1 M CH_3COONa

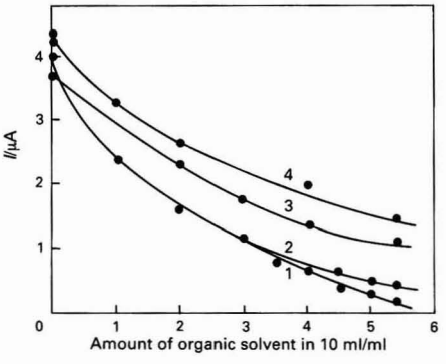

Fig. 6. Effect of solvent on the hydrogen catalytic wave of the solution: [MBT], 1.5×10^{-5} M; [Co^{2+}], 1×10^{-3} M; [NH_4Cl] 1 M; and [NH_3], 1×10^{-1} M. 1, *N*,*N*-Dimethylformamide; 2, propan-2-ol; 3, ethane-1,2-diol; and 4, methanol

Effect of Acetic Acid Concentration

Fig. 5 shows the variation of the catalytic current as a function of acetic acid concentration. A strong increase of the current is produced up to a 0.015 M concentration of CH_3COOH. For higher concentrations the curve tends to form a pseudo-plateau and the wave height becomes stable to fluctuations in the composition of the medium. Concentrations of CH_3COOH higher than 0.015 M are, therefore, suitable for analytical determinations.

Solvent Effect on Hydrogen Catalytic Wave

Unlike Co-cysteine complexes,[1,13,14] the Co-MBT complexes produce catalytic waves that are very sensitive to the presence of various organic solvents in solution. Such behaviour is to be expected as the water molecules are a rich source of protons and a decrease of this source must be reflected in the rate of the protonation reaction. It has been found that this explanation holds for catalytic waves produced by Co-MBT complexes, as is shown in Fig. 6. In this figure the variation of the catalytic current as a function of the amount of organic solvent is given for a set of four solvents: 1 dimethylformamide; 2, propan-2-ol; 3, ethane-1,2-diol; and 4, methanol. The first two solvents produce the same decrease in current when less than 30% of the solvent volume is used in the medium investigated; at higher volume percentages the dimethylformamide is the most active. Ethane-1,2-diol and methanol are less active, but for a 50% volume, the waves are reduced by more than half of their initial height.

Effect of Ionic Strength

The effect of the presence of different salts in solution was studied for alkali (Na^+ and K^+) and alkaline-earth (Ca^{2+}, Mg^{2+} and Ba^{2+}) ions. It was found that a visible influence is produced at lower salt concentrations, less than 0.2 M for alkali ions and 0.05 M for alkaline-earth ions. For higher concentrations, the hydrogen catalytic wave is modified to a lesser extent.

Intensity - Time Graphs

In the most dilute solutions of 2×10^{-6} M MBT the Co^{II} catalytic pre-wave prevails over the hydrogen wave. At the foot of the catalytic cobalt pre-wave (−0.88 V), in the moment of drop growth, the predominant role is played by the adsorption of complex ions. As the drop surface increases, the current increases, showing a rapid increase, characteristic of a catalysed discharge process. At a higher voltage of −0.94 V, corresponding to the limiting current range of the Co^{II} pre-wave, the inhibition period produced by adsorption is short and the current increases faster, also showing a catalysed discharge. After 5 s the process becomes diffusion controlled.

If the MBT concentration is increased to 0.6×10^{-5} M, the catalytic hydrogen discharge becomes intense enough for the process to be determined until −1.09 V when desorption of the active molecules begins. The incipient hydrogen discharge is located at −0.88 V, where the i-t curve shows a rapid increase of the current, characteristic of a kinetic process. At −0.9 V the hydrogen evolution is more intense and the corresponding i-t curve displays an extension of the induction period over a larger interval of about 10 s; the current increases more rapidly showing the kinetic nature of the wave. At −1.00 V the limiting current of the Co^{II} pre-wave is reached and the current is now diffusion controlled.

At still higher MBT concentrations more complex processes occur. An interesting oscillatory behaviour of adsorption-desorption processes can be observed, where the oscillation period is 1.21 s in the range 0–5 s and 1.36 s in the range 5–10 s, where the drop surface is larger.

Spectrometric Data

The polarographic waves alone cannot give direct information on the structure of complexes that produce the kinetic and catalytic effects described previously.

A characteristic of MBT in ethanol solution is the presence of a maximum at 332 nm in the ultraviolet(UV) absorption spectra. A second maximum at 236 nm has also been observed.[19]

Similar behaviour of MBT in UV absorption for the aqueous solutions was found, where this compound is soluble in the range of 1×10^{-5}–1×10^{-4} M (Fig. 7). In this instance the absorption maxima are located at 332 and 230 nm, respectively (curve 1, Fig. 7). In an ammoniacal medium (curve 2) a visible modification of the UV spectrum is produced owing to the displacement of the thione to the thiole form. Thus, the maximum at 332 nm is displaced to 318 nm, the height is decreased and there is an enlargement of the absorption peak width. At the same time the maximum at 230 nm is displaced to 233 nm, a strong increase of maximum height and an enlargement of the absorption band width is observed.

The presence of Co^{2+} ions at a concentration of *ca.* 1.5×10^{-5} M has no visible effect on the UV spectra in both the neutral and ammoniacal media, however, small displacements of absorbance intensity are produced at certain wavelengths.

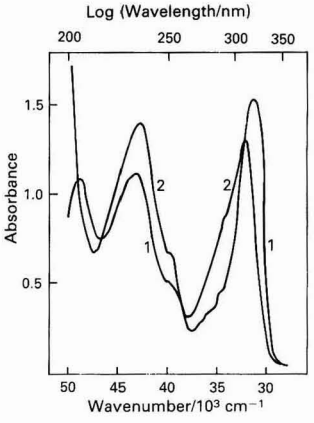

Fig. 7. Ultraviolet spectra of MBT (5×10^{-5} M) in the presence of Co^{2+} (1.5×10^{-5} M). 1, Without additive; and 2, in the presence of 1×10^{-1} M NH_4Cl and 1×10^{-2} M NH_3

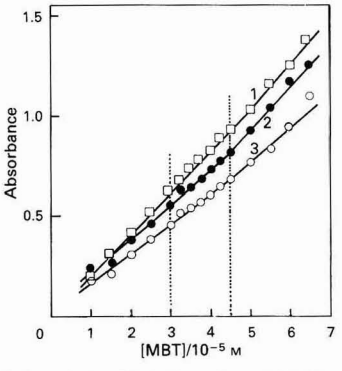

Fig. 8. Variation of the UV absorption of MBT at a cobalt concentration of 1.5×10^{-5} M in aqueous solution. 1, $\lambda_1 = 31\,080$ cm^{-1}; 2, $\lambda_2 = 48\,000$ cm^{-1}; and 3, $\lambda_3 = 43\,350$ cm^{-1}. Temperature, 25 °C

For structural purposes the slight variation of the intensity of a few of the UV absorption bands of MBT at constant cobalt concentration can be used to carry out a spectrophotometric titration (the mole ratio method could also be used). The difficulty arises owing to the very small difference in UV absorption between MBT in the free and complexed form, which lies within the range of the experimental errors. To overcome this difficulty computer processing of the data has been carried out for both registration spectra and separate data, corresponding to a defined amount of MBT at constant cobalt concentration and a selected wavelength. The microprocessor of the spectrophotometer commands the record point by point for steps of about 185 nm (20 cm^{-1} at 54 000 cm^{-1}). For each point, at the integration time of 0.5 s, the median of values was calculated and stored in the microprocessor memory. Finally, the whole spectrum was constructed from an average of these median values.

Fig. 8 shows the variation of the optical absorbance as a function of MBT concentration in the presence of 1.5×10^{-5} M Co^{2+} at 20 °C. The optical absorbance was taken from the UV spectrum at the wavelengths indicated in the figure legend. Similar results were obtained in the presence of ammonia and also at higher temperatures (50 °C).

For the interpretation of the polarographic data, it is interesting to note the obvious formation of a series of complexes. At a lower MBT:Co^{2+} ratio, Co^{II}-$(MBT)_2$ complexes are formed in both pure water and ammoniacal media. However, the polarographic data show considerable difference between these two systems, leading to the conclusion that the other components of the solution are included in the composition of the six-coordinate complex. For this type of complex the participation of other ligands from solution is supported also by the data obtained by McCleverty and co-workers[20–26] for Zn and Cd complexes formed in solutions containing acetate.

In this way we can consider that in aqueous solution the following complex is formed in very dilute solutions and a small ratio of MBT-Co^{2+}: $[Co^{II}(C_7H_4NS_2)_2(H_2O)_2]^0$. In ammoniacal media two complexes can be formed under the same conditions *i.e.*, $[Co^{II}(C_7H_4NS_2)_2(NH_3)(H_2O)]^0$ and $[Co^{II}(C_7H_4NS_2)_2(NH_3)_2]^0$. According to the data of McCleverty and co-workers,[20–26] in acetic acid media the complex must be of the form: $\{[Co^{II}(C_7H_4NS_2)_2(H_2O)]_2(CH_3COO)\}^-$, therefore one molecule of acetic acid binds two Co - MBT complex groups by means of oxygen bridges.

In dilute solutions, no direct evidence of this problem is available, but in the solid state for Zn and Cd McCleverty and co-workers,[20–26] showed, by rigorous determination of bond lengths and angles, that the binding involves the donor atom set formed by sulphur and nitrogen atoms. In the MBT heterocycle the strongest protonation ability corresponds to the N atom site. One can therefore expect that in the MBT complexes the protonation - deprotonation process occurs at this atom.

Mechanism of Polarographic Processes

A general feature of the polarographic catalytic waves is the lack of direct evidence to indicate which are the active species, *i.e.*, those that can be protonated. However, considering the available spectrometric data reasonable proposals for the reaction mechanism can be put forward.

Except for the nitrohydroxylaminic system,[1,7–10] where the catalytic reactions are established and a rigorous mathematical treatment has been deduced,[15,16] the interpretation for the other systems is based on the correlation of the data obtained from several methods. In spite of nitrohydroxylaminic complexes and other compounds that do not contain sulphur, producing a catalytic discharge, for instance Co - histidine[27] and Co - cycloserine, much emphasis has been placed, even recently, in assigning an essential role to –SH groups in the process of catalytic hydrogen discharge.[1]

For the Co catalytic pre-waves, the reaction scheme according to which an intermediate one-ligand complex (a dynamic step) adsorbed on the mercury surface produces the Co^{2+} catalytic discharge by a bridge mechanism, is generally used.[5–10] In this one-ligand complex the Co ions would have a lower discharge potential that allows pre-wave formation.

In the over-all complexing reaction the slowest step determining the rate of reaction is probably the Co^{II} catalytic discharge produced as follows:

$$[Co^{II}(C_7H_4NS_2)(H_2O)_3(NH_3)]^{+}_{ads} + 2e^- \rightarrow Co^0 + (C_7H_4NS_2)^{-}_{ads} + 3H_2O + NH_3 \quad .. \quad (1)$$

In this way the species $(C_7H_4NS_2)^{-}_{ads}$, where ads refers to adsorbed species, is again available for the first step of complexation and afterwards for the electrochemical step expressed by reaction (1).

At higher concentrations of MBT a new discharge process is superimposed on the first Co catalytic pre-wave. At concentrations of MBT greater than 1.5×10^{-5} M the cobalt discharge is completely displaced in the region of the catalytic pre-wave ($E_{\frac{1}{2}} = -0.89$ V) and the second discharge process appears in the form of a separate wave. It has already been shown that this wave definitely corresponds to a catalytic discharge of the hydrogen, as indicated by the microscopical observation of H_2

bubbles, this process ceases suddenly after the desorption of the active species which occurs at potentials between −1.09 and −1.22 V.

It can be assumed that the most stable form of Co^0 is the di-ligand MBT form as indicated by the spectrophotometric data given for Co^{II} (Fig. 8). As these processes occur as described in the presence of ammonia and also at a very low MBT concentration, the reactions involved in the catalytic proton discharge can be written as follows, considering that the Co^0 is bound in a complex of this structure:

$$[Co^0(C_7H_4NS_2)_2(H_2O)(NH_3)]^{2-}_{ads}$$

The first reaction step is then a protonation process at the N atom, a site that is easily protonated in the MBT structure:

$$[Co^0(C_7H_4NS_2)_2(H_2O)(NH_3)]^{2-}_{ads} + 2H^+ \rightarrow [Co^0(C_7H_4NHS_2)_2(H_2O)(NH_3)]^0_{ads} \quad . . \quad . . \quad (2)$$

As Co^0 remains coordinated to the N atom for some time, a fast electron transfer may be produced by quantum tunnelling to the proton species,[28] where a low unoccupied electron level occurs, owing to the positive charge of this site. After the electron transfer to the protonated group, a deprotonation process is produced by hydrogen evolution:

$$[Co^0(C_7H_4NHS_2)_2(H_2O)(NH_3)]^{2-}_{ads} \rightarrow [Co^{II}(C_7H_4NS_2)_2(H_2O)(NH_3)]^0_{ads} + 2H \quad . . \quad (3)$$

As the potential of the electrode is in the limiting current region the electrochemical discharge of Co^{II} can occur rapidly:

$$[Co^{II}(C_7H_4NS_2)_2(H_2O)(NH_3)]^0_{ads} + 2e^- \rightarrow [Co^0(C_7H_4NS_2)_2(H_2O)(NH_3)]^{2-}_{ads} \quad . . \quad . . \quad (4)$$

The zero-charge Co complex formed can re-enter the protonation reaction (2) and the entire cycle of reactions (2)–(4) is repeated, resulting in a catalytic hydrogen discharge at a very low potential, determined by the potential energy of the unoccupied level of the electron in the protonated form of the active species.

Analytical Applications

Although electrochemically inactive, MBT can be determined by polarographic techniques using its property of forming catalytic waves in ligand form with Co^{2+} ions. The chemical reaction, superimposed on the electron transfer reaction, is a kinetic process of the protonation - deprotonation type.

Calibration graph and sensitivity

According to the study presented above, the following media can be used: 2×10^{-6}–2×10^{-5} M MBT, 1×10^{-4}–1×10^{-3} M Co^{2+}, 5×10^{-1}–4 M NH_4Cl and 1×10^{-1}–5×10^{-1} M NH_3.

The calibration graph is quasi-linear at very low MBT concentrations (5×10^{-7}–4×10^{-5} M); for higher concentrations of the order of 1×10^{-4} M MBT a pseudo-plateau tends to be formed (Fig. 9). Therefore, for quantitative determination a calibration graph must be constructed. The suitable amount of sample can obviously be determined by successive trials. For higher cobalt content, concentrations of the order of 1×10^{-7} M MBT can also be detected. The method is therefore highly sensitive.

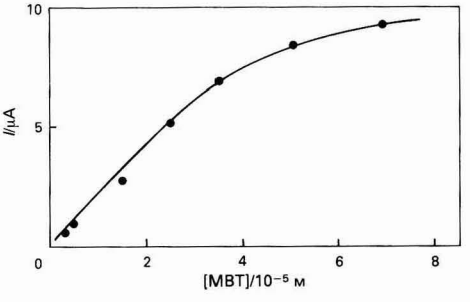

Fig. 9. Calibration graph for the polarographic determination of MBT by means of the catalytic hydrogen wave obtained for the Co^{2+} - MBT complex. Electrolyte: 1×10^{-3} M Co^{2+}; 1 M NH_4Cl; and 1×10^{-1} M NH_3

Interferences

Owing to the very low potential discharge of the protons in this system, the interferences from a large series of compounds, which also produce other types of hydrogen catalytic discharge are in general avoided. However, similar waves are produced by alkyldithiocarbamates and by uracil. In the presence of these substances the determination is subject to extreme interference.

The dimeric form containing –S–S– groups can be differentiated by the formation of a polarographic two-electron diffusion wave at potentials lower than −0.8 V. By simple suitable correlations of the –S–S– reduction wave with the hydrogen catalytic wave, the dimeric fraction in MBT can be determined using this simple polarographic technique. The diffusion wave, having a linear calibration graph, can be used for the determination of the dimeric form. By subtracting the corresponding part from the catalytic wave, the concentration of the simple form of MBT can be determined.

Accuracy and precision

The catalytic waves produced by the MBT - Co complexes are of surface character and therefore the adsorption process is the main parameter that determines the reproducibility of the shape and wave height. In order to ensure accurate conditions of determination the purity of the vessel, water and reagents is of particular significance, to ensure the lowest possible level of concentration of surface-active impurities, which can alter the height of the hydrogen catalytic wave. In general, the normal precision of the polarographic method of less than 5% can be observed if the determinations are carried out under accurate working conditions. The time elapsed also has visible effects on the reproducibility of the determination; a time interval of between 1 and 2 d, in general gives satisfactory results.

Application to N-cyclohexylbenzylsulphonamide fabrication

A practical example of the application of this method in an industrial process is in the control of the production of *N*-cyclohexylbenzylsulphonamide, which is used as an accelerator of rubber vulcanisation. In an aqueous medium the cyclohexylamine undergoes a condensation reaction with MBT in the presence of sodium hypochlorite. The MBT can remain as an impurity both in the final product and in residual waste water. Therefore, this method could be applied to the determination of MBT in the final product and also in residual water. Likewise, it is also useful to be able to determine the dimercaptobenzothiazole in the raw MBT material used for the condensation reaction.

The precision of determination, under suitable conditions, is a characteristic of the polarographic method, *i.e.*, the error is less than 5% and the sensitivity is very high: in the range of 1×10^{-6}–1×10^{-5} M or even 1×10^{-7} M MBT for higher cobalt concentrations, as indicated by the calibration graph. Under these conditions there are no interference problems.

Applications in water and environmental analysis

The determination of MBT in water poses no special problem, except in instances where there is a degree of impurity caused by surface-active products, such that corrections are necessary in the construction of the calibration graph.

For solid samples an extraction procedure must be used for the separation and concentration of trace amounts of MBT.

The most rapid and complete dissolution of MBT can be achieved in acetone. However, as shown in Fig. 6 other solvents can be used depending on the matrix submitted to the determination. The solvent is slowly removed by evaporation and the solid residue is dissolved in ammoniacal buffer and transferred into a calibrated flask; the solution can then be used directly for the determination. The concentration of the product by the extraction procedure allows a considerable increase in the sensitivity of the determination.

A range of practical examples can be investigated with this analytical method of MBT determination where kinetic phenomena allow a considerable increase in the sensitivity of determination.

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Continuous-flow Chemiluminescence Determination of Isoniazid by Oxidation With *N*-Bromosuccinimide

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A rapid and precise continuous-flow method is described for the determination of isoniazid (0.050–20.0 μg ml^{-1}) based on the chemiluminescence produced during its reaction with *N*-bromosuccinimide in alkaline medium. The emission intensity is greatly enhanced by the presence of ammonia. The procedure is automated and samples can be analysed at a rate of 112 solutions per hour with a relative error of about 2%. When applied to tablets, the method is relatively free from interferences from common excipients and co-existing compounds. The results obtained for the assay of commercial preparations compared well with those obtained by an official chemical method and demonstrated good accuracy and precision.

Keywords: N-*Bromosuccinimide; chemiluminescence; continuous flow; isoniazid determination; pharmaceutical preparation*

Chemiluminescence (CL) has been successfully extended to reactions involving organic compounds of pharmaceutical or biological interest. These reactions usually utilise inorganic oxidants, such as peroxodisulphate[1] or bromine[2] for tetracycline, permanganate for morphine,[3] cerium(IV) for acetaminophen[4] and hexacyanoferrate(III) for thiamine.[5]

Chemiluminescence reactions that occur by the action of oxidants containing positively charged bromine atoms are restricted to hypobromite[6] and 1,3-dibromo-5,5-dimethylhydantoin.[7] A well-known example of this group of compounds is *N*-bromosuccinimide (NBS), which has not yet been reported to participate in CL reactions.

Recently, NBS has been used for the kinetic - potentiometric determination of ascorbic acid, biotin, pyridoxine hydrochloride and thiamine hydrochloride.[8] A bromide-selective electrode was used to follow the rate of generation of bromide, which is one of the products of the reaction. The method was applied successfully to the assay of these vitamins in pharmaceutical preparations. *N*-Bromosuccinimide also oxidises isoniazid, which is an important drug for the chemotherapy of tuberculosis. The reaction has been used for the manual[9,10] or potentiometric[11] titration of the compound. Many other techniques have been proposed for the determination of isoniazid, such as electrochemistry,[12,13] spectrophotometry,[14,15] polarography,[16] spectrofluorimetry[17] and gasometry.[18]

It was discovered that CL is generated during oxidation of isoniazid by NBS in alkaline medium. The emission intensity and the sensitivity are greatly enhanced when ammonia is also present in the reaction medium. This work describes the development of an analytical CL method which was applied successfully to the determination of isoniazid in pharmaceutical preparations.

Experimental

Apparatus

All measurements were made by using the continuous-flow CL analyser shown in Fig. 1. It consisted of two basic units, the detector housing and the flow-through system.

The detector housing included a coiled glass flow cell situated in front of the photomultiplier tube (PMT). The coil consisted of 3.5 turns of glass tubing (i.d. 2 mm) and its total height was 22 mm. The coil volume was 300 μl. The distance of the coil from the PMT was 2 mm for greatest sensitivity. The coil was backed by a mirror for maximum light collection by the PMT. High voltage (−720 V) was supplied to the PMT (EMI 9783R, S-5 response) by two Heath Universal Power Supplies (0–500 V) connected in series. The output of the PMT was connected to an operational amplifier (RCA CA 3140) which served as a current to voltage converter (I/V). Damping was provided by inserting an RC circuit between the output of the I/V converter and the recorder. The output of the CL analyser was recorded with a Sargent - Welch (Model XKR) recorder.

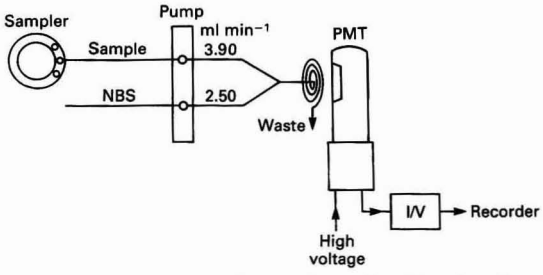

Fig. 1. Schematic diagram of the continuous-flow CL analyser (not to scale)

The solutions of reactants were supplied by a Technicon Proportioning Pump III and were mixed at a Y-junction, 20 mm before entering the flow cell. The final solution was carried into the flow cell by a Tygon tube with an i.d. of 2 mm. Samples were supplied to the manifold by a Technicon Sampler II with a 40-sample capacity.

Reagents

All solutions were prepared from analytical-reagent grade materials with distilled, de-ionised water.

Stock isoniazid solution, 100.0 μg ml^{-1}. Dissolve 0.1000 g of isoniazid (Sigma) in water, transfer the solution into a calibrated flask and dilute to 1 l with water. The solution was stable for at least 1 week.

Stock N-bromosuccinimide solution, 0.0500 M. Dissolve 2.225 g of NBS (Serva) in water, transfer the solution into a calibrated flask and dilute to 250 ml with water. The solution was prepared daily and kept at 4 °C in an amber-coloured bottle in the dark.

More dilute solutions were prepared daily by the minimum number of dilution steps possible.

All other chemicals used were of the best available grade and were used without further purification.

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Procedures

Measurement procedure

The instrument was set at the optimised conditions shown in Fig. 1 except that the sampling needle was kept in the "wash" position until the base line on the recorder had been established. The sampler was activated and the analysis proceeded automatically. The sampler was adjusted to allow analyte or standard solution and washing water to enter the manifold for 15 and 17 s, respectively. The calibration graph of emission intensity (I/mV) *versus* concentration of isoniazid (c/μg ml^{-1}) was constructed and the isoniazid content of the samples was determined. A standard solution should be included after every 12 samples.

Determination of isoniazid

A 25.00-ml volume of 4.0 M sodium hydroxide solution and 5.00 ml of 0.010 M ammonia solution were transferred into a calibrated flask with the appropriate volume of stock isoniazid solution and diluted to 100 ml with water. The final solution should contain 0.050–20.0 μg ml^{-1} of isoniazid in 1.0 M sodium hydroxide and 5.0×10^{-4} M ammonia.

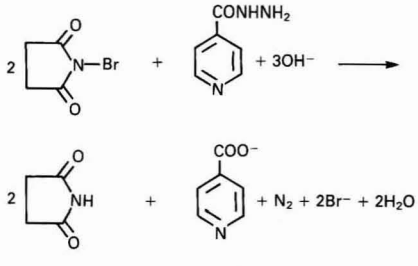
Sample preparation

Not less than 20 tablets were weighed, ground to a fine powder and mixed. A sample equivalent to approximately 200 mg of isoniazid was weighed accurately, transferred into a 1-l calibrated flask and made up to volume with water. The mixture was sonicated for 10 min to aid dissolution and then filtered. An appropriate volume of the filtrate was diluted further with water so that the concentration of isoniazid in the final solution was within the working range. The final solution should also contain 1.0 M sodium hydroxide and 5.0×10^{-4} M ammonia.

