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Acetic acid glacial
Acetone
Acetonitrile
Benzene
n-Butyl alcohol
Carbon tetrachloride
Chloroform (stabilized with amylene)
Chloroform (stabilized with ethyl alcohol)
Cyclohexane
Diethylenedioxide
Diethyl ether
Ethyl acetate
Ethyl alcohol
n-Heptane
n-Hexane
Methyl alcohol
Methylene chloride (stabilized with amylene)
Methylene chloride (stabilized with ethyl alcohol)
Methylethylketone
iso-Octane
n-Propyl alcohol
iso-Propyl alcohol
Tetrahydrofuran
Toluene
Water (in preparation)

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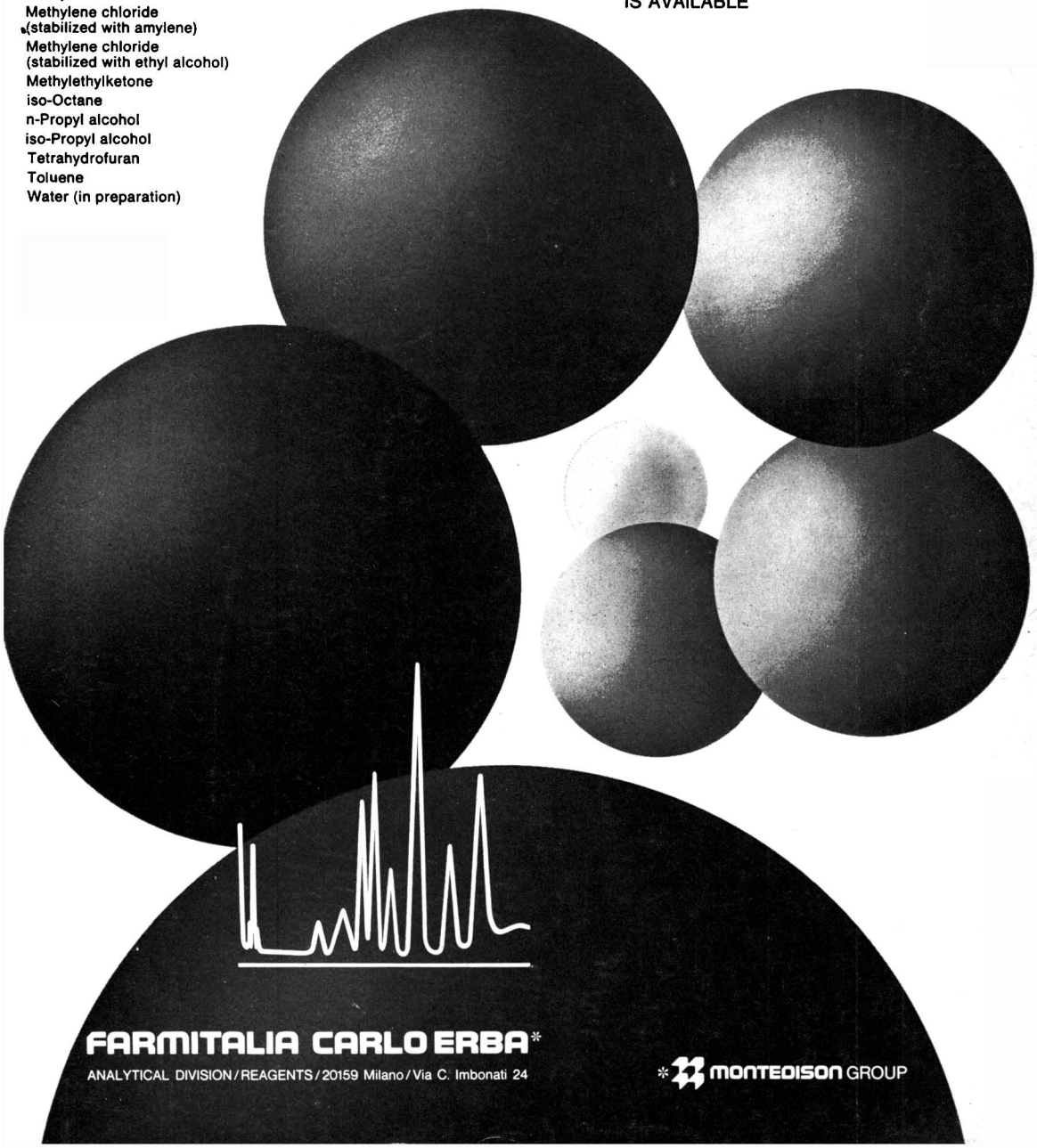

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Summaries of Papers in this Issue

Preservation of Inorganic Arsenic Species at Microgram Levels in Water Samples

A preservation study was carried out to determine the stability of inorganic species of arsenic as arsenic(III) and arsenic(V) in distilled and natural waters for a duration of 125 d. Various conditions of storage, such as pH, level of arsenic, type of container material and size of container, were studied. It was found that arsenic(III) and arsenic(V) are satisfactorily preserved for this time in both distilled and natural water samples at 1 and 10 $\mu g\ l^{-1}$ levels at room temperature if they are stored in polyethylene or Pyrex bottles with 0.2% V/V sulphuric acid.

Keywords: *Arsenic(III) and arsenic(V)*; *preservation and stability of solutions*; *distilled and natural water samples*

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Analyst, 1980, **105**, 737–743.

Automated Procedure for the Determination of Soluble Arsenic Using Hydride Generation Atomic-absorption Spectroscopy

An automated procedure for the determination of soluble arsenic, using hydride generation atomic-absorption spectroscopy, is described and optimised operating conditions are derived. Interferences are observed in the presence of silver(I), gold(III), iron(III), platinum(IV), antimony(III), strontium(II), fluoride and sulphide but can be overcome by suitable pretreatment procedures. The detection limit (based on twice the standard deviation for 15 blank measurements) is 0.90 ng ml^{-1} of arsenic for arsenic(III), arsenic(V) and methylarsenic species. A 10% negative bias of results is observed in the determination of dimethylarsenic species.

Keywords: *Arsenic determination*; *hydride generation*; *atomic-absorption spectroscopy*; *interferences*

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Analyst, 1980, **105**, 744–750.

Arsenic Speciation: Limitations with Direct Hydride Analysis

Speciation of inorganic arsenic(III) by hydride evolution directly into an atomic-absorption system was found to be subject to error when organic forms of arsenic (such as dimethylarsinic acid) were present. Organic forms of arsenic can produce an underestimation of total arsenic when the hydride response from concentrated acid is quantitated against inorganic arsenic. Hydride responses from solutions of various acidities are reported for dimethylarsinic acid, monomethylarsonic acid, inorganic arsenic(III) and inorganic arsenic(V). Even simultaneous equations did not provide a means of resolving mixtures of the four arsenic species investigated by using direct analysis of the evolved hydrides.

Keywords: *Arsenic speciation*; *arsine generation*; *atomic-absorption spectrophotometry*; *environmental analysis*

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Analyst, 1980, **105**, 751–755.

Simultaneous Determination of Trace Concentrations of Arsenic, Antimony and Bismuth in Soils and Sediments by Volatile Hydride Generation and Inductively Coupled Plasma Emission Spectrometry

Trace amounts of arsenic, antimony and bismuth in soils and sediments are determined simultaneously by an inductively coupled plasma - volatile hydride method after rapid attack with concentrated hydrochloric acid in sealed tubes. The samples are treated with the acid at 150 °C for 2 h in capped test-tubes. After addition of potassium iodide solution, the hydrides are formed by mixing the solution with sodium tetrahydroborate(III) solution in a continuous-flow system, and are swept into the plasma by a stream of argon for determination by atomic-emission spectrometry. Acceptable precision and accuracy are obtained, and the detection limits for all three analytes are about 0.1 $\mu g\ g^{-1}$. Approximately 200 samples can analysed by one person in a 2-d cycle.

Keywords: *Arsenic, antimony and bismuth determination*; *hydride generation*; *inductively coupled plasma emission spectrometry*; *geochemical samples*

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Analyst, 1980, **105**, 756–761.

Specific and Sensitive Spectrophotometric Determination of Cobalt with 3-(2′-Thiazolylazo)-2,6-diaminotoluene

Cobalt and 3-(2′-thiazolylazo)-2,6-diaminotoluene react in an acidic sodium acetate medium in the presence of vanadate to give several complexes. The soluble blue complex in a strong perchloric acid medium ($H_o = -0.5$) obeys Beer's law between 0.05 and 0.60 p.p.m., with a molar absorptivity of 9.74×10^4 l mol^{-1} cm^{-1} at 590 nm, a Sandell sensitivity index of 0.60 ng cm^{-2} and a relative error of 0.13%. The method is highly specific and has been applied to the spectrophotometric determination of cobalt in low-alloy steels, hydrofining catalysts and high-purity nickel salts.

Keywords: 3-(2′-*Thiazolylazo*)-2,6-*diaminotoluene reagent*; *cobalt determination*; *spectrophotometry*

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Analyst, 1980, **105**, 762–767.

Spectrophotometric Determination of Anionic Surfactants in River Waters Using 1-(4-nitrobenzyl)-4-(4-diethylaminophenylazo)-pyridinium Bromide

Nitro, bromo and methyl derivatives of 1-(benzyl)-4-(4-diethylaminophenylazo)pyridinium bromide were synthesised and evaluated as new cationic reagents for the determination of anionic surfactants. These reagents were very stable and reacted with anionic surfactants, such as alkylbenzenesulphonate and alkylsulphate, to form a 1:1 stable ion associate, which was extracted into chlorobenzene in a single extraction. The apparent molar absorptivity of the ion associate of the 4-nitro derivative with sodium di-(2-ethylhexyl)sulphosuccinate was 6.10×10^4 l mol^{-1} cm^{-1} (at 573 nm) in chlorobenzene. 1-(4-Nitrobenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide was used in the determination of μg l^{-1} levels of anionic surfactants in river water. The results were compared with the methylene blue method (Japanese Industrial Standard method) for river waters. This method is designed to determine anionic surfactant concentrations in solution.

Keywords: *Anionic surfactant determination*; *water analysis*; *spectrophotometry*; 1-(*benzyl*)-4-(4-*diethylaminophenylazo*)*pyridinium bromide derivatives*

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Analyst, 1980, **105**, 768–773.

Use of Charge-transfer Complexation in the Spectrophotometric Assay of Antazoline and Naphazoline in Combination

A simple and accurate spectrophotometric method is described for the assay of antazoline and naphazoline, combined in eye-nose drops in a ratio of 20:1. The major component, antazoline, is determined, without interference from naphazoline, by charge-transfer complexation with σ-acceptor iodine. After precipitation of antazoline with sodium carbonate, the minor component, naphazoline, can be determined using the π-acceptor chloranil. The accuracy of the method is assessed by the assay of a standard mixture of the two components containing the same proportions as the commercial preparation.

Keywords: *Spectrophotometry*; *antazoline determination*; *naphazoline determination*; *eye - nose drops*

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Analyst, 1980, **105**, 774–778.

Voltammetric Determination of Pharmaceuticals at the Tubular Graphite Electrode

Many pharmaceuticals, including phenothiazines, sulpha drugs, purines, phenolic acids, local anaesthetics and antifertility compounds, have been determined quantitatively by oxidative voltammetry at the tubular graphite electrode under hydrodynamic conditions. The data obtained fully establish the reliability of the technique for the determination of these compounds with ease, precision and speed.

A simple empirical correlation that permits the direct determination of the concentration of any unknown electroactive substance in one stage without using a standard reference solution has been derived and verified.

Keywords: *Voltammetry*; *tubular graphite electrode*; *sulpha drugs*; *phenothiazine drugs*

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Analyst, 1980, **105**, 779–786.

Determination of Dinitrotoluene Isomers in Sea Water and Industrial Effluent by High-resolution Electron-capture Gas Chromatography with a Glass Capillary Column

A method for the rapid determination, with base-line separation, of dinitrotoluene isomers in sea water and industrial effluent using gas chromatography with glass capillary columns and electron-capture detection is described. The samples were extracted with benzene and the extract was injected into the gas chromatograph using a support-coated open-tubular (SCOT) glass capillary column coated with Apiezon L grease. The chromatogram was completed in 8 min. Sea water control samples were spiked at the levels of 0.8 and 8 $\mu g\ l^{-1}$ of 2,6-, 2,3- and 2,5-dinitrotoluene, at 3.2 and 32 $\mu g\ l^{-1}$ of 2,4- and 3,5-dinitrotoluene and at 1.6 and 16 $\mu g\ l^{-1}$ of 3,4-dinitrotoluene. Recoveries of 91–102% ($n = 5$) were obtained for each isomer.

The limits of detection were 0.059, 0.045, 0.031, 0.13, 0.12 and 0.17 $\mu g\ l^{-1}$ for 2,6-, 2,3-, 2,5-, 2,4-, 3,4- and 3,5-dinitrotoluene, respectively.

The method described in this work was applied successfully to the determination of dinitrotoluenes in sea water and industrial effluent without requiring further clean-up procedures.

A Silar 10C column also gave base-line separation of the dinitrotoluenes.

Keywords: *Dinitrotoluene isomer determination*; *gas chromatography*; *electron-capture detection*; *sea water analysis*; *industrial effluent analysis*

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Analyst, 1980, **105**, 787–793.

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The Analyst

Preservation of Inorganic Arsenic Species at Microgram Levels in Water Samples

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A preservation study was carried out to determine the stability of inorganic species of arsenic as arsenic(III) and arsenic(V) in distilled and natural waters for a duration of 125 d. Various conditions of storage, such as pH, level of arsenic, type of container material and size of container, were studied. It was found that arsenic(III) and arsenic(V) are satisfactorily preserved for this time in both distilled and natural water samples at 1 and 10 $\mu g\ l^{-1}$ levels at room temperature if they are stored in polyethylene or Pyrex bottles with 0.2% *V/V* sulphuric acid.

Keywords: Arsenic(III) and arsenic(V); preservation and stability of solutions; distilled and natural water samples; pH; container sizes

Preservation conditions that maintain integrity and stability of environmental water samples are a prerequisite to the successful execution of analytical determinations and inter-laboratory quality control studies. Our laboratory has initiated a series of studies on key parameters that have recently been of environmental importance. These include mercury,[1] seven phenoxy herbicidal acids[2] and selenium.[3] These studies have served as a basis for the confident preparation of stable aqueous solutions in National Interlaboratory Quality Control Studies.[4–6] This study was undertaken with the same purpose as the above and deals with the preservation and stability of arsenic in water.

It was believed for a long time that arsenic exhibited great toxicity but it has recently been suggested to have, along with selenium, nutritional value owing to its electron-transfer capability.[7] Arsenic is one of the many constituents routinely analysed by most analytical-services laboratories. It is, therefore, an important parameter to be investigated so that the optimum conditions are found that will demonstrate its stability in environmental samples. These optimum conditions are not only necessary for the planning and designing of inter-laboratory comparison studies and for accurate and precise determinations, but are also vital information for the preparation of bulk reference samples.

There is no comprehensive documentation on the stability of arsenic at natural levels and it is not clear whether a preservative is needed. The Analytical Methods Manual of the Water Quality Branch[8] makes no special recommendation for the preservation and storage of arsenic. In the past, samples have been stored unacidified but at 4 °C. This procedure satisfactorily maintains arsenic in solution and at the same time prevents the formation of algae that occurs when unacidified natural samples with bacterial activity are stored at room temperature. As will be shown later, algae are a serious interferent. Low-temperature storage is, however, impractical as it requires special facilities and could be expensive for large numbers of samples. Furthermore, transportation of samples becomes a real problem. Samples must be kept preserved in transit from field sampling stations to the laboratory. Also, inter-laboratory quality control samples must retain their original integrity from the time of shipment to participating laboratories until time of analysis.

The United States Environmental Protection Agency[9] has recommended preservation with nitric acid to a pH of 2. Nitric acid, however, has been reported to be a serious interferent[10,11] in the reduction reactions commonly used in arsenic determinations. In inter-laboratory quality control studies, where many different methodologies are used by participating laboratories, the use of nitric acid as a preservative could result in a negative bias

for those laboratories using methods sensitive to the above interference. Therefore, this acid could not be considered. Hydrochloric acid has no interfering effects but it is very volatile and somewhat impractical for use in the field. Sulphuric acid was chosen because it is a common acid used in analytical chemistry, and is stable and non-volatile and therefore is easy to use in the field. Furthermore, it has no serious interfering effects on the reduction reactions used in the analysis of arsenic.

In our Water Quality Laboratories, arsenic and selenium are normally analysed from the same solution, as their analytical methodologies are similar. Cheam and Agemian[3] reported that 0.2% *V/V* sulphuric acid satisfactorily stabilises aqueous solutions (distilled and natural waters) of selenium(IV) and selenium(VI) for the duration of 4 months in both glass and plastic containers. This study was designed in a similar way so that common preservation conditions could be found for the two metals. It can be seen that 0.2% *V/V* sulphuric acid is a common preservative for the two metals at the parts per billion (10^9) levels (micrograms per litre) and for seven phenoxy herbicidal acids too.[2] Optimum conditions are discussed in terms of pH, water types and container material and size. Two types of water are used: distilled water representing very clean sample types and Hamilton Harbour water, which is heavily loaded with nutrients, bacterial activity and major ions, which is at the other extreme. Most water samples analysed in Water Quality laboratories lie within these two extremes. The preservation conditions recommended in this study take into account practical considerations for both routine monitoring of a large number of samples as well as large scale inter-laboratory quality control studies.

Experimental

Reagents

Analytical-reagent grade chemicals were used throughout.

Stock solutions of arsenic, 1000 mg l^{-1}. Prepared using the sodium salts $NaAsO_2$ and $Na_2HAsO_4.7H_2O$ (Baker Analyzed Reagents and Ventron Corporation/Alfa Division). The solutions were preserved with 1% *V/V* sulphuric acid.

Sodium tetrahydroborate(III). Fisher Scientific Co.

Sodium hydroxide pellets. Fisher Scientific Co.

Concentrated sulphuric acid. Baker Analyzed Reagent.

Concentrated hydrochloric acid. Baker Analyzed Reagent.