Results and Discussion

N-Bromosuccinimide has been used extensively as a brominating and oxidising agent.[19,20] In aqueous solutions, its oxidising properties were attributed to hypobromous acid generated by hydrolysis of the reagent. Chemiluminescence reactions with hypobromite are known[6] but the reagent disproportionates easily to bromate and bromide ions.[19,20] As NBS has similar oxidising properties to hypobromite, but is more stable, it was decided to investigate its use as a CL reagent.

Preliminary experiments showed that the oxidation of isoniazid by NBS in alkaline medium was chemiluminescent. The reaction which occurs is:



The radical of the isonicotinate ion might be the emitting species due to its similarity to the aminophthalate radical formed during the oxidation of luminol.[21]

The CL behaviour of compounds similar to NBS, such as *N*-chlorosuccinimide and *N*-iodosuccinimide, was also investigated. *N*-Chlorosuccinimide, which is a stronger oxidant than NBS, generated CL when mixed with sodium hydroxide. It was not possible to examine the CL properties of *N*-iodosuccinimide because this compound is very unstable in aqueous solutions.

A series of experiments was conducted to establish the optimum analytical conditions for the chemiluminescent oxidation of isoniazid by NBS. The parameters studied were reagent and sample flow-rates, and oxidant and alkali concentrations.

Effect of Flow-rate

The effect of flow-rate on the emission intensity is shown in Fig. 2. The optimum values for reagent and sample flow-rates were 2.50 and 3.90 ml min^{-1}, respectively. These flow-rates allow the solutions to react for about 0.6 s before entering the cell, according to the manifold configuration. Higher flow-rates of sample had no significant effect on the CL intensity. The sampling and wash times of the sampler were chosen so as to give sharp and smooth peaks. The response was equivalent to 90% of that of the steady state.

Effect of NBS and Alkali Concentrations

The oxidation of isoniazid by NBS is accompanied by radiation only if the reaction is carried out in alkaline medium. However, NBS is unstable in this medium and hence sodium hydroxide must be added to the analyte solution.

The effect of NBS concentration on 20.0 and 50.0 μg ml^{-1} of isoniazid at various sodium hydroxide concentrations (0.050–3.0 M) is shown in Fig. 3. No significant difference in CL intensity from 50.0 μg ml^{-1} of isoniazid was observed at concentrations of NBS and sodium hydroxide ≥0.015 and

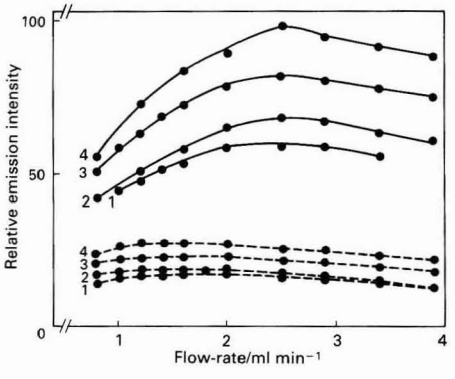


Fig. 2. Effect of the flow-rate of 0.020 M NBS in 0.20 M NaOH on the CL intensity, from 20.0 (broken lines) and 50.0 (solid lines) μg ml^{-1} of isoniazid supplied to the manifold at 1, 2.00; 2, 2.50; 3, 2.90; and 4, 3.90 ml min^{-1}

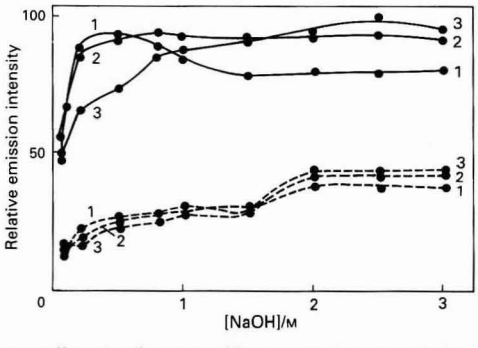


Fig. 3. Effect of sodium hydroxide concentration on the CL intensity from 20.0 (broken lines) and 50.0 (solid lines) μg ml^{-1} of isoniazid with 1, 0.0050; 2, 0.015; and 3, 0.050 M NBS

0.80 M, respectively. However, in the presence of 20.0 μg ml^{-1} of isoniazid, the most intense radiation was obtained at concentrations of NaOH ≥2.0 M.

The chosen concentration of NBS was 0.015 M as higher concentrations do not increase the emission intensity markedly. This concentration ensures that the emission intensity depends only on the concentration of isoniazid.

Effect of Surfactants and Other Compounds

Surfactants are commonly used to increase the efficiency of CL and the sensitivity of the measurement.[22] Therefore, the effect of some typical surfactants on the CL emission from the reaction of NBS with isoniazid was investigated (Table 1).

When the non-ionic surfactants examined were present in the solution, emulsions or precipitates appeared and the CL intensity was severely reduced. When the cationic surfactant cetylpyridinium chloride was evaluated, a precipitate was formed in the solution within 4 h. However, the solutions can be measured soon after their preparation.

The CL intensity was increased only by hexadecyltrimethylammonium bromide [cetyltrimethylammonium bromide (CTAB)] (Table 1). The slope of the calibration graph increased and the limit of detection decreased when 0.0010 M CTAB was added to the analyte solution (Table 2). However, the presence of CTAB in the final solution caused extensive foaming. Hence, the reproducibility of the method was severely reduced and this observation made the use of CTAB problematic.

Counter ions have been used successfully to increase the intensity in fluorescence spectrometry.[23] The effect of two counter ions on the NBS CL intensity was also investigated (Table 1). Sodium tetrafluoroborate increased the CL efficiency whereas tetramethylammonium bromide decreased the intensity, probably due to quenching. These preliminary results indicate that counter ions might be as useful as micellar systems in CL.

Effect of Ammonia

During the study of interferences from compounds which co-exist with isoniazid in pharmaceutical preparations, nicotinamide was found to increase the CL intensity (Table 3). The interference increased with time. When pure solutions of nicotinamide were introduced into the manifold, the intensity of the CL emission generated increased with time. It was established that the behaviour of nicotinamide was due to ammonia which was produced by alkaline hydrolysis. *N*-Bromosuccinimide oxidises ammonia to nitrogen[24,25] and the reaction was found to be chemiluminescent. When ammonia was added to the isoniazid solution, the CL emission was more intense than the sum of the intensities from each of the

Table 1. Effect of various compounds and surfactants on the CL emission from 20.0 and 50.0 μg ml^{-1} of isoniazid

Compound (type of interaction)	Concentration/M	Isoniazid/μg ml^{-1} 20.0	Isoniazid/μg ml^{-1} 50.0
		Change in emission intensity, %	
Hexadecyltrimethylammonium bromide			
(cationic micelle, CMC*: 0.0013 M)	1.0×10^{-5}	+9.0	+5.0
	1.0×10^{-4}	+55.0	+32.0
	1.0×10^{-3}	+108.0	+53.0
	1.0×10^{-2}	+58.0	−10.0
Cetylpyridinium chloride			
(cationic micelle, CMC: 1.2×10^{-4} M)	1.0×10^{-5}	+14.0	+3.0
	1.0×10^{-4}	0	−7.0
	1.0×10^{-3}	−38.0	−58.0
	1.0×10^{-2}	−70.0	−88.0
Sodium lauryl sulphate			
(anionic micelle, CMC: 0.0081M)	1.0×10^{-6}	+15.0	−8.0
	1.0×10^{-5}	+21.0	−15.0
	1.0×10^{-4}	−24.0	−31.0
	1.0×10^{-3}	Formation of precipitate	
Sodium dioctyl sulphosuccinate (Aerosol OT)			
(anionic micelle, CMC: 6×10^{-4} M)	1.0×10^{-6}	0	0
	1.0×10^{-5}	+9.0	+2.0
	1.0×10^{-4}	−19.0	−24.0
	1.0×10^{-3}	−62.0	−66.0
Bion-NE-9			
(non-ionic micelle)	1‰ (v/v)†	−9.0	−5.0
Polyoxyethylene (20) sorbitan mono-oleate (Tween-80)			
(non-ionic micelle, CMC: 0.013 g l^{-1})	1‰ (v/v)	Formation of emulsion	
Sodium tetrafluoroborate			
(ion pair)	1.0×10^{-5}	−6.0	0
	1.0×10^{-4}	+21.0	+11.0
	1.0×10^{-3}	+450.0	+243.0
	1.0×10^{-2}	+240.0	+155.0
Tetramethylammonium bromide			
(ion pair)	1.0×10^{-5}	−17.0	0
	1.0×10^{-4}	−16.0	−3.0
	1.0×10^{-3}	−43.0	−21.0

* CMC = critical micellar concentration.
† 1 ml diluted to 10000 ml.

Table 2. Effect of hexadecyltrimethylammonium bromide (CTAB) on the isoniazid calibration graph

CTAB/M	Isoniazid/μg ml^{-1} Linear range	LOD*	Slope (±SD†)	Intercept (±SD†)	r	n‡
0	3.00–50.0	2.34	0.68 ± 0.01	−1.53 ± 0.33	0.9992	7
1.0×10^{-4}	1.00–10.0	0.60	0.86 ± 0.02	0.12 ± 0.15	0.999	5
1.0×10^{-3}	1.00–10.0	0.38	1.15 ± 0.02	−0.24 ± 0.13	0.9995	5

* LOD = analyte concentration giving a signal equal to the intercept of the calibration graph plus three times the standard deviation of y-residuals.
† SD = standard deviation.
‡ n = number of samples, each measured three times.

Table 3. Effect of ammonia and various amino compounds (5.0×10^{-4} M) on the CL emission from 10.0 μg ml^{-1} of isoniazid (emission intensity from pure isoniazid = 100)

Compound	Relative emission intensity, % Without isoniazid	With isoniazid
Ammonia	119.1	3500
Nicotinamide	170.2	3000
Hydroxylamine hydrochloride	ND*	8.5
Propylamine	ND	6.4
Dimethylamine	ND	4.3
Aniline	ND	ND
p-Phenylenediamine hydrochloride	ND	ND
o-Phenylenediamine hydrochloride	ND	3.2
Sulphanilic acid	ND	2.1
Sulphanilamide	ND	6.4
Salicylamide	ND	17.0
Sulphamic acid	ND	27.7
4-Nitroaniline	ND	ND
Urea	40.4	89.4
Amino acids:		
Glycine	17.0	1100
Alanine	11.1	316.7
β-Alanine	9.7	38.9
Phenylalanine	11.1	325.0
Serine	12.5	1200
Cysteine	13.9	161.1
Lysine	8.1	41.9
Tryptophan	32.3	64.5
Glutamic acid	ND	458.1
Histidine	ND	45.2
Proline	3.2	19.4

* ND = No detectable emission.

components of the mixture. Therefore, ammonia enhances the emission from the NBS - isoniazid reaction, probably due to a synergistic effect.

The investigation was extended to a variety of compounds with amino groups (Table 3). Most of these compounds reduce the emission intensity probably because they give rise to quenching effects. Alternatively, the effect might be due to a reduction of the reaction rate caused by the formation of hydrogen bonds. These bonds can occur between the amino group of the interferent and the isonicotinate radical.[21]

Although salicylamide is similar to nicotinamide, it does not increase the emission intensity because it is not hydrolysed to ammonia under these conditions.

The effect of amino acids is interesting, as some of them increase the intensity whereas others reduce it severely (Table 3). Amino acids are oxidised by NBS to an aldehyde or nitrile by reaction (1) and/or (2)[20,25]:

$$RCH(NH_2)COOH + 2NBS + H_2O \rightarrow RCHO + CO_2 + NH_3 + Br_2 + 2NHS \quad (1)$$

$$RCH(NH_2)COOH + 2NBS \rightarrow RCN + CO_2 + 2NHS + 2H^+ + 2Br^- \quad (2)$$

where NHS = succinimide.

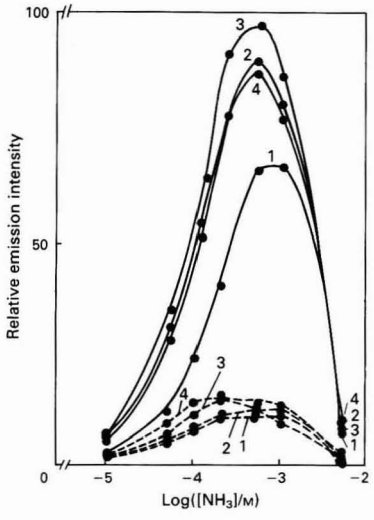


Fig. 4. Effect of ammonia concentration on the CL intensity from 2.00 (broken lines) and 10.0 (solid lines) μg ml^{-1} of isoniazid at 1, 0.5; 2, 0.8; 3, 1.0; and 4, 2.0 M sodium hydroxide ([NBS] = 0.015 M)

If reaction (1) predominates, then the emission intensity increases due to ammonia which is one of the products. Reaction (2) reduces the intensity due to consumption of the oxidant.

As the addition of ammonia to the analyte solution allows the determination of very low concentrations of isoniazid, its effect was investigated further (Fig. 4); 5.0×10^{-4} M ammonia and 1.0 M sodium hydroxide were the best concentrations for maximum emission intensity.

Analytical Parameters

Fig. 5 shows a typical recording for a series of isoniazid standards obtained using the proposed procedure. The calibration graph [I (mV) *versus* isoniazid concentration c (μg ml^{-1})] was linear in the range 0.050–1.00 μg ml^{-1} of isoniazid: $I = (3.34 \pm 0.18) + (19.4 \pm 0.3)c$, $r = 0.9995$ ($n = 6$); and in the range 1.00–20.0 μg ml^{-1} of isoniazid: $I = (-16.2 \pm 3.6) + (30.2 \pm 0.3)c$, $r = 0.9996$ ($n = 10$).

The limit of detection (blank plus three times its standard deviation) was 0.024 μg ml^{-1} of isoniazid and the coefficients of variation for 0.200 and 1.00 μg ml^{-1} of isoniazid were 3.0 and 0.9%, respectively ($n = 10$). Aqueous solutions of isoniazid (0.050–20.0 μg ml^{-1}) were analysed as samples of unknown concentration with a mean relative error of 1.2% (range 0–3.8%). The correlation coefficient (r) was 0.99993 ($n = 13$).

Interference Studies

As NBS disproportionates to bromide ion in alkaline medium,[8] the effect of this anion on the emission intensity was investigated. No interference was observed, even when bromide was present at concentrations up to 0.10 M.

In order to assess possible analytical applications of the proposed method, the effect of some common excipients used in pharmaceutical preparations was studied by analysing synthetic sample solutions containing 0.200 μg ml^{-1} of isoniazid and 2.00 μg ml^{-1} of each excipient. The undissolved material, if any, was filtered before measurement. The recovery results are given in Table 4. No interference was observed from any of the excipients tested, which showed recoveries in the range 97.5–103.9%.

The effect of some common co-existing compounds on the recovery of 0.200 μg ml^{-1} of isoniazid was studied by analysing synthetic sample solutions, as for excipients, but with various amounts of each co-existing compound (Table 5). No interference was observed from calcium pantothenate, nicotinic acid and thiamine hydrochloride even when present in a 10-fold excess. Nicotinamide does not interfere because the ammonia produced by its hydrolysis is negligible compared with the excess of ammonia already added to the solution.

Pyridoxine hydrochloride interferes, when present at a concentration ratio >0.1, due to the hydrogen bonds formed between its hydroxyl groups and isoniazid. Riboflavin interferes when present at a concentration ratio >0.1, due to its CL reaction with NBS.

Ascorbic acid interferes when present at high concentrations. The reducing action of ascorbic acid may be responsible for its enhancing effect on the measurements. However, no CL was observed from the reaction of NBS with ascorbic acid. The interference of ascorbic acid was eliminated when the synthetic sample solution was measured after 1 h, due to rapid oxidation of ascorbic acid by atmospheric oxygen in alkaline medium.[8,26]

The interference from *p*-aminosalicylic acid and glutamic acid when present at a concentration ratio >1 is probably due to a quenching effect or hydrogen bonding.

Table 4. Recovery of 0.200 μg ml^{-1} of isoniazid from solutions with a 10-fold concentration of various additives used as excipients

Additive	Recovery, % (n = 3)
Glucose	97.9
Galactose	98.7
Lactose	97.9
Sugar	100.2
Sorbitol	97.5
Talc	103.9
Starch	99.7
CAHP*	102.1
EDTA†	98.7
Carbowax‡	102.3
Carbopol§	99.6
Magnesium stearate	100.0
Sodium lauryl sulphate	102.5
$CaSO_4$	100.3
Sodium citrate	102.3

* CAHP = cellulose acetate hydroxyphthalate.
† EDTA = ethylenediaminetetraacetic acid.
‡ Polyethylene glycol 4000.
§ Carboxypolymethylene.

Table 5. Recovery of isoniazid (0.200 μg ml^{-1}) from solutions with some co-existing compounds

Compound	Concentration ratio (compound to isoniazid)	Recovery, % (n = 3)
Thiamine hydrochloride	10	99.3
Calcium pantothenate	10	103.2
Nicotinic acid	10	100.7
Nicotinamide	10	100.2
Pyridoxine hydrochloride	10	51.0
	1	75.9
	0.1	101.0
Riboflavin	10	563.2
	1	164.6
	0.1	98.9
Ascorbic acid	10	122.8
	1	119.4
	0.1	111.1
p-Aminosalicylic acid	10	70.2
	1	95.6
	0.1	99.6
Glutamic acid	10	63.6
	1	95.1
	0.1	102.8
Hydrazine sulphate	10	131.2
	1	103.2

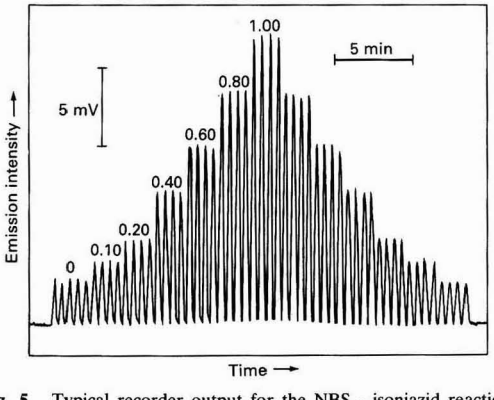


Fig. 5. Typical recorder output for the NBS - isoniazid reaction under the recommended conditions (the numbers above each set of peaks are μg ml^{-1} of isoniazid)

Table 6. Recovery experiments for isoniazid added to sample solutions of commercial formulations

Formulation	Isoniazid/μg ml^{-1} Initially present	Added	Recovered	Recovery, % (n = 3)
Rimifon	0.199	0.200	0.192	96.0
		0.400	0.388	97.0
		0.800	0.834	104.2
Dianicotyl	0.209	0.200	0.193	96.5
		0.400	0.387	96.8
		0.800	0.822	102.8
			Mean:	98.9

Table 7. Determination of isoniazid in commercial formulations with the proposed CL method and the BP official method

Formulation	Isoniazid/mg per tablet: Claimed	Found: CL ± SD*	Found: Official method†	Relative difference (CL − official), %
Rimifon	50	49.5 ± 0.1	48.9	+1.2
Dianicotyl	100	97.2 ± 0.9	93.8	+3.6
				Average: 2.4

* SD = standard deviation (n = 3).
† See reference 28.

The effect of hydrazine sulphate on the determination of isoniazid was also studied as it is one of the degradation products of this drug.[27]

It is important to note that rifampicin interferes, even when present at a concentration ratio of <0.1. Therefore, the method cannot be applied to samples that also contain rifampicin.

Accuracy

The accuracy of the proposed continuous-flow CL method was examined by performing recovery experiments on solutions prepared from isoniazid tablet formulations. A mean recovery of 98.9% (range 96.0–104.2%) was found (Table 6).

The proposed method was also evaluated by analysing commercial formulations of isoniazid. The results were compared with those obtained by the British Pharmacopoeial (BP) official method.[28] A satisfactory agreement between the results was obtained (Table 7) with a mean relative difference of 2.4%.

Conclusions

The potential application of NBS as an organic CL oxidising reagent has been demonstrated in this work. The main advantage of NBS over other common inorganic oxidants (*i.e.*, Ce^{IV} and $KMnO_4$) and coloured organic oxidising reagents is the absence of an absorption band. The investigation revealed that ammonia acts as an enhancer of the emission. Ammonia is probably a selective enhancer of NBS reactions in alkaline medium. However, the nature of the emitting species and the effect of the enhancer have not been clearly established, as equipment for monitoring CL emission spectra was not available.

The proposed automated CL method is very simple, fast, fairly sensitive and sufficiently accurate and precise. The results are reproducible and show that the method can be applied to the sensitive determination of isoniazid in pharmaceutical preparations without severe interferences.

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Determination of Calcium in Waters, Milk and Wine by Discontinuous-flow Analysis

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The flow-based analysis method, discontinuous-flow analysis (DFA), was used for the determination of total calcium in drinking water, milk and wine by titration with ethyleneglycoltetraacetic acid. The detector is a coated-wire calcium-selective electrode. The titration can be cycled continuously with a cycle time of about 1 min. This can be carried out with a single sample or with different samples using an autosampler. The method for waters and wine is simple, fast and highly reproducible. For milk, a back-titration method was used because of the complex matrix of the sample.

Keywords: *Discontinuous-flow analysis; calcium titration; calcium in waters, wine and milk*

Discontinuous flow-analysis (DFA) is an automated analytical method[1] for flow-based chemical analysis developed by the Australian company, Ionode Pty (Brisbane, Queensland, Australia). Conventional flow-based methods of analysis such as continuous flow and flow injection rely on peristaltic or piston pumps operating in the discharge mode to propel samples to the detector. In the simplest version of DFA, where the analyser is used as an automatic titrator, two syringe pumps are used, one operating in the discharge mode, and the other in the suction mode. The syringes are operated by a cam and by selecting the appropriate cam profile the flow-rates of either or both pumps can be varied in a reproducible way. In a typical titration pure titrant flows initially to the detector followed by a rapid flow of pure sample (sample flush). At this point sample is rapidly aspirated into the titrant until the ratio of sample to titrant is 2 : 1 (controlled by the particular cam used). Sample is then continually aspirated into the titrant by a varying sample to titrant flow ratio until the sample to titrant ratio is 1 : 2. The end-point occurs at some point between these two limits at a particular ratio of sample to titrant depending on the concentration of sample and titrant. A unique method employing a reed mixer[1] is used to mix sample and titrant continuously. The potential of the calcium-selective electrode is monitored and plotted as a titration curve.

Discontinuous-flow analysis, being a very fast and reproducible procedure, is ideally suited to routine analysis and is easily automated. This paper describes the first application of DFA to a routine analysis, *i.e.*, the determination of total calcium in waters, milk and wine. This analysis is most commonly performed by atomic absorption spectrometry (AAS).

Experimental

Reagents and Chemicals

Standard calcium solutions were prepared from calcium chloride (BDH, Poole, Dorset, UK, AnalaR grade). Ethylenebis(oxyethyleneimino)tetraacetic acid (ethyleneglycoltetraacetic acid, EGTA, Titriplex VI, Merck, Darmstadt, FRG, analytical-reagent grade) was used as the titrant. Lanthanum chloride (Ajax, Sydney, New South Wales, Australia, analytical-reagent grade) was used as a releasing agent for the determinations made using AAS. The adjustment of the pH of samples and titrant was made using sodium borate (Mallinckrodt, Paris, Kentucky, USA, analytical-reagent grade) and potassium hydroxide (Ajax, analytical-reagent grade). Casein (BDH), potassium dihydrogen phosphate (May and Baker, Dagenham, Essex, UK), citric acid (Ajax) and lactose (BDH) were all of laboratory-reagent grade and were used in the preparation of the model milk solutions. Sodium chloride (May and Baker, analytical-reagent grade) was used as an ionic strength adjuster and magnesium nitrate (Ajax, analytical-reagent grade) was used as a complexing agent in the back-titration of milk. All solutions were made using NANOpure water (16 MΩ, Barnstead, Dubuque, Iowa, USA).

Discontinuous-flow Analyser

The instrument was obtained from Ionode Pty. The construction of the instrument and flow cell has been described by Arnold *et al.*[1] In this application the flow cell contained a calcium-selective coated-wire[2] electrode and a silver - silver chloride reference electrode with a glass frit junction. The calcium electrode was made by casting a poly(vinyl chloride) (PVC) membrane on to the flattened end of a copper wire fixed in a PVC casing which was threaded to screw into the flow cell. The membrane had the following composition (m/m): 64.0% *o*-nitrophenyl octyl ether (Fluka, Glossop, Derbyshire, UK), 34.5% PVC (ICI, Melbourne, Victoria, Australia), 1.0% (−)-(*R,R*)-*N,N'*-bis[(11-ethoxycarbonyl)-undecyl]-*N,N'*-4,5-tetramethyl-3,6-dioxaoctane-diamide (ETH 1001, Fluka) and 0.5% potassium tetrakis(*p*-chlorophenyl)borate (Fluka) and was prepared from a tetrahydrofuran solution.[3]

Data Acquisition

The cam drive shaft of the instrument is fitted with an encoder which divides a complete revolution of the cam into 1000 pulses. This serves to locate the position of the cam at any point in time as it rotates. The electrodes are connected to a differential amplifier and the millivolt signal for each pulse number is sent via a 12-bit analogue to digital converter to an IBM compatible microcomptuer (XT Turbo, Imagineering, Melbourne, Victoria, Australia). A potential reading is only taken when the system is triggered by the incremental encoder. Thus, each data point corresponds to a pulse number (*x*-coordinate) and a potential measured at that pulse number (*y*-coordinate). The software, written in QuickBasic, enables the data to be processed in a number of ways. Data can be plotted, smoothed and derivatised, end-points can be located,

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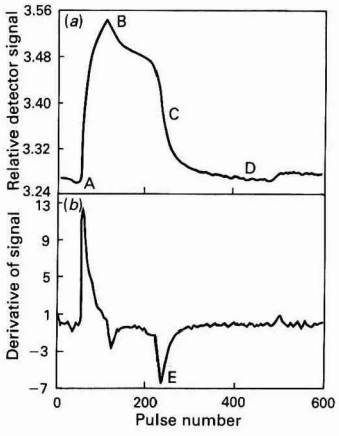

Fig. 1. (*a*) DFA curve for the titration of calcium with EGTA; and (*b*) derivative plot. For details, see text

and hard copies obtained (Epson LX-800 dot matrix printer Seiko Epson, Nagano, Japan).

Atomic Absorption Spectrometry

Total calcium was determined by AAS (λ = 422.3 nm) using an air - acetylene flame and standards in the concentration range 1–15 mg l^{-1}. Water, milk and wine samples were diluted where necessary with NANOpure water to bring them within the range of the standards. All solutions contained 1% m/v lanthanum chloride.

Results and Discussion

A typical curve for a calcium standard titrated with EGTA is shown in Fig. 1(*a*). Initially, the electrode responds to pure titrant in the flow cell. At point A calcium standard rapidly enters the flow cell and the electrode potential rises to a maximum at B. The flow profile for the particular cam used in this work is such that sample and titrant are then rapidly delivered to the flow cell until the ratio of sample to titrant is 2:1, which leads to a small drop in potential. This is followed by small increases in the amount of titrant which yields the potentiometric titration curve with an inflection at the end-point, C. The titration is complete at point D where the sample to titrant ratio is 1:2. The remainder of the cam cycle is used to refill the titrant syringe and to expel solutions to waste. The derivative plot of the titration curve is shown in Fig. 1(*b*) with the end-point located at E. This point corresponds to a particular rotation position of the cam, which can be located by the pulse number.

As the cam profile is cut using a mathematical relationship of the flow-rate ratio of sample to titrant, each pulse number corresponds to a particular ratio of sample to titrant, and so, theoretically, it should be possible to determine the concentration of a sample by using the concentration of the titrant and the ratio of sample to titrant at the end-point. Initially, the instrument and flow cell would need to be calibrated as there is a small delay between actual mixing of the sample and titrant and sensing of the analyte.[1] For the work in this paper, however, a calibration graph was used which yielded better accuracy and precision than did the above procedure. A typical calibration graph of pulse number against concentration is shown in Fig. 2. The graph is non-linear as expected in this version of the instrument[1] and conforms to a second-order polynomial equation. The actual calcium concentration range covered depends on the titrant concentration, however, with the particular cam used in this work, the range cannot span more than twice or less than half the titrant concentration, otherwise, no end-point is obtained and an "out of range" titration curve is observed.

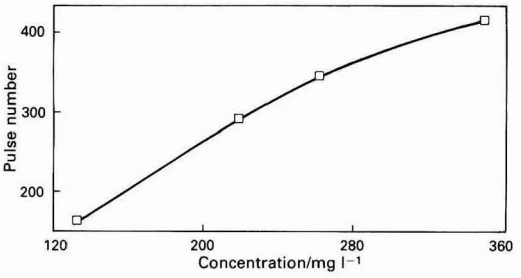

Fig. 2. Calibration graph for calcium

Table 1. Total calcium content of tap water, mineral water and wine samples

Sample	Total calcium content/mg l^{-1} DFA	AAS
Tap water 1	30.5 (3.0)*	31.5 (1.0)
Tap water 2	4.1 (2.4)	4.6 (1.1)
Mineral water 1	76.2 (2.0)	76.1 (1.2)
Mineral water 2	10.0 (4.0)	9.1 (1.1)
Wine 1 (white)	67.3 (1.8)	66.8 (0.3)
Wine 2 (white)	59.5 (0.8)	60.6 (0.7)
Wine 3 (red)	64.7 (1.4)	64.7 (0.3)

* RSD, n = 4 (waters) or 5 (wines), given in parentheses.

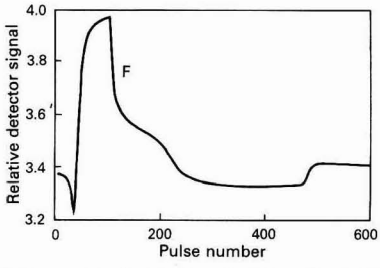

Fig. 3. DFA curve for the titration of whole milk with EGTA. For details, see text

Waters and Wine

The total calcium contents of several samples of tap water, mineral water and wine were determined by DFA and the results compared with those obtained using AAS. The results are shown in Table 1. The water samples were titrated without pH adjustment whereas the wine samples were adjusted to pH 10 by a 1 + 1 dilution with 0.1 M sodium borate solution. Two calibration graphs were necessary, one covering the calcium concentration range 2 × 10^{-3}–7 × 10^{-4} M which used an EGTA titrant concentration of 1 × 10^{-3} M, and the other covering the range 1 × 10^{-4}–3 × 10^{-4} M calcium with an EGTA concentration of 2 × 10^{-4} M. The EGTA solutions were adjusted to pH 12 with a potassium hydroxide - potassium chloride solution. The results show the excellent agreement in both accuracy and precision between the DFA and AAS methods. Recoveries of spiked water samples were between 96 and 102%.

Table 2. Total calcium content of three types of milk

Sample	Total calcium content of milk/mg l^{-1} DFA	AAS
Whole	1325 (1.4)*	1360 (0.7)
Fat-reduced	1510 (1.9)	1495 (1.1)
Long-life	1420 (1.9)	1430 (0.8)

* RSD, $n = 5$, given in parentheses.

Milk

The direct titration of milk with EGTA using DFA produced the titration curve shown in Fig. 3, which has a very different shape to that observed in Fig. 1(*a*) for the calcium standard. This was true for several different types of milk tested, whole milk, fat-reduced milk and long-life milk. In each instance, the total calcium content determined by DFA was underestimated, being only about 80% of the value obtained by the AAS method. For example, the value for fat-reduced milk by DFA was 1090 (8.3) mg l^{-1} compared with 1362 (1.1) mg l^{-1} by AAS and 1370 (2.2) mg l^{-1} by a conventional batch titration method with EGTA. The numbers in parentheses are the relative standard deviations (RSDs) for $n = 4$.

Whole milk is a complex mixture with the approximate composition, water (86%), fat (5%), lactose (5%), casein (2.5%), phosphate (0.2%), citrate (0.2%) and vitamins, inorganic ions, other proteins and trace components (1.1%).[4] A number of these constituents are known to bind calcium and measurements carried out with a calcium ion-selective electrode have shown that only about 7% of the total calcium in milk is ionised. Thus, one explanation for the very large drop in electrode potential seen at the start of the DFA titration in Fig. 3 (region F) is that, in this region of the titration, the ionised portion of the calcium is titrated. The fact that there is no discernible end-point is because the titration is essentially "out of range" as the titrant concentration is too high. The complexed calcium titrates more slowly giving rise to the different shape of the curve compared with the pure calcium solution and to the underestimation of the end-point.

Experiments were carried out to mimic this effect using synthetic milk compositions which contained the various components of milk that complex calcium. A solution containing calcium, casein and citrate gave 100% recovery of the calcium by DFA, but on the addition of phosphate only about 80% of the total calcium was determined and the DFA trace resembled very closely that of milk. It is well known[5] that casein in milk exists as micelles and that calcium phosphate acts in some way as a cementing agent for the micelles. Thus, the inability to obtain 100% recovery of calcium in milk by DFA is thought to be associated with these complex casein structures. The fact that a conventional EGTA titration of calcium in milk gives 100% recovery suggests that the effect is a kinetic one. In DFA, the time interval between mixing of the EGTA with the sample and detection of the calcium is only a few seconds which may not be long enough for the complexation reaction to reach equilibrium. Thus, the direct titration of calcium in milk with EGTA is not possible using this configuration of DFA.

A back-titration method, however, was found to be satisfactory for the determination. The procedure consisted of adding 10 ml of 0.1 M EGTA to 10 ml of milk and stirring magnetically for 1 min. Then 2 ml of 3.0 M magnesium nitrate were added and stirred for a further 10 min. The solution was centrifuged and the excess of EGTA was titrated with a 3×10^{-2} M solution of calcium chloride using DFA. A calibration graph was constructed using EGTA solutions in the range 2×10^{-2}–5×10^{-2} M. The addition of magnesium nitrate was necessary in order to precipitate phosphate and remove other complexing agents which otherwise were found to react with the calcium titrant producing a second end-point in the DFA titration curve. Results for three types of milk are shown in Table 2 and the excellent agreement with AAS in both accuracy and precision can clearly be seen.

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Simultaneous Determination of Hydroxylamine and Cyanide in Formulations Containing Pralidoxime Salts by Flow Injection

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A flow injection method is described for the simultaneous determination of cyanide and hydroxylamine which are known decomposition products of formulations containing pralidoxime salts used in the treatment of anticholinesterase poisoning. By using the diffusion of HCN from the carrier stream followed by amperometric detection, high selectivity and sensitivity and a wide dynamic range can be achieved. Hydroxylamine is determined by its oxidation with iodine to nitrite which can then be determined colorimetrically. The gas diffusion unit effectively acts as a stream splitter for the two analytes allowing their simultaneous determination from a single sample injection. The performance of the system and its applicability to thermally stressed pralidoxime solutions are described.

Keywords: *Cyanide determination; hydroxylamine determination; flow injection; pralidoxime mesylate; pharmaceutical analysis*

Formulations containing pralidoxime (2-hydroxyiminomethyl-1-methylpyridinium) salts have been used for the treatment of anticholinesterase poisoning for many years with the drug being delivered as an intra-muscular injection in aqueous solution. Problems have been encountered with the stability of these formulations and there have been many studies both of the extent of the degradation and the nature of the products.[1] Recently most of the organic products have been identified, high-performance liquid chromatographic methods for their analysis devised and the degradation pathways elucidated.[2–4] In addition to the organic degradation products two inorganic species are produced, hydrogen cyanide and hydroxylamine. These are of interest not only because of their toxicity but also because it has been shown that they participate in secondary reactions with other products.[3] Detailed studies of the pralidoxime degradation reactions therefore require that methods for the determination of these species be available.

Although many methods for the determination of cyanide have been described in the literature[5] there are three problems in their direct application to pralidoxime formulations; the high concentration of pralidoxime salt, typically 250 mg ml^{-1}, decomposition products which interfere directly with the method and lastly the conditions used in the analytical method can cause a change in the cyanide concentration. For example, a method requiring alkaline conditions will generate free cyanide from the 2-cyano-1-methylpyridinium salt (P2S-cyanide) present as a decomposition product.[6] To overcome these problems the cyanide has usually been separated from the formulation either by a gas stream[7] or by diffusion in a Conway dish[8] and collected in alkali. These methods can be both time consuming and difficult to control. By using the same physical principle but configured as a gas diffusion unit in flow injection (FI), rapid and closely controlled stripping of HCN from a sample can be achieved.[9] This approach has been used for the determination of cyanide in wastewaters with the determination being carried out by either an ion-selective electrode[10] or by a colour reaction.[11] In the present application gas diffusion is linked with an amperometric method of cyanide determination in order to achieve the necessary sensitivity and wide dynamic range.

Hydroxylamine has been determined in pralidoxime salt formulations by its oxidation to nitrite with iodine, reduction of excess of iodine with sodium arsenite and subsequent determination of the nitrite produced by a diazo coupling reaction.[12,13] The literature contains little information on how these reactions were applied or on the characteristics of the method. The technique involves several reagents in a sequence of reaction steps which could with advantage be configured for FI. This paper describes the application of FI to the simultaneous determination of cyanide and hydroxylamine in pralidoxime formulations.

Experimental

Apparatus

The system is based on the Tecator 5020 FIA-star system with a 5024 photometer fitted with a Helma 178.713 cell and 540-nm filter. The V-200 valve was modified to give an injection volume of 20 µl. The FI manifold used Tecator type III and type V (gas diffusion) solvent-resistant chemifolds. The diffusion membrane was microporous PTFE film. Reaction tubes were 0.5 mm i.d. PTFE and the pearl string reactor (PSR)[14] was made from 40 mesh glass beads packed into a 0.8 mm i.d. PTFE tube. Pump tubing was solvent-resistant poly(vinyl chloride) (Watson-Marlow) except for the iodine reagent channel which was 0.5 mm i.d. isoversinic (Anachem).

The amperometric detector was a Metrohm 656 electrochemical flow cell fitted with a purpose-built silver working electrode 2 mm in diameter, a glassy carbon auxiliary electrode and a silver - silver chloride reference electrode connected to a 641 detector. The applied voltage was zero. The FI manifold is shown in Fig. 1.

Reagents

Carrier stream. 0.2 M citrate buffer (BDH), pH 3.2, prepared from 0.2 M trisodium citrate (BDH) (20 ml) and 0.2 M citric acid (80 ml).

Cyanide collection stream. 0.1 M sodium borate prepared by dissolving 6.18 g of boric acid (BDH) in 1 l of 0.1 M sodium hydroxide solutions, pH 10.5.

Iodine reagent. Acetic acid (20 ml, BDH) 1 M sodium bromide solutions (80 ml, BDH), sulphanilamide (0.2 g, Aldrich) and iodine (3 g, BDH) stirred for 3 h and filtered before use.

Sodium arsenite. 0.0705 M prepared from a standard ampoule (BDH).

N-(1-*Naphthyl)ethylenediamine.* 0.2 g of the amine hydrochloride (Aldrich) and 2 g of phosphoric acid (BDH) made up to 100 ml.

Pralidoxime mesylate (P2S, prepared at CDE). The solution for the degradation experiment was 250 mg ml^{-1} P2S in a 0.1 M citrate buffer of pH 3.2.

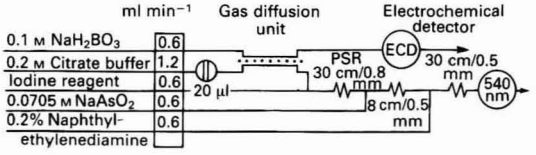

Fig. 1. Manifold for the simultaneous determination of cyanide and hydroxylamine

Cyanide calibration solutions. Diluted from 2.50 mg ml^{-1} potassium cyanide solution with 0.2 M citrate buffer.

Hydroxylamine calibration solutions. Diluted from 2.1 mg ml^{-1} hydroxylamine hydrochloride solutions with 0.2 M citrate buffer.

P2S degradation products. Prepared at the Chemical Defence Establishment: 2-formyl-1-methylpyridinium iodide (P2S-aldehyde); 2-cyano-1-methylpyridinium iodide (P2S-cyanide); 2-carbamoyl-1-methylpyridinium iodide (P2S-amide); 2-hydroxymethyl-1-methylpyridinium iodide (P2S-alcohol); 2-hydroxyaminoiminomethyl-1-methylpyridinium methanesulphonate (P2S-amidoxime); 2-carboxyl-1-methylpyridinium chloride (P2S-acid); and 1-methyl-2-pyridone (P2S-pyridone).

Procedures

Reagent solutions were filtered before use. After assembly of the FI system the integrity of the gas diffusion membrane was checked by injecting the 1 mg ml^{-1} hydroxylamine solution and observing the response on the electrochemical detector over its most sensitive range. Hydroxylamine is electroactive under the conditions used and any gross leakage in the membrane can be detected. The system was satisfactory if no signal was detected above the base line noise.

The detection limit, linear range and error structure were determined for each of the channels from the calibration process. The validity of using calibration standards in citrate buffer rather than in P2S solution was checked for both hydroxylamine and cyanide by standard additions to fresh and degraded P2S. The effect of known organic degradation products was assessed by injecting samples of the products in citrate buffer at 25 mg ml^{-1}.

The applicability of the method was assessed by the analysis of P2S solutions which had been stored at 80 °C for up to 14 d. The solutions were diluted 10-fold with 0.1 M citrate buffer before analysis. The validity of using the electrochemical response as a measure of cyanide concentration was checked by using an independent method of cyanide determination using Conway dish diffusion followed by colorimetric analysis[8] of solutions degraded for 5 h and 5 d.

Results and Discussion

Determination of Hydroxylamine

The FI system for the determination of hydroxylamine was based on established chemistry that had previously been used with pralidoxime formulations in batch methods. The direct transcription to FI however presented several problems. The P2S formulation is so concentrated, 250 mg ml^{-1}, that attempts at direct analysis by FI either produced a large refractive index peak if a low density carrier stream was used or, if attempts were made to balance this with a high refractive index stream containing a high concentration of dissolved salts, a noisy base line resulted from poor mixing. Further, in this instance the colour reaction was suppressed. The problem was overcome by using a 0.2 M citrate carrier stream and a preliminary 10-fold dilution of the samples with 0.1 M citrate. The refractive indices were sufficiently close to produce only a minimal blank response. The other problems were associated with the iodine reagent. In the batch method iodine was dissolved in glacial acetic acid but when this reagent was used in FI incomplete mixing with the aqueous carrier caused streaming in the photometer cell and a high noise level. Although the reagents were de-gassed by filtration there was sufficient residual dissolved air to give problems with bubble formation when the streams mixed. Further, the iodine tended to precipitate. Attempts to use potassium iodide as the iodine solvent led to the precipitation of the P2S as an ion pair with the hydrophobic triiodide ion. The mixed acetic acid - sodium bromide solvent overcame these problems and gave a saturated solution typically 0.05 M in iodine.

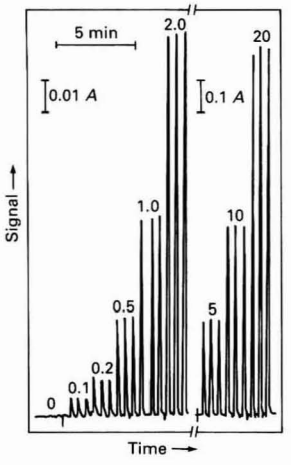

Fig. 2. Hydroxylamine calibration. The hydroxylamine concentration in µg ml^{-1} is shown above each group of peaks. A = absorbance

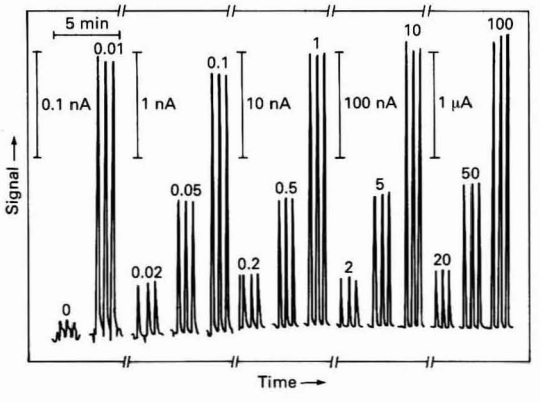

Fig. 3. Cyanide calibration. The cyanide concentration in µg ml^{-1} is shown above each group of peaks

The use of a pearl string reactor for the iodine oxidation step gave a combination of lower noise level, higher signal and less dispersion than could be obtained with an open tube. Comparison with the response obtained from injected sodium nitrite indicated that the oxidation of hydroxylamine to nitrite was quantitative. Stopped flow showed that the final colour development reaction was 80% complete.

Hydroxylamine calibration over the range 0.1–20 µg ml^{-1} is shown in Fig. 2. The detection limit [three times the standard deviation (SD) of the blank] was 0.03 µg ml^{-1}. The error structure showed a substantially constant coefficient of variation (CV) of 0.7% over much of the range but degraded near the detection limit, *e.g.*, at 0.1 µg ml^{-1} CV = 4.3% (n = 5).

Calibration standards were not made up in P2S solution because even at room temperature the oxime hydrolyses to give measurable concentrations of hydroxylamine within 1 h. The validity of using calibration solutions in 0.2 M citrate was demonstrated by analysing a freshly prepared P2S solution which showed no response above the blank and by comparing the 5 μg ml^{-1} standard with a P2S solution (25 mg ml^{-1}) to which 5 μg ml^{-1} of hydroxylamine had been added. The responses were within 2%.

The dispersion (D) of the hydroxylamine manifold was determined by comparing the response of an injected 2 μg ml^{-1} standard with the response of the same standard pumped through the carrier channel. A D value of 5.1 was obtained and an analysis rate of 120 samples h^{-1} could easily be achieved. It was found that the 18 μl photometer cell supplied with the FIA-star system was a major cause of dispersion; the 8 μl cell used in this work was much more satisfactory.

Determination of Cyanide

Calibration over the range 10 ng ml^{-1}–100 μg ml^{-1} of cyanide is shown in Fig. 3. The method has excellent sensitivity and linearity over a wide range. The CV was substantially constant throughout the range at 1.0% (mean of 13 standards with five injections of each). A sampling rate of 120 h^{-1} could easily be achieved.

The transfer efficiency of HCN across the PTFE membrane was assessed by injecting the 100 μg ml^{-1} standard and collecting the borate stream before the amperometric detector. The sample was diluted to 5 ml with citrate buffer and the concentration measured by re-injection into the system. A diffusion efficiency of 13.6% was found. Both the diffusion efficiency and the response of the electrochemical detector were temperature dependent; however the effect was small and no attempt was made to control the temperature of these components. The slight drift in response could be compensated for by the normal calibration process.

The calibration solutions were prepared in citrate buffer rather than in P2S solution for the same reasons as for the hydroxylamine channel, *i.e.*, there is the possibility of cyanide being formed in the calibration standards from P2S or indeed cyanide could be lost from solution by reaction with P2S-aldehyde which is known to be formed.[3] The validity of this approach was confirmed by the analysis of freshly prepared cyanide standards in P2S (25 mg ml^{-1}) and comparison with standards in citrate. Differences throughout the concentration range were less than 1%. Although the pH of the sample and calibration solutions was well below the pK_a of HCN the solutions could be handled normally in stoppered flasks without any apparent loss.

Table 1. Hydroxylamine and cyanide in P2S solutions degraded at 80 °C. The concentrations refer to the original, undiluted solution

Storage time	Hydroxylamine/ μg ml^{-1}	Cyanide/ μg ml^{-1}	pH
0 h	0.84	0.0065	3.20
0.5 h	22.7	0.015	3.17
1 h	25.1	0.042	3.24
2 h	21.3	0.163	3.70
5 h	10.2	0.83	3.13
8 h	9.0	1.76	3.36
12 h	22.6	3.24	3.38
24 h	28.0	7.2	3.50
2 d	9.9	110	3.70
5 d	8.8	820	3.56
14 d	296	1020	3.13

Applicability of the Method to P2S Degradation Studies

The FI response from the simultaneous determination of hydroxylamine and cyanide in P2S solutions heated at 80 °C for various times is shown in Fig. 4 and the results are summarised in Table 1. Hydroxylamine was not detected in freshly prepared P2S solutions but it became measurable after 1–2 h at room temperature. Over the degradation period the hydroxylamine concentration varied in a complex way indicative of the many competitive reactions. For example, the initial hydrolysis of P2S is pH dependent and the measured pH varies with time, the equilibrium can be displaced by the removal of P2S-aldehyde and hydroxylamine itself is known to react with P2S-cyanide in the degraded formulation.[3]

The P2S solution became darker in appearance with storage time, changing from colourless, through a pale straw colour to

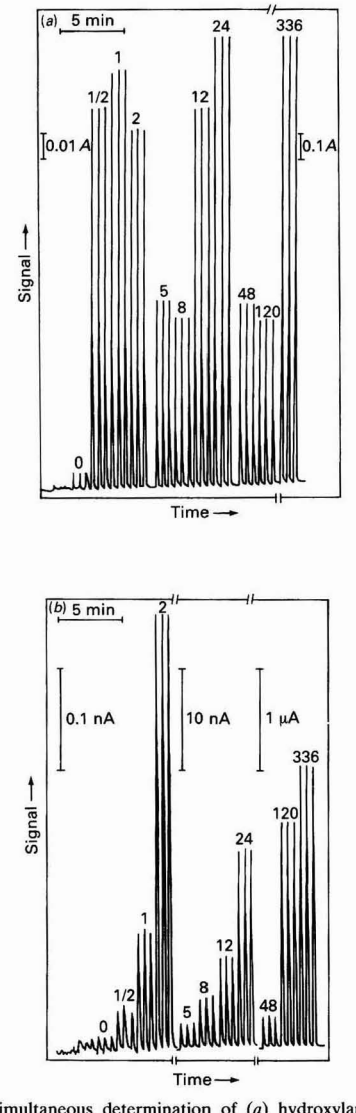

Fig. 4. Simultaneous determination of (*a*) hydroxylamine (A = absorbance) and (*b*) cyanide in P2S stored at 80 °C. The storage time in hours is indicated above each group of peaks

Table 2. Interfering responses of organic decomposition products

Product	Molar response relative to hydroxylamine	Molar response relative to cyanide
P2S-aldehyde	2.9×10^{-5}	32×10^{-5}
P2S-cyanide	5.6×10^{-5}	12×10^{-5}
P2S-amide	—*	2.4×10^{-5}
P2S-alcohol	—*	3.5×10^{-5}
P2S-amidoxime	1.1×10^{-3}	—†
P2S-acid	—*	—†
P2S-pyridone	—*	—†

* Differential peak due to refractive index mis-match of the same order as the blank.