Containers

Pyrex and polyethylene containers. Two sizes of containers were considered: 25-gal polyethylene barrels with spigots, purchased from CANBAR Products Ltd., and 500-ml Nalgene and Pyrex bottles.

Pyrex calibrated flasks. For the stock, intermediate and standard solutions.

All containers were cleaned with chromic acid, and rinsed five times with hot tap water and finally three times with de-ionised distilled water.

Hamilton Harbour Water

A comparison of some chemical composition data for Hamilton Harbour water with other major water systems is given in Table I. The data substantiate the fact that Hamilton Harbour water represents the highly contaminated extreme of water sample types used in Water Quality Laboratories.

Bulk and specific sample preparation

The bulk preservation samples were prepared using eight 25-gal barrels, four of which were filled with de-ionised distilled water and the other four with unfiltered Hamilton Harbour water. They were then spiked with arsenic(III) and arsenic(V) species at concentrations near 1 and 10 p.p.b. Each solution was then homogenised by a closed circuit mixing for 3 h using a magnetic drive pump (Fasco Industry Inc.). A control barrel containing de-ionised distilled water was monitored for possible arsenic release; after 3 months no arsenic was detected, and therefore further monitoring or discussion was deemed unnecessary.

Specific preservation samples were prepared by sub-sampling each spiked bulk sample into 3 Pyrex and 3 polyethylene 500-ml bottles. The pH of these samples were then adjusted to 1.5 (corresponding to about 0.2% *V/V* sulphuric acid), 5.4 (the approximate pH of distilled water) and 7.2 (the approximate pH of Hamilton Harbour water).

All preservation samples were kept at room temperature throughout the study.

TABLE I

SOME WATER QUALITY PARAMETERS REPORTED FOR HAMILTON HARBOUR LOWER AND UPPER GREAT LAKES AND WORLD LAKE AND RIVER WATER

Constituent	Hamilton Harbour[12]*	Lake Ontario[12]	Lake Erie[12]	Lake Huron[12]	Lake Superior[12]	Mean of World lake and river waters[14]
Calcium/mg l^{-1}	54	40.1	38	26.0	13.1	15
Magnesium/mg l^{-1}	11.6	8.2	8	7.2	2.8	4.1
Sodium/mg l^{-1}	30	13.1	11.4	3.1	1.2	6.3
Potassium/mg l^{-1}	5.37	1.4	1.2	0.8	0.5	2.3
Chloride/mg l^{-1}	61.2	28.3	24.5	5.6	1.2	7.8
Sulphate/mg l^{-1}	63	28.6	25.0	16.0	2.7	11.2
Hardness, total $CaCO_3$/mg l^{-1}	183	134	128	95	44	54
Specific conductance/μohm^{-1} cm^{-1}	511	344	292	207	97	149 (Calculated, ref. 12)
Complexing capacity/μg l^{-1} Cu	200[15]	55[15]	—	—	—	—
Total phosphorus/μg l^{-1}	73	24	28	5.5	5.0	—
Nitrate/mg l^{-1} N	1.89	0.14	0.337	0.282	0.308	0.23
Ammonia/mg l^{-1} N	1.13	0.01	—	0.003	0.002	—
Iron/mg l^{-1}	0.28	0.018	0.003	0.003	0.0022	0.67
Manganese/mg l^{-1}	0.09	0.0006	0.0005	0.0004	0.0003	—
Copper/mg l^{-1}	0.02	0.0012	0.0025	0.0005	0.001	0.010†
Zinc/mg l^{-1}	0.06	0.0022	0.010	0.001	0.0037	0.010†

* The values reported for Hamilton Harbour are mean results observed in 1975.
† Mean copper content of ordinary fresh waters is about 0.010 mg l^{-1} (ref. 14, p. G–47).
Mean zinc content of ordinary lake and river waters is about 0.010 mg l^{-1} (ref. 14, p. G–48).

Arsenic Monitoring

The monitoring of inorganic arsenic was carried out for a period of 4 months using the automated technique reported by Agemian and Cheam.[16] The relative standard deviations at 1 and 10 μg l^{-1} of arsenic were estimated to be around 10 and 5%, respectively. During the study many runs were made, and each consisted of about 60 actual analyses in addition to those for calibration graphs, bracketing about every 12 analyses. The intermediate and standard solutions were prepared freshly on the day each run was carried out.

Results and Discussion

The levels of arsenic in the North American Great Lakes[17] are in the range <0.1–1.20 μg l^{-1}. Schramel *et al.*[18] have reported a range of 2.2–8.1 μg l^{-1} of arsenic in some locations in Germany. Arsenic levels as high as 70 μg l^{-1} have been reported, such as in the Allegheny River, Pittsburgh.[19] In this study the stability of 1 and 10 μg l^{-1} levels of arsenic were studied, as this range represents the lower end of the range of natural levels. As the higher the level of an analyte the greater is its solution stability, it would follow that if low-level solutions preserve well then higher level solutions will also preserve well.

Figs. 1 (*a*) and (*b*) show the stability of 1 μg l^{-1} of arsenic(III) solutions under various conditions of storage. It is apparent that pH 1.5 is the most favourable condition for preservation for both types of container and water. It is interesting to note that in distilled water and at the higher pH values of 5.4 and 7.2, preservation was satisfactory for 500-ml polyethylene containers while substantial losses occurred in 500-ml Pyrex bottles. Because distilled water contains no interfering ions and no species with complexing ability or bacterial activity and as volatilisation is not a common problem with arsenic under these conditions, the low recoveries observed above are predominantly due to a surface interaction. Comparison of Figs. 1 (*a*) and (*b*) shows that at pH 5.4 and 7.2 the stability of arsenic(III) in distilled water and in polyethylene bottles was satisfactory for the duration of the experiment, but the stability in Hamilton Harbour water decreased considerably after about 2 months.

There was, however, an interesting observation in the middle of the experiment. Traces of algal growth were observed after 6 weeks and by 2 months had become substantial. The appearance of algae corresponds well with the drop in arsenic(III) recovery at around 2 months. For the Pyrex containers the behaviour of the graphs is different for the two

types of water. In the distilled water a steady loss is observed and as indicated above is most probably due to a surface interaction. However, for the Hamilton Harbour water, glass containers are satisfactory for up to 2 months but stability declines as algae appear.

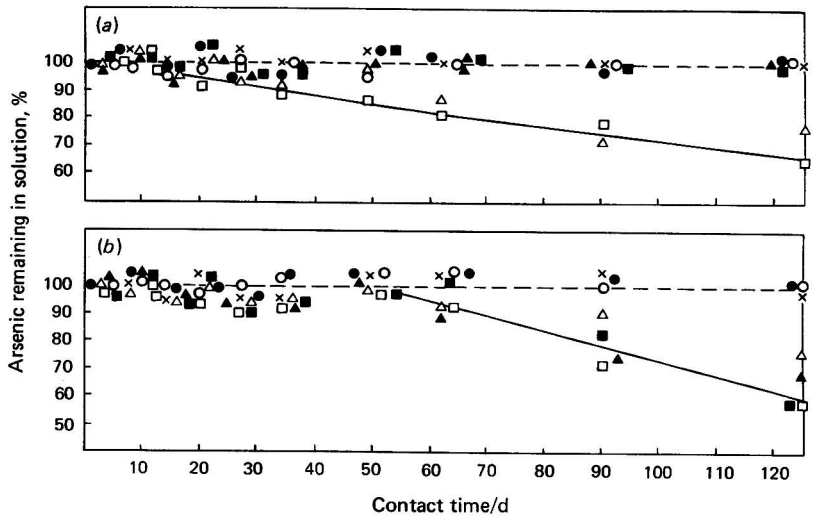

Fig. 1. Time dependence of arsenic(III) species, 1 μg l^{-1}, remaining in solution: (*a*) distilled water; (*b*) Hamilton Harbour water. Polyethylene bottles: ● pH 1.5, ▲ pH 5.4, ■ pH 7.2. Pyrex bottles: ○ pH 1.5, △ pH 5.4, □ pH 7.2. Polyethylene barrel: × pH 5.4 for distilled water or 7.2 for Hamilton Harbour water.

Evidently the higher ionic load of this water compared with distilled water removes the effect of the container material on the stability but the bacterial activity causes algal interference after 2 months. For those containers preserved at pH 1.5, no algae were observed in the Hamilton Harbour water samples. The data indicate that acidification to pH 1.5 with sulphuric acid effectively stops bacterial activity and removes the above-mentioned interference as well as the effect of container material.

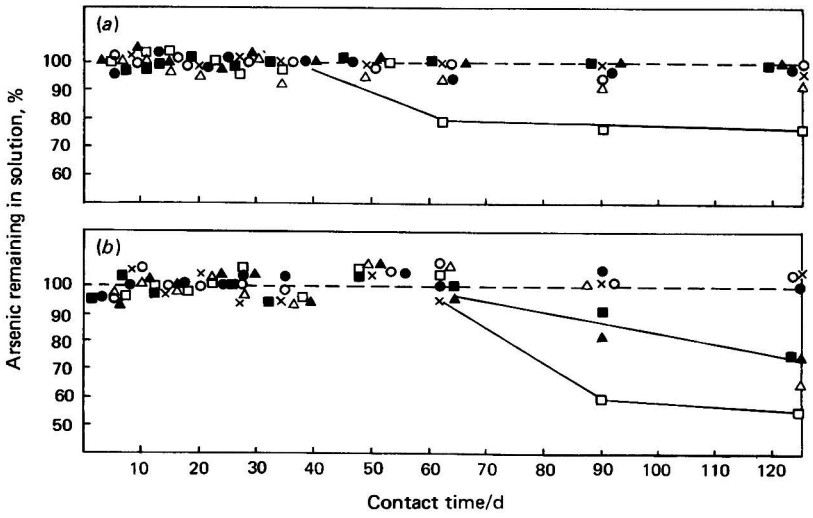

Fig. 2. Time dependence of arsenic(III) species, 1 μg l^{-1}, remaining in solution: (*a*) distilled water; (*b*) Hamilton Harbour water. Polyethylene bottles: ● pH 1.5, ▲ pH 5.4, ■ pH 7.2. Pyrex bottles: ○ pH 1.5, △ pH 5.4, □ pH 7.2. Polyethylene barrel: × pH 5.4 for distilled water or pH 7.2 for Hamilton Harbour water.

Figs. 2 (*a*) and (*b*) show similar graphs to the above for arsenic(III) at a 10-fold greater concentration. Polyethylene containers are again preferred to Pyrex bottles at high pH in distilled water. The negative effect of algae in Hamilton Harbour water is similarly seen after 2 months of storage at pH 5.4 or 7.2. Acidification with 0.2% sulphuric acid to a pH of 1.5 again inhibits bacterial activity and the consequent growth of algae, thus satisfactorily preserving arsenic(III), irrespective of container material or water type.

Arsenic has two stable oxidation states, three and five. Of these the former is probably the dominant state in the natural environment owing to the reducing effects of the abundant organic matter. It is, however, necessary to study the behaviour of both species in water samples in order to obtain a better understanding of their role in solution stability. Figs. 3 (*a*) and (*b*) show the behaviour of arsenic(V) at 1 μg l^{-1} for the same conditions as studied above. The effect of container material is similar to arsenic(III), where polyethylene containers were shown to be better than those made of Pyrex. There is, however, a significant difference with the distilled water. While with arsenic(III) and plastic bottles satisfactory stability is obtained at pH 1.5, 5.4 and 7.2, for arsenic(V) this is only true at pH 1.5 at the end of the experiment. This signifies that the stability of arsenic(V) is much more dependent on pH than that of arsenic(III). In the Hamilton Harbour water, the stability again deteriorates after 2 months when algae appear, but the loss is more severe than for arsenic(III). Acidification to pH 1.5 removes all interfering effects and gives 100% recovery after 4 months.

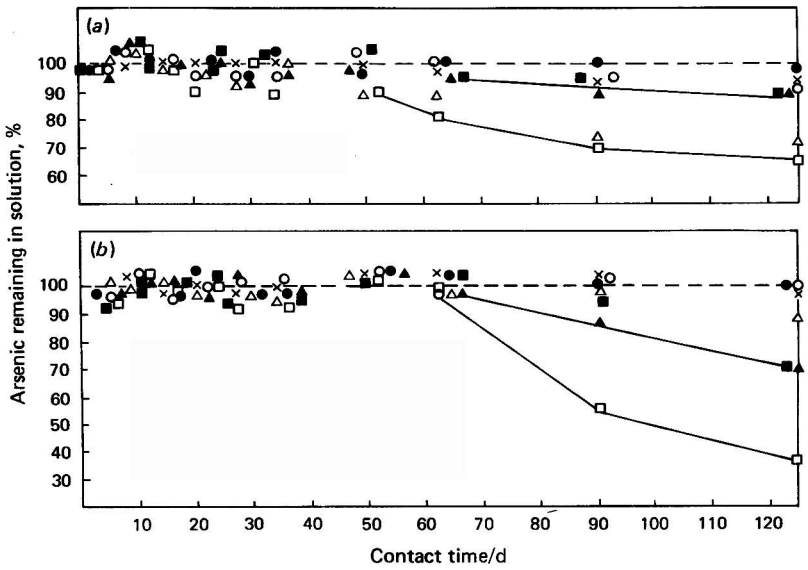

Fig. 3. Time dependence of arsenic(V) species, 1 μg l^{-1}, remaining in solution: (*a*) distilled water; (*b*) Hamilton Harbour water. Polyethylene bottles: ● pH 1.5, ▲ pH 5.4, ■ pH 7.2. Pyrex bottles: ○ pH 1.5, △ pH 5.4, □ pH 7.2. Polyethylene barrel: × pH 5.4 for distilled water or pH 7.2 for Hamilton Harbour water.

Figs. 4 (*a*) and (*b*) show the behaviour of arsenic(V) at a concentration of 10 μg l^{-1} under the above conditions. An increase in concentration has a slight stabilising effect for all conditions, unlike arsenic(III), for which there was no detectable change. The behaviour of arsenic(V) at 10 μg l^{-1} is otherwise similar to that of 1 μg l^{-1} solutions.

The experiments discussed so far were carried out in 500-ml bottles, which is a typical size for routine monitoring or for inter-laboratory quality control studies. Some experiments were also carried out in 25-gal polyethylene barrels to determine the effect the volume to surface area ratio has on the stability of unacidified arsenic solutions. It is apparent from Figs. 1–4 (polyethylene barrel) that no deterioration in stability occurred after 4 months of storage for any of the various conditions studied. Even at pH 5.4 for distilled water, or 7.2 for Hamilton Harbour water, no significant loss occurred. A calculation of the

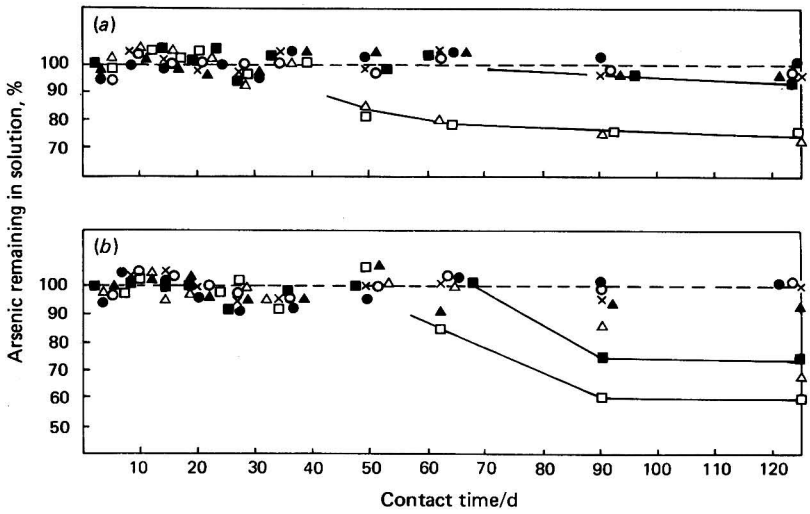

Fig. 4. Time dependence of arsenic(V) species, 1 μg l^{-1}, remaining in solution: (*a*) distilled water; (*b*) Hamilton Harbour water. Polyethylene bottles: ● pH 1.5, ▲ pH 5.4, ■ pH 7.2. Pyrex bottles: ○ pH 1.5, △ pH 5.4, □ pH 7.2. Polyethylene barrel: × pH 5.4 for distilled water or pH 7.2 for Hamilton Harbour water.

surface area to volume ratio showed that this ratio is 7 times higher for the 500-ml bottles than for the 25-gal barrels. Therefore, container size effectively changes the available surface area and substantially affects solution stability. Even for Hamilton Harbour water where algae form, no loss occurred. Evidently the algae surface area is not large enough to cause any appreciable loss. It is therefore obvious that while a pH of 1.5 achieved by adding sulphuric acid is indeed necessary for proper preservation and for the inhibition of algal growth in 500-ml bottles, no preservative is required for 25-gal barrels. This type of observation was also made earlier with selenium species,[3] which were stable in the barrels but unstable in the 500-ml bottles for both distilled and natural waters.

The above observations on the effect of container size could lead to some practical implications. For large scale inter-laboratory quality control studies large bulk samples must be obtained, homogenised, spiked and stored until sub-divided into smaller bottles for shipment to participating laboratories. The above data indicate that 25-gal barrels are suitable as storage vessels without any chemical treatment and thus are directly applicable in such round-robin studies.

The 0.2% sulphuric acid preservative, by inhibiting algal growth, makes the samples suitable for analysis by an automated system. If samples are not acidified and algae are produced in the sample, the only way that a meaningful determination could be carried out would be to digest and oxidise the whole content of the bottle and release all the arsenic back into solution. The inclusion of such a step is not desirable as this leads to a manual step being introduced, which is more expensive and time consuming to carry out, introduces possible errors and a blank value due to the reagents used, and destroys the whole sample.

The preferred and suggested preservation procedure is acidification with 0.2% *V*/*V* sulphuric acid (pH 1.5) and storage at room temperature in polyethylene bottles. Although glass is equally suitable under these conditions, it is excluded for practical reasons. This preservation method was tested in a National Interlaboratory Quality Control Study.[6] Ten samples, concentrations ranging from 0 to 1000 μg l^{-1}, were distributed to 41 laboratories. Statistical analyses of all returned analytical data support the effectiveness of the preservation system described, particularly for those data determined by the hydride generation technique.