† Response of the same order as the blank.

Table 3. Comparison of cyanide concentration in degraded P2S by FI and the Conway diffusion - colorimetric methods. Solutions were generated during a different storage run from results given in Table 1. Results are mean ± SD, $n = 5$ (FI), $n = 4$ (Conway)

	[CN]/μg ml^{-1}	
Storage time	FI	Conway - colorimetric
5 h	0.82 ± 0.014	0.83 ± 0.07
5 d	707 ± 10	719 ± 34

Table 4. Standard additions. Concentrations given are those of the solution diluted 9 + 1 with 0.1 M citrate buffer

Hydroxylamine		Cyanide	
Sample	Concentration/ μg ml^{-1}	Sample	Concentration/ μg ml^{-1}
$t = 0$	0.084	$t = 0$	0.0038
$t = 0 + 5$ μg ml^{-1}	5.03	$t = 0 + 1$ μg ml^{-1}	1.00
$t = 5$ h	1.10	$t = 5$ h	0.090
$t = 5$ h + 5 μg ml^{-1}	6.28	$t = 5$ h + 100 ng ml^{-1}	0.189
$t = 5$ d	0.88	$t = 5$ d	80.3
$t = 5$ d + 5 μg ml^{-1}	4.62* 2.31†	$t = 5$ d + 50 μg ml^{-1}	131.1

* 1 min after addition.

† 10 min after addition.

dark brown. To assess the degree of interference at the photometer from this coloration, samples were injected with no iodine in the first reagent. The 14 d old solution gave a response equivalent to 0.5 μg ml^{-1} of hydroxylamine, the 5 d solution was equivalent to 0.1 μg ml^{-1} and the others gave no response greater than the blank. The interference from the colour was therefore small, occurred only in solutions which had been extensively degraded and could be allowed for by subtraction.

In contrast to hydroxylamine the cyanide concentration increased monotonically with storage time. The response shows the need for high sensitivity and wide dynamic range if undesirable changes in dilution are to be avoided. In both these respects the amperometric method was superior to the colorimetric or potentiometric method. The method is suitable for the determination of cyanide in the production of P2S, for quality control purposes, where determination at the p.p.m. level is required.[15]

Interference by the known organic degradation products, summarised in Table 2, was low. On the hydroxylamine channel the greatest interference was from P2S-amidoxime. This may be a genuine interference response or, because the amidoxime was prepared from P2S and hydroxylamine, a reflection of some residual free hydroxylamine in the sample. Further, the response from the amidoxime increased with time after preparation of the solution indicating that hydrolysis was occurring. The response ratio, therefore, represents the maximum level of interference. On the cyanide channel interference was low but did indicate that the diffusion membrane allowed some non-volatile material to pass. Because most of the organic products were available only as their iodide salts the response to sodium iodide was measured to check if the interference signal could have been due to iodide. The response was very low. The comparison of results obtained by FI with those found using the Conway diffusion - colorimetric method is shown in Table 3 and confirms the validity of the FI technique.

The standard additions results, Table 4, showed no interference on the cyanide channel. The hydroxylamine recovery was quantitative with lightly degraded solutions but with the 5 d sample hydroxylamine concentrations were much lower even 1 min after mixing and the continuing fall with time could be monitored and reached a steady value after 10 min. This was probably due to the known rapid reaction of hydroxylamine with P2S-cyanide present in the degraded solution giving a different equilibrium concentration.[3] The results therefore reflect a genuine change in the hydroxylamine concentration rather than an interference with the method of determination.

Conclusions

Flow injection allows the determination of hydroxylamine in P2S solutions to be automated. The method is sensitive, precise and rapid and covers the required concentration range. The use of FI gas diffusion followed by amperometric detection allows the determination of cyanide in P2S formulations. The method is adequately free from interference and allows a wide concentration range to be covered. The two methods can be integrated to allow the determination of both hydroxylamine and cyanide from a single injection.

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Enzymic Determination of Ammonia in Food by Flow Injection

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Ammonia in food samples was determined by its reaction in an immobilised enzyme reactor containing glutamate dehydrogenase (GlDH) in a flow injection system, by measuring the decrease in the absorbance of ultraviolet radiation by reduced nicotinamide adenine dinucleotide (NADH). There was a linear relationship ($r = 0.9995$) between peak height and ammonia concentration over the range 0.05–0.6 mM. The detection limit was 0.005 mM for an injection volume of 19 μl. Sampling frequency was 60 h^{-1} and the precision was better than 1.09% for 11 successive assays. The interference effect of urea and ascorbic acid at concentrations greater than 100 mg per 100 g of product should be taken into account. The interference caused by glycine, creatinine and amino acids is negligible. Only a 20% loss in the activity of the GlDH column was observed after 500 determinations during a 3-month period.

Keywords: *Ammonia; food; enzyme; flow injection*

The presence of high levels of ammonia in food such as meat or fish, is an indication of the freshness of a product.[1–4] Ammonia has also been found to have a role in the organoleptic properties of foods.[5–7] The level of NH_4^+ in meat derivatives depends on the thermal treatment, additives, temperature and storage time.[8]

The determination of ammonia in foods poses an analytical problem which has to be solved because of the importance of this determination in food technology. The classical methods for the determination of ammonia are based on modifications of the Kjeldahl method and yield values that are higher than expected, the reason being the hydrolysis of the amino groups of the proteins.[9] Other more simple methods, such as the use of ion-selective electrodes, determine the volatile amines together with NH_3, thus specificity is lost.[10]

The use of dissolved enzymes is a valid alternative as a high specificity is obtained, however, it results in a high cost per analysis. The advantages of immobilised enzymes (higher stability, longer life span, lower analytical cost and higher selectivity) over dissolved enzymes make them suitable for routine analysis.[11]

The coupling of an immobilised enzyme reactor (IMER) with flow injection (FI) provides increased rapidity, precision and convenience compared with classical enzymic procedures[12] and decreases the cost per assay. A number of immobilised enzymes have been coupled with an FI methodology for the analysis of foods.[13–16]

The enzyme glutamate dehydrogenase (GlDH) catalyses the following reaction:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADH} \overset{\text{GlDH}}{\rightleftharpoons} \text{L-glutamic acid} + \text{NAD}^+ + H_2O$$

where NAD^+ = nicotinamide adenine dinucleotide and NADH = the reduced form of NAD. The NAD^+ generated is monitored in a flow-through spectrophotometric detector by measuring the decrease in the absorbance of the NADH.

The purpose of this research is to develop an FI method for the determination of ammonia in foods, using GlDH immobilised on controlled porosity glass (CPG) and a flow-through spectrophotometric detector, thus combining the high specificity of an enzymic method with the advantages of an FI method, *i.e.*, rapidity, precision, simplicity, cost saving and a minimum of sample treatment.

Experimental

Reagents

3-Aminopropyltriethoxysilane, 99%; L-amino acids, L-AA21; and controlled pore glass, CPG 240-200, were obtained from Sigma (St. Louis, MO, USA). The pore size and particle size of the CPG were 242A and 100–200 mesh, respectively. Reduced nicotinamide adenine dinucleotide, grade I, 100%; L-glutamate dehydrogenase E.C. 1.4.1.3, ex-bovine liver, 3000 U; and adenosine 5′-diphosphate disodium salt were purchased from Boehringer Mannheim (Barcelona, Spain). Glutaraldehyde solution, 25%, for electron microscopy, was obtained from Taab Laboratory Equipment (Aldermaston, Berkshire, UK). All other chemicals were commercially available and of analytical-reagent grade.

Stock solutions of 0.01 and 0.1 M NH_4Cl were prepared by dissolving the appropriate, weighed amount of solid in distilled, de-ionised water. Working solutions were prepared just prior to use by appropriate dilution of the stock solutions in 0.1 M phosphate buffer of pH 8.0 with 1 mM ethylenediaminetetraacetic acid (EDTA); NADH was prepared each week in 1% m/v sodium hydrogen carbonate. All solutions were prepared with distilled, de-ionised water; water quality was controlled by ion chromatography (Dionex, CA, USA).

Procedure

Enzyme immobilisation

The activated glass was prepared as follows: the porous glass was washed in boiling 5% v/v nitric acid, silanised with 3-aminopropyltriethoxysilane to give the alkylamino glass, and treated with 2.5% v/v glutaraldehyde in an aqueous system.[17] It was then added to the GlDH dissolved in 3 ml of 0.1 M phosphate buffer (pH 7.0). The solution was kept at 4 °C for 2.5 h, and then washed with cold distilled water and cold phosphate buffer to remove any unlinked enzyme. The enzyme-coated glass beads were packed into a 7.5 cm long glass column (1.2 mm i.d.), capped with polytetrafluoroethylene (PTFE) end fittings and stored, filled with phosphate buffer, at 4 °C. The void volume of the enzyme reactor was 8 mm^3.

The protein concentration of the enzyme was determined by a spectrophotometric method (Bradford protein assay, Bio-Rad Labs., Richmond, CA, USA) both before and after the immobilisation reaction. The coupling yield was then calculated as the percentage disappearance of the amount of protein initially added to the reaction mixture. Immobilisation resulted in the attachment of 95.4% of the protein initially present. The activity of the immobilised enzyme was determined according to Korenbrot *et al.*[18] The specific activity per mg of immobilised enzyme was 12% of that of the soluble enzyme and the specific acitivity of immobilised enzyme present in the reactor was 130 U.

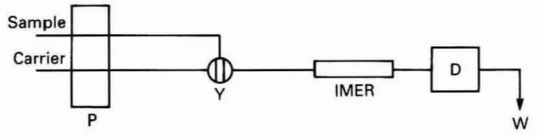

Fig. 1. Schematic diagram of the flow system for the determination of ammonia: P, pump; V, injection valve; IMER, immobilised enzymic reactor; D, detector; and W, waste

Flow system

A schematic diagram of the flow system is shown in Fig. 1. A four-channel peristaltic pump, Minipuls III (Gilson, Worthington, OH, USA), a Rheodyne Model 5041 sample injection valve (Rheodyne, Cotati, CA, USA) and PTFE tube (0.5 mm i.d.) were used for this work. The carrier, 0.1 M phosphate buffer of pH 8.0, containing 1 mM EDTA, 0.2 mM NADH and 0.01 M oxoglutarate was pumped at a flow-rate of 1.0 ml min^{-1}. The 19-μl sample was directly aspirated into the system and passed through the immobilised GlDH column. The decrease in the absorbance intensity caused by the disappearance of NADH was measured at 340 nm with a Spectronic 2000 ultraviolet - visible spectrophotometer (Bausch & Lomb, Rochester, NY, USA), equipped with an 18-μl flow cell (Hellma, Jamaica, NY, USA; 178.12 QS) and a Hewlett-Packard (Avondale, PA, USA) HP 3392A integrator. The flow system was operated at room temperature (20 °C). The peak height (arbitrary units) was used for the quantification of the ammonia.

Sample preparation

Food samples (cheese and baked ham) were extracted using a modification of the method of Parris and Foglia.[19] The modification consisted of sonicating for 10 min after the aqueous extraction step in order to disperse any clots and break any cells present, thus liberating their contents.

Study of interferences

Independent assays were performed with different aminated substances that were present in the analysed foods. Different concentrations of these interferents were added to 5 p.p.m. NH_4^+ standard solutions and these were then injected into the FI system both with and without the enzymic reactor, to assess if the interference was due to the enzymic reaction or to the inherent characteristics of the interfering agent at the monitoring wavelength.

The interfering substances tested were the following: a mixture of glycine, phenylalanine, arginine, proline, glutamic acid, serine and asparagine (the major amino acids existing in ham) each at a concentration of 5 mg per 100 g of product; asparagine and glycine at concentrations from 10 to 100 mg per 100 g of product; urea and creatinine from 10 to 250 mg per 100 g of product; and ascorbic acid from 10 to 200 mg per 100 g of product.

Results and Discussion

The conditions for the determination of ammonia were optimised by studying the effect of various parameters such as pH, the composition and concentration of the buffer, NADH and oxoglutarate concentration, alternative use of adenosine 5'-diphosphate activator and FI variables. Standard solutions of 100 p.p.m. NH_4^+ (the normal value found in meats[9]) were used in all of the optimisation stages.

The pH of the carrier must be carefully chosen to ensure maximum sensitivity and stability. At pH 9.0 the destruction of NAD^+ was rapid[20] and below pH 7.0 NADH was unstable.[21] The effect of pH between 8.0 and 9.0 on ammonia measurements was examined by adding 0.1 M KOH to the carrier (Fig. 2). A gradual decrease in the analytical signal was observed with increasing pH. A pH of 8.0 was, therefore, used for subsequent experiments to ensure greater sensitivity and NADH stability.

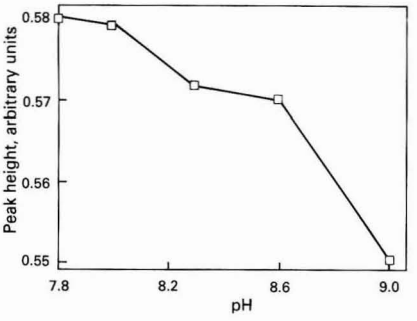

Fig. 2. Effect of pH on the response to 100 p.p.m. of NH_4^+

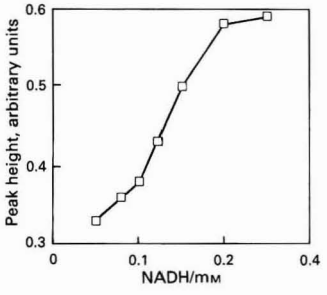

Fig. 3. Effect of NADH concentration on the response to 100 p.p.m. of NH_4^+

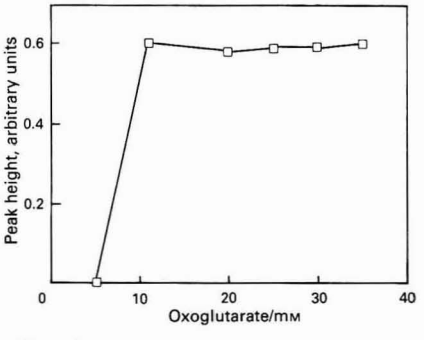

Fig. 4. Effect of oxoglutarate concentration on the response to 100 p.p.m. of NH_4^+

The study of buffer composition was performed with the following two buffers: phosphate[22] and triethanolamine chlorohydrate[9] at pH 8.0. The analytical signal was higher when phosphate buffer was used. The 0.1 M potassium phosphate buffer pH 8.0 was retained for all experiments.

The effect of NADH concentration on the response of the system is shown in Fig. 3; the signal increased with NADH concentration, hence, 0.2 mM NADH was subsequently chosen; at higher concentrations a decrease in the sensitivity of the method was observed. This conclusion is in agreement with that of Tabata *et al.*,[23] who used the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). The oxoglutarate concentration has no influence on the results

Table 1. Effect of the interfering agents on the determination of ammonia

Interfering agent	Concentration of interfering agent/mg per 100 g of sample	Interference, % With enzymic reactor	Interference, % Without enzymic reactor
Amino acids (various)	5 (each)	0.2	0.9
Asparagine	7	0.2	−0.9
	10	0.2	−1.0
	60	1.2	−2.4
	100	0.9	−3.1
Glycine	10	−0.6	−0.3
	60	1.9	−0.6
	100	3.6	−0.8
Urea	10	0.3	0.7
	60	1.2	0.0
	100	0.2	6.8
	250	3.0	8.4
Creatinine	10	0.8	0.0
	60	0.5	1.6
	100	0.5	2.8
	250	0.3	9.8
Ascorbic acid	10	1.0	2.7
	100	4.8	6.6
	200	5.3	9.1

Table 2. Recoveries obtained with the modified extraction method in ham samples

	Concentration of NH_4^+/mM			
NH_4^+ added/mM	Total found	Originally present	Amount recovered	Recovery, %
0.333	5.028	4.727	0.301	90.96
0.133	4.861	4.727	0.134	100.40

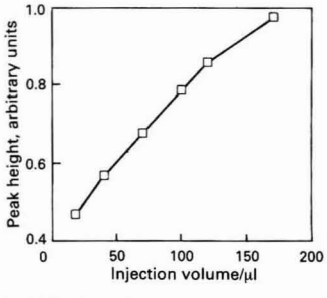

Fig. 5. Effect of injection volume on the response to 100 p.p.m. of NH_4^+

when concentrations greater than 0.01 M (Fig. 4) are used, therefore, this value was chosen as optimum. When adenosine 5′-diphosphate (ADP), disodium salt (from 500 to 4000 μM), was added to the carrier stream as an enzymic activator as described by Bruce *et al.*,[24] no significant difference in the analytical signal was observed.

Under these conditions (0.1 M phosphate buffer of pH 8.0, 1 mM EDTA, 0.2 mM NADH and 0.01 M oxoglutarate) sample injection volumes from 19 to 143.4 μl were investigated. The peak height increased markedly with injection volume (Fig. 5). Therefore, a 19-μl sample injection volume was selected for higher sensitivity and sampling frequency and was used in all subsequent runs.

The flow parameters were optimised by varying the flow-rate of the carrier. Increasing the flow-rate from 0.85 to 2.50 ml min^{-1} does not influence the signal (Fig. 6). The choice of a flow-rate of 1.0 ml min^{-1} was a compromise between the sample output rate and the elimination of over-pressures in the system.

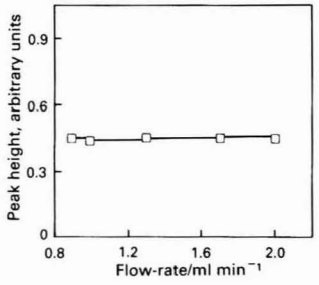

Fig. 6. Effect of flow-rate on analytical signal

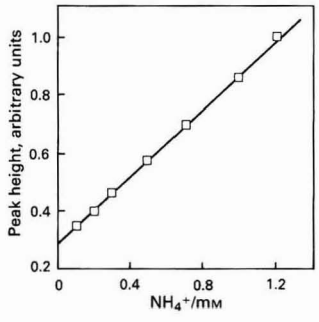

Fig. 7. Calibration graph for ammonia

System Performance

Standard solutions of ammonia were prepared in a 0.1 M phosphate buffer of pH 8.0 and 1 mM EDTA, and injected in triplicate.

Under the working conditions previously stated, the calibration graph (Fig. 7) was linear between 0.05 and 0.60 mM ammonia, with a correlation coefficient (r) of 0.9995. The reproducibility was established by repeated determinations using 0.25 mM NH_4^+. The relative standard deviation of the peak heights was 1.09% ($n = 11$) with a sampling frequency of 60 h^{-1}. When the enzymic reactor was stored at 4 °C, a 20% loss of the initial activity occurred after 500 determinations, over a 3-month period.

Interferences

The effect of the interferents on the results for the determination of ammonia is shown in Table 1. The interferences observed are spectral in most instances and they can be reduced to a minimum by the use of the enzymic reactor. The interference found is less than 1% in nearly every instance and, therefore, negligible as this is similar to the precision of the method. Only the interference caused by urea (at concentrations greater than 100 mg per 100 g of sample) and ascorbic acid (from 100 to 200 mg per 100 g of sample) should be taken into account. The urea interference could be because of the instability of NAD^+ at high pH values. In addition, the presence of urea drives the equilibrium of the NADH/NAD^+ reaction to the right. The interference caused by ascorbic acid is probably due to its reducing capacity, which causes a non-specific consumption of NADH. Amino acids interfere at concentrations greater than 100 mg per 100 g of sample, which do not occur in normal samples.

Application to Food Samples

In order to apply this method to the determination of ammonia in cheese and baked ham, the extracts of the samples were diluted, taking into account the average concentration of ammonia in meats[9] and cheeses,[6] as well as the linear range of the method.

The matrix effect can be considered negligible in the analysed samples, as the differences between the slopes of the calibration graphs run with internal and external references were very small, being 0.19 and 0.21% for ham and cheese, respectively. This, therefore, avoids the necessity to work with an internal reference, thus making the analysis easier.

The recoveries obtained with samples of ham using the modified Parris and Foglia extraction method (Table 2) range between *ca.* 91 and 100%, which is acceptable for these types of samples that contain low levels of ammonia.

Conclusions

The determination of ammonia in foods can be automated by the use of FI; 60 analyses per hour can be carried out. Linearity, recovery and precision parameters obtained confirm the reliability of the results achieved with this technique.

Substances that, according to their labile amino groups, potentially liberate ammonia, such as urea, creatinine and amino acids, do not exert any positive influence. The specificity of the enzymic method facilitates the determination of ammonia, in the presence of other nitrogenous substances common in foods, without previous distillation.

Non-specific reducing agents, such as ascorbic acid, do not exert an effect as they do not consume NADH owing to the reduced analysis times involved in FI systems. As a consequence, the use of NADPH[25] is not necessary.

The FI - immobilised enzyme combination has proved to be excellent for the determination of ammonia in foods, the use of enzyme activators being unnecessary.

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Coulometric Detector Cell for Use With Flow Injection

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A two-electrode cell for constant potential coulometric detection in flow injection systems has been developed. The cell has a saturated calomel electrode with a large surface area and a porous working electrode made of platinum particles. The calomel electrode is not polarised, the potential is kept constant and the electrode serves as both reference and counter electrode. This design of the calomel electrode provides a detector that is simple to construct and easy to use. The cell was tested with the coulometric determination of thallium(I) by means of its oxidation reaction to thallium(III) in a 0.1 M hydrochloric acid medium. A 1.025 V constant potential was applied. Amounts of 0.02–0.17 μequiv. of thallium(I) were determined at flow-rates of 0.2–1.0 ml min^{-1}, respectively. The detection limit was 8 pequiv. of thallium(I) at a signal to noise ratio of 2 : 1 and a flow-rate of 1.0 ml min^{-1}. Amounts between 0.006 and 5.80 μg of thallium(I) were determined by flow injection with a relative standard deviation of 3–6%. A procedure is proposed for calculation of the dispersion in a flow-injection system with coulometric detection.

Keywords: *Coulometric detector cell; flow injection; thallium(I) determination; dispersion calculation*

At the present stage of development of flow injection, coulometric detectors are as yet used only to a limited extent. The coulometric requirement for complete conversion of the electroactive species, *i.e.*, a working electrode with a large surface area and small dimensions, characteristic of the flow injection system, creates difficulties in the designing of the coulometric cell. The constructional refinement of the coulometric cells is, probably, one of the ways to increase their application.

A successful coulometric cell has been described by Johnson and Laroshelle.[1] The basic constructional feature in this cell is the serial position, in the direction of the flow, of the working and counter electrodes. Schieffer[2] has proved experimentally that the radial position of the working and counter electrodes alters the shape of current - potential curves and improves the selectivity of the coulometric detector. A similar configuration of the electrodes has been adopted by other workers.[3]

Another constructional solution is a coulometric cell having two electrodes instead of the usual three.[4–6] In these cell constructions the reference electrode also fulfils the function of a counter electrode. The advantages, in this instance, are simplification of cell construction, reduction of the dead volume and that a potentiostat is no longer necessary (a constant voltage source is sufficient). The disadvantage of these cells is that the maintenance of a constant potential at the reference/counter electrode is achieved by admitting an appropriate electrolyte. The potential of this electrode, though constant, is not always known[6] because the potential of the working electrode is not identical with the voltage applied externally.

The purpose of this work is to create a two-electrode coulometric detector cell that does not suffer the disadvantages mentioned above.

Experimental

Solutions

Use was made of 0.1 M HCl as both carrier solution and supporting electrolyte. The 0.1 M stock standard solution of $TlNO_3$ was prepared directly. The solutions for analysis were obtained by appropriate dilution of the stock solution. All reagents were of analytical-reagent grade, and distilled water was used throughout. De-aeration of the solutions was not necessary.

Apparatus

The experiments are carried out with a flow-injection system, a schematic diagram of which is shown in Fig. 1. The flow is generated by an Ismatec peristaltic pump. Two types of laboratory-constructed injector valves are used. One of these has loops of volume 8.3 and 23 μl. The other, combined with an Eppendorf pipette, is used for other volumes as appropriate; when a polarograph is used as the measuring device this injection valve is used for checking and calibration. The coulombic content of the flow-injection peak is determined by the integrating device of an OH-404 coulometer (Radelkis, Hungary). This instrument can be used for the determination of μg amounts of thallium(I). This corresponds to the maximum amounts that can be determined by coulometry when the suggested detector is used. The measured electric charges in this instance are of the order of mC, which is at the lower limit of the capacity of the device. For the determination of smaller amounts of thallium(I), use is made of an OH-105 polarograph (Radelkis). The electric charges, in this instance, are calculated from the peak area, determined by weighing the chart recorder paper. An EP20A potentiostat (Elpan, Poland) and a PM8251 A recorder (Philips, The Netherlands) are used, when the recorded currents are of the order of mA. Two types of coulometric cells are used: the two-electrode cell discussed here and a cell of analogous construction but with three electrodes. The latter is used for the determination of electrochemical conversion efficiency in a steady-state regime. The currents recorded with this study are, in some instances, of the order of mA.

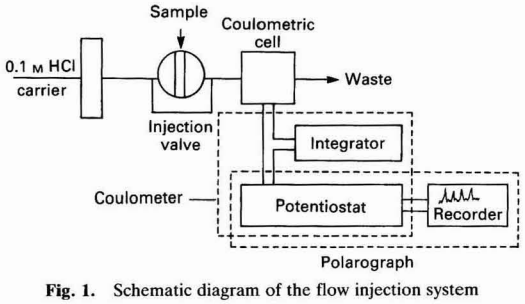

Fig. 1. Schematic diagram of the flow injection system

Electrochemical Conversion Efficiency

Determination of the electrochemical conversion efficiency, under both flow injection and steady-state conditions, is described elsewhere.[3]

Coulometric Cell Design

The coulometric cell (Fig. 2) is fabricated from a Plexiglas block (1) 60 mm in diameter and 70 mm in length. In the body of the cell is drilled a horizontal channel, 12.5 mm in diameter, as a working electrode compartment and a vertical cylindrical chamber (13), 20 mm in diameter and 50 mm in length, which serves as a container for the saturated calomel electrode. In a Plexiglas cylinder (2), 12 mm in diameter and 28 mm in length, is drilled a lengthwise channel, 6 mm in diameter. Perpendicular to this channel 24 holes of 1.0 mm diameter are arranged in four lines. An ion-exchange membrane sheet is formed as a cylinder (3) and inserted into the cylinder (2) described above. The seam formed by the edges of the membrane sheet must be aligned between two of the lines of holes with 1.0 mm diameter mentioned above. Then the cylinder (2) containing the membrane is set in water for several minutes until the membrane sheet swells and sticks tightly on to a Plexiglas channel of the cylinder (2). The interior of the resultant channel (15) is firmly packed with platinum chips (*ca.* 0.1 mm in length) to form the working electrode. Small plugs made of platinum wire with a diameter of 0.1 mm are inserted into each end of the packed channel to prevent the platinum chips from being forced out by the flowing stream. Then cylinder (2), containing the working electrode is set in the horizontal channel of the cell and attached to the body by means of O-rings (11) and Plexiglas fittings (4). A piece of platinum wire (9) and a bronze jack (6) are used to make an electrical contact to the porous working electrode. PTFE fittings (5) and tubing (14, 16) are used as the inlet and outlet. The vertical chamber (13) is filled with mercury until it covers the cylinder (2). A mixture of calomel, mercury and saturated potassium chloride solution, is added to form a saturated calomel electrode. The cell is closed by use of a rubber gasket (10) and a Plexiglas plug (8). The mercury of the calomel electrode makes contact with the ion-exchange membrane (3) by means of the 1.0-mm holes in the Plexiglas cylinder (2). The membrane separates the calomel and porous working electrode allowing contact between them via the cell solution. A stainless-steel jack (7), attached to the body of the cell by means of the O-ring (12), is used as an electrical contact to the mercury of the saturated calomel electrode.

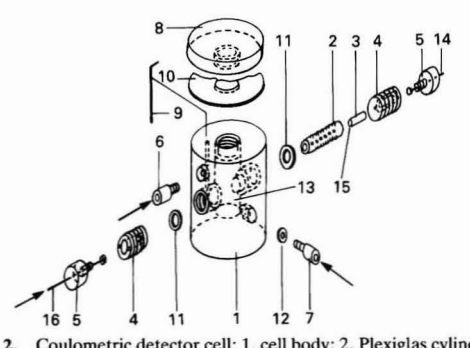

Fig. 2. Coulometric detector cell: 1, cell body; 2, Plexiglas cylinder; 3, ion-exchange membrane; 4, Plexiglas fittings; 5, PTFE fittings; 6, bronze jack; 7, stainless-steel jack; 8, Plexiglas plug; 9, platinum wire; 10, rubber gasket; 11 and 12, O-rings; 13, saturated calomel electrode chamber; 14 and 16, PTFE tubing; and 15, porous platinum working electrode

Results and Discussion

In the two-electrode detector cell constructed here, a saturated calomel electrode with a large surface area acts simultaneously as both reference and counter electrode. Data from the literature[1–6] and our experience with the detector, indicate that under conditions of flow injection with coulometric detection, the current values vary from nA to μA. Such currents are unable to produce a noticeable polarisation of a saturated calomel electrode with a large surface area. Such electrodes are used in polarography and in amperometric titration. An additional flow line through the electrode is not necessary. In the coulometric cell a radial contact between the working porous platinum electrode and calomel electrode is achieved. This provides advantages with regard to the hydrodynamic current - potential curves and selectivity of the cell. The cell can be constructed very simply and is easy to use.

The optimum potential of the working electrode for thallium(I) oxidation is accepted to be 1.025 V, established by the dependence of the flow injection coulometric signal on the potential, when all other conditions (flow-rate, concentration and volume of the sample) are equal (Fig. 3).

In order to perform the determination coulometrically it is necessary that the electroactive compounds be completely converted as the sample passes through the cell. Hence, the maximum amount of substance that can be determined will depend on the flow-rate. Fig. 4 shows the dependence of the electrochemical conversion efficiency on the amount of substance determined and the flow-rate. This means that the maximum amount of sample that can be determined for a given flow-rate can be calculated. Thus, for flow-rates of 0.2 and 1.0 ml min^{-1} the amounts that can be determined are ≤0.17 μequiv. and ≤0.02 μequiv., respectively. Flow-rates

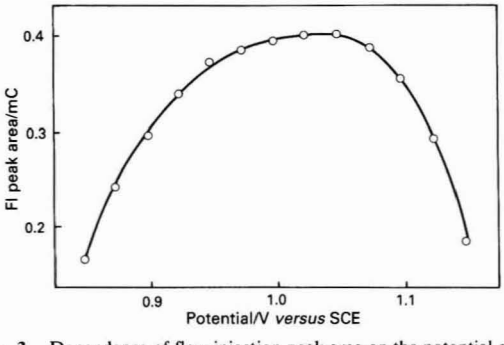

Fig. 3. Dependence of flow injection peak area on the potential of the working electrode. $TlNO_3$, 1.0×10^{-4} M; sample volume, 23 μl; and flow-rate, 1.0 ml min^{-1}

Table 1. Coulometric determination of thallium(I) by flow injection

Amount of Tl^I present/ μg	Number of determinations, n	Amount Tl^I found $\bar{X} \pm \frac{t_\alpha * s}{\sqrt{n}}$ /μg	RSD, %
5.80	10	5.77 ± 0.17	4
5.20	14	5.15 ± 0.09	3
2.60	13	2.62 ± 0.09	6
1.70	5	1.76 ± 0.09	3
0.85	5	0.858 ± 0.006	5
0.20	6	0.184 ± 0.006	3
0.075	5	0.0725 ± 0.0034	4
0.006	7	0.0057 ± 0.0003	5

* t_α = 0.95 (Students t value at 95% probability); s = standard deviation; n = number of determinations.

Table 2. Determination of dispersion by means of a coulometric detector

	Flow-rate/ml min^{-1} (V_s = 8.3 μl)						V_s/μl (flow-rate = 1.0 ml min^{-1})	
Dispersion	0.2	0.5	0.75	1.0	1.5	2.0	10	50
Calculated using equation (3)	7.4 ± 0.4	10.8 ± 0.9	14.1 ± 1.3	17.1 ± 0.9	23.8 ± 0.5	31.7 ± 0.9	13.3 ± 0.2	2.8 ± 0.1
Calculated using equation (1)*	7.2 ± 1.0	11.1 ± 1.1	14.5 ± 0.3	17.5 ± 0.9	23.8 ± 0.3	29.8 ± 2.1	13.2 ± 0.7	2.8 ± 0.1

* Reference 8.

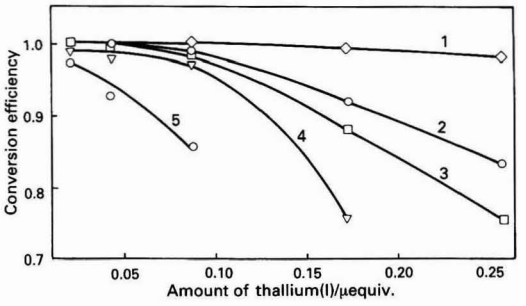

Fig. 4. Dependence of the electrochemical conversion efficiency under flow injection conditions on the amount of thallium(I) (μequiv.). Flow-rate: 1, 0.2; 2, 0.5; 3, 0.75; 4, 1.0; and 5, 1.5 ml min^{-1}

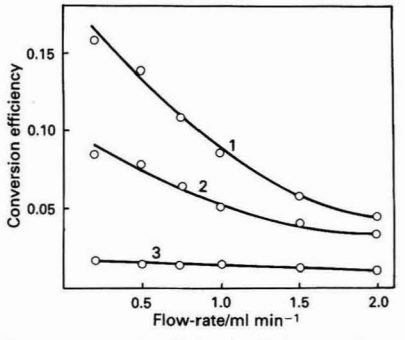

Fig. 5. Dependence of the electrochemical conversion efficiency under steady-state conditions on the flow-rate (ml min^{-1}). Concentration of thallium(I): 1, 1.0 × 10^{-3}; 2, 5.0 × 10^{-4}; and 3, 1.0 × 10^{-4} mol l^{-1}

>1.0 ml min^{-1} show no particular advantages but they reduce considerably the amount of substance that can be determined by coulometry.

The detection limit at a signal to noise ratio of 2:1 and a flow-rate of 1.0 ml min^{-1} is 8 pequiv. It should be pointed out that the fluctuations of the baseline, due to pump pulsations, are counted as noise. To remove these fluctuations use is made of the damping device of the polarograph, however, it lowers the height of the peak. The data about the maximum and minimum determinable amounts are obtained from the determination of thallium(I), but they also apply to any other electrochemical reaction and in this respect they appear to be a characteristic of the cell and flow-injection system used.

Table 1 presents the data from the coulometric determination of various amounts of thallium(I). There appears to be a good correlation between the amount of substance known to be present and the amount determined. The relative standard deviation (RSD) is approximately the same for any substance being determined. This indicates that the coulometric detector works similarly over the range of substances that can be determined.

Another basic characteristic feature concerning the coulometric cell is dispersion, D. It is calculated[7] in relation to the concentration of the injected sample and the concentration corresponding to the maximum of the flow injection signal. For photometric detectors D is determined in relation to the signal resulting from continuous passing of the sample (steady-state signal) and peak height. With the working conditions of the cell, suggested here (Fig. 4), the flow injection signal is coulometric, *i.e.*, the efficiency of the electrochemical conversion is 1 (100%). Under steady-state conditions the effectiveness of electrochemical conversion for different flow-rates and solution concentrations is low, as is seen in Fig. 5 and the conditions differ very much from the coulometric ones. Hence, the calculation of D by the method mentioned above would give incorrect results.

A method is suggested[8] for determining D in electrochemical detectors, by which, instead of the experimentally obtained steady-state signal use is made of the integral of the flow injection current - time curve, which is determined by means of a personal computer. By this method D is calculated using the equation:

$$D = \frac{q \int i \, \mathrm{d}t}{0.06 \, i_{\max.} \, V_s} \quad \ldots \ldots \ldots \quad (1)$$

where q is the flow-rate in ml min^{-1}; $i_{\max.}$ is the peak height in μA; $\int i \, \mathrm{d}t$ is the coulombic content of the flow injection peak area in μC; and V_s is the volume of injected sample in μl. For the coulometric detectors, the following is obtained from Faraday's Law

$$\int i \, \mathrm{d}t = c \, n \, F \, V_s \quad \ldots \ldots \ldots \quad (2)$$

where c is the concentration of the sample in mol l^{-1}; F is the Faraday constant (96 486.4 C equiv.$^{-1}$); and n is the number of electrons transferred per molecule. The following is obtained from equations (1) and (2):

$$D = \frac{q \, c \, n \, F}{0.06 \, i_{\max.}} \quad \ldots \ldots \ldots \quad (3)$$

Hence, to determine the dispersion using equation (3), particularly in the coulometric cells, only experimental data about the peak height are required and it is not necessary to determine the electric charges. With the suggested coulometric detector cell, D is determined using equations (1) and (3) and the data are presented in Table 2. There are no statistical differences in the D values calculated using the various equations.

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Flow Injection of Lithium Ion Using Chromogenic 14-Crown-4 Derivatives as Extraction - Spectrophotometric Reagents

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A system for flow injection of Li^+ has been designed, with use of proton-dissociable chromogenic 14-crown-4 derivatives as the extraction - spectrophotometric reagents, and the analytical conditions have been optimised. This flow injection system showed high selectivity for Li^+ reflecting the cation-complexing property of the chromogenic crown ethers. The determination of Li^+ in the clinical range in blood under a high Na^+ background of 130–160 mM was feasible, with a small sample size (50 μl) and high sampling rate (more than 100 injections per hour), with this method. The proposed extraction - spectrophotometric flow injection system was, therefore, found to be promising for the efficient determination of Li^+ in biological samples, such as blood sera, with a high Na^+ background.

Keywords: *Flow injection; lithium ion; 14-crown-4; chromogenic crown ether; extraction spectrophotometry*

Spectrophotometry, combined with solvent extraction, with use of crown ether derivatives, is convenient for enhancing sensitivity and selectivity in spectrophotometry. Extraction spectrophotometry is, however, likely to be time-consuming if batch systems are adopted. Flow injection (FI) could solve the significant problem in corresponding batch systems to realise rapid extraction spectrophotometry. In most instances, ion-pair extraction systems haye been applied to FI. There are only a few examples of FI, based on solvent extraction, involving use of proton-dissociable (potentially anionic) crown compounds.[1,2]

The small-size crown ether, 1,4,8,11-tetraoxacyclotetradecane (14-crown-4), is an excellent component for Li ionophores.[3] Incorporation of a proton-dissociable chromogen, such as nitrophenol or nitroazophenol, in the 14-crown-4 molecule, as shown in compounds (**1**)–(**6**) (Fig. 1), affords attractive properties, induced by the cation complexation of the 14-crown-4 ring. The proton-dissociable chromogenic crown ethers can bind Li^+ selectively. Under conditions basic enough to dissociate a proton on the chromogen, the resulting anion interacts strongly with the crown-complexed Li^+ in such a way as to compensate the electrical charge intramolecularly. As a result, under these conditions, the proton-dissociable crown ethers can extract Li^+ selectively from an aqueous into an organic phase, the extractability being independent of the type of counter anion of the salt used. Further, the proton dissociation and cation complexation of the chromogenic crown ethers causes significant perturbation of the chromogen, bringing about drastic spectral changes to the crown ethers.

Under acidic conditions, the intramolecular Li^+ complexes of the chromogenic crown ethers are easily protonated, releasing the metal ion. Hence, the 14-crown-4 derivatives carrying a proton-dissociable chromogen were found to be promising Li^+-selective reagents for metal ion indication, extraction spectrophotometry,[4] and separation and enrichment by uphill membrane transport.[5,6]

Our study on extraction spectrophotometry with the chromogenic 14-crown-4 derivatives prompted us to develop further the Li^+-selective extraction system into an FI system. We have already reported that one of the chromogenic 14-crown-4 derivatives is applicable to Li^+ FI based on extraction spectrophotometry.[7] Here, we report the Li^+ extraction - spectrophotometric FI of synthetic blood serum samples, which possess a high background of Na^+, by using several proton-dissociable chromogenic 14-crown-4 derivatives [(**1**)–(**6**)] (Fig. 1). The application of this FI method to the determination of Li^+ in blood serum, in the clinical range,[8] is described.

Experimental

Reagents

The chromogenic crown ethers used were prepared as reported previously.[4] The organic solvents for the extraction were distilled. The metal salts were of analytical-reagent grade. The water was demineralised and distilled. Unless specified otherwise, the synthetic serum samples contained 145 mM NaCl, 4.5 mM KCl, 5.0 mM $CaCl_2$, 1.6 mM $MgCl_2$, 2.5 mM urea, 4.7 mM glucose and an appropriate amount (0.5–2.0 mM) of LiCl.

Apparatus

A diagram of the FI manifold is given in Fig. 2. The polytetrafluoroethylene (PTFE) tubing used was of 0.5 mm i.d., unless specified otherwise. An organic solvent for the organic carrier stream and a buffer solution (H_3BO_3 - KOH) of appropriate pH for the aqueous carrier stream were delivered individually at a constant flow-rate by a quadruple-plunger pump (JASCO RP-4F). Aliquots of an aqueous sample solution and a crown ether (generally 3×10^{-4} M) solution were injected simultaneously into the aqueous and organic carrier streams, respectively, through six-port rotary valves, and then merged synchronously at a T-shaped segmentor. The aqueous phase was caused to flow straight through the segmentor, and the organic phase was merged vertically with the aqueous phase stream. The organic phase was separated by a flow-through phase separator (Hitachi 155-0820) fitted with a microporous membrane filter (Fluoropore, pore size 0.8 μm) and was then introduced into a spectrophotometric detector (UVIDEC-100-VI) via a 0.2-m PTFE tube of 0.5 mm i.d. The absorbance was monitored at 410 nm for systems (**1**)–(**4**), at 488 nm for system (**5**) and at 582 nm for system (**6**). The PTFE tubes for the back-pressure coils BC_1 and BC_2 were 4 m (0.5 mm i.d.) and 0.5 m (0.25 mm i.d.) in length, respectively. Four sets of injections were performed for each sample.

Extraction Equilibria and Calibration Graph

The extraction equilibria have been described in detail in a previous paper.[4] If one assumes that the chromogenic crown ether (HL) loses a proton (H^+) to yield a corresponding

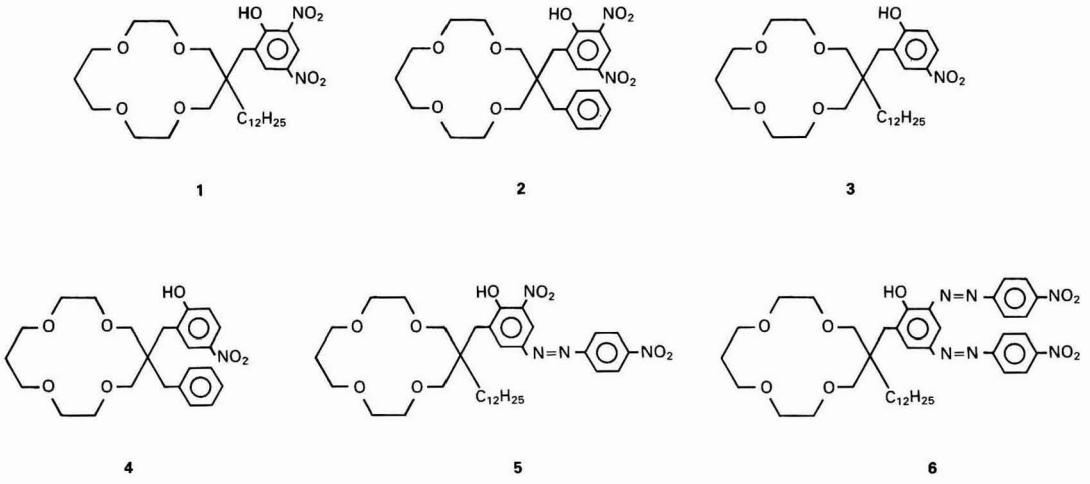

Fig. 1. Structures of the 14-crown-4 derivatives studied

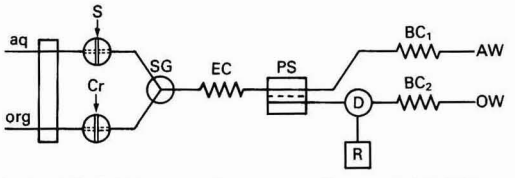

Fig. 2. Manifold for extraction - spectrophotometric FI of Li^+. aq, Aqueous carrier stream; org, organic carrier stream; AW, aqueous waste; OW, organic waste; SG, segmentor; EC, extraction coil; PS, membrane phase separator; BC_1 and BC_2, back-pressure coils; D, spectrophotometric detector; and R, recorder

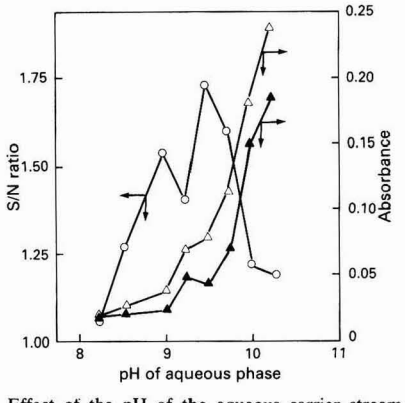

Fig. 3. Effect of the pH of the aqueous carrier stream on the absorbance and S/N ratio for synthetic blood sera with a high Na^+ background using the chromogenic crown ether (**1**) as extraction - spectrophotometric reagent. ○, S/N ratio for 1 mM Li^+; △, absorbance for 1 mM Li^+; and ▲, absorbance in the absence of Li^+. Na^+ background concentration, 145 mM; concentrations of the other components of the synthetic serum samples are as given under Experimental. Organic carrier stream, $CHCl_3$; flow-rate, 0.75 ml min^{-1} (both aqueous and organic carrier streams); sample, synthetic blood serum (Na^+ background, 145 mM) with and without 1 mM Li^+ (50 μl per injection); reagent, 3×10^{-4} M crown ether solution in $CHCl_3$ (50 μl per injection); and extraction coil, PTFE tube (1 m)

anionic species (L^-) and then forms a 1 : 1 complex (ML) with a metal ion (M^+), the extraction equilibrium constant K_{ex} is defined as follows:

$$K_{ex} = ([ML]_o[H^+]_a)/([HL]_o[M^+]_a) \quad . . \quad (1)$$

where the subscripts "o" and "a" denote the organic and aqueous phases, respectively.

As the following assumptions are reasonable, *i.e.*, $[HL]_i \approx [HL]_o + [ML]_o$, $[M^+]_i \approx [M^+]_a$ ("i" represents "initial"), $[HL]_a \approx 0$, $[ML]_a \approx 0$, and $[L^-]_o \approx 0$, equation (1) can be transformed into equation (2):

$$[H^+]/[M^+]_i = K_{ex}(\varepsilon_{ML} - \varepsilon_{HL})A/ \varepsilon_{HL}(A - A_i) - K_{ex}\varepsilon_{ML}/\varepsilon_{HL} \quad (2)$$

Here, ε is the molar absorptivity of the respective species, and A_i and A are the absorbances at a given wavelength for the organic phase before and after extraction of the cation, respectively. Equation (2) can be applied to the extraction systems by using the chromogenic crown ether (**4**). If the chromogenic crown ether (HL species) is negligibly distributed in the aqueous phase, then equation (2) can be simplified to equation (3). The latter equation is valid for extraction systems of the comparatively lipophilic crown ethers (**1**), (**2**), (**3**), (**5**) and (**6**):

$$[H^+]/[M^+]_i = K_{ex}\varepsilon_{ML}[HL]_i/A - K_{ex} \quad . . \quad (3)$$

Hence, graphs of $A/(A - A_i)$ or $1/A$ *versus* $1/[M^+]_i$ yield straight lines. Therefore, these linear plots were adopted as calibration graphs throughout this FI study.

Results and Discussion

The aim of this study was to establish conditions for the clinical range determination of Li^+ (0.5–2 mM) in synthetic blood sera with high Na^+ concentrations by extraction - spectrophotometric FI, using the crown nitrophenols or azophenols (**1**)–(**6**) as proton-dissociable chromogenic crown ethers. In order to preserve the crown ether reagents, a merging-zone method was adopted (Fig. 2), *i.e.*, aqueous and organic carrier streams were pumped at an identical flow-rate. The same amounts of a sample and a chromogenic crown ether solution were injected into the aqueous and organic streams, respectively, and then merged at a segmentor. Extraction of Li^+ was achieved with an extraction coil. The organic phase was then separated and

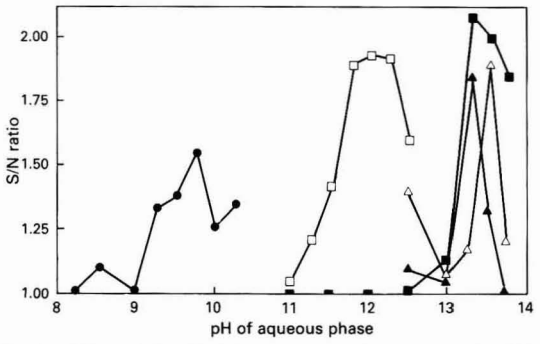

Fig. 4. Effect of the pH of the aqueous carrier stream on the S/N ratio using chromogenic crown ethers (**2**)–(**6**). Chromogenic crown ether: ●, (**2**); △, (**3**); ▲, (**4**); □, (**5**); and ■, (**6**). Other FI conditions as in Fig. 3

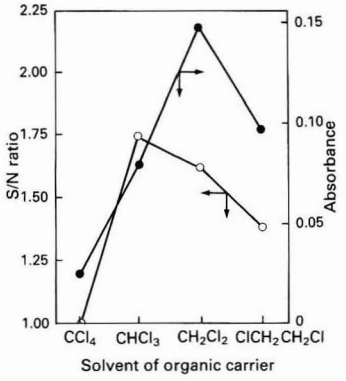

Fig. 5. Selection of solvent for the organic carrier stream using the chromogenic crown ether (**1**). ○, S/N ratio; and ●, absorbance. Aqueous carrier stream, H_3BO_3 - KOH (pH 9.5). Other FI conditions as in Fig. 3

the metal ion complex of the chromogenic crown ether was detected spectrophotometrically. Optimisation of various FI conditions for Li^+ FI was therefore attempted.