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Automated Procedure for the Determination of Soluble Arsenic Using Hydride Generation Atomic-absorption Spectroscopy

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An automated procedure for the determination of soluble arsenic, using hydride generation atomic-absorption spectroscopy, is described and optimised operating conditions are derived. Interferences are observed in the presence of silver(I), gold(III), iron(III), platinum(IV), antimony(III), strontium(II), fluoride and sulphide but can be overcome by suitable pretreatment procedures. The detection limit (based on twice the standard deviation for 15 blank measurements) is 0.90 ng ml^{-1} of arsenic for arsenic(III), arsenic(V) and methylarsenic species. A 10% negative bias of results is observed in the determination of dimethylarsenic species.

Keywords: *Arsenic determination*; *hydride generation*; *atomic-absorption spectroscopy*; *interferences*

Arsenic is a widespread element that has found extensive use in medicinal, agricultural and industrial fields. Although arsenic does not appear to be accumulated by man[1] it has been implicated in the development of hyperkeratosis and skin cancer,[2] lung cancer[3] and arteriosclerosis.[4] In natural waters it is considered to represent a significant health hazard[5] and as such it is important that suitable sensitive methods of analysis are available for its routine determination.

Although many techniques have been applied to the analysis of arsenic,[6] only spectrophotometry and atomic-absorption spectroscopy have been widely used. The standard method of the American Public Health Association[7] is based on the colorimetric reaction between arsine and silver diethyldithiocarbamate (SDDC). This method is slow, comparatively insensitive, of poor precision in inexperienced hands and is limited to the determination of inorganic arsenic(III) and arsenic(V) species. Although other colorimetric procedures are available,[8–11] few are superior to the SDDC procedure.

Owing to the inherent insensitivity of the flame atomic-absorption spectroscopy of arsenic,[12] the conversion of arsenic compounds into arsines, followed by atomisation in a heated atomiser tube, has been widely adopted.[13–15] Using the hydride generation techniques very high sensitivity can be achieved, but only at the expense of a comparatively long analysis time and constant attention from the operator. Procedures for the automation of arsenic analysis by hydride generation atomic-absorption spectroscopy have been described by several workers.[16–18] Major interferences have been observed from those elements which consume reductant and thereby reduce the efficiency of arsine production,[19] but little information is available on overcoming such problems.

In this paper the optimised automation of the hydride generation atomic-absorption spectroscopic determination of arsenic is described; interferences have been investigated and treated. The procedure retains much of the inherent sensitivity of the manual hydride generation technique whilst allowing the efficient processing of large numbers of samples.

Experimental

Reagents and Glassware

Unless otherwise stated all chemicals were of analytical-reagent grade. Glassware was soaked in nitric acid (1 + 9), rinsed with distilled water and dried before use.

Stock 1000 μg ml^{-1} solutions of arsenic were prepared from arsenic trioxide, disodium hydrogen arsenate, methylarsonic acid (disodium salt) and dimethylarsenic acid (sodium salt), and were checked for total arsenic content by atomic-absorption spectroscopy using

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an air - acetylene flame. Solutions of lower concentration were prepared immediately before use. Control of pH was achieved using potassium dihydrogen orthophosphate (0.1 M) - orthophosphoric acid for pH 1–3, sodium acetate (0.2 M) - acetic acid for pH 4–7 and borax (0.25 M) adjusted to give pH values between 8 and 11.

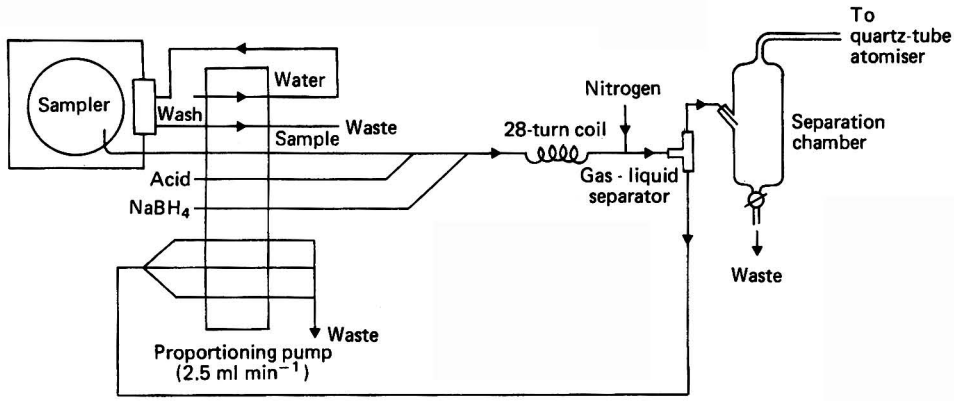

Fig. 1. Schematic diagram of the arsine generation manifold.

Apparatus

The apparatus consisted of a Hook and Tucker A40 Mk II automatic sampler, a Technicon AutoAnalyzer proportioning pump fitted with 2.18 mm i.d. silicone-rubber tubing giving a flow-rate of approximately 2.5 ml min^{-1} on all channels, and standard AutoAnalyzer components. The manifold arrangement, the major role of which is the reduction of the arsenic compounds to arsines and separation of the gas phase from the reactants, is shown schematically in Fig. 1. Samples from the autosampler are mixed with hydrochloric acid and then with sodium tetrahydroborate(III) solution. Rapid evolution of hydrogen from the decomposition of the sodium tetrahydroborate(III) leads to the formation of gas pockets that segment the liquid stream whilst it travels through the 28-turn mixing coil (Technicon No. K105–0083). A flow of nitrogen carrier gas takes the liquid - gas mixture rapidly to a preliminary gas - liquid separator (Technicon No. K116–0122) and from there to the separation chamber, where residual solvent droplets sediment out. The gas stream is then swept into a quartz tube (14 cm × 8 mm i.d.) atomiser aligned in the optical path of a Varian-Techtron AA175 AB background-corrected atomic-absorption spectrometer. In order to achieve atomisation of the arsine, the quartz tube atomiser is heated with an air - acetylene flame.[20] The following spectrometer conditions were used throughout the work: lamp current, 7.0 mA; wavelength, 197.3 nm; spectral band pass, 1 nm; and damping, B.

Sample Digestion

Acid procedure

A sample aliquot (less than 1 g) is boiled with 10 ml of concentrated sulphuric acid until the sample is dry. After cooling, concentrated sulphuric acid (10 ml) and concentrated nitric acid (10 ml) are added and the mixture is heated almost to boiling. The digestion is completed by the careful dropwise addition of 10 ml of hydrogen peroxide solution (30% *m/V*) to the sample and evaporation of the sample to a final volume of approximately 5 ml.

Alkaline fusion

An accurately weighed sample aliquot (less than 1 g) is fused in a zirconium crucible with sodium hydroxide (1 g). The melt is heated for 10 min and, after cooling, is dissolved in 10 ml of distilled water.

Results

Optimisation of Experimental Conditions

Reduction pH

The redox potential of sodium tetrahydroborate(III) is highly dependent on pH and the reduction of the four arsenic species was therefore studied over the pH range 1–9 and at high acid concentrations. For these experiments the "acid" reagent reservoir was filled with buffer solution of the appropriate pH or with dilute hydrochloric acid, and the sodium tetrahydroborate(III) concentration was maintained at 2%. The results of these experiments are shown in Fig. 2 as a graph of response due to 200 ng ml^{-1} arsenic solutions *versus* acidity. The pH of samples of low buffer capacity had no effect on the response of the instrument.

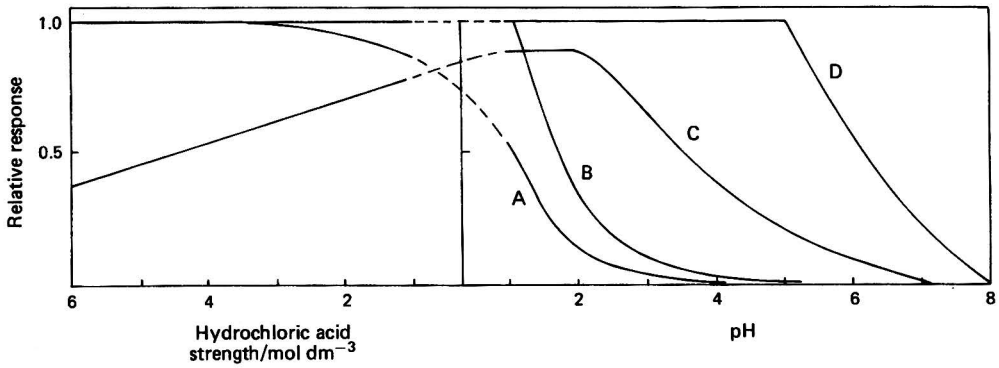

Fig. 2. Effect of reduction acidity on arsine yield, 200 ng ml^{-1} arsenic solutions with sodium tetrahydroborate(III) concentration held at 2% *m/V*. A, Arsenic(V); B, methylarsenic; C, dimethylarsenic; and D, arsenic(III).

Sodium tetrahydroborate(III) concentration

The concentration of the sodium tetrahydroborate(III) solution was varied from 1 to 5% *m/V* in distilled water, whilst maintaining the acid concentration at 3 M. The peak heights due to 200 ng ml^{-1} arsenic solutions were monitored as a function of sodium tetrahydroborate(III) concentration and are presented in Fig. 3.

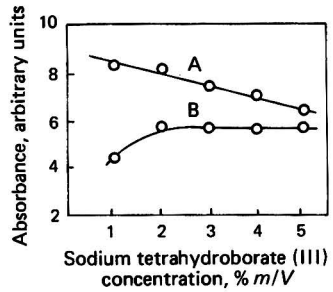

Fig. 3. Effect of sodium tetrahydroborate(III) concentration on arsine yield using 200 ng ml^{-1} arsenic solutions and 3 M hydrochloric acid. A, Arsenic(III), arsenic(V) and methylarsenic; B, dimethylarsenic.

Carrier gas flow-rate

The effect of altering the carrier gas flow-rate through the atomiser tube is influenced by both the instrument response time and the steady-state arsenic concentration in the atomisa-

tion cell. In order to investigate the effect of the nitrogen carrier gas flow-rate on instrument sensitivity the nitrogen flow-rate was varied from 0 to 900 ml min^{-1} and the response due to 200 ng ml^{-1} arsenic solutions was monitored (Fig. 4).

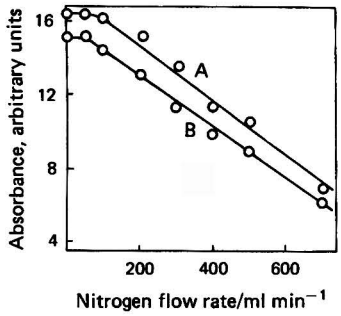

Fig. 4. Influence of carrier gas flow-rate on peak height obtained for 200 ng ml^{-1} arsenic solutions. A, Arsenic(III), arsenic(V) and methylarsenic; B, dimethylarsenic.

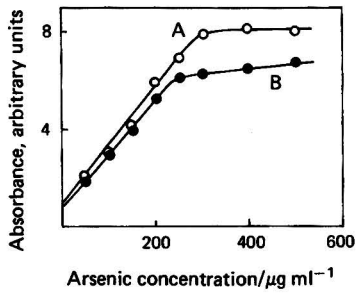

Fig. 5. Typical calibration graphs. A, Arsenic(III), arsenic(V) and methylarsenic; B, dimethylarsenic.

Optimised conditions

The following conditions were considered to give optimum instrument response: sampling time, 30 s; wash time, 40 s; sodium tetrahydroborate(III) concentration, 2% *m/V*; hydrochloric acid concentration, 3 M; and nitrogen flow-rate, 100 ml min^{-1}. A typical calibration graph is shown in Fig. 5.

When samples containing relatively high arsenic concentrations are interspersed between the low-concentration samples, a small amount of carry-over can be observed using the 40-s wash interval. Under such conditions, wash intervals of 150 and 250 s have been found to be sufficient to overcome carry-over from 10 and 100 μg ml^{-1} arsenic samples, respectively.

Based on 20 replicate analyses, the relative standard deviation of the technique was 1.7% for 100 ng ml^{-1} arsenic samples, increasing to 8.1% for 10 ng ml^{-1} arsenic samples. The detection limit (based on twice the standard deviation of 15 blank measurements) is 0.90 ng ml^{-1} of arsenic as arsenic(III), arsenic(V) or methylarsenic.

Interferences

Identification

Possible interference effects were assessed by the analysis of standard 200 ng ml^{-1} arsenic solutions in the presence of an excess of foreign ions. The results of these analyses are presented in Table I. Interference effects were observed in the presence of silver(I), iron(III), gold(III), platinum(IV), antimony(III), strontium(II), fluoride and sulphide. No effects (*i.e.*, less than 2% enhancement or depression of response) were observed for up to 10 μg ml^{-1} of aluminium(III), boron(III), bismuth(III), calcium(II), cadmium(II), cobalt(II), chromium(VI), copper(II), germanium(IV), mercury(II), potassium(I), lanthanum(III), magnesium(II), manganese(II), molybdenum(VI), nickel(II), lead(II), selenium(VI), tin(II), tellurium(IV), tellurium(VI), vanadium(IV) or zinc(II) or 100 μg ml^{-1} of nitrite, bromide, iodide or cysteine hydrochloride. In addition, 1000 μg ml^{-1} of calcium, potassium, hydrogen carbonate, chloride, nitrate, perchlorate and sulphate did not affect the analytical result.

Treatment

Antimony(III). Add 5 ml of 0.5 M pyrogallol solution to 25 ml of a 0.5 M sodium tartrate solution of the sample. Leave the sample for 2 h prior to analysis.

Gold(III). Make the sample solution (pH 2) 2.5 mM in thiosemicarbazide. Alternatively, extract the sample (pH 2) with a 0.005 M solution of dithizone in dichloromethane.

TABLE I

ASSESSMENT OF INTERFERENCES

Element	Concentration/ $\mu g\ ml^{-1}$	Depression of signal due to 200 ng ml^{-1} of arsenic, %			
		Arsenic(III)	Arsenic(V)	Methylarsenic	Dimethylarsenic
Ag(I)	0.1				
	1.0			5	10
	10			32	43
Au(III)	0.1				
	1.0	16			
	10	41	17	19	50
Fe(III)	0.1				
	1.0				
	10	19	10	10	8
Pt(IV)	0.1				
	1.0	9	10	18	25
	10	60	60	57	68
Sb(III)	0.1				
	1.0	34	30	50	45
	10	77	84	72	83
Sr(II)	0.1				
	1.0				
	10				17
F^-	10	48	52	50	55
S^{2-}	1	18	20	17	22

Platinum(IV) and strontium(II). Add 1% *m/V* 1,10-phenanthroline to the 3 M hydrochloric acid or sample. For all species except dimethylarsenic, 0.1% *m/V* 1,10-phenanthroline solution is sufficient.

Iron(III). Add 1 ml of 0.1 M cupferron to 10 ml of the sample solution (pH 1).

Fluoride. Add 0.01 M lanthanum nitrate and 0.01 M alizarin fluorine blue to the 3 M hydrochloric acid or sample.

Sulphide. Fit a lead acetate-impregnated glass-wool scrubber to the exit tube of the separation chamber.

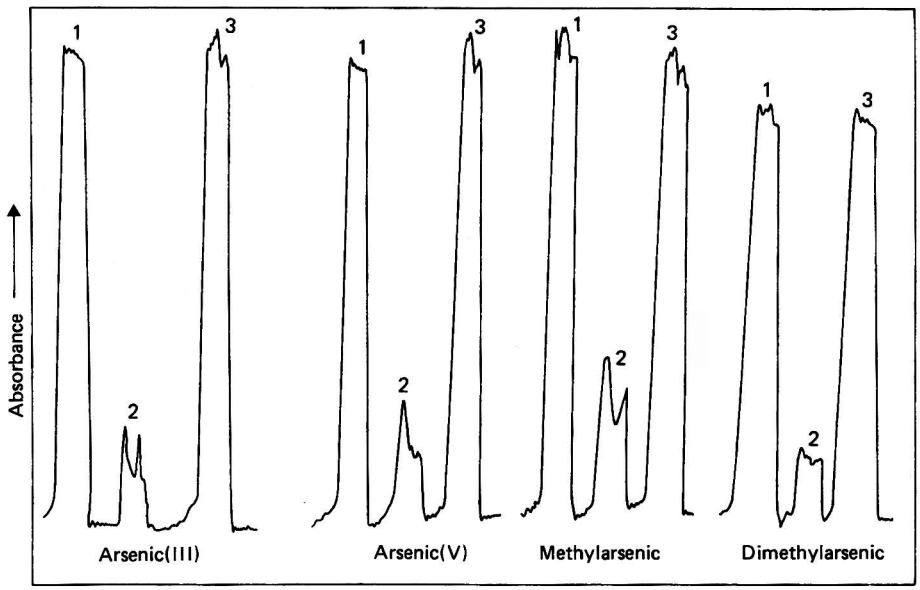

Fig. 6. Treatment of interferences. 1, Arsenic alone; 2, arsenic + interferents; and 3, arsenic + interferents after treatment.

The efficiency of these treatments was assessed by the analysis of solutions containing 200 ng ml^{-1} of arsenic together with 10 μg ml^{-1} of silver(I), gold(III), platinum(IV), antimony(III) and fluoride with and without treatment. Those analyses carried out with treatment involved extraction of the sample (pH 2) with dithizone followed by the addition of a reagent "cocktail" containing 1,10-phenanthroline, pyrogallol and alizarin fluorine blue reagents to the sample. Typical traces are shown in Fig. 6.

As washing powders are a potentially large source of arsenic in effluent and river waters, the analysis procedure has been applied to the determination of arsenic in several washing powder samples. The analyses were carried out using both acid and alkali fusion digestions and the results are presented in Table II.

TABLE II

ANALYSIS OF WASHING POWDERS

Values are the means of 5 determinations.