Chromogenic Crown Ethers and pH of Aqueous Carrier Stream

In the extraction of metal ions by proton-dissociable crown ethers, the extractability depends considerably on the pH of the aqueous phase. The pH of the aqueous carrier stream is, therefore, an important factor for extraction - spectrophotometric FI, using the proton-dissociable crown ethers. The pH of the aqueous phase was changed continuously by H_3BO_3 - KOH buffer systems, while the other FI conditions were fixed as given in Fig. 3. This figure illustrates the effect of the pH of the aqueous phase on the absorbance and signal to noise (S/N) ratio observed at the detector for the organic phase of the FI system when the 14-crown-4 dinitrophenol (**1**) is used.

The absorbance is measured at the wavelength of the chromogen for proton-dissociated species or for its metal ion complex of the crown ether. The S/N ratio is defined here as the ratio of the absorbance for synthetic serum samples with and without Li^+. The "noise" consists primarily of a background signal derived from high concentrations of Na^+ in the samples. The absorbance and S/N ratio are useful indicators of the sensitivity and selectivity of the determination of Li^+ in blood sera by extraction - spectrophotometric FI. At a lower pH, the crown ether cannot proton-dissociate to any great extent, so the metal ion extractability of the crown ether is low. Increasing the pH promotes the proton-dissociation of the chromogen and hence extraction of the cation, which increases the absorbance at the detector. This is true for the synthetic serum samples both with and without 1 mM Li^+. This is partly because the serum samples contained a high concentration (145 mM) of Na^+. Further, the aqueous carrier stream of higher pH, which was adjusted by means of the H_3BO_3 - KOH buffer system, included a larger amount of K^+. Considerable amounts of Na^+ and K^+ and also of Li^+ can, therefore, be extracted under these aqueous carrier stream conditions, despite the high Li^+ selectivity of the chromogenic 14-crown-4 derivative against Na^+ and K^+ in the extraction (Li^+ : Na^+ selectivity ratio of *ca.* 100).[4] As a result, the Li^+ extraction - spectrophotometric FI of the blood sera still suffered from the significant background signal, mainly due to Na^+ in the sera, as shown in Fig. 3. Accordingly, the S/N ratio is a crucial factor for the successful determination of Li^+ in blood sera by use of this FI system. The highest S/N ratio was attained in the pH range 9–10 for the aqueous carrier stream in the FI system, with use of the chromogenic crown ether (**1**).

For the crown dinitrophenol system (**2**), a similar tendency was observed in the relationship between the S/N ratio and pH (Fig. 4). For the crown nitrophenol and azophenol systems (**3**)–(**6**), the S/N ratio peak shifted to the higher pH values. The pH of the aqueous carrier stream that allows the highest S/N ratio appears to be closely related to the acidity constants of the chromogenic crown ethers.[4] The optimum pH of the aqueous carrier stream was, consequently, 9.5, 9.8, >13, >13, 12 and >13 for the crown ethers (**1**)–(**6**), respectively. If mild pH conditions for the aqueous carrier stream are desired in the determination of Li^+ using extraction - spectrophotometric FI, the crown dinitrophenols (**1**) and (**2**) are recommended as the chromogenic extracting reagents. The chromogenic crown ether (**1**) is superior to (**2**) with respect to the maximum S/N ratio. For the optimisation of the other FI conditions, the 14-crown-4 dinitrophenol (**1**) was, therefore, selected.

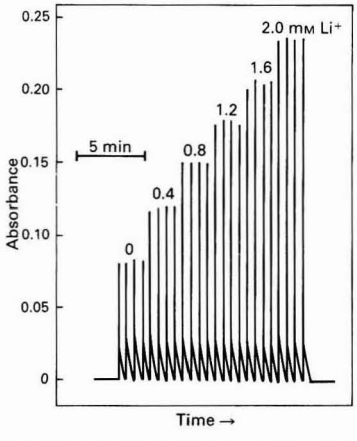

Fig. 6. Typical FI profile for extraction - spectrophotometric FI of Li^+ for synthetic sera containing a high Na^+ background. Reagent, chromogenic crown ether (**1**) under optimised FI conditions

Organic Carrier Stream

Four chlorinated hydrocarbons, *viz.*, chloroform, dichloromethane, 1,2-dichloroethane and carbon tetrachloride, were tested as the organic carrier stream for the extraction - spectrophotometric FI of Li^+, taking into account the solubility of the chromogenic crown ethers, the water immiscibility and the phase separation characteristics. With respect to S/N ratio, chloroform is the best solvent, while in terms of

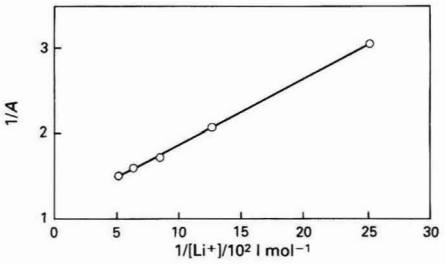

Fig. 7. Calibration graph for extraction - spectrophotometric FI of synthetic sera using the chromogenic crown ether (**1**)

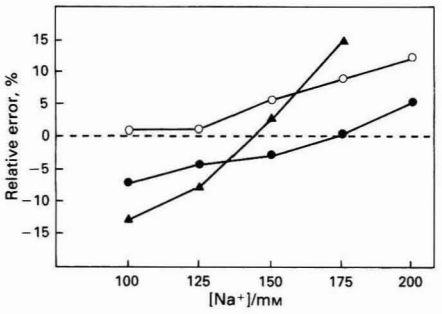

Fig. 8. Dependence of the reliability of the found Li^+ value on the Na^+ concentration of synthetic blood sera. Li^+ concentration: ▲, 0.7; ○, 1.0; and ●, 1.5 mM. Flow injection conditions as in Fig. 6. The calibration graph is based on synthetic sera with an Na^+ background of 145 mM

absorbance, dichloromethane is the best (Fig. 5). Chloroform is preferable to dichloromethane in terms of the reproducibility of absorbance readings.

Flow-rates of Carrier Streams

The flow-rate of the aqueous and organic carrier streams was varied from 0.5 to 1.5 ml min^{-1} in increments of 0.25 ml min^{-1}. Flow-rates of 0.75 and 1.0 ml min^{-1} gave fairly high absorbance values, but 0.75 ml min^{-1} was the best flow-rate in terms of S/N ratio. With an initial flow-rate of 0.75 ml min^{-1}, the flow-rate of the organic phase beyond the phase separator was controlled to be *ca.* 0.6 ml min^{-1} by means of the back-pressure coils (see under Experimental).

Extraction Coil Length

The effects of extraction coils 0.5, 1.0, 2.0 and 5.0 m in length on the sensitivity and selectivity of extraction - spectrophotometric FI were investigated. Coil lengths ranging from 0.5 to 2.0 m afforded comparatively high absorbance values, and it was considered reasonable to adopt an extraction coil of length 1.0 m from the viewpoint of selectivity (S/N ratio). Longer extraction coils (>2 m) decreased the sensitivity, probably as a result of the diffusion of the crown ether reagent, and also resulted in a long analysis time per sample. More than 100 injections of the sample were possible in the FI system with a carrier stream flow-rate of 0.75 ml min^{-1} and an extraction coil length of 1 m.

Calibration Graph

The Li^+ extraction - spectrophotometric FI of the synthetic blood sera by use of chromogenic crown ether (**1**) was carried out under the following optimised conditions. Aqueous carrier stream: pH 9.5 (H_3BO_3 - KOH buffer); organic carrier stream: $CHCl_3$; flow-rate of the carrier stream: 0.75 ml min^{-1}; and extraction coil length: 1.0 m. Fig. 6 depicts the typical FI profile for synthetic blood sera containing 0–2 mM of Li^+, showing the good reproducibility of the FI signals in spite of the high Na^+ background concentration (145 mM). Also, a plot of $1/A$ *versus* $1/[Li^+]$ gave a straight line with a correlation coefficient of 0.9995, providing an excellent calibration graph for the proposed method (Fig. 7). The FI of Li^+ with use of the other chromogenic crown ethers (**2**)–(**6**) was also performed under the respective optimum pH values for the aqueous carrier stream and under the same FI conditions employed for system (**1**). The calibration graphs (plots of $1/A$ *versus* $1/[Li^+]$) showed good linearity for systems (**2**), (**3**), (**5**) and (**6**). On the other hand, the plots of $A/(A - A_i)$ *versus* $1/[Li^+]$ afforded linear calibration graphs for the chromogenic crown ether system (**4**).

Effect of Variation of Na^+ Concentration in Sera

Despite the high selectivity of the chromogenic crown ethers for Li^+ against Na^+, the background signal obtained with high concentrations of Na^+ in blood sera cannot be ignored in the FI determination of Li^+ in blood serum in the clinical range. It is, therefore, of interest to see how the variation of the Na^+ concentration in blood sera affects the determination of Li^+ using extraction - spectrophotometric FI. Attempts were made to determine Li^+ in the clinical range in synthetic blood sera with varying concentrations of Na^+ from 100 to 200 mM using the proposed FI method. The results are presented in Fig. 8, which shows the relationship between the Na^+ concentration and the relative error in Li^+ concentration obtained with extraction - spectrophotometric FI for synthetic serum samples spiked with 0.7, 1.0 and 1.5 mM Li^+. As expected, in the samples containing the lower Li^+ concentrations, the variation in Na^+ concentration affected considerably the values found for the Li^+ concentration. In the Na^+ concentration range 130–160 mM, however, the relative errors were within several per cent. These results clearly indicate that the proposed extraction - spectrophotometric FI method is useful for the determination of Li^+ in blood sera.

Conclusions

Extraction spectrophotometry with use of the proton-dissociable chromogenic crown ethers (**1**)–(**6**) has been extended to FI in combination with a merging-zone method and membrane-phase separator. High efficiency in extraction spectrophotometry, *i.e.*, small sample size and high sampling rate, was attained under the optimised FI conditions. The determination of Li^+ in the clinical range in synthetic blood sera containing a high Na^+ background was performed successfully using the proposed FI method, which afforded reliable data for samples containing 130–160 mM Na^+ by using a calibration graph obtained for synthetic sera containing 145 mM Na^+. Hence, extraction - spectrophotometric FI is very promising for the determination of Li^+ in human blood sera during lithium therapy of manic-depressive patients. As the detection limit is 1×10^{-7} M Li^+ for aqueous solutions (without Na^+) of pure Li^+, this extraction - spectrophotometric FI method could also be applicable as a highly sensitive method for the determination of Li^+.

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Kinetic Determination of Tellurium Based on Its Inhibitory Effect on the Palladium(II)-catalysed Reaction Between Pyronine G and Hypophosphite Ion

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A kinetic method for the determination of Te based on its inhibitory effect on the Pd^{II}-catalysed reaction between pyronine G and $H_2PO_2^-$ is described. The influence of experimental variables on the rate of the process and the potential interfering effect of a large number of ions has been studied. Under the selected experimental conditions: 6×10^{-5} M pyronine G; 0.6 M $H_2PO_2^-$; pH 2.6, adjusted with Britton - Robinson buffer; 0.80 μg ml^{-1} of Pd^{II}; and a temperature of 22 ± 0.2 °C, Te was determined in the concentration range 0.08–0.85 μg ml^{-1}. The method was applied to the determination of Te in waters and lead concentrates.

Keywords: *Kinetic determination; tellurium; palladium(II) - pyronine G - hypophosphite*

In earlier work[1] it was found that the hypophosphite ion ($H_2PO_2^-$) does not reduce pyronine G over a wide pH range, whereas in the presence of Pd^{II} it is reduced owing to the catalytic effect of Pd^{II}. It was also found that some ions exert a strong inhibitory effect on this catalysed system. From this, sensitive kinetic methods have been developed for the determination of Pd^{II}, Ag^{I}, I^- and As^{III}.[1,2]

This paper reports on the modifying effect of Te on the Pd^{II}-catalysed reaction between pyronine G and $H_2PO_2^-$. Tellurium which, by itself, does not act on the reduction of pyronine G by $H_2PO_2^-$, does inhibit the catalysed reaction, the rate of which is decreased in proportion to its concentration. The proposed method was applied with excellent results to the determination of Te in different types of samples containing various amounts of this element.

Tellurium finds use in the metallurgical industry as a constituent of alloys; it improves the acid resistance of lead used in batteries. Other uses include the manufacture of heat and abrasion resistant rubber. It appears as a by-product in the electrolytic refining of copper where its determination in the presence of several other elements is important. In the semiconductor industry its determination at ultra-trace levels in Te-doped single crystals is often required.

Several analytical methods for the determination of Te have been described, among them electroanalytical,[3–7] spectrophotometric,[8–14] spectrofluorimetric[15–17] and atomic absorption and fluorescence spectrometric methods many of them using the hydride generation technique.[18–24] There are a number of kinetic methods for the determination of Te. In these methods Te acts either as a catalyst in reactions in which $SnCl_2$ is a reducing agent for substrates such as tartrate[25] or the complex of Fe^{III} with tartrate[26] or as an activator in the reduction of Co^{III} - ethylenediaminetetraacetic acid by $SnCl_2$ catalysed by colloidal Au.[27]

Experimental

Apparatus

Absorbance *versus* time (*A* - *t*) graphs were recorded on a Perkin-Elmer 550SE ultraviolet - visible spectrophotometer furnished with 1-cm path length cells thermostated by means of a Minicryostat MCIII apparatus. Measurements of pH were made with a Radiometer PHM63 pH-meter.

Reagents

Pyronine G. An aqueous solution containing 0.1816 g l^{-1} was prepared from 3,6-bis(dimethylamino)xanthene [Colour Index (C.I.) 45005].

NaH_2PO_2, 3 M. This solution should be renewed frequently.

$PdCl_2$, 5×10^{-3} M. Made in 0.2 M HCl and titrated as described elsewhere.[28]

Tellurite stock solution, 1×10^{-2} M. This was prepared by dissolving previously dried K_2TeO_3 (Merck) in water. Working standards were subsequently prepared by appropriate dilution with water.

Britton - Robinson buffers of different pH values between 1.7 and 4.4 were used.

Solutions of various metal nitrates and the sodium salts of different anions were prepared for the study of interferents. All the solutions were prepared using analytical-reagent grade reagents and doubly distilled water. Solutions of lower concentrations were prepared by accurate dilution as required.

Recommended Procedure for the Determination of Tellurium

The thermostated spectrophotometer cells were filled with 0.3 ml of 6×10^{-4} M pyronine G, 0.5 ml of buffer of pH 2.60, 0.2 ml of 12.0 μg ml^{-1} Pd^{II} and different volumes of 2.54 μg ml^{-1} Te^{IV} to give a final concentration between 0.08 and 0.85 μg ml^{-1}. The samples were then diluted to 2.40 ml with distilled water and the cells were placed in the thermostat at 22 ± 0.2 °C for 10 min. Then 0.6 ml of 3 M $H_2PO_2^-$ was added and the spectrophotometer recorder started in order to measure the time accurately.

After the sample had been homogenised by stirring, the cell was placed in the spectrophotometer compartment and the *A* - *t* graph recorded at 548 nm. The data obtained were used to construct a calibration graph by plotting the reciprocal of the time required for the initial absorbance to decrease by 0.1 decrements against the Te concentration.

Procedure for the Determination of Tellurium in Lead Concentrates

The sample (0.5–1.0 g) was treated with 5 ml of HNO_3 (1 + 1) and heated to near dryness. Then 20 ml of water, 3 ml of H_2SO_4 (1 + 2) and 2–3 drops of HCl (1 + 1) were added to precipitate lead and silver. The precipitate was filtered off and the filtrate heated until persistent white fumes appeared. Then 5 ml of water and 5 ml of HCl (1 + 1) were added and the solution was heated repeatedly to produce white fumes to eliminate Se.[29] The solution thus obtained was diluted to 50 ml with doubly distilled water in a calibrated flask and Te determined in different aliquots by the procedure described above. To determine Te in synthetic concentrates of lead, different volumes of 253.8 μg ml^{-1} Te^{IV} were added to the samples previously weighed and they were dissolved as

Table 1. Determination of Te in lead concentrates

Sample	Te added/ mg g^{-1}	Te found*/ mg g^{-1}	Recovery, %
1	0	ND†	—
	0.0735	0.0730	99.3
	0.1472	0.1483	100.7
	0.2208	0.2119	99.9
2	0	0.0319	—
	0.0709	0.1025	99.7
	0.1419	0.1738	100.0
	0.2129	0.2454	100.2

* Average of three determinations.
† ND = not determined.

Table 2. Determination of Te in synthetic lead concentrates

Sample	Te added/ mg g^{-1}	Te found*/ mg g^{-1}	Recovery, %
1	0	ND†	—
	0.3556	0.3540	99.5
	0.4375	0.4364	99.7
	0.5198	0.5150	99.1
2	0	0.0380	—
	0.3724	0.4110	100.1
	0.4584	0.4961	99.9
	0.5444	0.5820	99.9

* Average of three determinations.
† ND = not determined.

described above. The results obtained are listed in Tables 1 and 2.

Procedure for the Determination of Tellurium in Waters

The determination of Te in spring waters was carried out with no sample pre-treatment. For soda waters 3 ml of HNO_3 (1 + 1) and 3 ml of H_2SO_4 (1 + 2) were added to 25 ml of sample and the mixture was heated to near dryness, cooled and diluted to 50 ml with doubly distilled water in a calibrated flask. In both samples Te was determined by applying the recommended procedure to several aliquots. The experimental results obtained are given in Table 3.

Results and Discussion

Previous work demonstrated that over a wide pH range $H_2PO_2^-$ does not reduce pyronine G at an appreciable rate, but that Pd^{II} is a sensitive catalyst for this process. It was found that Te^{IV} has no effect on the $H_2PO_2^-$ - pyronine G system. On the other hand, it has a strong inhibitory effect on the Pd^{II}-catalysed reaction. For a given fixed amount of Pd^{II} present in the medium, the rate of the inhibited process is proportional to the Te^{IV} concentration. The process is monitored spectrophotometrically at 548 nm by measuring the decrease in the absorbance of pyronine G; then, the reciprocal of the time required for such an absorbance to decrease by 0.1 decrements is plotted against the Te concentration. Fig. 1 shows the A - t graphs at pH 2.6 with 6×10^{-5} M pyronine G, 0.6 M $H_2PO_2^-$, 0.80 μg ml^{-1} Pd^{II} and variable concentrations of Te^{IV} between 0 (curve A) and 0.85 μg ml^{-1} (curve H). As can be seen, the rate of the process decreases with an increase in the Te^{IV} concentration, even at low concentrations of this ion.

Influence of the pH

The influence of the pH on the rate of the process was studied over the range 1.7–4.4; the different pH values assayed were adjusted with the Britton - Robinson buffers. Fig. 2 shows the results obtained with samples containing 6×10^{-5} M pyronine G, 0.6 M $H_2PO_2^-$ and 0.80 μg ml^{-1} Pd^{II} (curve A) or 0.80 μg ml^{-1} Pd^{II} plus 0.50 μg ml^{-1} Te^{IV} (curve B).

Table 3. Recoveries of Te from spring and soda waters

Sample	Te added/ μg ml^{-1}	Te found*/ μg ml^{-1}	Recovery, %
1†	0	ND§	—
	0.254	0.251	98.8
	0.508	0.511	100.6
	0.762	0.756	99.3
2†	0	ND	—
	0.254	0.252	99.2
	0.508	0.505	99.4
	0.762	0.758	99.5
3‡	0	ND	—
	0.254	0.252	99.2
	0.508	0.503	99.0
	0.762	0.757	99.3

* Average of three determinations.
† Spring water.
‡ Soda water.
§ ND = not determined.

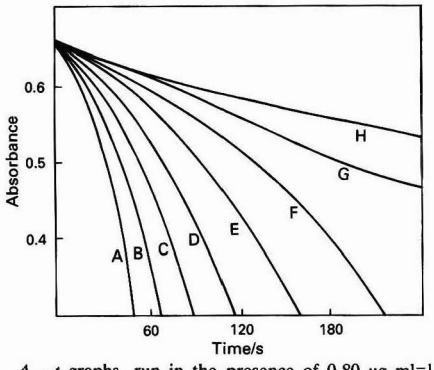

Fig. 1. A - t graphs, run in the presence of 0.80 μg ml^{-1} Pd^{II}, Pyronine G, 6×10^{-5} M; $H_2PO_2^-$, 0.6 M; pH, 2.6; and temperature, 22 ± 0.2 °C. Te^{IV} concentration: A, 0; B, 0.08; C, 0.17; D, 0.25; E, 0.34; F, 0.50; G, 0.68; and H, 0.85 μg ml^{-1}

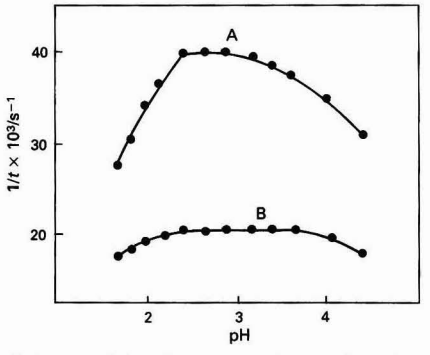

Fig. 2. Influence of the pH on the reaction rate. Pyronine G, 6×10^{-5} M; and $H_2PO_2^-$, 0.6 M. A, 0.80 μg ml^{-1} Pd^{II}; and B, 0.80 μg ml^{-1} Pd^{II} plus 0.50 μg ml^{-1} Te^{IV}

As can be seen the rate of the inhibited process remained virtually constant over the pH range 2.2–3.6; the differences between the rate of the catalysed and the inhibited processes were at a maximum in the range 2.2–2.8. A pH of 2.6 was chosen for all subsequent experiments. The pyronine G shows an absorption maximum at 548 nm at this pH.

The partially inhibited reaction order, in hydrogen ion, was −¼ between pH 1.7 and 2.2, 0 in the range 2.2–3.6 and ⅕ between pH 3.6 and 4.4.

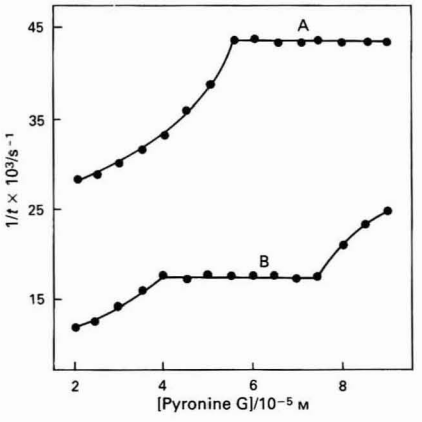

Fig. 3. Influence of the pyronine G concentration on the reaction rate. $H_2PO_2^-$, 0.6 M; and pH, 2.6. A, 0.80 μg ml^{-1} Pd^{II}; and B, 0.80 μg ml^{-1} Pd^{II} plus 0.50 μg ml^{-1} Te^{IV}

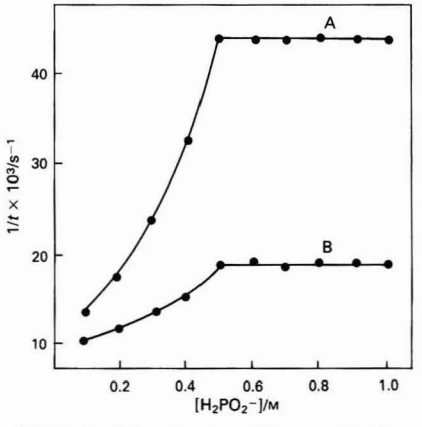

Fig. 4. Influence of the hypophosphite concentration on the inhibitory effect of Te. Pyronine G, 6×10^{-5} M, pH 2.6. A, 0.80 μg ml^{-1} Pd^{II} and B, 0.80 μg ml^{-1} Pd^{II} + 0.50 μg ml^{-1} Te^{IV}

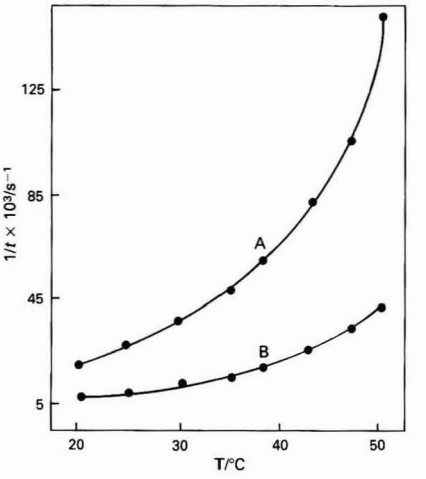

Fig. 5. Influence of the temperature on the reaction rate. Pyronine G, 6×10^{-5} M; $H_2PO_2^-$, 0.6 M; and pH, 2.6. A, 0.80 μg ml^{-1} Pd^{II}; and B, 0.80 μg ml^{-1} Pd^{II} plus 0.50 μg ml^{-1} Te^{IV}

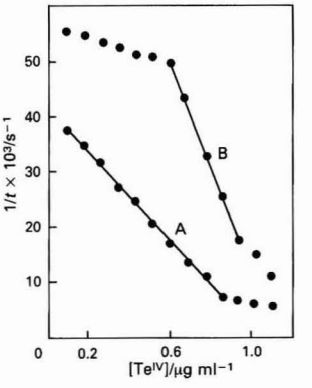

Fig. 6. Influence of Pd^{II} concentration on the reaction rate. Pyronine G, 6×10^{-5} M; $H_2PO_2^-$, 0.6 M; pH, 2.6; and temperature, 22 ± 0.2 °C. A, 0.80; and B, 1.15 μg ml^{-1} of Pd^{II}

Influence of Other Variables

The influence of the dye concentration was studied by preparing samples containing fixed amounts of $H_2PO_2^-$ and Pd^{II} at pH 2.6 and variable concentrations of pyronine G in the range 2×10^{-5}–9×10^{-5} M, both in the absence and in the presence of 0.50 μg ml^{-1} of Te^{IV}. The results obtained are shown in Fig. 3. Curve A corresponds to the catalysed process and curve B to the inhibited process.