Sample	Arsenic content/μg g^{-1}	
	Acid digest	Alkaline fusion
A	1.23 ± 0.03	1.25 ± 0.04
B	1.70 ± 0.05	1.67 ± 0.05
C	0.24 ± 0.03	0.27 ± 0.02

Discussion and Conclusions

Optimised conditions have been developed for the automated determination of arsenic by atomic-absorption spectroscopy following conversion of arsenic compounds into their corresponding arsines. Below pH 1, reduction and atomisation of arsenic(III), arsenic(V) and methylarsenic are essentially complete and the instrument response is related to concentration irrespective of the arsenic species tested. Dimethylarsenic compounds, however, give a lower response than would be expected based on arsenic content and may represent incomplete reduction, condensation of dimethylarsine in the transport tubes or incomplete atomisation. The optimum sodium tetrahydroborate(III) concentration is 2% m/V, efficient reduction of dimethylarsenic being obtained whilst the intrinisic alkalinity of the sodium tetrahydroborate(III) is sufficiently low to maintain effective reduction of the other arsenic species. Although at high sodium tetrahydroborate(III) concentrations the responses due to the four arsenic species converge, destruction of dimethylarsenic by digestion prior to analysis is recommended.

The nitrogen carrier gas plays an important role in determining the sensitivity and speed of analysis. As can be seen from Fig. 4, an increase in the flow-rate decreases the sensitivity by reducing the residence time of atomic arsenic in the absorption cell. However, at the same time high carrier gas flow-rates give rise to improved equilibration of the system towards sample changes and help to cool the gas transit lines. A compromise between these two factors led to the selection of a carrier gas flow-rate between 100 and 200 ml min^{-1}.

Interferences that have so far been identified are silver(I), gold(III), iron(III), strontium(II), platinum(IV), antimony(III), fluoride and sulphide. By the addition of a "cocktail" of pyrogallol, thiosemicarbazide, 1,10-phenanthroline, cupferron, lanthanum nitrate and aliazarin fluorine blue to the sample and by fitting a lead acetate scrubber to the separation chamber, all of the identified interference effects can be overcome without a significant increase in the reagent blank.

The technique described provides a rapid and sensitive method for the assessment of arsenic levels in a wide range of sample types. With samples believed to contain dimethylarsenic, and where a maximum under-estimate of approximately 10% is unacceptable, preliminary destruction of dimethylarsenic species must be carried out prior to the determination step. Although most wet-ashing procedures fail to destroy organoarsenic compounds efficiently, two digestion procedures that have proved suitable are fusion with sodium hydroxide and wet ashing with nitric acid - sulphuric acid - hydrogen peroxide.

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Arsenic Speciation: Limitations with Direct Hydride Analysis

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Speciation of inorganic arsenic(III) by hydride evolution directly into an atomic-absorption system was found to be subject to error when organic forms of arsenic (such as dimethylarsinic acid) were present. Organic forms of arsenic can produce an underestimation of total arsenic when the hydride response from concentrated acid is quantitated against inorganic arsenic. Hydride responses from solutions of various acidities are reported for dimethylarsinic acid, monomethylarsonic acid, inorganic arsenic(III) and inorganic arsenic(V). Even simultaneous equations did not provide a means of resolving mixtures of the four arsenic species investigated by using direct analysis of the evolved hydrides.

Keywords: *Arsenic speciation*; *arsine generation*; *atomic-absorption spectrophotometry*; *environmental analysis*

Aggett and Aspell[1] reported the application of an arsenic speciation method for the determination of inorganic arsenic(III) and total arsenic in environmental water samples. In our experience the presence of methylated arsenic compounds can produce errors in speciation methods, like that described by Aggett and Aspell,[1] where hydrides of arsenic are generated directly into a detector. Some environmental water samples have been reported[2–4] to contain methylated arsenic compounds in amounts as high as 16–54% of the total arsenic. Even where methylated arsenic compounds have not been used agriculturally, inorganic arsenic can be converted into methylated forms in the environment.[5–8] Most of the inorganic arsenic ingested by humans is reportedly[9,10] excreted in the urine as methylated forms, which could add organic arsenic to environmental water systems. Dimethylarsinic acid is expected by some investigators[8] to be "the most abundant methylated arsenic compound in both freshwater and seawater."

Experimental

Apparatus†

A commercial hydride generation cell[11] (Instrumentation Laboratory Inc.) was used with a Perkin-Elmer, Model 403, atomic-absorption spectrophotometer (set to 193.7 nm) equipped with a deuterium arc background corrector, a nitrogen-entrained air - hydrogen flame and a 10-mV chart recorder with a rapid pen response (requiring less than 0.5 s for full-scale response).

A three-slot burner (length 11.2 cm) was used with flow-rates of 4 l min^{-1} of hydrogen and 23 l min^{-1} of nitrogen. The slit width on the spectrophotometer was set to 0.7 nm.

Reagents†

Arsenious oxide for inorganic arsenic(III). US National Bureau of Standards, 83c.
Arsenic pentoxide for inorganic arsenic(V). Fisher Scientific, A-54.
Disodium salt of monomethylarsonic acid (MMA). Alpha Products, 12134.
Sodium salt of dimethylarsinic acid (DMA). Fisher Scientific, S-257.
Sodium tetrahydroborate(III). Fisher Scientific, S-678. Solutions of 1 and 2% *m/V* in de-ionised water were prepared just before use.

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† Use of brand names does not constitute endorsement by the US Environmental Protection Agency.

Acetate buffer solution, 2 M (*pH* 4.8). A 28.6-ml volume of glacial acetic acid (Fisher Scientific, A-38) plus 68.04 g of sodium acetate trihydrate (Fisher Scientific, S-209) were made up to 500 ml with de-ionised water.

Concentrated hydrochloric acid. ACS reagent grade.

Reducing solution mixture. An 8.30-g amount of potassium iodide (Matheson, Coleman and Bell, CB 626) plus 5.0 g of L-ascorbic acid (Fisher Scientific, A-61) were made up to 50 ml with de-ionised water (after Siemer *et al.*[12]).

Procedure

For hydride generation at pH 4.8, 4 ml of acetate buffer were combined in the hydride cell with the sample solution and water to provide a total volume of 8 ml. With a septum covering the top port of the hydride generation cell, nitrogen was directed through the solution in the hydride generation cell for 30 s to remove air. The flow of nitrogen was then directed so as to bypass the generator solution. With a PTFE-coated magnet stirring the solution in the hydride generation cell and with the chart recorder pen tracing, 2 ml of the 1% sodium tetrahydroborate(III) solution were injected (using a syringe with a bevelled 23-gauge needle) through the septum in 2–3 s. At 30 s after the start of this injection, the flow of nitrogen was quickly directed through the hydride generating solution for 5 s before the nitrogen was re-directed to bypass the hydride generating solution. With the recorder pen still tracing, the injection of 1% sodium tetrahydroborate(III) solution was repeated, and the response recorded after 30 s by again flushing the hydride generating solution with nitrogen. The generator solution was drained and the hydride generation cell was rinsed with de-ionised water.

The two absorbance peak-height measurements for each solution were corrected for reagent blank contributions before being summed. Electronic integration values for these transient signals were not as reproducible as the peak heights. Use of a single injection of more sodium tetrahydroborate(III) solution instead of the two injections produced a premature release of arsenic hydrides into the nitrogen bypass stream to the detector. Purging the hydride generating solution with nitrogen while the sodium tetrahydroborate(III) solution was injected reduced the analytical sensitivity. The optimum signal to noise ratio for arsenic hydrides was obtained by positioning the burner just below the height at which light from the arsenic lamp began to be obstructed.

Treatment with the reducing agent mixture involved mixing 0.5 ml with a 2-ml portion of sample and 1.5 ml of 6 M hydrochloric acid, and allowing at least 30 min for reaction at room temperature before analysis.

For hydride generation from hydrochloric acid solution, varous acidities were obtained by using different volumes of concentrated hydrochloric acid combined with the sample solution and water, and the generation procedure was the same as for the studies using acetate buffer except that 2% sodium tetrahydroborate(III) solution was used instead of the 1% solution (and no acetate buffer was used).

Results and Discussion

With the system described above, a detection limit of 3 ng was obtained for inorganic arsenic(V) in hydrochloric acid, which agrees with the results of other workers[11,13,14] obtained using flame atomic-absorption analysis. For a reagent blank standard deviation corresponding to 0.9 ng ($n = 10$), a detection limit of 3 ng excludes 99.9% of the individual blank values. For the pulse-purged system described (providing an acceptable detection limit), reaction competition and the generator design prevented complete reaction of arsenic with a single treatment with sodium tetrahydroborate(III) solution, and the double treatment improved the precision of the measurement. With the pulse purge of the hydride generating solution, a 5-s flush was sufficient because the arsenic hydrides were driven into the detector beam rapidly, as indicated by the return of the recorder pen to the base-line level in 2 s.

Aggett and Aspell[1] attributed the response from environmental water samples buffered near pH 5 solely to inorganic arsenic(III) when the contribution from inorganic arsenic(V) was insignificant. They evaluated total arsenic in environmental water samples with the response from 5 M hydrochloric acid using an inorganic arsenic(III) calibration. Braman and Foreback[15] reported the use of pH adjustment in the selective hydride generation for inorganic arsenic(III).

Table I shows the relative hydride-forming behaviour observed for the arsenic compounds investigated. With the system described the hydride response from acetate buffer (pH 4.8) for the dimethylarsinic form (DMA) was found (Table I) to be 85 ± 2% of the response for an equal amount of arsenic in the form of inorganic arsenic(III). A mixture prepared to contain 12 ng ml^{-1} of arsenic in each of the forms inorganic arsenic(III), inorganic arsenic(V) and MMA, plus 60 ng ml^{-1} of arsenic as DMA, produced a hydride response from acetate buffer, indicating the presence of 66 ng ml^{-1} of arsenic in the form of inorganic arsenic(III) when the response was attributed solely to this arsenic species.

TABLE I

ATOMIC-ABSORPTION RESPONSES FOR HYDRIDES FROM FOUR ARSENIC SPECIES

Linear regression slopes ± standard errors for several days and levels.

Generating solution	Response relative to inorganic arsenic(III) = 100%			
	As(III)	As(V)	MMA	DMA
pH 4.8*	100 ± 2	3.3 ± 0.07	12 ± 1	85 ± 2
2 M HCl	100	99	99	44
4 M HCl	100	101	88	15
6 M HCl	100 ± 6	99 ± 3	69 ± 2	7 ± 0.3
8 M HCl	100	96	50	6
10 M HCl	100 ± 2	103 ± 2	29 ± 0.3	6 ± 0.2

* Acetate buffer at 1 M in total acetate after dilution by sample.

Both our measurements and the measurements by Aggett and Aspell[1] near pH 5 were conducted with strong buffering. Not more than a 0.25 pH unit change occurred during hydride generation according to Aggett and Aspell.[1] The buffering condition described by Braman and co-workers[2,15] is not comparable in strength to those used by us or by Aggett and Aspell.[1] When weak buffering was used, the hydride response from DMA relative to inorganic arsenic(III) was reduced from 84% to 12%, but both the precision and magnitude of the inorganic arsenic(III) response suffered with weak buffering. The buffering capacity is relevant because the sodium tetrahydroborate(III) reagent forms a basic solution at pH 11 or more (by hydrolysis when an alkali metal hydroxide is not added). Hydride formation from DMA (with pK_a 6.19)[2] at a stable pH near 5 is consistent with the contention by Braman and Foreback[15] that the undissociated molecules are reactive.

DMA may not be the only organoarsenic compound that can contribute large amounts of hydride, relative to inorganic arsenic(III), from a strong buffer at pH 4.8. When MMA was treated with the reducing agent mixture before hydride generation from acetate buffer, the response at 103 ± 2.4% (n = 26) relative to inorganic arsenic(III) differed markedly from the 12 ± 1% for untreated MMA shown in Table I. Neither inorganic arsenic(III) nor DMA showed a significant change in hydride response from acetate buffer after treatment with the reducing agent mixture. It is conceivable (but not proved) that the change in behaviour observed with MMA is the result of the conversion of the pentavalent arsenic in MMA to the trivalent (and less dissociated) arsonous [$CH_3As(OH)_2$] or arsenosomethane (CH_3AsO) forms.

The hydride response from hydrochloric acid (4 M and above) for DMA was found (Table I) to be 15% or less of the response for an equal amount of arsenic in the form of inorganic arsenic(III). The mixture specified three paragraphs above (with a total arsenic concentration of 96 ng ml^{-1}) produced a hydride response from 10 M hydrochloric acid, indicating the presence of only 37 ng ml^{-1} of total arsenic when the response was attributed solely to inorganic arsenic, *i.e.*, evaluated with an inorganic arsenic calibration. When sufficient sodium tetrahydroborate(III) reagent is used, inorganic arsenic(III) and inorganic arsenic(V) respond similarly from hydrochloric acid in our system. The use of 10 M hydrochloric acid provided a means of minimising the hydride contribution from the methylated arsenic compounds as part of a speciation investigation. Judging from Table I, the results for the determination of total arsenic in samples containing methylated arsenic using the hydride response from 5 M hydrochloric acid would also be an underestimate when evaluated with an inorganic arsenic calibration. Table I shows that the relative hydride response for both

methylated arsenic species decreased with increasing acidity of the hydride generating solution. While the decreasing hydride response with increasing acidity of the hydride generating solution may simply result from proton competition for the sodium tetrahydroborate(III) reagent, the formation of arsenic cations by protonation may also play a role.

Even samples subjected to acid digestion may still contain DMA, as it is reportedly[16] "a remarkably stable compound, remaining undecomposed by the action of fuming nitric acid, aqua regia or potassium permanganate even upon heating." If DMA is not decomposed to inorganic arsenic by a digestion procedure, underestimation of the total arsenic could occur when the evaluation is based on an inorganic arsenic calibration. Other investigators[17] have commented on the digestion procedure suggested by Aggett and Aspell[1] for plant materials in terms of the inorganic arsenic species.

When synthetic mixtures were limited to the two inorganic arsenic species, the practicality of compensating (by calculation) for the hydride contribution from inorganic arsenic(V) in determining inorganic arsenic(III) from acetate buffer responses was confirmed by a statistical evaluation. Inorganic arsenic(V) was evaluated for these two-component mixtures by the difference between the total arsenic (from the hydrochloric acid response) and the inorganic arsenic(III). Although Table I shows that the hydride response from acetate buffer for inorganic arsenic(V) is only 3.3% of the inorganic arsenic(III) response, the bulk of the hydride response from acetate buffer can stem from inorganic arsenic(V). For example, 60% of the hydride response from acetate buffer observed for a mixture prepared to contain 18 ng ml^{-1} of inorganic arsenic(III) and 600 ng ml^{-1} of inorganic arsenic(V) was attributable to the latter species. The hydride response from acetate buffer for inorganic arsenic(V) was not merely contamination of the inorganic arsenic(V) by inorganic arsenic(III) because the response did not decrease with additional treatments with sodium tetrahydroborate(III) solution. Some other investigators[1,3] (but not all) have observed a contribution from inorganic arsenic(V) under the conditions used to determine inorganic arsenic(III), and the extent of the contribution from inorganic arsenic(V) may depend on the buffering capacity of the hydride generating solution. With weak buffering, the pH of the hydride generating solution can increase by several units on addition of the sodium tetrahydroborate(III) reagent, which can alter the hydride response by altering the dissociation (and thereby the reduction potential) of the arsenic species.

While overlapping hydride responses for mixtures limited to inorganic arsenic(III) and inorganic arsenic(V) did not prevent speciation, resolving mixtures of all four arsenic compounds (specified in Table I) by simultaneous equations (using four generating conditions) proved unreliable, even though the statistical analysis showed less than 1% interaction among the arsenic species.

Conclusion

The warning offered in this paper concerns the application of a method, not the method *per se* (*i.e.*, in the absence of methylated arsenic compounds the published method[1] could provide acceptable data). Arsenic speciation methods[2,3,18] that involve separation of the hydrides before the measurement step are not subject to the potential errors described here, except that any inorganic arsenic(V) contributions under the conditions used to determine inorganic arsenic(III) are not physically separable from the inorganic arsenic(III) because both inorganic arsenic species yield the same hydride.

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Simultaneous Determination of Trace Concentrations of Arsenic, Antimony and Bismuth in Soils and Sediments by Volatile Hydride Generation and Inductively Coupled Plasma Emission Spectrometry

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Trace amounts of arsenic, antimony and bismuth in soils and sediments are determined simultaneously by an inductively coupled plasma - volatile hydride method after rapid attack with concentrated hydrochloric acid in sealed tubes. The samples are treated with the acid at 150 °C for 2 h in capped test-tubes. After addition of potassium iodide solution, the hydrides are formed by mixing the solution with sodium tetrahydroborate(III) solution in a continuous-flow system, and are swept into the plasma by a stream of argon for determination by atomic-emission spectrometry. Acceptable precision and accuracy are obtained, and the detection limits for all three analytes are about 0.1 $\mu g\ g^{-1}$. Approximately 200 samples can be analysed by one person in a 2-d cycle.

Keywords: Arsenic, antimony and bismuth determination; hydride generation; inductively coupled plasma emission spectrometry; geochemical samples

The analytical characteristics of a procedure for the simultaneous determination of arsenic, antimony, bismuth, selenium and tellurium in aqueous solution has been described previously.[1,2] The elements were reduced to their hydrides by mixing the test solution with a solution of sodium tetrahydroborate(III) in a continuous-flow system. The hydrides were carried into an inductively coupled plasma (ICP) and determined by atomic-emission spectrometry. The detection limits obtained were comparable to the best reported for atomic absorption - volatile hydride methods, but the linear calibration ranges were greater. A few transition metal ions (mainly copper and nickel) were found to interfere with the reduction of bismuth, selenium and tellurium, causing reduced efficiency in the production of the hydrides, whereas arsenic and antimony were virtually free from interference problems.

The initial purpose of the work partially reported here was to produce a rapid ICP - volatile hydride method by which the five analytes could be determined simultaneously in rock, soil and sediments. However, no rapid method of sample attack has been found that was suitable for bringing the elements into solution simultaneously. A variety of mineral acids and mixtures have been investigated. Strongly oxidising acids are required to effect the dissolution of selenium and tellurium, which would otherwise be reduced to the elements [by trace amounts of reducing agents such as organic matter or iron(II)] and subsequently lost. However, the use of oxidising acids was found to give rise to very low recoveries of antimony, possibly owing to the production of a hydrolysed species of antimony(V) that was not amenable to reduction and hydride formation.

In view of this difficulty, the two periodic groups of elements have been treated separately. The determination of selenium in soils and sediments has already been reported[3]; this paper reports a simple and rapid method of sample attack for the simultaneous determination of arsenic, antimony and bismuth.

Experimental

Equipment

Hydride generator - plasma system

The system consisted of a continuous-flow hydride generator, a Radyne R50 plasma generator and an Applied Research Laboratories 29000B quantometer. The operating conditions and procedure were as previously described.[1]

Screw-capped test-tubes

Sample attacks were carried out in Sovirel screw-capped borosilicate-glass test-tubes (160 × 16 mm), with specially made cap liners consisting of a layer of silicone-rubber (Esco type SR70, 3.2 mm thickness) covered with a chemically resistant film (Du Pont Teflon FEP Type A, 0.36 mm thickness). These were more effective than the original liners in preventing leakages, and were designed by Dr. J. Lovell, Barringer Research Inc., Toronto, Canada.

Heating block

Batches of tubes were heated in a thermostatically controlled aluminium block to a depth of about 65 mm. The Bakelite caps and the tops of the tubes were kept cool by means of a blast of air from a domestic vacuum cleaner.

Materials

Analytical-reagent grade chemicals and purified water were used throughout.

Sample Attack

The powdered samples (0.250 g) were weighed into test-tubes and 5 ml of concentrated hydrochloric acid were added to each tube. (For samples containing carbonate minerals, the acid should be added cautiously, in small portions, and the tubes set aside until evolution of gas has ceased.) The tubes were capped and placed in the heating block at 150 °C for 2 h, then they were removed cautiously, cooled rapidly in a cold water-bath and unsealed. A 5-ml volume of 0.2% *m*/*V* potassium iodide solution was added to each tube and the contents were mixed by thorough shaking. The solid residue was allowed to settle (for about 4 h) and the solutions were used directly for the determinations.

Determination

The arsenic, antimony and bismuth were determined simultaneously in the analyte solution. A calibrating solution was made containing the analytes [as arsenic(III), antimony(III) and bismuth(III)] at a concentration of 100 ng ml^{-1} in 1 + 1 hydrochloric acid, and was run, together with a blank solution, after every tenth sample solution. Sample solutions containing an analyte at a concentration above the limit of linear calibration (arsenic 800 ng ml^{-1}, antimony 1500 ng ml^{-1}, bismuth 500 ng ml^{-1}) were diluted 10-fold and re-run.

Safety Precautions

Although no instance of a tube bursting has occurred in the operation of this procedure, the possibility must be guarded against. The operator must be protected from flying glass and fumes while the tubes are hot and care must be exercised to prevent the temperature of the hot block exceeding the required level.

Results and Discussion

Selection of Sample Digestion Conditions

In primary magmatic rocks, arsenic, antimony and bismuth are highly concentrated in sulphide phases, although bismuth has some tendency to replace calcium in apatite and certain silicates. However, in sedimentary rocks, soil and sediment, the three elements are largely bound to precipitated iron(III) oxide minerals. Hot, concentrated hydrochloric acid is a good solvent for this type of material, but has not been used for the determination of arsenic and antimony because of the volatility of their lower chlorides. A wider range of minerals including many silicates are decomposed by hydrochloric acid at elevated temperatures and pressures, but the normal methods for carrying out the attack (in PTFE bombs or heavy-walled sealed glass tubes) are not suitable for the rapid, high-throughput work required in applied geochemistry. The use of hydrochloric acid as a reagent for decomposition of minerals has been reviewed by Dolezal *et al.*[4] and Bock.[5]

Sovirel capped test-tubes, when used as described above, can withstand the pressure generated by concentrated hydrochloric acid up to a block temperature of at least 180 °C. Above this temperature the caps have an increasing tendency to fail. The liquid phase adopts a temperature slightly lower than block temperature: with the block at 150 °C the liquid equilibrates at 140 °C within a few minutes. With the cap liners described and a moderate finger torque for fastening, loss of the analytes from the tubes is insignificant. When the tubes were tested under the conditions of the proposed method with 5-ml portions of concentrated hydrochloric acid containing 25, 250 or 1250 ng ml^{-1} of the analytes, no significant loss (*i.e.*, greater than the uncertainty of the determination) could be detected. Where the sealing of the tube is imperfect, an obvious loss of acid volume occurs, which enables the analyst to test for adequacy of sealing. Thorough shaking after the attack is necessary as arsenic tends to accumulate in the cool upper part of the tube.

The effect of different block temperatures for a 2-h attack with concentrated hydrochloric acid was tested for a wide variety of samples within the range 25–180 °C. All samples show increasing proportions of the analytes up to 150 °C, with no further increase at 180 °C. However, at the lower temperatures the proportions of the elements extracted varied markedly from sample to sample, presumably depending on the mode of occurrence of the analytes and the resistance to dissolution of the host minerals.

Effect of the Attack on Various Minerals

The efficacy of the attack on a variety of minerals was tested by subjecting samples to the procedure described, where necessary subjecting the separated residues to identification by X-ray diffraction. It was found that the iron minerals magnetite, haematite, siderite and geothite, in which the analytes would normally be expected to be concentrated, were virtually completely dissolved, as was the sample of apatite. Some silicates (montmorillonite, biotite) were also completely destroyed, leaving only a residue of amorphous silica. In other silicate minerals (pyroxene, kaolinite, hornblende, muscovite, albite, orthoclase) the lattice was apparently unaffected by the treatment, although Foster[6] has shown that hydrochloric acid (under milder conditions) is an effective reagent for the extraction of trace amounts of a number of base metals from some of these minerals.

The attack was also attempted on small (about 20-mg) samples of various sulphide minerals, although these are not normally encountered in stream sediments or soils. It was found that galena, sphalerite, chalcopyrite and pyrrhotite were almost completely dissolved, whereas pyrite and marcasite were hardly affected.

Effect of Organic Matter on Recovery of Bismuth

Initial work suggested that low recoveries of bismuth were occurring from samples that contained appreciable amounts of organic matter. However, no reliably analysed standard samples with suitable levels of bismuth and organic carbon were available to test this possibility. Accordingly, three samples of soils containing a range of organic carbon contents were treated by the standard method, both alone and after spiking with 0.25-, 2.5- or 12.5-μg amounts of arsenic, antimony and bismuth. The solutions were then analysed in the normal manner. A low recovery of any analyte would be indicated by the differences between the original samples and the respective spiked sample, compared with the concentration added.

It was found that recoveries of arsenic and antimony were consistently good, no significant losses being observed. Bismuth, however, gave low recoveries, the loss being roughly in proportion to the organic matter content, and almost independent of the concentration of bismuth. This loss amounted to about 50% for a soil sample containing 19.9% of organic carbon. Addition of 0.10 ml of bromine to the reaction mixture before the heating stage resulted in much better recoveries of bismuth, suggesting that bismuth may be partially reduced to the element in the presence of the organic matter. However, the use of bromine brought about very low recoveries of antimony and therefore was not adopted for general use. Reaction conditions milder than those in the proposed method might be suitable for optimising the recovery of bismuth in organic-rich samples.

Effect of Potassium Iodide on Formation of the Hydrides

Potassium iodide has the effect of slightly enhancing the analytical response of arsenic(III) and antimony(III) and slightly suppressing the response of bismuth(III) in the ICP - hydride system.[2] It also brings about the reduction of arsenic(V) and antimony(V) to the +3 state, which is necessary in this system because of the diminished response given by the higher oxidation states, especially antimony(V).

Finally, the potassium iodide is required to reduce the interference with bismuth due to copper. Arsenic and antimony can be determined by the ICP - hydride system with no interference from the other elements at concentrations likely to be encountered in the analysis of soils and stream sediments.[2] Bismuth is affected by copper in concentrations above 2 mg l^{-1} in the test solution, equivalent to 80 mg kg^{-1} of copper in the solid sample when it is attacked by the method given. In the presence of potassium iodide, however, the critical level of copper (at which the onset of interference is apparent) is increased to about 80 mg l^{-1} in solution (Fig. 1), equivalent to 3200 mg kg^{-1} in the sample. This level of copper is rarely exceeded in soil and sediment samples.

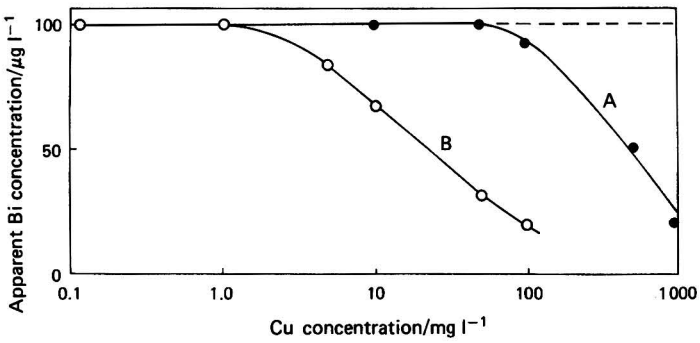

Fig. 1. Effect of copper concentration on the recovery of bismuth from A, solutions containing 0.1% potassium iodide (closed circles) and B, solutions with no added potassium iodide (open circles).

Accuracy and Precision of the Method

The method described has been applied to a variety of standard analysed materials, mostly soils and rocks. Table I shows a comparison of values obtained by the proposed method and the values obtained for a series of soil standards from the Canadian Certified Materials Project (CCMP). The proposed method produced comparable although generally slightly lower values than those currently recommended, but the number of laboratories returning data for arsenic, antimony and bismuth was small, and most of the results obtained for bismuth are close to the detection limit of the proposed method and apparently the CCMP method.

TABLE I

RESULTS PRODUCED ON THE CANADIAN CERTIFIED MATERIALS PROJECT STANDARD SOILS COMPARED WITH THE RECOMMENDED VALUES

SO-1, SO-2 and SO-3 are single determinations; SO-4 is the mean and standard deviation of eight determinations on separate portions of the material.

Sample	Result	As/mg kg^{-1}	Sb/mg kg^{-1}	Bi/mg kg^{-1}
SO-1	Found	1.94	0.12	0.24
	Recommended	1.9 ± 0.3	0.2	0.5
SO-2	Found	0.77	0.1	0.03
	Recommended	1.2 ± 0.2	0.1	0.1
SO-3	Found	2.32	0.22	0.03
	Recommended	2.6 ± 0.1	0.3	0.1
SO-4	Found	6.45 ± 0.07	0.25 ± 0.03	0.19 ± 0.02
	Recommended	7.1 ± 0.7	0.7	0.1

Results obtained on some of the US Geological Survey (USGS) rock standards are compared with recommended values[7] in Table II. Although the proposed method might be expected to produce low values on igneous rocks (because of the resistance of many silicates to the attack), agreement is reasonably close for arsenic and antimony, considering the low levels involved. Values obtained for bismuth are around the detection limit and not strictly comparable to the recommended values, which were obtained by a neutron-activation method with a much lower detection limit.

TABLE II

RESULTS OBTAINED BY THE PROPOSED METHOD ON SOME USGS STANDARD ROCKS COMPARED WITH ACCEPTED VALUES[7]

Sample	Result	As/mg kg^{-1}	Sb/mg kg^{-1}	Bi/mg kg^{-1}
W-1	Found	0.89	1.18	<0.04
	Accepted	1.9	1.0	0.046
G-1	Found	0.50	0.55	0.12
	Accepted	0.5	0.31	0.065
G-2	Found	0.22	0.12	0.12
	Accepted	0.25	0.1	0.043
GSP-1 ..	Found	0.12	3.20	0.18
	Accepted	0.09	3.1	0.037

The method has also been applied to the Geochemical Exploration Series (GXR) standards, which were produced by the USGS in conjunction with the Association of Exploration Geochemists, and which contain a higher range of analyte values. The GXR standards consist of a variety of rocks and soils. In Table III the results obtained are compared with those of other workers. However, the comparisons are not completely satisfactory because of the dearth of reliable analyses. The "extracted values" cited are based on a tabulation of results produced by many laboratories on a world-wide basis.[8] They were produced by one of the authors (M.T.) by a weighted average of the median result obtained for each given method. In view of the large variations between laboratories and between methods in the tabulation, especially for antimony and bismuth, the "extracted values" must be regarded as suspect. The other results that are cited are independent of the tabulation.

TABLE III

COMPARISON OF THE RESULTS OBTAINED BY THE PROPOSED METHOD AND BY OTHER WORKERS ON THE GEOCHEMICAL EXPLORATION SERIES STANDARD ROCKS AND SOILS

Results by the proposed method show the mean and standard deviation (in parentheses) of eight replicated samples taken through the entire procedure. All results in mg kg^{-1}.

Element	Source of data	Standard sample GXR1	GXR2	GXR3	GXR4	GXR5	GXR6
Arsenic	This work	395 (6.4)	23.4 (0.30)	4638 (124)	107 (2.9)	10.7 (0.26)	398 (10.8)
	"Extracted value"	390	22	4200	95	11	306
Antimony ..	This work	159 (5.8)	46.5 (0.19)	66.0 (2.7)	4.6 (0.05)	1.15 (0.04)	3.36 (0.08)
	"Extracted value"	115	41	30	4.5	1.5	3
	Hannaker and Hughs[9]	154	49	—	6.1	3.4	2.3
Bismuth	This work	3419 (70)	0.59 (0.09)	0.29 (0.06)	17.8 (0.07)	0.32 (0.04)	0.27 (0.06)
	"Extracted value"	1600	8	20	24	7	11
	Hannaker and Hughs[9]	939	49	—	16	3	1
	Viets[10]	1725	0.4	<0.2	21.2	0.4	0.2

Agreement with the "extracted value" is close for arsenic, less so for antimony and negligible for bismuth. For antimony the results of Hannaker and Hughs[9] (produced by solvent extraction and flame atomic-absorption spectrometry) agree reasonably well, but less well for bismuth. Apart from the value for GXR1 the bismuth results obtained by the proposed method agree with those of Viets,[10] also produced by solvent extraction and flame

atomic-absorption spectrometry. The high result produced by the proposed method on GXR1 is noteworthy. GXR1 is a sample of jasperoid, high in iron and silica, and possibly more amenable to the attack with hydrochloric acid than to the attacks used by other workers.

The precisions obtained by replicate analysis of the GXR samples (Table III) and one of the soil samples (Table I) are satisfactory for most purposes, and suggest that the practical detection limits for all three elements is of the order of 0.1 $\mu g\ g^{-1}$. Approximately 200 samples can be analysed by one person in a 2-d cycle.

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Specific and Sensitive Spectrophotometric Determination of Cobalt with 3-(2′-Thiazolylazo)-2,6-diaminotoluene

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Cobalt and 3-(2′-thiazolylazo)-2,6-diaminotoluene react in an acidic sodium acetate medium in the presence of vanadate to give several complexes. The soluble blue complex in a strong perchloric acid medium ($H_0 = -0.5$) obeys Beer's law between 0.05 and 0.60 p.p.m., with a molar absorptivity of 9.74×10^4 l mol^{-1} cm^{-1} at 590 nm, a Sandell sensitivity index of 0.60 ng cm^{-2} and a relative error of 0.13%. The method is highly specific and has been applied to the spectrophotometric determination of cobalt in low-alloy steels, hydrofining catalysts and high-purity nickel salts.

Keywords: *3-(2′-Thiazolylazo)-2,6-diaminotoluene reagent*; *cobalt determination*; *spectrophotometry*

Cobalt(II) and cobalt(III) react readily with organic compounds containing donor atoms such as oxygen, nitrogen or sulphur.[1–3] In recent years very sensitive reagents derived from *o*-aminoazopyridine have been introduced,[4] the cobalt complexes of which have molar absorptivities ranging from 1.07×10^5 l mol^{-1} cm^{-1} for 4-(2′-pyridylazo)-1,3-diaminobenzene to 1.42×10^5 l mol^{-1} cm^{-1} for 5-(3′,5′-dichloro-2′-pyridylazo)-2,4-diaminotoluene. The spectrophotometric determination of cobalt using these reagents suffers from interference mainly by iron and palladium, respectively.[5,6]

In a previous paper[7] we have described the synthesis of 3-(2′-thiazolylazo)-2,6-diaminotoluene (2,6-TADAT) and studied its reaction with palladium. Cobalt reacts with 2,6-TADAT only after oxidation to cobalt(III). It has been shown[8] that when solutions of cobalt(II) and 2,6-TADAT are left in solution at pH > 11 for at least 30 mins, and then acidified with perchloric acid, several 1:3 complexes are formed, the most interesting originating at $H_0 = -1.5$ ($\epsilon_{590} = 8.50 \times 10^4$ l mol^{-1} cm^{-1}). However, the spectrophotometric determination of cobalt by this method suffers interference from those ions which form precipitates in very alkaline media and are difficult to redissolve, and from ions that complex strongly with the cobalt.

In order to overcome these problems, this work establishes the experimental conditions required for the determination of cobalt in an alkaline complexing medium, where the cobalt is oxidised by atmospheric oxygen, or in an acidic medium, where sodium vanadate is used as a mild oxidant. Very sensitive and selective methods have been developed for the spectrophotometric determination of cobalt. One of these methods has been used to assay several cobalt-containing materials.

Experimental

Apparatus

The apparatus used included a Beckman 25 and a Hitachi Perkin-Elmer 200 recording spectrophotometer with 1-cm path length glass or silica cells. A Radiometer PHM25 pH meter with glass and saturated calomel electrodes, a Pye Unicam 1900 atomic-absorption spectrophotometer and a Sartorius MPR35 balance were also used.

Reagents

Chemicals of analytical-reagent grade and de-ionised water were used throughout, without further purification.