A concentration of pyronine G of 6×10^{-5} M, at which the inhibitory effect of Te^{IV} was at a maximum and constant was selected. Moreover, this concentration provided suitable absorbance values. The partial order of the reaction in pyronine G was ¾ between 2.0×10^{-5} and 4.0×10^{-5} M, 0 between 4.0×10^{-5} and 7.5×10^{-5} M, and ½ between 7.5×10^{-5} and 9.0×10^{-5} M pyronine G.

The influence of the $H_2PO_2^-$ concentration was studied similarly over the range 0.1–1.0 M. The rates of the catalysed and inhibited processes increased with increasing $H_2PO_2^-$ concentration up to 0.5 M, above which they remained virtually constant. Fig. 4 reflects the variation of the rate as a function of the $H_2PO_2^-$ concentration; as can be seen, the inhibitory effect of Te^{IV} was at a maximum for $H_2PO_2^-$ concentrations above 0.5 M, hence 0.6 M $H_2PO_2^-$ was chosen for further experiments.

The partial order of the reaction in $H_2PO_2^-$ was ½ between 0.1 and 0.5 M and 0 between 0.5 and 1.0 M $H_2PO_2^-$.

Influence of the Temperature

Energy of activation

Fig. 5 shows the influence of the temperature on the rate of the catalysed (curve A) and inhibited reactions (curve B), determined at the reactant concentrations stated above and with 0.50 μg ml^{-1} of Te. A temperature of 22 ± 0.2 °C was chosen.

The energy of activation found for the Pd^{II}-catalysed reaction in the absence of Te^{IV} and for the inhibited reaction in its presence, was 57.74 and 71.96 kJ mol^{-1}, respectively.

Influence of the Pd^{II} Concentration

An important variable is the concentration of Pd. It was observed that when the concentration of Te^{IV} is constant the rate of the process increases considerably with increasing Pd^{II} concentration.

Table 4. Effect of various foreign ions on the determination of Te

Foreign ion (FI)	Maximum tolerated [FI]/[Te^{IV}] molar ratio
Co^{II}*	10 000
Ni^{II}*, Zn^{II}*	2 000
F^-*	500
Mg^{II}*, ClO_4^-*	100
Mn^{II}*, Cl^-†, SO_3^{2-}†	50
Pb^{II}*, Mo^{VI}*, Se^{IV}*	10
Cu^{II}*, W^{VI}*	1
Ag^I*, U^{VI}*	0.5
Cd^{II}*, Hg^{II}*, Fe^{III}†, Ru^{III}*, $S_2O_3^{2-}$*, V^V*	0.1
Au^{III}†, Bi^{III}*, As^{III}*, I^-*, Cr^{VI}*, Br^-*, S^{2-}*	<0.1
SO_4^{2-}, NO_3^-, HPO_4^{2-}, Ca^{II}, Na^I, K^I	No interference

* Positive error.
† Negative error.

Fig. 6 corresponds to two series of experiments carried out with the concentrations of pyronine G, $H_2PO_2^-$ and pH mentioned, containing 0.8 μg ml^{-1} (graph A) or 1.15 μg ml^{-1} (graph B) of Pd^{II} and various amounts of Te^{IV}.

In agreement with these results the intervals of determination of Te and the slopes of the graphs obtained depend on the concentration of the catalyst. A concentration of 0.80 μg ml^{-1} of Pd^{II} was chosen as the most suitable as this gave a much wider linear interval for the determination of Te^{IV}.

Mechanism of the Inhibited Reaction

In previous work[1] it was found that the catalytic effect of Pd^{II} on the reduction of pyronine G by $H_2PO_2^-$ seems to be due to heterogeneous catalysis attributed to the formation of elemental Pd in the fast reduction of Pd^{II} by $H_2PO_2^-$. This Pd catalytically decomposes the excess of $H_2PO_2^-$, yielding active hydrogen, which rapidly reduces the pyronine G, as occurs in some processes involving $H_2PO_2^-$ and dyes catalysed by Pd^{II}.[30,31]

The mechanism of the process inhibited by Te^{IV} is thought to be similar to that suggested for an analogous process,[31] the $H_2PO_2^-$ reduction of Pd^{II}, which induces the simultaneous reduction of Te^{IV}. The Te formed is in close contact with the Pd catalyst, thereby reducing the active surface of the latter, giving rise to the inhibitory effect of Te^{IV}.

Calibration Graph

Rate equation

Under the selected experimental conditions, 6×10^{-5} M pyronine G, 0.6 M $H_2PO_2^-$, 0.80 μg ml^{-1} Pd^{II}, pH 2.6 and 22 ± 0.2 °C, the rate of the process was linearly related to the Te^{IV} concentration over the range 0.08–0.85 μg ml^{-1}.

The regression equation found was $y = 0.0411 - 0.0395x$, with a correlation coefficient $r = 0.9959$. The coefficient of variation for ten determinations of 0.34 and 0.68 μg ml^{-1} of Te^{IV} was ±2.9 and ±1.3%, respectively, while the relative error was ±2.1 and 0.94%, respectively. Under the recommended experimental conditions, the kinetic equation representative of the process is

$$-d[\text{pyronine G}]/dt = k\,[Pd^{II}][Te^{IV}]^{-1}$$

where k is the rate constant.

Interference From Foreign Ions

The interference of foreign ions with the proposed procedure was studied in experiments with 0.50 μg ml^{-1} of Te^{IV} and the ion in question. The results obtained are listed in Table 4, which states the type of error caused. A given ion was considered to interfere when its presence resulted in an error of greater than ±3% in the determination of Te^{IV}.

Applications

The proposed method for the determination of Te was applied to the analysis of some Pb concentrates. The first two samples of Pb concentrates were dissolved and the standard additions method was applied. The results obtained are shown in Table 1. The second two synthetic Pb concentrate samples were prepared by adding different amounts of standard solutions of Te to the solid Pb concentrates. The results obtained are shown in Table 2.

This method has also been applied to the determination of Te in spring and soda waters using the method of standard additions. The results are shown in Table 3.

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Simultaneous Kinetic Determination of Zineb and Maneb by the Continuous Addition of Reagent Technique

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The continuous addition of reagent technique was applied to the simultaneous kinetic determination of zinc ethylenebis(dithiocarbamate) (zineb) and manganese ethylenebis(dithiocarbamate) (maneb) in mixtures. The method developed for this purpose is based on the continuous addition of zincon to a solution containing zinc(II) and manganese(II) released in the acid decomposition of the pesticides. The sequential formation of the zinc and manganese complexes with the reagent is monitored spectrophotometrically. Two consecutive kinetic curves are obtained, the slope of the straight portion of which is related to the concentration of each pesticide. The method allows the resolution of zineb - maneb mixtures at the μg ml^{-1} level over the ratio range 1 : 12–10 : 1, with an average relative standard deviation of 1.3%, and was applied satisfactorily to the determination of both pesticides in commercial formulations.

Keywords: *Continuous addition of reagent; simultaneous kinetic determination; zineb; maneb; fungicide*

Ethylenebis(dithiocarbamate) pesticides containing metal ions [*e.g.*, zinc ethylenebis(dithiocarbamate) (zineb) and manganese ethylenebis(dithiocarbamate) (maneb)] can be determined on the basis of their organic or inorganic component contents. Owing to their joint use in commercial agricultural fungicide formulations, a method for the determination of one of them in the presence of the other and the simultaneous determination of both is of relevance.

As these compounds are not volatile, they cannot be analysed directly by gas chromatography. However, they can be determined after thermal decomposition into various fragments such as ethylenethiourea[1] and carbon hydrogen sulphide.[2–4] On the other hand, relatively few high-performance liquid chromatographic methods for the determination of these pesticides have been reported to date.[5,6] Thin-layer chromatography has also been used for the separation and colorimetric determination of these and other pesticides such as tetramethylthiuram disulphide (thiram) and zinc dimethyldithiocarbamate (ziram).[7,8] Polarographic techniques have been applied to the resolution of thiram and maneb,[9] and even a qualitative assay for the separation of zineb, maneb and mancozeb (co-ordination product of maneb containing 16–20% manganese and 2–2.5% zinc) in mixtures has been reported recently.[10] However, these methods are generally time consuming and not very sensitive.

The proposed method is based on the inorganic component of the pesticide and allows the determination of zineb and maneb at concentrations of a few μg ml^{-1}. In an earlier paper[11] Quintero *et al.* reported the kinetic determination of zineb based on the complex formation reaction between the zinc(II) released upon acid treatment of the pesticide and zincon (2-carboxy-2′-hydroxy-5′-sulphoformazylbenzene), a classical chromogenic reagent. This fast reaction was monitored spectrophotometrically by the continuous addition of reagent (CAR) technique,[12,13] which is particularly useful for kinetic measurements on reactions on the millisecond time scale. The method was extended in the present work to the resolution of zineb and maneb in mixtures by the addition of zincon at a constant rate to the solution containing the zinc(II) and manganese(II) ions previously obtained by mineralising the sample. The singular features of the CAR technique, the affinity of these metal ions for the ligand and their different reaction rates allow two sequential kinetic curves to be obtained; their slopes are related to the concentration of each metal ion in the sample. The proposed method allows the automated, simultaneous kinetic determination of these pesticides without the need for any of the mathematical treatments typically involved in differential reaction-rate methods.[14,15] The same approach was applied to the resolution of copper(II) - iron(III) mixtures using pyridoxal thiosemicarbazone as the reagent.[16]

Kinetic methods have rarely been applied to the individual determination of pesticides[17,18] and the method proposed here is the first attempt at the kinetic resolution of pesticide mixtures.

Experimental

Reagents

Zineb and maneb (Chem. Service, West Chester, PA, USA) solutions (250 mg l^{-1}) were made by dissolving 25 mg of each pesticide in 2.0 ml of concentrated nitric acid, heating to complete dissolution and diluting with distilled water in a 100-ml calibrated flask. Dilute solutions were prepared immediately prior to use. A 2.95×10^{-3} M zincon solution was prepared by dissolving 130.0 mg of the chemical in 2.0 ml of 0.1 M sodium hydroxide and diluting to 100 ml with distilled water. A 0.1 M sodium borate - boric acid buffer of pH 9.0 was also prepared.

Apparatus

The instrumental set-up used for implementation of the CAR technique has been described elsewhere.[12] A Radiometer PHM62 pH-meter equipped with a combined glass - calomel electrode was used for pH measurements.

Procedures

Simultaneous determination of zineb and maneb

Synthetic samples containing various trace concentrations of zineb and maneb were analysed in a single kinetic run. Thus, the reaction vessel of the CAR system was filled with a solution containing between 15 and 75 μg of zineb, 15 and 90 μg of maneb and 5.0 ml of buffer of pH 9.0, and diluted to 60 ml with distilled water. Both complex-formation reactions were developed by adding an 8.85×10^{-4} M zincon solution from the autoburette at a rate of 40 ml min^{-1} while stirring at 200 rev min^{-1}. The absorbance was monitored at 620 nm at a data collection rate of 100 ms per point. The reaction rate of zineb and maneb was determined from the straight portion of the kinetic curve and the concentration of both pesticides in

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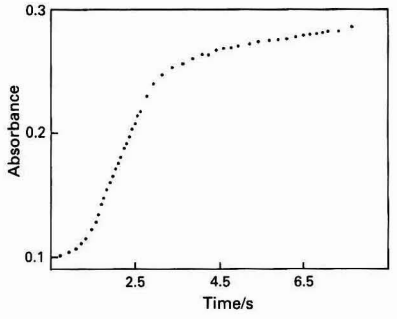

Fig. 1. Absorbance - time curve obtained by the CAR technique for a mixture of 1.2 μg ml^{-1} of zineb and maneb. Other conditions as described under Experimental

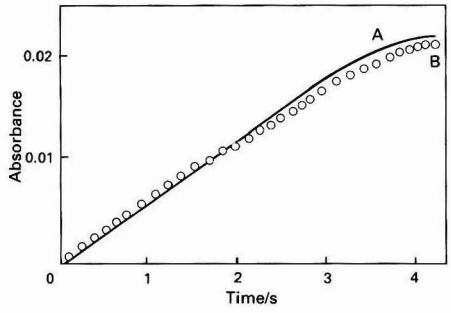

Fig. 2. A, Experimental and B, simulated kinetic curves for the formation of the manganese(II) - zincon complex. Conditions as described in text and under Experimental

the sample was determined from their corresponding individual calibration graph.

Simultaneous determination of zineb and maneb in commercial formulations

The procedure used to release zinc(II) and manganese(II) ions from the formulation was as follows: 200 mg of sample were suspended in 1 l of distilled water in a calibrated flask. This solution was continuously stirred and 0.8-ml aliquots were treated with 3.0 ml of concentrated nitric acid as described above for the preparation of zineb and maneb standards, and diluted with 10 ml of distilled water. This mixture was placed in the reaction vessel together with 5.0 ml of buffer of pH 9.0 and 0.45 ml of 100 μg ml^{-1} potassium cyanide solution. The mixture was then diluted to 60 ml with distilled water and the CAR determination was performed as described above.

Results and Discussion

The CAR technique, described elsewhere,[12] allows the kinetic resolution of mixtures of substances in a single sample aliquot more readily than differential reaction-rate methods, and is very simple to automate, which makes it particularly suitable for routine analyses.[13]

The proposed method involves the continuous addition of a common reagent, zincon, at a constant rate to the zinc(II) and manganese(II) sample solution, thus allowing the sequential determination of each metal ion on the basis of its kinetic behaviour. The absorbance at the maximum absorption of the metal - zincon complex is recorded as a function of time as a response curve with two adjacent linear segments (Fig. 1) from which each reaction rate can be calculated and related to the concentration of each component in the sample. As the zinc(II) reacts faster with zincon than does manganese(II), the first linear segment corresponds to the gradual formation of the zinc(II) - zincon complex.

Individual Determination of Maneb

The influence of variables on the kinetic determination of zineb alone, by this approach, has been studied in detail.[11] Therefore, in order to undertake the simultaneous determination of these pesticides the effect of chemical and instrumental variables on the determination of maneb under the same conditions established for the determination of zineb was examined initially.

Preliminary results showed maneb to behave in a similar manner to zineb with respect to all the variables; however, its initial rate was considerably smaller. Therefore, the kinetic determination of maneb was carried out at a maximum rate of reagent addition (40 ml min^{-1}) from the autoburette and using a slightly higher zincon concentration than the optimum used for zineb (8.85×10^{-4} M). Under these conditions, the reaction rate was higher, which in turn allowed a data acquisition rate of 60 ms per point and the curve to be recorded in only 5 s. The optimum values of the other chemical and instrumental variables were the same as for the determination of zineb and maneb described under Experimental.

Under these optimum conditions the slopes of the initial straight portions of the absorbance - time curves were linearly related to the maneb concentration over the range 0.1–1.5 μg ml^{-1}. The sample throughput was about 95 samples per hour, which shows the suitability of the proposed method for routine analyses for this pesticide.

Determination of the Pseudo Second-order Rate Constants

The pseudo first-order rate constants of the formation of the zinc(II) and manganese(II) complexes with zincon cannot be determined, even by the stopped-flow technique[19] because the reaction rates are too high; this explains why these reactions have not yet been applied to the individual or simultaneous kinetic determination of these metal ions.

The CAR technique involves a pseudo second-order kinetic dependence, the rate constant of which can be obtained readily. Thus the pseudo second-order rate constant of the manganese(II) - zincon complex was calculated from the integrated equation following this approach.[12] The average value obtained was $k_{Mn} = 1.9 \pm 0.1 \times 10^4$ l mol^{-1} s^{-1} for a zincon concentration of 8.85×10^{-4} M, an addition rate of 40 ml min^{-1} and an initial volume of the solution in the reaction vessel of 60 ml. From the integrated equation mentioned above and by using the rate constant obtained, a theoretical kinetic curve was constructed and compared with the experimental curve. Fig. 2 shows the good fit between the two, which indicates the validity of the approach applied.

Although the pseudo second-order rate constant of the formation of the zinc(II) - zincon complex has been calculated elsewhere ($k_{Zn} = 9.9 \pm 0.5 \times 10^4$ l mol^{-1} s^{-1}),[11] as the experimental conditions were slightly modified, a new value, $k_{Zn} = 1.05 \pm 0.05 \times 10^5$ l mol^{-1} s^{-1}, was determined here. Therefore, the pseudo second-order rate constant ratio, k_{Zn}/k_{Mn}, was 5.4. Mixtures of other metal ions such as copper(II) and iron(III), the pseudo second-order rate constant ratio of which is 4.6, have been resolved satisfactorily using the CAR technique by sequential complexation with pyridoxal thiosemicarbazone.[16] Studies in progress are aimed at finding the minimum rate constant ratio allowing the simultaneous resolution of a binary mixture with a minimum error.

Simultaneous Determination of Zineb and Maneb

When the zincon solution is automatically added at a constant rate to the sample solution containing the zinc(II) and

manganese(II) ions released from a mixture of zineb and maneb after mineralisation, the zinc(II) - zincon complex is formed initially. Only when this complex has almost completely formed does the manganese(II) - zincon complex develop as the zinc(II) ion reacts more rapidly with zincon than does the manganese(II) ion. The sequential formation of these complexes is monitored spectrophotometrically at 620 nm and the changes in the absorbances as a function of time are recorded by the computer unit. The response curve shows two adjacent kinetic portions (see Fig. 1). The slopes of the first and second portions (initial-rate method) are proportional to the zinc(II) and manganese(II) concentration, respectively. The respective reaction rates were confirmed to be independent of each other. From the respective initial rates and the corresponding calibration graphs the concentration of each component in the mixture could be computed readily.

The concentration of zincon was a critical variable in the resolution of the mixture. In order to find the concentration of this reagent that resulted in the best possible resolution in terms of accuracy, three synthetic zineb - maneb mixtures were analysed in ratios between 1:5 and 5:1, with zincon at concentrations in the range 2.95×10^{-4}–8.85×10^{-4} M. The results obtained are listed in Table 1. The errors in the determination of zineb were fairly small in general and always <5% at all of the concentrations of zincon tested. However, determining maneb with an acceptable accuracy required the zincon concentration to be equal to or greater than *ca.* 6.0×10^{-4} M. This can be attributed to the higher affinity of zincon for the zinc(II) ion. The concentration ratio of the two metal ions in the mixture appears to have no influence on the results. A zincon concentration of 8.85×10^{-4} M was thus selected for subsequent experiments.

Table 1. Influence of the zincon concentration on the resolution of zineb - maneb mixtures

Zincon concentration/ 10^{-4} M	Added		Found			
	Zineb/ μg ml^{-1}	Maneb/ μg ml^{-1}	Zineb/ μg ml^{-1}	Error, %	Maneb/ μg ml^{-1}	Error, %
2.95	0.25	0.25	0.24	−4.0	0.26	4.0
	0.25	1.25	0.24	−4.0	0.97	−22.4
	1.25	0.25	1.26	0.8	0.21	−16.0
4.43	0.25	0.25	0.25	0.0	0.23	−8.0
	0.25	1.25	0.25	0.0	1.16	−7.2
	1.25	0.25	1.25	0.0	0.21	−16.0
5.90	0.25	0.25	0.25	0.0	0.26	4.0
	0.25	1.25	0.25	0.0	1.25	0.0
	1.25	0.25	1.24	−0.8	0.24	−4.0
7.38	0.25	0.25	0.24	−4.0	0.25	0.0
	0.25	1.25	0.24	−4.0	1.19	−4.8
	1.25	0.25	1.25	0.0	0.26	4.0
8.85	0.25	0.25	0.25	0.0	0.25	0.0
	0.25	1.25	0.25	0.0	1.25	0.0
	1.25	0.25	1.24	−0.8	0.26	4.0

Table 2. Calibration graphs and analytical features of the individual determination of zineb and maneb

Analytical feature	Zineb	Maneb
Linear dynamic range/μg ml^{-1} . .	0.05–1.25	0.1–1.5
Sensitivity/10^3 ml μg^{-1} s^{-1}	81.8	3.29
Detection limit/ng ml^{-1}	20	55
Precision (RSD), %	1.25	1.21

Table 3. Resolution of synthetic zineb - maneb mixtures

Added/μg ml^{-1}		Found			
Zineb	Maneb	Zineb/ μg ml^{-1}	Error, %	Maneb/ μg ml^{-1}	Error, %
0.250	0.250	0.248	−0.8	0.249	−0.4
0.250	0.500	0.249	−0.4	0.501	0.2
0.250	1.250	0.249	−0.4	1.250	0.0
0.125	1.250	0.124	−0.8	1.254	0.3
0.125	1.500	0.122	−2.4	1.492	−0.5
0.500	0.250	0.509	1.8	0.253	1.2
1.250	0.250	1.280	2.4	0.248	−0.8
0.875	0.125	0.880	0.6	0.125	0.0
1.250	0.125	1.237	−1.0	0.122	−2.4

Resolution of Synthetic Mixtures

Various synthetic zineb - maneb mixtures were resolved by the procedure described under Experimental. The concentration of each component was calculated from its respective initial rate and calibration graphs were plotted for the determination of each pesticide. The analytical features of the individual determinations are summarised in Table 2. As can be seen the sensitivity was fairly high, with detection limits as low as 20 and 55 ng ml^{-1} for zineb and maneb, respectively.

In Table 3 the results obtained for various synthetic mixtures of these pesticides are given. It can be inferred from these data that mixtures of zineb and maneb can be resolved in the range of ratios from 1:12 to 10:1 with an error equal to or less than 3%. This range of ratios is very wide, its only constraint being imposed by the limits of the calibration graph rather than by the errors made in the determination. The method is also fairly precise as the relative standard deviation for a mixture containing 0.5 μg ml^{-1} of each component was 1.4 and 1.2% for zineb and maneb, respectively.

The selectivity study was limited to the tolerated amount of copper(II) ion, which is often associated with zineb and maneb in commercial formulations. As reported elsewhere,[11] it requires the addition of cyanide ion to raise the tolerated level of copper(II) in order to avoid its interference with the determination of these fungicides. In the presence of 0.75 μg ml^{-1} of cyanide the tolerated copper(II) concentration (as oxychloride) limit is four times that of the two pesticides at a concentration of 0.5 μg ml^{-1} each. The potential interference from other organic pesticides was not tested as the pesticide samples must be pre-treated with nitric acid to release the zinc(II) and manganese(II) ions.

Determination of Zineb and Maneb in Commercial Formulations

The simultaneous determination of these fungicides was carried out in "ZZ-Azul Triple Micro," a commercial formulation of wide use as an agricultural fungicide. The procedure applied is described under Experimental. The results obtained for this sample (15.6 ± 0.6% of zineb and 19.6 ± 0.8% of

Table 4. Analysis for zineb and maneb in "ZZ-Azul Triple Micro"

Found*/μg ml^{-1}		Added/μg ml^{-1}		Recovery/μg ml^{-1}		Recovery, %	
Zineb	Maneb	Zineb	Maneb	Zineb	Maneb	Zineb	Maneb
0.42	0.53	—	—	—	—	—	—
0.42	0.53	0.25	0.25	0.639	0.749	95.4	96.0
0.42	0.53	0.375	0.50	0.796	1.044	100.1	101.4
0.42	0.53	0.50	1.00	0.921	1.478	100.1	96.6

* Average of three determinations.

maneb) were consistent with those claimed by the manufacturer (dust, 20% maneb, 15% zineb and 15% copper as oxychloride). Table 4 includes the recoveries achieved (close to 100% in all instances).