3-(2′-Thiazolylazo)-2,6-diaminotoluene solution, 10^{-3} M *in* 1 M *perchloric acid.*

Cobalt(II) nitrate solution, 10^{-1} M. Standardised by EDTA titration.[9]

Sodium vanadate solution, 10^{-1} M.
Sodium acetate solution, 1 M.
These solutions were diluted with water as required.

Recommended Procedures for the Determination of Cobalt

Method 1

To a solution containing 2.5–17.5 μg of cobalt in a 25-ml calibrated flask, add 2 ml of a 10^{-3} M solution of 2,6-TADAT in 1 M perchloric acid and 5 ml of 1 M ammonia solution. After 1 h add 2 ml of 70% perchloric acid and make up to volume with water. Measure the absorbance of the solution at 590 nm against a reagent blank, in 1-cm path length cells.

Method 2

To a solution containing 1.25–15.0 μg of cobalt in a 25-ml calibrated flask, add 2 ml of a 10^{-3} M solution of 2,6-TADAT in 1 M perchloric acid, 2 ml of 1 M sodium acetate and 1 ml of 10^{-2} M sodium vanadate solution. Wait for 1 h and then add 2 ml of 70% perchloric acid and make up to volume with water. Measure the absorbance of the solution at 590 nm against a reagent blank, in 1-cm path length cells.

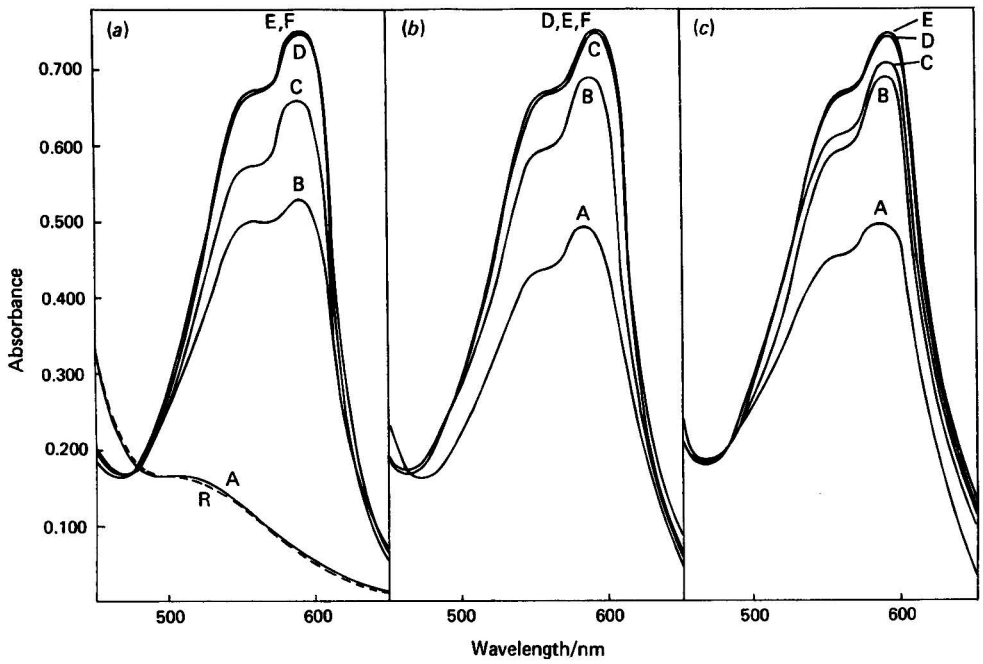

Fig. 1. Influence of initial pH, vanadate concentration, C_V, and time, t, on the formation of the Co(III) - 2,6-TADAT complex. $C_L = 4.0 \times 10^{-5}$ M, $C_{Co} = 8.0 \times 10^{-6}$ M, final $H_0 = -0.5$. (*a*) Influence of initial pH: $C_V = 4.0 \times 10^{-4}$ M, $t = 1$ h. A, pH = 1.5; B, pH = 2.9; C, pH = 3.5; D, pH = 4.2; E, pH = 5.2; F, pH = 8.4; R = reagent alone at pH = 4.0. (*b*) Influence of the vanadate concentration: initial pH = 4.85; $t = 1$ h. C_V = A, 0.0; B, 8.0×10^{-6} M; C, 4.0×10^{-5} M; D, 2.0×10^{-4} M; E, 4.0×10^{-4} M; F, 8.0×10^{-4} M. (*c*) Influence of time: initial pH = 4.85; $C_V = 4.0 \times 10^{-4}$ M. A, 0 min; B, 15 min; C, 30 min; D, 60 min; E, 120 min.

Determination of cobalt in steels and hydrofining catalysts

Weigh accurately 0.1–1.0 g of the steel or catalyst sample and dissolve in hydrochloric acid (1 + 1) while heating on a water-bath. Evaporate the solution to dryness. Dissolve the residue in 1 M perchloric acid in a 100-ml calibrated flask and make up to volume with 1 M perchloric acid. Assay suitable aliquots of the final solution as described under *Method* 2.

Determination of cobalt in high-purity nickel salts

Weigh accurately 1–10 g of the nickel salt, dissolve and dilute to 100 ml with water in a calibrated flask. Assay 5-ml aliquots of the final solution as described under *Method* 2.

Results and Discussion

Reaction with Cobalt

In ammoniacal medium

The absorption spectra, graphs of absorbance *versus* hydrogen concentration and the stoicheiometry of the solutions obtained when cobalt and 2,6-TADAT are mixed in an ammoniacal medium of pH about 10, left for 1 h, and then acidified with perchloric acid until $H_0 = -0.5$, are all similar to those obtained by the same procedure when mixing is carried out in sodium hydroxide solution at pH > 11.[8]

If method 1 is followed, then in a final medium of $H_0 = -0.5$ (1.35 M perchloric acid) and at 590 nm, Beer's law is obeyed for 0.10–0.70 p.p.m. of cobalt. The minimum error range of 0.18–0.47 p.p.m. was evaluated from a Ringbom plot. The molar absorptivity is 8.83×10^4 l mol^{-1} cm^{-1}, with a Sandell sensitivity index of 0.66 ng cm^{-2}. The relative error of the method is 0.13% (95% confidence interval). Solutions are stable for more than 5 d.

The effects of several cations and anions were examined by using method 1 to assay solutions containing 0.237 p.p.m. of cobalt and various concentrations of other ions (Table I).

In acidic medium

In order to avoid the interferences found when the determination of cobalt with 2,6-TADAT is commenced in alkaline media, it would be necessary to begin the determination in acidic media. However, when cobalt(II) and 2,6-TADAT are mixed in acidic media a reddish brown colour develops, which becomes bluish violet on acidifying to 1–3 M in perchloric acid. Lower and non-reproducible absorbance values are obtained in this way, probably owing to incomplete oxidation of cobalt. Therefore, the use of an oxidising agent for cobalt(II) in acidic media would appear to be necessary.

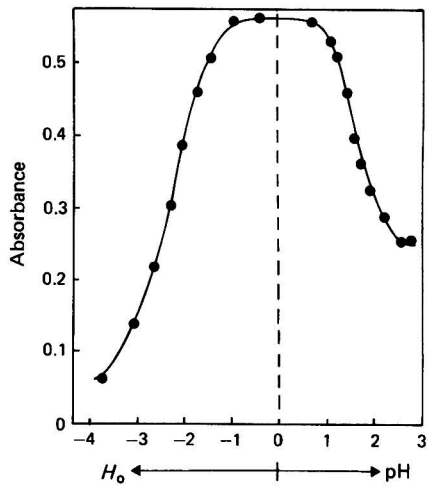

Fig. 2. Influence of the final acidity on the formation of the Co(III) - 2,6-TADAT complex. $C_L = 4.0 \times 10^{-5}$ M, $C_{Co} = 8.0 \times 10^{-6}$ M, initial pH = 4.85, $C_V = 4.0 \times 10^{-4}$ M, $t = 1$ h.

Action of Oxidising Agents

As 2,6-TADAT is destroyed by some oxidising agents, several were tried in order to find one mild enough to oxidise cobalt(II) to cobalt(III) in acidic media, without destroying the

reagent. A 2×10^{-5} M solution of 2,6-TADAT is quickly decolorised by peroxidisulphate, bromate, periodate, etc., even in the cold; however, vanadate appeared satisfactory and was therefore chosen for subsequent use.

Initial pH, Vanadate Concentration and Time

Experiments were performed on solutions with a 2,6-TADAT concentration of $C_L = 4.0 \times 10^{-5}$ M and a cobalt concentration of $C_{Co} = 8.0 \times 10^{-6}$ M. As the complex develops in 0.5–2.0 M perchloric acid medium, in which cobalt and 2,6-TADAT do not react directly, in this work the initial pH and vanadate concentration to be studied were adjusted, and the mixture left for a measured time, and then the required volume of 70% perchloric acid was added so as to give a final perchloric acid concentration of 1 M. The spectra were then recorded.

From Fig. 1, it is clear that to obtain the highest molar absorptivities it is necessary to start with an initial pH near to 4.8, with a vanadate to cobalt ratio of about ten, and to wait for 1 h before adding the perchloric acid.

Final Acidity

In Fig. 2, the variation of absorbance with the final hydrogen ion concentration is shown. It is clear that the measurements of absorbance must be made between pH = 0.5 and $H_0 = -1.0$. At pH > 3 solutions develop a slight turbidity owing to the formation of less soluble complex species similar to those previously described.[8] Absorption spectra and Job's plots show that $CoH_xL_3^{(x+3)+}$ is the bluish violet soluble complex[8] (x being the number of protonated amino groups in the reagent).

TABLE I

DETERMINATION OF COBALT IN THE PRESENCE OF OTHER IONS

The concentration of cobalt present in each determination was 0.237 p.p.m.

Ion	Molar ratio, ion : Co(II)	Co(II) found, p.p.m.*	
		Alkaline medium	Acidic medium
Cl^-	150	0.238	0.237
NO_3^-	150	0.236	0.236
SO_4^{2-}	150	0.237	0.237
CH_3COO^-	150	0.237	0.237
Cu(II)	20	0.239	0.238
	100	0.240	0.319
Ni(II)	150	0.232	0.234
Zn(II)	150	0.240	0.235
Pb(II)	100	0.238	0.237
Pt(II)	1	0.239	0.235
	10	0.205	0.238
Hg(II)	1	0.103	0.113
Pd(II)	1	0.402	0.438
Ca(II)	100	0.233	0.236
Ba(II)	100	0.234	0.237
Mg(II)	100	0.234	0.237
Mn(II)	10	0.230	0.239
Fe(II)	100	0.239	—
Fe(III)	100	0.236	0.235
Al(III)	100	0.232	0.238
Cr(III)	1	0.180	0.238
	100	—	0.237
V(V)	50	0.230	—
Mo(VI)	100	0.234	0.239
W(VI)	1	0.098	0.238
	25	—	0.240

* Mean of three determinations.

Determination of Cobalt

In solutions with final $H_0 = -0.5$ (1.35 M perchloric acid) and at 590 nm, the complex obeys Beer's law in the range 0.05–0.60 p.p.m. A Ringbom plot shows that 0.14–0.44 p.p.m. of cobalt is the optimum concentration range. The molar absorptivity is $9.74 \times$

TABLE II

DETERMINATION OF COBALT IN LOW-ALLOY STEELS

Steel	Certified cobalt content, %	Cobalt found,* %
BCS 251 (Mn 0.16%, Ni 5.15%)	0.070	0.070
BCS 252 (Mn 0.016%, Ni 4.10%)	0.040	0.041
BCS 254 (Mn 0.525%, Ni 2.08%)	0.029	0.019

* Mean of three determinations.

10^4 l mol^{-1} cm^{-1}, with a Sandell sensitivity index of 0.60 ng cm^{-2}, and the relative error of the method is 0.14% (95% confidence interval). Solutions are stable for at least 5 d.

The influence of several ions was examined by applying *Method* 2 to the assay of solutions containing 0.237 p.p.m. of cobalt and various concentrations of other ions (Table I).

TABLE III

DETERMINATION OF COBALT IN HYDROFINING CATALYSTS

Sample	Estimated cobalt to nickel ratio	Cobalt found,* % 2,6-TADAT	Cobalt found,* % AAS†
1	0.02	0.053	0.056
2	65	2.080	2.085
3	75	2.355	2.355

* Mean of three determinations.
† Atomic-absorption spectrophotometry.

Determination of Cobalt in Different Materials

A comparison of the two methods developed for the spectrophotometric determination of cobalt with 2,6-TADAT, shows that method 1 is simpler, but that method 2 offers higher sensitivity and specificity (except for copper). Consequently, the determinations of cobalt in steels, hydrofining catalysts and high-purity nickel salts described below, were carried out by method 2.

The results obtained for three determinations of cobalt in each of the BCS 251, BCS 252 and BCS 254 low-alloy steels are shown in Table II. The interference due to manganese in the BCS 254 steel is apparent because the manganese to cobalt ratio is greater than the limit previously established.

TABLE IV

DETERMINATION OF COBALT IN HIGH-PURITY NICKEL SALTS

Salt	Cobalt found,* % 2,6-TADAT	Cobalt found,* % AAS†
$NiSO_4.6H_2O$	0.38×10^{-3}	0.41×10^{-3}
$NiCl_2.6H_2O$	0.59×10^{-3}	0.59×10^{-3}
$Ni(NO_3)_2.6H_2O$	0.65×10^{-3}	0.70×10^{-3}
$Ni(CH_3COO)_2.4H_2O$	0.88×10^{-1}	0.90×10^{-1}

* Mean of three determinations.
† Atomic-absorption spectrophotometry.

Spectrophotometric determinations of cobalt in several hydrofining catalysts, containing aluminium (40–45%), molybdenum (5–10%), nickel (0.05–3%), and cobalt (0.05–2.5%) were carried out. Results are shown in Table III where they are compared with those obtained by atomic-absorption spectrophotometry.[10] The direct determination of cobalt in high-

purity nickel salts may also be carried out using 2,6-TADAT. Results obtained for several nickel salts are shown in Table IV where they are compared with results obtained by atomic-absorption spectrophotometry.[10]

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Spectrophotometric Determination of Anionic Surfactants in River Waters Using 1-(4-Nitrobenzyl)-4-(4-diethylaminophenylazo)-pyridinium Bromide

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Nitro, bromo and methyl derivatives of 1-(benzyl)-4-(4-diethylaminophenylazo)pyridinium bromide were synthesised and evaluated as new cationic reagents for the determination of anionic surfactants. These reagents were very stable and reacted with anionic surfactants, such as alkylbenzenesulphonate and alkylsulphate, to form a 1:1 stable ion associate, which was extracted into chlorobenzene in a single extraction. The apparent molar absorptivity of the ion associate of the 4-nitro derivative with sodium di-(2-ethylhexyl)sulphosuccinate was 6.10×10^4 l mol^{-1} cm^{-1} (at 573 nm) in chlorobenzene. 1-(4-Nitrobenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide was used in the determination of μg l^{-1} levels of anionic surfactants in river water. The results were compared with the methylene blue method (Japanese Industrial Standard method) for river waters. This method is designed to determine anionic surfactant concentrations in solution.

Keywords: *Anionic surfactant determination*; *water analysis*; *spectrophotometry*; 1-(*benzyl*)-4-(4-*diethylaminophenylazo*)*pyridinium bromide derivatives*

A minute amount of anionic surfactants in river waters has been determined by an extraction - spectrophotometric method with Bindschedler's Green derivatives.[1] The dibutyl derivative can react with an anionic surfactant to form an ion associate that is extracted into toluene at pH 7 and the absorbance at 730 nm can then be measured. The apparent molar absorptivity is 7.1×10^4 l mol^{-1} cm^{-1}. However, the reagent is not very stable and the colour fades gradually, especially in the presence of an oxidant such as iron(III). To improve the stability of the reagent, some pyridinium azo compounds were synthesised and used for the determination of anionic surfactants. Of these compounds, 1-(4-nitrobenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide (NDP) forms a stable 1:1 ion associate with anionic surfactants, such as sodium dodecylsulphate, sodium dodecylbenzenesulphonate (DBS) and sodium di(2-ethylhexyl)sulphosuccinate (DESS), which are extracted into chlorobenzene by a single extraction.

This paper describes a simple and rapid method for the determination of μg l^{-1} levels of anionic surfactants in river waters with NDP, with 97–100% recovery levels.

Experimental

Apparatus

Spectrophotometric measurements were carried out using Hitachi, Model 139 and Model EPS-3T, spectrophotometers with 10-mm glass cells. Extractions were carried out by shaking with an Iwaki, Model KM, shaker. The pH measurements were carried out with a Hitachi-Horiba, Model F–5ss, pH meter. The centrifugation was performed with a Shimazu, Model CPN–005, centrifuge.

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Reagents

Syntheses of 1-(*benzyl*)-, 1-(4-*methylbenzyl*)-, 1-(4-*bromobenzyl*)- *and* 1-(4-*nitrobenzyl*)-4-(4-*diethylaminophenylazo*)*pyridinium bromides.*

4-(4-Diethylaminophenylazo)pyridine (DP)[2] was obtained by coupling diazotised 4-aminopyridine with *NN*-diethylaniline in phosphoric acid. A solution of 1.8 g (0.019 mol) of 4-aminopyridine in 10 ml of 85% phosphoric acid and 5 ml of concentrated nitric acid were mixed at 0 °C; 1.4 g (0.02 mol) of sodium nitrite and 25 g of ice were then added successively. The solution was poured into 20 ml of 30% phosphoric acid containing 3 g (0.02 mol) of *NN*-diethylaniline. After the reaction, the solution was neutralised with sodium carbonate, and the precipitate was filtered off and recrystallised from an ethanol - water solution (1 + 4) (yield 51%). The crystals were reddish yellow plates, m.p. 182–184 °C. Elemental analysis: found, C 69.87%, H 7.09% and N 21.30%; calculated (for $C_{15}H_{18}N_4$), C 70.84%, H 7.13% and N 22.03%.