Conclusions

The CAR technique opens new prospects for differential reaction-rate methods while avoiding the mathematical treatments usually required and using straightforward, automated instrumentation particularly suitable for routine analyses. The excellent performance of this approach was shown in the kinetic determination of zineb and maneb in commercial formulations in a single kinetic run.

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Improved Extraction Method for Avoiding the Interference of Ascorbic Acid in the Spectrophotometric Determination of Nitrite in Meat Products

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In minced meat products, ascorbic acid interferes in the usual spectrophotometric determination of nitrite by the diazotisation - coupling technique with sulphanilamide and *N*-(1-naphthyl)ethylenediamine. In a modified extraction method, the enzyme ascorbate oxidase was shown to diminish the interference of the acid. Within 10 min the enzyme was able to reduce the level of ascorbic acid in the minced meat specimens from 1000 p.p.m. down to the tolerance limit for interference. This appeared to be superior to the extraction method involving the use of water and was comparable to a previously described method based on the use of 0.01 M sodium hydroxide. As isoascorbic acid is not approved as a food additive in Norway no attempt was made to investigate the influence of this acid.

Keywords: *Interference; ascorbic acid; spectrophotometry; nitrite determination; diazotisation - coupling technique*

Nitrite and ascorbic acid are among the approved food additives for use in minced meat products in Norway. The maximum level of sodium nitrite that can be added to minced meat cakes, balls and sausages during processing is 30 mg kg^{-1}, to sandwich meats made from minced meat 60 mg kg^{-1} and to cured products the maximum level is 120 mg kg^{-1}. Some of the added nitrite is consumed during processing[1] and some is decomposed during storage, leaving only the remaining nitrite to be detected.

No maximum level has been laid down for ascorbic acid in either of these products; however, the amount found by analysis in our laboratory mostly lies between 150 and 500 p.p.m.

Ascorbic acid at levels higher than 4 p.p.m. in solution has been shown[2] to interfere with the determination of nitrite by reduction to nitrogen monoxide,[3] thereby preventing some of the formation of the diazonium salt from nitrite and sulphanilamide. A concentration of 4 p.p.m. in solution corresponds to 40 p.p.m. in the minced meat specimen.

The interference is shown to be dependent on pH, being only slight or moderate in basic solutions and very significant under neutral or acidic conditions.[2,3] When nitrite (and ascorbic acid) is extracted from the meat specimens with water the solution will be slightly acidic. Addition of Carrez reagents to remove proteins leaves the solution at pH 4.5. The first step in the diazotisation reaction, the formation of the diazonium salt, demands an even lower pH. At the same time reduction of diazonium salts can lead to arene or hydrazine formation.[4] A similar reaction with ascorbic acid as a reducing agent is likely to occur. Until the coupling reaction has occurred both nitrite and diazonium salt are vulnerable to attack from ascorbic acid.

In 1987, Norwitz and Keliher[3] stated that at pH 8, 10 and 12 no significant decomposition of nitrite occurred in standard solutions. They proposed a new extraction procedure to avoid the interference, whereby a slurry of 5 g of the sample in 0.01 M sodium hydroxide is digested at 80 °C for 90 min and the nitrite is determined in the filtrate.

However, for cured meat the results and recoveries were satisfactory in some instances and less satisfactory in others for reasons that were not clear. In addition the method increases the extraction time by more than 1 h.

Experimental

Reagents

High-quality distilled water was used. The organic chemicals were purchased from Merck or BDH. The nitrite and ascorbic acid test-strips were Merckoquant 10007 and 10023, respectively, from Merck. The ascorbate oxidase spatulas were purchased from Boehringer Mannheim.

Samples of minced meat pudding containing neither nitrite nor ascorbic acid were used in all the experiments in order to obtain reaction conditions as close as possible to those normally used in routine control work.

Standard nitrite solution A (1 ml = 100 μg *of* $NaNO_2$). Sodium nitrite (0.1000 g) was dissolved in water and diluted to 1000 ml in a calibrated flask.

Sulphanilamide - NED reagent. Sulphanilamide (2.50 g) was dissolved in 650 ml of 1 M hydrochloric acid at room temperature, 10 ml of 0.6% *N*-(1-naphthyl)ethylenediamine (NED) solution were added and the mixture was diluted to 1000 ml. This solution was stored in a brown bottle in a refrigerator and was stable for 1 month.

Carrez reagents. Prepared from 15% potassium hexacyanoferrate(III) and 30% zinc sulphate solutions.

Ascorbic acid (1 ml = 1.0 mg *of ascorbic acid*). Prepared fresh daily by dissolving 0.250 g of ascorbic acid in water and diluting to 250 ml in a calibrated flask.

Sulphanilic acid. Sulphanilic acid (0.54 g) was dissolved in 70 ml of water by heating, the solution was cooled to room temperature, 20 ml of concentrated hydrochloric acid were added and the solution was diluted to 100 ml.

NED solution (0.6%). *N*-(1-Naphthyl)ethylenediamine dihydrochloride (0.6 g) was dissolved in water and diluted to 100 ml. The reagent was stored in a brown bottle and was stable for 1 month.

Preparation of Calibration Graphs

Appropriate aliquots of standard nitrite solution A, diluted to 18 ml, were transferred into test-tubes containing 5 g of minced meat pudding (containing no nitrite), treated with 1 ml of each of the Carrez reagents and filtered before the addition of 2.5 ml of sulphanilamide - NED reagent. The absorbance of the reaction mixture was measured at 542 nm against a distilled water blank.

Instability of the Diazonium Ion

Appropriate aliquots of nitrite, together with the Carrez reagents, were added to the minced meat pudding and the slurry was shaken vigorously. After filtration, 2.5 ml of acetic acid and 0.5 ml of sulphanilic acid were added to 3.5 ml of each of the filtrates. The solutions were allowed to stand for 8 min before the addition of various amounts of ascorbic acid. After

a further 2 min, 0.5 ml of NED solution was added and the absorbance was measured as described above.

Study of the Disappearance of Ascorbic Acid due to Ascorbate Oxidase

Samples of minced meat pudding, together with ascorbate oxidase spatulas, were blended thoroughly in water before adding different amounts of ascorbic acid. After various time intervals, aliquots of the test slurry were filtered into a solution of 4% oxalic acid saturated with sodium chloride and were immediately titrated with 2,6-dichlorophenolindophenol. The time was calculated from the moment the ascorbic acid was added to the slurry. Comparative experiments were performed without, with one and with two ascorbate oxidase spatulas in the test slurry.

Table 1. Nitrite recovery (in %) after addition of various amounts of ascorbic acid after the diazonium ion had been formed

Starting concentration of nitrite, p.p.m.	Amount of ascorbic acid added, p.p.m.			
	0	500	1000	2000
30	100	42	31	27
60	100	57	34	24
120	100	45	30	21

Table 2. Effect of ascorbate oxidase spatula (AOS) and nitrite on ascorbic acid in solution

Addition	Ascorbic acid, p.p.m.	Recovery of ascorbic acid (%) added after		
		5 min	10 min	15 min
None	500	95	95	95
	1000	95	95	95
	2000	95	95	95
30 p.p.m. NO_2^-	500	70	60	—
	1000	85	85	—
	2000	95	95	—
1 spatula	500	40	*ca.* 4	0
	1000	30	*ca.* 3	0
	2000	50	30	*ca.* 1
2 spatulas	1000	5	0	—
	2000	25	*ca.* 1	0

Procedures

Minced meat samples, two ascorbate oxidase spatulas and water were mixed thoroughly for 10 min and treated as described for the preparation of the calibration graphs for the spectrophotometric determination.

When ascorbate oxidase was not used, mixing for 10 min was omitted, otherwise the procedure was the same.

A slurry of the sample in 0.01 M sodium hydroxide was digested at 80 °C for 1 h as previously described.[3]

A nitrite test, using test-strips for the detection and semi-quantitative determination of nitrite ions, was carried out as described by the manufacturer. This was also the situation with ascorbic acid test-strips.

Results and Discussion

Instability of Diazonium Salt in Solution due to Ascorbic Acid

The supposed decomposition of the diazonium salt formed[3] by ascorbic acid was confirmed as taking place within 2 min under the reaction conditions used during the normal determination of nitrite, as can be seen from Table 1. As expected, the percentage nitrite recovery was lowered when the concentration of ascorbic acid was increased.

Disappearance of Ascorbic Acid due to Ascorbate Oxidase

The enzyme ascorbate oxidase is available as a powder and in spatulas. The spatulas were preferred because they required no buffer solution and in the preliminary experiments their use gave better reproducibility than did the use of powder.

The fact that little if any interference was observed after the ascorbate oxidase treatment requires that the reaction causing the interference must be slower than the decomposition of ascorbic acid by the enzyme.

Table 2 shows the rate of disappearance of ascorbic acid due to ascorbate oxidase and due to nitrite. Two spatulas appeared to be adequate and necessary for the disappearance of the acid

Table 3. Comparison of yield from different methods for the determination of nitrite in 22 minced meat samples

Product No.	Ascorbic acid, p.p.m.	Nitrite, p.p.m.	Nitrite found by three methods as % yield of the extraction method with ascorbate oxidase		
		Enzyme/water	Water	NaOH solution	Test-strips
1	125	11.5	89	106	78
2	150	13.7	83	107	88
3	350	14.3	71	96	84
4	250	15.2	78	122	59
5	700	16.0	71	109	69
6	150	16.0	70	103	81
7	600	16.3	38	68	55
8	75	18.9	87	94	48
9	600	18.9	55	87	63
10	375	19.3	90	97	114
11	600	19.6	69	133	92
12	150	20.2	75	89	30
13	100	23.0	76	84	48
14	750	23.8	63	111	55
15	250	24.1	78	105	75
16	525	25.0	76	92	48
17	275	26.9	77	81	93
18	700	29.9	68	75	20
19	100	34.2	64	106	56
20	200	34.8	64	94	52
21	100	36.0	90	101	53
22	175	36.9	83	93	108
Average:			73.4	97.9	66.8

in 10 min. The use of only one spatula was enough to bring the acid content down to the tolerance limit when the starting concentration was 1000 p.p.m. or lower; however, the use of a single spatula did not appear to be sufficient when the starting concentration was *ca.* 2000 p.p.m. Even if our results showed that the usual content is lower than 500 p.p.m. of ascorbic acid in minced meat, as a precaution, two spatulas were used in the experiments for the spectrophotometric determination of nitrite.

The use of one or two spatulas in the test solution containing no ascorbic acid gave *ca.* 100% of the nitrite added, leading to the conclusion that the ascorbate oxidase spatulas themselves do not interfere with the spectrophotometric determination of nitrite.

Recovery of Added Nitrite Compared With the Amount of Ascorbic Acid Added to Minced Meat by Using Ascorbate Oxidase in the Extraction Mixture

The effect of various amounts of ascorbic acid, covering the range corresponding to 0–2000 p.p.m. in the minced meat, was studied. Two ascorbate oxidase spatulas were in contact with the meat sample and water before the nitrite and the interfering acid were added. The concentration of nitrite was chosen to correspond to 30, 60 and 120 p.p.m. in minced meat. The recoveries were all close to 100% of the nitrite added and the results showed good reproducibility with low standard deviations for the average of eight measurements of each concentration. It can be concluded that the use of two spatulas is sufficient to prevent ascorbic acid from interfering in the determination of nitrite.

Comparison of the Three Different Extraction Procedures

The extraction method with water and two ascorbate oxidase spatulas was compared with extraction methods involving the use of water only and of sodium hydroxide solution.[3]

All minced meat products arriving in the laboratory for routine control work were tested for both ascorbic acid and nitrite content with test-strips. The nitrite test-strips make use of the same diazotisation technique as the spectrophotometric determination and are thereby exposed to the same ascorbic acid interference; in addition they are subject to a great uncertainty when reading the results. Nevertheless, 22 specimens containing both food additives were selected. Each sample was analysed by using all three types of extraction on the same day to avoid the decomposition of nitrite (which occurs even when samples are stored in a freezer) influencing the results. The results were compared by calculating their yield in terms of percentage recovery from extraction with the enzyme, as can be seen from Table 3. Also included in the table are the results obtained with the nitrite test-strips.

The use of the enzyme allows more nitrite to be detected than without the enzyme. There is a tendency that high amounts of ascorbic acid give higher differences in the nitrite detected between the two extraction methods. The extractions with sodium hydroxide and with water and ascorbate oxidase are competitive. Nitrite strips can only be used as a screening method and will mostly give only two-thirds of the nitrite content.

The extraction with water and enzyme is labour saving and less time consuming than the extraction with sodium hydroxide. Therefore, the extraction method described here appears to be the easiest to use in routine work for determining the nitrite content of minced meat products and is highly recommended.

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SHORT PAPERS

Ultra-rapid High-performance Liquid Chromatographic Screening for Phenothiazines in Human Samples

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A high-performance liquid chromatographic method is presented for the simultaneous identification and quantification of six commonly prescribed phenothiazines. Single-step extraction was achieved from alkaline samples with heptane - isoamyl alcohol (98.5 + 1.5), using prochlorperazine as an internal standard. A Spherisorb CN column was used, with a mobile phase of acetonitrile - acetate buffer (95 + 5). Detection was carried out at 254 nm.

Keywords: *Phenothiazine; high-performance liquid chromatography*

Phenothiazines are widely used in the treatment of certain psychiatric disorders. Overdoses of these drugs are common, and are potentially life-threatening in patients admitted to emergency departments of hospitals.

There is a need to differentiate rapidly the phenothiazine overdose from others in the emergency unit population. Consequently, it has become useful to determine these neuroleptics. Although numerous methods, such as gas chromatography - mass spectrometry,[1] high-performance liquid chromatography (HPLC)[2] or radioimmunoassay,[3] exist for the individual analysis for phenothiazines, none was available for the determination of the most prescribed compounds according to medical statistics. This paper describes several modifications (in terms of mobile phase, internal standard and extraction procedure) to the technique of Midha *et al.*,[2] which allows the determination of six phenothiazines in human fluids and tissues.

Experimental

Samples (1 ml of fluid or tissue homogenate) were extracted with 5 ml of heptane - isoamyl alcohol (98.5 + 1.5) after rendering alkaline (with 500 µl of a saturated solution of Na_2CO_3) and the addition of prochlorperazine (100 mg l^{-1}) as an internal standard. After evaporating the organic extract to dryness, the residue was dissolved in acetonitrile (100 µl), and a 70-µl portion of this solution was then injected on to a column (250 × 3.2 mm i.d.) packed with 5-µm Spherisorb CN. The mobile phase consisted of 5% aqueous 0.015 M sodium acetate - acetic acid buffer (pH 6.5) and 95% acetonitrile. A flow-rate of 1.0 ml min^{-1} was used. Detection was effected by measuring the absorbance at 254 nm. Peak-area ratios were used to prepare calibration graphs and to calculate the concentrations.

Results

The results were linear over the range 0.02–20 µg ml^{-1} (n = 4). The retention times, extraction efficiencies, precisions and limits of detection in plasma for the phenothiazines

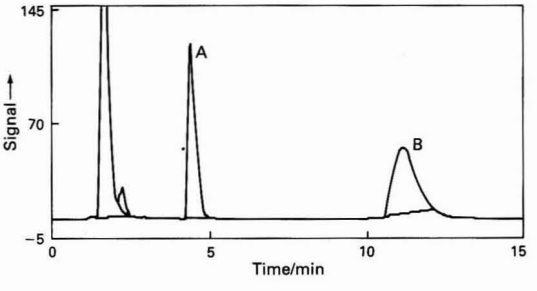

Fig. 1. Typical HPLC trace of plasma, obtained from a subject undergoing treatment with levomepromazine (424 ng ml^{-1}). A, Levomepromazine; and B, prochlorperazine

Table 1. Data for the phenothiazines tested in human plasma

Compound	Retention time/ min	Recovery,* %	Within-run precision,* %	Between-day precision* over 2 weeks, %	Limit of detection/ ng ml^{-1}
Cyamemazine	3.80	74.6	5.2	8.1	28
Levomepromazine	4.41	77.9	4.8	5.9	18
Alimemazine	4.98	69.4	3.9	6.1	20
Aceprometazine	5.49	67.8	4.6	7.0	24
Prometazine	6.21	74.4	4.1	6.5	14
Chlorpromazine	6.98	81.7	3.4	5.9	21

* The extraction recovery and precision (n = 9) were studied for 300 ng ml^{-1} of each component in plasma.

tested are summarised in Table 1. The absolute recovery was determined for each drug by comparing the representative peak areas of extracted plasma samples, at a concentration of 300 ng ml^{-1}, with the peak areas of the methanolic standards at the same concentration. The limit of detection was determined by spiking plasma with decreasing concentrations of phenothiazines until a response equivalent to three times the background noise was observed. A typical chromatogram for plasma is shown in Fig. 1.

No interference from human fluids or tissue components was observed. This technique was also found to be suitable for forensic analysis.[4]

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Determination of Diethanolamine and Morpholine in Systems Containing Copper(II) Using Liquid Chromatography

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A method is described for determining 2,2′-iminobisethanol (diethanolamine) and tetrahydro-1,4-isoxazin (morpholine) in a system containing copper(II). By using a more powerful complex-forming agent, copper(II) is removed from the sample, and the compounds to be determined are extracted with acid and separated on a strongly acidic sulphonated cation-exchange column with phosphate buffer as the mobile phase. The relative error of the method is less than 5%, the detection limit being 20 p.p.m. for each amine.

Keywords: *Liquid chromatography with conductivity detection; diethanolamine and morpholine determination; copper-containing system*

2,2′-Iminobisethanol (diethanolamine) and tetrahydro-1,4-isoxazin (morpholine) find wide applications in the chemical industry and in power plants. The requirements for their determination appear also in connection with their application in poly(phenylene oxide) (PPO) production.[1]

Chromatographic methods include thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography. The simultaneous determination of diethanolamine (DEA) and morpholine (MP) has been described by *e.g.*, Kaiger *et al*,[2] using TLC, and Bazylak and Maslowska[3], by means of GC.

The direct determination of DEA and MP by liquid chromatography is complicated due to the poor chromatographic properties of these amines. Derivatisation procedures are questionable in some instances, because of the low reactivity of the amines under the conditions used.

The method described here has been developed for the analysis of process streams containing amines and copper in PPO production, according to the original Czechoslovak process.[1] As the determination of DEA and MP, based on simple extraction of the amines with acid followed by chromatographic separation, led to erroneous results, a modified method had to be developed.

Experimental

Chemicals

Monoethanolamine (MEA) and potassium dihydrogen phosphate were obtained from Merck (Darmstadt, FRG), MP from Texaco (Spain), and Kupral (sodium diethyldithiocarbamate), DEA, ethyl acetate, acetic acid, hydrochloric acid, sulphuric acid, methanol and toluene from Lachema (Brno, Czechoslovakia).

Standard solutions were prepared by dissolving suitable amounts of MEA, DEA and MP in water.

For the determination, *ca.* 0.1 g of solid Kupral, 2 ml of water and 10 ml of ethyl acetate are added to 1–2 ml of the mother liquor, after polymerisation, in a 50-ml separating funnel. The mixture is shaken thoroughly and allowed to stand. A 0.1% aqueous solution (0.5 ml) of MEA (internal standard) is gradually added, together with 1 ml of 2 M hydrochloric acid and the mixture is shaken and allowed to stand. The aqueous layer is run off and passed through an Acrodisc CR filter (0.45 μm). The filtered sample is flushed for 5 min under a slow stream of nitrogen, and a 10-μl aliquot injected into the liquid chromatograph.

Liquid Chromatography

A Perkin-Elmer LC-2 liquid chromatograph, equipped with a Rheodyne 7010 sampling valve, an LC-21 conductivity detector (Perkin-Elmer) and a Philips PM 8202 recorder, was used. The detector sensitivity was 0.3 mS cm^{-1}, and the recorder output was 10 mV.

The column (25 × 0.4 cm i.d.) used was of Partisil PXS 10/25 SCX (Whatman) particle size 10 μm, protected by a pre-column (3 × 0.4 cm i.d.) packed with LiChrosorb KAT

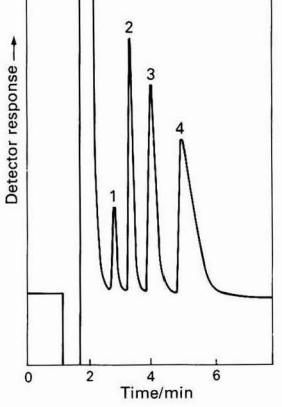


Fig. 1. Typical chromatogram of amines. For conditions see text. 1, Diethylamine; 2, monoethanolamine; 3, diethanolamine; and 4, morpholine

Table 1. Determination of DEA and MP in a model mixture

	Component			
	DEA		MP	
No. of measurement	Dosed/mmol	Found/mmol	Dosed/mmol	Found/mmol
After decomposition of the complexes with H_2SO_4—				
1	0.0	0.017	0.69	0.67
2	0.0	0.015	0.69	0.68
3	0.0	0.017	0.69	0.67
4	0.0	0.013	0.69	0.68
After decomposition with Kupral—				
1	0.025	0.024	0.69	0.67
2	0.025	0.024	0.69	0.71
3	0.025	0.025	0.69	0.68
4	0.025	0.024	0.69	0.70

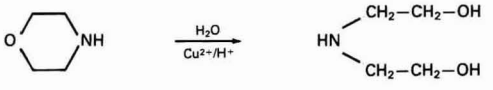
(Merck), particle size 10 μm; 0.045 M potassium dihydrogen phosphate, adjusted to pH 2.5 with glacial acetic acid, was used as the mobile phase. The flow-rate of the mobile phase was 1.5 ml min^{-1} and the over-pressure at the entrance of the column was *ca.* 8 MPa. Separation of the amines was achieved in 8 min (Fig. 1).

Results and Discussion

For the simultaneous determination of DEA and MP in various process streams in PPO production, it is necessary to decompose the complex $[Cu(DEA)_2]^{2+}$ and to modify the sample in such a way that would allow its injection on to the liquid chromatography column.

The first method used was that involving the decomposition of $[Cu(DEA)_2]^{2+}$ with strong acids.

From the results given in Table 1 it is obvious that, during decomposition of $[Cu(DEA)_2]^{2+}$, hydrolysis of MP occurs according to the reaction:



The influence of Cu^{2+} concentration on the course of hydrolysis is shown in Fig. 2. The observations made above show that direct decomposition of complexes with strong acids cannot be used for determination of DEA and MP.

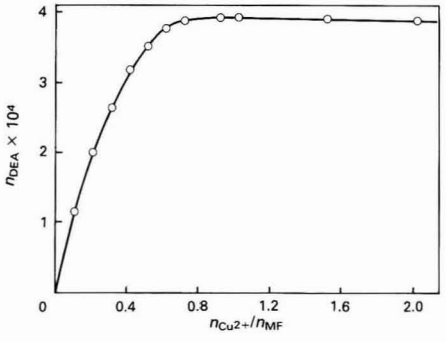


Fig. 2. Influence of Cu^{2+} concentration on the course of morpholine hydrolysis

Table 2. Analysis of model polymerisations

	Component					
	MF			DEA		
No. of analysis	Dosed/g	Found/g	Relative error,%	Dosed/g	Found/g	Relative error,%
1	0.368	0.386	4.9	0.105	0.109	3.8
2	0.368	0.375	1.9	0.105	0.106	0.9
3	0.368	0.384	4.3	0.105	0.103	1.9
4	0.368	0.377	2.4	0.105	0.104	0.9

Evidently, it is necessary to liberate DEA from complexes with Cu^{2+} and to eliminate simultaneously the influence of Cu^{2+}, *e.g.*, by competition with such analytical agents that would form stronger Cu complexes. Such a compound could be, *e.g.*, Kupral, which forms a brown complex with copper(II) ($\log \beta_2 = 28.8$). The cumulative constant for the DEA complex with Cu^{2+} was found to be $\beta_4 = 16.0$ (see reference 4).

Validation of the application of Kupral is given in Table 1. From the reported results it follows that Kupral can be used to release DEA quantitatively from the $[Cu(DEA)_2]^{2+}$ complex. Hence the determination of DEA and MP is possible.

By adding hydrochloric acid, the amines to be determined will pass quantitatively into the aqueous phase as their hydrochlorides. The excess of Kupral that is not bound to the complex with copper(II) will decompose to form diethylamine:

The carbon disulphide formed can react theoretically with secondary amines to form the corresponding dithiocarbamides. This reaction, however, does not occur if the amines are bound in the form of their hydrochlorides.

The conductivity of the test compounds is, in comparison with the conductivity of the mobile phase, very low, which, together with the optimisation of the chromatographic process, leads to a sufficiently low detection limit.

In order to assess the accuracy of the method, samples of model polymerisation were analysed. The results of the analyses are summarised in Table 2. Relative errors in the determination are less than 5%.

Conclusions

The proposed method was applied to the determination of amines in all of the process streams in PPO production.[1] The principle of the method is extraction of the amines with acid, removal of the influence of copper(II) and separation of the amines by liquid chromatography, with conductivity detection. In view of the possibility of changing the pH of the mobile phase, the method is also expected to be viable for the determination of other alkanolamines.

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