The DP was quaternised by refluxing with benzyl, 4-methylbenzyl, 4-bromobenzyl or 4-nitrobenzyl bromide in benzene over an oil-bath. The reagents obtained, respectively, 1-(benzyl)-4-(4-diethylaminophenylazo)pyridinium bromide (BDP), 1-(4-methylbenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide (MDP), 1-(4-bromobenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide (BrDP) and 1-(4-nitrobenzyl)-4-(4-diethylaminophenylazo)-pyridinium bromide (NDP), were washed with benzene until the washings were no longer coloured and then dried at reduced pressure and at 50 °C to a constant mass. A 2.6×10^{-4} M solution of each reagent was prepared by dissolving each one in distilled water.

Anionic surfactants. The anionic surfactant used was sodium di(2-ethylhexyl)sulphosuccinate (DESS), certified as 96.3% by the Japan Oil Chemists' Society. It was dried at 50 °C under reduced pressure (about 3 mmHg) before weighing.

Buffer solution (pH 6). Prepare the buffer solution by adding 2 M sodium dihydrogen orthophosphate solution, with mixing to 2 M potassium monohydrogen orthophosphate solution until a pH of 6 is obtained as measured by a pH meter.

Procedure

Place 100 ml of a sample solution (surfactant content below 7.0×10^{-7} M), in a 100-ml separating funnel, add 5 ml of phosphate buffer solution (pH 6) and 1 ml of 2.6×10^{-4} M NDP solution. Add 5 ml of chlorobenzene and shake the funnel mechanically for 5 min, 2–3 times per second, in order to extract the ion associate that has formed; stand for 10 min. Transfer the chlorobenzene into a test-tube and centrifuge for 1 min at 2000 rev min^{-1}. Measure the absorbance of the organic phase at 573 nm against chlorobenzene using 10-mm glass cells.

Results and Discussion

Molar Absorptivity of the Ion Associate

The ion associate formed between the reagent cation and the anionic surfactant was extracted completely into chlorobenzene. Fig. 1 shows the absorption spectrum of a solution containing 6.3×10^{-7} M DESS using the described procedure. The wavelength of maximum absorption occurs at 573 nm. The molar absorptivities of the reagent solution and the ion associate are shown in Table I. NDP is the preferred reagent for the determination of anionic surfactants. The pK_a values of the reagents were determined spectrophotometrically at ionic strength, $I = 0.1$, adjusted using potassium nitrate.

Extraction Solvents

Eleven organic solvents were examined. Generally, as the dielectric constant of the solvent increases, the amount of reagent extracted increases, *e.g.*, the order of increasing extractability of the solvents is chloroform, chlorobenzene, *o*-dichlorobenzene, 1,2-dichloroethane and nitrobenzene, and the last (dielectric constant 35) can extract all the reagents. Although the dielectric constant of chlorobenzene (5.5) is between chloroform and *o*-dichlorobenzene, the reagent was little extracted into this solvent. On the other hand, solvents useful for the extraction of the ion associates are carbon tetrachloride, benzene, toluene,

TABLE I

pK_a AND MOLAR ABSORPTIVITY OF THE REAGENTS AND THEIR ION ASSOCIATES

Reagent	pK_a	Reagent in water		Ion associate with DESS in chlorobenzene	
		Molar absorptivity/ l mol^{-1} cm^{-1}	Wavelength/ nm	Molar absorptivity/ l mol^{-1} cm^{-1}	Wavelength/ nm
BDP	3.8	6.60×10^4	576	5.27×10^4	566
MDP	3.8	6.47×10^4	574	5.50×10^4	560
BrDP ..	3.8	6.90×10^4	576	5.70×10^4	565
NDP	3.8	7.36×10^4	578	6.10×10^4	573

xylene, diisopropyl ether, isoamyl acetate and chlorobenzene. From the results of the solvents tested, chlorobenzene was preferred because it gave a small blank value and the largest absorbance difference at 573 nm between the ion associate and the blank.

Stoicheiometry of the Ion Associate

DESS reacts with NDP rapidly and quantitatively to form the ion associate when the amount of NDP is more than twice that of the DESS. Job's curves were plotted for the ion associate in chlorobenzene. The results are shown in Fig. 2 and the stoicheiometric ratio of NDP to DESS was found to be 1:1. The ratio of NDP to DBS or DS was also found to be a 1:1.

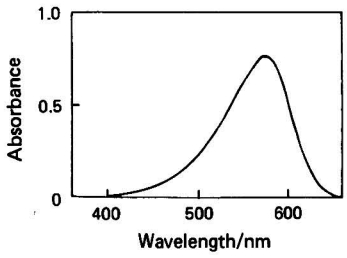

Fig. 1. Absorption spectrum of DESS - NDP ion associate. Concentration of DESS 6.3×10^{-7} M. Ion associate extracted into chlorobenzene at pH 6.

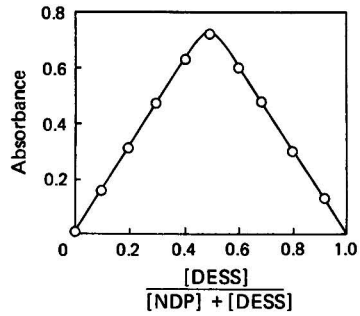

Fig. 2. Composition of DESS - NDP ion associate by the continuous variation method. [DESS] + [NDP] = 1.30×10^{-6} M.

Effect of pH on Extraction

The absorbance was a maximum between pH 4 and 8 and was constant throughout this range (Fig. 3). The absorbance decreased above pH 9 and below pH 4. The extraction of the ion associate into chlorobenzene was therefore carried out at pH 6.

Effect of Reagent Concentration, Shaking and Standing Time

The effect of an excess of the reagent was examined; the addition of a 2–30 times molar excess of NDP against DESS gave a constant maximum absorbance.

The extraction of the ion associate into chlorobenzene was examined. The separating funnel was shaken mechanically and then left to stand. When the shaking time was varied between 3 and 30 min, the absorbances obtained remained constant. The standing time was varied between 5 and 90 min, and the absorbances were found to be constant. A standing time of 10 min was therefore considered to be sufficient. The organic phase was still slightly turbid after standing when no phosphate buffer was present, so the buffer solution was needed to hasten the phase separation and the organic phase should additionally be centrifuged for 1 min at 2000 rev min^{-1}.

Effect of Volume of Water

The effect of the volume of water on extraction was examined. The absorbance of the ion associate in 5 ml of chlorobenzene was constant when the aqueous phase was varied between 50 and 500 ml (Fig. 4). From the results, the enrichment of anionic surfactants by 10–100 times was possible from sample waters into the organic phase.

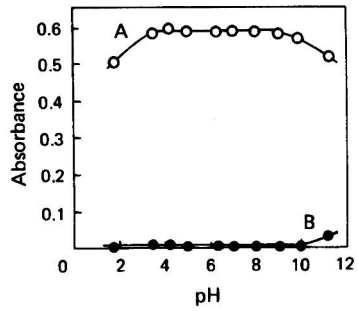

Fig. 3. Effect of pH on extraction. A, DESS - NDP ion associate; B, reagent blank; [DESS] = 4.83×10^{-7} M; [NDP] = 2.55×10^{-6} M.

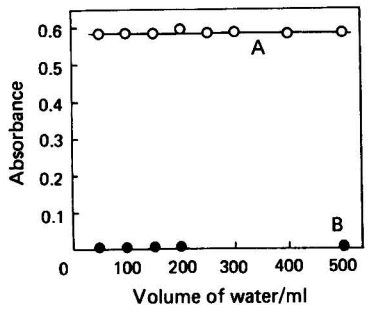

Fig. 4. Effect of the volume of water on extraction. A, DESS - NDP ion associate; B, reagent blank; [DESS] = 4.83×10^{-7} M; [NDP] = 2.55×10^{-6} M; pH 6.

Calibration Graph

The calibration graphs obtained by the procedure were found to show a good linear relationship over the range 0 to 1.3×10^{-5} M of an anionic surfactant. The molar absorptivity of DESS with NDP was 6.10×10^{4} l mol^{-1} cm^{-1} at 573 nm.

TABLE II

INTERFERING IONS

The test solution contained 4.83×10^{-7} M DESS in 100 ml of water.

Ion	Ion concentration/ M	Absorbance of ion associate
None	—	0.582
Cl^-	10^{-3}	0.580
HCO_3^-	10^{-3}	0.585
NO_3^-	5×10^{-4}	0.582
	10^{-3}	0.599
SO_4^{2-}	10^{-3}	0.583
SiO_3^{2-}	10^{-3}	0.584
Na^+	10^{-3}	0.580
K^+	10^{-3}	0.585
NH_4^+	10^{-3}	0.582
Mg^{2+}	10^{-3}	0.578
Ca^{2+}	10^{-3}	0.579
Fe^{3+}	10^{-3}	0.538
Fe^{3+} + EDTA	—	0.582
Sodium stearate	10^{-4}	0.965
	10^{-5}	0.582
Humic acid*: 1 g l^{-1}		0.678
0.1 g l^{-1}		0.602
0.01 g l^{-1}		0.582

* Humic acid concentrations are expressed as grams per litre of soluble matter.

Effect of Diverse Ions

The interferences due to various ions were examined in 100 ml of sample solution containing 4.83×10^{-7} M of DESS. The concentration of the diverse ions tested was 10–100 times higher than the normal content of the ions in river waters in Japan. The results obtained are shown in Table II. The common cations and anions present in river water did not interfere. Nitrate ion above 10^{-3} M and stearate ion above 10^{-5} M caused positive errors. Iron(III) above 10^{-3} M caused a negative error but the interference was effectively masked with EDTA. Humic acid solution at a concentration of more than 0.01 g l^{-1} of soluble matter caused a positive error.

TABLE III

PRECISION OF MEASUREMENTS WITH THE PROPOSED METHOD

Results obtained using a 100-ml river water sample.

(*a*) *Repeatability tests*—

Absorbance of ion associate	Ion associate content as DESS/μg l^{-1}
0.235	85.2
0.246	89.2
0.231	83.8
0.228	82.7
0.235	85.2
0.244	88.5
0.230	83.4
0.238	86.3
0.239	86.7
0.246	89.2
Mean value	86.0
Standard deviation	2.3
Relative standard deviation ..	2.7%

(*b*) *Recovery tests*—

DESS concentration/μg l^{-1}			
Added	Found	Recovered	Recovery, %
None	86	—	—
89	177	91	102
89	176	90	101
89	175	89	100
89	172	86	97
89	176	90	101

Accuracy and Precision

The accuracy of the procedure was evaluated by recovering experiments, in which known amounts of DESS were added to river water and the samples were treated as described in the procedure. In all instances, theoretical recoveries were obtained, within experimental error (Table III). The precision of the procedure was evaluated by analysing ten samples of river water. The mean result obtained was 86.0 p.p.b. with a standard deviation of 2.3 p.p.b. and a relative standard deviation of 2.7%.

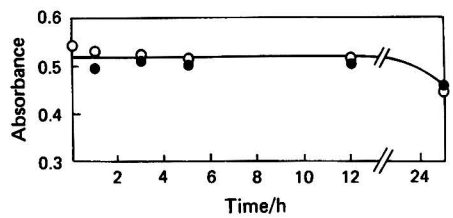

Fig. 5. Loss of anionic surfactant on storage. ○, Glass container; ●, polyethylene container. Results obtained using a 100-ml sample of river water.

Loss of Anionic Surfactants on Storage

A sample solution of river water was filtered and stored and possible losses of anionic surfactants owing to adsorption on to the walls of glass or polyethylene containers were examined; the results are shown in Fig. 5. There was no loss within 12 h in either container, but a loss of about 15% was found after 24 h.

Determination of Anionic Surfactants in River Water

The concentration of anionic surfactants in river water can be measured by the above procedure. The river water samples should be filtered through a membrane filter (0.45 μm), and 1 ml of 0.1 M EDTA solution added to remove the interference due to iron(III). Table IV shows results for anionic surfactants in river waters compared with those by the methylene blue method (Japanese Industrial Standard method).[3] Each value was the average of three determinations. The recovery test [Table III, part (*b*)] was carried out in each instance, and the recovery was found to be from 97 to 100%. Determination by the methylene blue method was impossible with samples 1–4, because their concentrations were below the lower limit of the methylene blue method. With samples 5–7, the results obtained by the proposed method agreed approximately with those obtained by the methylene blue method.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY THE PROPOSED METHOD WITH THE METHYLENE BLUE METHOD FOR RIVER WATERS IN OKAYAMA PREFECTURE

Values in parentheses are recoveries (per cent.), determined as in Table III.

	Proposed method		Methylene blue method	
River water sample No.	Absorbance	Anionic surfactants/* μg l^{-1}	Absorbance	Anionic surfactants/* μg l^{-1}
1	0.079	29 (98)		
2	0.064	23 (97)		
3	0.172	62 (98)		
4	0.149	54 (98)		
5	0.226	82 (98)	0.027	70
6	0.422	153 (98)	0.053	130
7	0.552	200 (100)	0.076	190

* The amounts of anionic surfactants were calculated from the calibration graph of DESS.

Conclusion

A new cationic azo dye, 1-(4-nitrobenzyl)-4-(4-diethylaminophenylazo)pyridinium bromide, can react with anionic surfactants, such as alkylbenzenesulphonate and alkylsulphate, to form ion associates that can be extracted into chlorobenzene by a single extraction. The ion associates formed are very stable in the organic phase and the stoicheiometric ratios are 1 : 1.

The calibration graphs are straight lines over the range 0 to 1.3×10^{-5} M of DESS, and the apparent molar absorptivity is 6.10×10^4 l mol^{-1} cm^{-1} at 573 nm.

The method is simpler and faster than the methylene blue method for the determination of anionic surfactants in river waters.

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Use of Charge-transfer Complexation in the Spectrophotometric Assay of Antazoline and Naphazoline in Combination

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A simple and accurate spectrophotometric method is described for the assay of antazoline and naphazoline, combined in eye-nose drops in a ratio of 20:1. The major component, antazoline, is determined, without interference from naphazoline, by charge-transfer complexation with σ-acceptor iodine. After precipitation of antazoline with sodium carbonate, the minor component, naphazoline, can be determined using the π-acceptor chloranil. The accuracy of the method is assessed by the assay of a standard mixture of the two components containing the same proportions as the commercial preparation.

Keywords: *Spectrophotometry*; *antazoline determination*; *naphazoline determination*; *eye - nose drops*

Assay of two-component mixtures either simultaneously[1–3] or by independent methods[4,5] has attracted the attention of many analysts. An example of such a mixture is the combination of naphazoline [2-(1-naphthyl)-2-imidazoline] and antazoline (2-*N*-phenylbenzylaminomethyl-2-imidazoline) in the ratio of 1:20 as used in commercial eye - nose drops. The determination of a minor component in the presence of a major component is often difficult; in this instance the interference from preservatives in the eye - nose drop solution increases the severity of the problem.

The gas-chromatographic determination of antazoline, naphazoline and other antihistamines in pharmaceutical preparations has been described.[6,7] Being imidazoline derivatives, antazoline and naphazoline can be assayed colorimetrically with alkaline nitroprusside.[8] The reaction, being dependent on the presence of the imidazoline ring, measures the total imidazolines when applied to a mixture of antazoline and naphazoline. After precipitation of antazoline with sodium carbonate,[9] naphazoline, the minor component, was determined by the orthogonal function method[3,10] or the least-squares method.[11,12]

In this work, the antazoline - naphazoline mixture is assayed for both components by charge-transfer complex formation. A σ-acceptor,[4,13] iodine, is used to determine the major component and a π-acceptor,[14,15] chloranil, is used for the determination of the minor component naphazoline. Compared with the other methods mentioned above, the procedure described in this work is simpler, faster and more economical.

Experimental

Reagents and Materials

The reagents used were of analytical-reagent grade and the solvents were of spectroscopic grade.

Standard solutions of free bases of antazoline and naphazoline. Standard solutions were drepared by treating an amount of the salt corresponding to the required mass of free base with ammonia solution[9] and then quantitatively extracting into chloroform. The extract was washed with 20 ml of water and then dried with anhydrous sodium sulphate before diluting with chloroform to the required concentration.

Commercial eye - nose drops. Antistin-Privine eye - nose drops labelled to contain 0.5 g of antazoline sulphate and 0.025 g of naphazoline nitrate and supplied by Ciba were used.

Iodine solution, 10^{-3} M. Prepared by dissolving iodine in chloroform.

Chloranil solution. Chloranil (purified by crystallisation from redistilled benzene) was dissolved in chloroform to obtain 2×10^{-3} M solution.

Instrument

A Beckman double-beam spectrophotometer, Model 24, with 1-cm silica or glass cells was used.

Assay of Antazoline - Naphazoline Drops (Antistin-Privine)

Antazoline content

Transfer 2.0 ml of the drop solution into a separating funnel and dilute to about 15 ml with water. Make the solution alkaline with ammonia solution and then extract into three 20-ml portions of chloroform. Wash each extract with the same 20-ml of water in another separating funnel. Pool the chloroform extracts, after drying with anhydrous sodium sulphate, into a 100-ml calibrated flask and dilute to volume with chloroform.

Transfer 5 ml of the chloroform solution into a 25-ml calibrated flask, add 2 ml of iodine solution, mix and dilute to volume with chloroform. Leave the solution to stand at room temperature for 30 min and then measure the absorbances at 300 and 365 nm, against a blank prepared by diluting 2 ml of iodine solution to 25 ml with chloroform. Calculate $\Delta A_{(300-365)}$ by subtracting the absorbance at 365 nm from that at 300 nm.

Naphazoline content

Transfer 1.0 ml of the drop solution into a small beaker and dilute to about 10 ml with water. Add slowly with stirring 5.0 ml of a 5% m/V sodium carbonate solution. Allow to stand for 10 min and then filter the supernatant liquid into a separating funnel. Wash the precipitate with three 10-ml portions of water. Extract the combined filtrate and washings with a 10- and then three 5-ml portions of chloroform, washing each extract with the same 10-ml of water. Pool the extracts into a 25-ml calibrated flask and dilute to volume with chloroform. Pipette 4.0 ml of the resulting solution into a 10-ml calibrated flask and add 5.0 ml of chloranil solution. Maintain the flask at 40 $\pm$ 1 °C in a water-bath for 45 min. Finally, cool the solution, dilute to volume with chloroform and measure the absorbance at 515 nm against a blank prepared by diluting 5.0 ml of chloranil solution to 10.0 ml with chloroform.

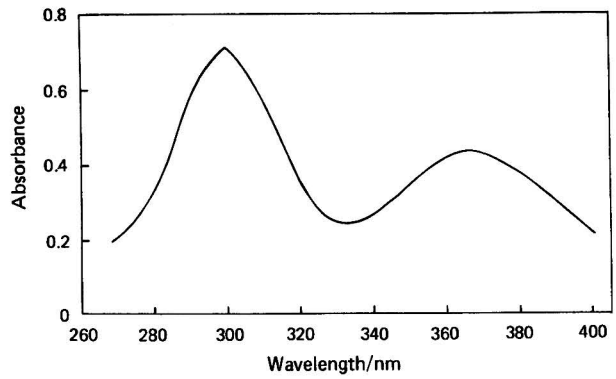

Fig. 1. Absorption spectrum of antazoline - iodine complex in chloroform (1.6 mg per 100 ml).

Results and Discussion

Determination of Antazoline

The spectrum of the chloroform solution of antazoline mixed with iodine exhibits intense bands at 300 and 365 nm (Fig. 1). The appearance of these bands is attributed to charge-transfer transitions from n-donor antazoline to σ-acceptor iodine with consequent formation of iodide ion, which subsequently combines with iodine to give the triiodide ion.[16–18] In order to make use of this complex formation for the determination of antazoline two experimental conditions are necessary: (*a*) an iodine concentration suitable for quantitative

complexation; this concentration should not be much higher than the antazoline concentration in order to avoid the formation of termolecular complexes with a consequent deviation from Beer's law; in this work 2 ml of a 10^{-3} M iodine solution was found suitable; (*b*) the absorbance should be measured 30 min after the addition of the reactants in order to minimise changes in the absorbance with time due to conversion of the outer to the inner charge-transfer complex, the latter form being common for n-donor complexes with iodine.[19]

Under these experimental conditions the absorbance of the complexed antazoline was measured at 300 and 365 nm. The difference between the absorbance values at 300 and 365 nm [$\Delta A_{(300-365)}$] was then calculated. A linear relationship was found betwen $\Delta A_{(300-365)}$ and the concentration of antazoline, C, in the range 0.32–1.92 mg ml^{-1} in the final dilution, *i.e.*, when the sample solution is mixed with iodine in the 25-ml calibrated flask. Using the method of least squares[20] the regression equation was found to be

$$\Delta A_{(300-365)} = -0.0160 + 0.18077C \quad .. \quad .. \quad .. \quad (1)$$

with a regression coefficient, r, of 0.9985.

No interference from naphazoline at one twentieth of the concentration of antazoline (the same ratio as in commercial drops) was found experimentally. This is due to the very low concentration of naphazoline and the mathematical correction for any residual interference obtained by calculating the $\Delta A_{(300-365)}$ value.

Determination of Naphazoline

Naphazoline, the minor component, was determined by formation of a charge-transfer complex (1 : 1, as shown by the continuous variation method) with chloranil. This complex exhibits a maximum at 515 nm (Fig. 2). Because the reaction with chloranil at room temperature is known to be slow,[21] the reactants were kept at 40 °C for 45 min. Under such conditions the formation of the complex was complete and quantitative, as the maximum absorbance (measured at $\lambda_{max.}$) was given under these conditions. The absorbance of the complex was found to be stable for 1 h. The optimum amount of chloranil to be used was found to be 5 ml of 2×10^{-3} M solution; smaller amounts gave lower absorbances.

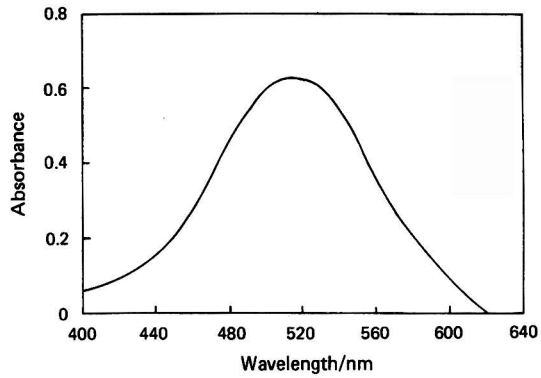

Fig. 2. Absorption spectrum of naphazoline - chloranil complex in chloroform (5 mg per 100 ml).

Under the optimum conditions, a linear correlation was obtained between absorbance and naphazoline concentration, C, from 1.68 to 5.04 mg per 100 ml, calculated in the final dilution, *i.e.*, when the sample solution is mixed with chloranil in the 10-ml calibrated flask. On extrapolation to zero concentration, the graph had a small positive intercept on the absorbance axis. Using the method of least squares the calibration graph was described by the regression equation

$$A_{515} = -0.0127 + 0.1264C \quad .. \quad .. \quad .. \quad .. \quad (2)$$

for which the regression coefficient, r, was 1.0003.

In deriving equations (1) and (2) the reproducibility was assessed using three different amounts of naphazoline from which at least five concentrations were prepared. The validity of equations (1) and (2) was checked occasionally during the work by assaying standards.

After precipitation with sodium carbonate solution about 6 mg of antazoline per 100 ml still remained in the assay solution. No absolute loss of the minor component, naphazoline, has been reported during the precipitation.[10,12] This residual antazoline was found not to interfere in the determination of naphazoline; this was confirmed by experiments on antazoline solutions containing about 6 mg per 100 ml, which revealed no complex formation with chloranil in chloroform. The failure of antazoline to interact with chloranil may be related to the weak basicity of antazoline ($pk_{a_1} = 2.5$ and $pk_{a_2} = 10.1$ at 25 °C),[22] which is insufficient to induce the ionisation of the relatively weak π-acceptor chloranil as compared with the σ-acceptor iodine and also to the absence of a naphthalene ring in the antazoline molecule. It was found experimentally that naphthalene in chloroform solution gave a colour with chloranil.

Assay of Eye - Nose Drop Solution

To assess the accuracy of the method a standard mixture of antazoline and naphazoline, prepared in the laboratory to contain the same concentrations as commercial eye - nose drops (Antistin-Privine drops) was assayed. The results obtained are given in Table I.

Table I

Determination of antazoline and naphazoline in a standard mixture

The standard solution contains the same concentrations of antazoline sulphate and naphazoline nitrate as the commercial eye - nose drop solution, *i.e.*, 0.5 and 0.025 g per 100 ml, respectively.

	Antazoline sulphate	Naphazoline nitrate
Number of experiments	5	5
Mean recovery, %	99.65	100.38
Standard deviation, %	0.35	0.45
Calculated value of t	2.21	1.88
Theoretical value of t ($p = 0.05$) ..	2.776	2.776

Applying a t-test at the 95% confidence level, the calculated value of t does not exceed the theoretical value, which indicates that the proposed method gives results not significantly different from the true values, thereby confirming the high accuracy of the method. Substances that are likely to be present as preservatives, antioxidants or buffering agents exhibit no interference during the assay procedure as it is based on the extraction of the free bases prior to complexation. The low standard deviation proved the good reproducibility of the method.

The use of the method for the analysis of commercial samples (Antistin-Privine drops) was also checked, and the results are presented in Table II.

Table II

Determination of antazoline and naphazoline in commercial eye - nose drops (Antistin-Privine)

The solution (supplied by Ciba, Batch No. 085p) is labelled to contain 0.5 g of antazoline sulphate and 0.025 g of naphazoline nitrate per 100 ml.

	Antazoline sulphate	Naphazoline nitrate
Number of determinations	5	5
Mean recovery, %	103.30	102.24
Standard deviation, %	0.25	0.68

Conclusion

Naphazoline, the minor component in the eye - nose drop solution, was previously determined using Glenn's orthogonal function method to correct for the unprecipitated antazoline.[10] As stated by the authors, this method suffers from three sources of error, *i.e.*, wavelength setting error, non-zero coefficient on measuring zero concentration and over-all shifts in wavelength calibration. To minimise these errors use of the least-squares method was recommended.[12] Nevertheless, the method does not give precise results unless 15 or more absorbance measurements are made and the calculation required is not simple; most analysts would need to study the subject further in order to use the method.

The method proposed in this work is easy to follow, direct and uses only simple calculations to give very accurate and precise results.

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Voltammetric Determination of Pharmaceuticals at the Tubular Graphite Electrode

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Many pharmaceuticals, including phenothiazines, sulpha drugs, purines, phenolic acids, local anaesthetics and antifertility compounds, have been determined quantitatively by oxidative voltammetry at the tubular graphite electrode under hydrodynamic conditions. The data obtained fully establish the reliability of the technique for the determination of these compounds with ease, precision and speed.

A simple empirical correlation that permits the direct determination of the concentration of any unknown electroactive substance in one stage without using a standard reference solution has been derived and verified.

Keywords: *Voltammetry*; *tubular graphite electrode*; *sulpha drugs*; *phenothiazine drugs*

The tubular graphite electrode (TGE), fabricated, studied and standardised by Sharma and co-workers,[1–7] has been successfully employed for voltammetric determinations of a wide variety of aromatic amino and phenolic compounds.[8,9] As most pharmaceuticals contain either easily oxidisable functional groups or ring structures, it was decided to investigate the oxidative voltammetric behaviour of some important pharmaceuticals at this electrode to see if these compounds could be determined voltammetrically with adequate ease, precision and speed.

The analytical methods being used for the determination of these compounds at present[10] are both laborious and time consuming.

Experimental and Results

Samples

Forty different pharmaceuticals, including *N*-substituted phenothiazines, sulpha drugs, purines, phenolic acids, local anaesthetics and antifertility compounds (Table I), were obtained in pure form either from the manufacturer or from the Pharmacology Department of the Postgraduate Institute of Medical Education and Research, Chandigarh. All of the compounds were purified again before use by standard methods.[11]

Electrode Assembly

The electrode assembly and the details of its operation are essentially the same as described earlier.[12] For convenience, however, the assembly is shown in Fig. 1.

The electrodes were made of Specpure compressed graphite rods (Johnson Matthey Chemicals Ltd., Royston, Herts., England) after impregnation with ceresin wax.[13]

Current - Voltage Graphs

Current - voltage graphs for the electro-oxidation of various compounds were obtained by automatic scanning on a Sargent, Model XXI, polarograph under the following conditions: concentration of the electroactive material, 10^{-4} M; supporting electrolyte, 0.1 M sulphuric acid; volumetric flow-rate of electrolytic solution, 10 ml min^{-1}; electrode, length 1.2 cm and internal diameter 1 mm; and temperature, 25 $\pm$ 1 °C.

Extremely well defined voltammograms with well defined diffusion plateaux were obtained in most instances. In a few instances the waves obtained were rather extended. In two of the forty examples the waves exhibited maxima. The values of the limiting currents and the half-wave potentials, corrected for *IR* drop, are given in Table I.

The reproducibility of the current - voltage graphs was checked by scanning three or four successive waves for each substance using the same electrode. If the variation in the

current was within ±2%, the voltammograms were taken to be reproducible. Following this procedure, remarkably reproducible waves (current variation less than ±2%) were obtained for compounds 1–13, 18–28, 30 and 32–40 listed in Table I.

For the remaining compounds, however, the reproducibility was not as good. Successive waves exhibited a noticeable decrease in the values of the limiting currents. This was obviously because of filming of the electrode surface, a common phenomenon in organic voltammetry.

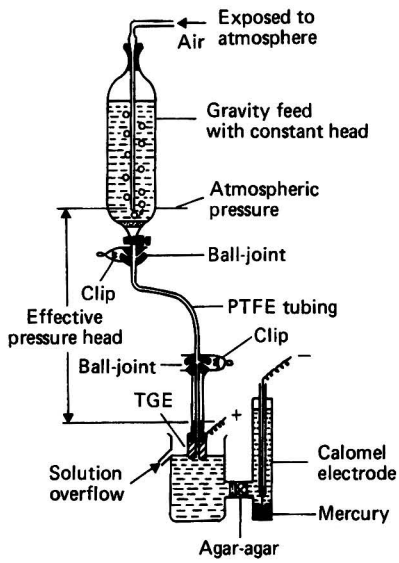

Fig. 1. The tubular graphite electrode (TGE) assembly.

Establishing Current - Concentration Relationship

Based on principles of physico-chemical hydrodynamics, Levich[14] derived the following equation for describing the limiting current in the case of a tubular electrode:

$$I_l = 2.01nCFD^{\frac{2}{3}}R^{\frac{1}{3}}X^{\frac{1}{3}}U_{max.}^{\frac{1}{2}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

Because for practical purposes it is more convenient to measure the volumetric flow-rate (V_f) than the maximum axial velocity ($U_{max.}$), the latter is therefore expressed in terms of the former,[15] and the following expression is obtained:

$$I_l = 5.24 \times 10^5 nCD^{\frac{2}{3}}V_f^{\frac{1}{2}}X^{\frac{1}{3}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

In subsequent discussion equation (2) will be referred to as the Levich equation. In equations (1) and (2), I_l A is the limiting current, C mol ml^{-1} is the concentration, V_f ml s^{-1} is the volumetric flow-rate, X cm is the length of the electrode, n is the number of electrons involved in the oxidation process, D cm^2 s^{-1} is the diffusion coefficient of the electroactive material, F is the Faraday constant and R cm is the radius of the bore of the tubular electrode.

In order to establish the validity of the voltammetric technique for the determination of various electroactive substances, it was necessary to establish the linear relationship between current and concentration predicted by the Levich equation for anodic oxidation. Various solutions with concentrations varying from 10^{-6} to 5×10^{-4} M in 0.1 M sulphuric acid were therefore prepared by appropriate dilutions of 10^{-2} M stock solutions. About twelve compounds were examined in this way, at least two being selected from each series of pharmaceuticals under investigation.

TABLE I

VOLTAMMETRIC DATA FOR ANODIC OXIDATION OF VARIOUS PHARMACEUTICALS

Concentration of analyte, 10^{-4} M; supporting electrolyte, 0.1 M sulphuric acid; electrode length, 1.2 cm; volumetric flow-rate, 10 ml min^{-1}.

No.	Compound	Limiting diffusion current/μA	Half-wave potential/V *vs.* S.C.E.
1	Promethazine hydrochloride	11.0 and 9.0	0.622 and 0.912
2	Promazine hydrochloride	10.6 and 8.8	0.526 and 0.952
3	Chlorpromazine hydrochloride	10.0 and 9.0	0.562 and 0.912
4	Diethazine hydrochloride	11.0 and 10.2	0.580 and 0.952
5	Prochlorperazine methanesulphonate	10.4 and 6.8	0.572 and 0.992
6	Ethopropazine hydrochloride	9.6 and 5.6	0.652 and 0.942
7	Trifluoperazine hydrochloride	10.0 and 9.0	0.616 and 0.962
8	Triflupromazine hydrochloride	11.0 and 7.0	0.652 and 1.020
9	Trimeprazine tatrate	9.6 and 7.9	0.482 and 0.836
10	Sulphanilamide	20.60	1.020
11	Sulphadiazine	20.20	1.070
12	Sulphaguanidine	21.20	1.070
13	Sulphamethazine	20.80	0.990
14	Sulphapyridine*	20.80	0.970
15	Sulphathiazole*	20.40	1.010
16	Sulphamerazine*	21.00	0.990
17	Sulphasomidine*	20.80	0.950
18	Adenine	20.30	0.995
19	Guanine	20.20	1.010
20	Isoguanine	20.40	1.035
21	Xanthine	20.10	1.000
22	Hypoxanthine	20.20	1.055
23	1-Methylxanthine	20.60	1.060
24	Uric acid	21.10	0.620
25	Tyrosine	20.20	0.965
26	Hydroxytyrosine (DOPA)	21.00	0.590
27	Methylhydroxytryosine	21.20	0.580
28	Caffeic acid	19.60	0.580
29	Ferulic acid†	19.80	0.695
30	Protocatechuic acid	21.10	0.625
31	β-Resorcylic acid†	21.20	0.990
32	Amethocaine hydrochloride	21.20	0.890
33	Procaine hydrochloride	21.20	1.015
34	Benzocaine hydrochloride	20.80	1.040
35	Procaine penicillin	21.00	1.005
36	4-Hydroxydiphenyl	19.40	0.765
37	3-Piperidino-4-hydroxydiphenyl	20.00	0.860
38	3-Morpholino-4-hydroxydiphenyl	19.60	0.960
39	3-Pyridino-4-hydroxydiphenyl	21.00	0.905
40	3-Dimethylino-4-hydrodiphenyl	21.20	0.760

* Compounds causing electrode filming.
† Compounds exhibiting maxima in their voltammograms.

The solutions were gravity fed using a constant head (Fig. 1) and allowed to pass through the electrode at a constant flow-rate (10 ml min^{-1}). The tubular graphite electrode was exposed to a potential within the diffusion plateau of the current - voltage graph previously scanned for the substance under test. After 1–2 min, the current was recorded. The value obtained was corrected for the residual current. Limiting currents for solutions of different concentrations of the various substances examined were determined, all other parameters being kept constant and the results obtained were plotted as current - concentration graphs. The results showed a linear current - concentration relationship up to a concentration of 2×10^{-4} M for all of the substances tested, and in a few instances this linear relationship could be extended to higher concentrations. In these investigations, therefore, the concentrations of the various compounds were kept at or below 2×10^{-4} M.

Voltammetric Determination of Phenothiazines

During scanning of voltammograms for various pharmaceuticals, it was observed that in 0.1 M sulphuric acid solution, phenothiazines invariably oxidise in two steps. However, the height of the second wave is less than that of the first. This is due to partial decomposition of the intermediate cation radical formed during anodic oxidation of the phenothiazine.[16] The total current in each instance fell to about 18 μA. In 0.01 M sulphuric acid solution or in buffers of pH 2 and above, phenothiazines oxidise in a single step with a half-wave potential of about 0.6 V *versus* S.C.E., giving a total current of about 20 μA. The waves in

TABLE II

VOLTAMMETRIC DETERINATION OF VARIOUS PHARMACEUTICALS

Supporting electrolyte, Britton - Robinson buffer of pH 2 for phenothiazines and 0.1 M sulphuric acid in all other instances; electrode length, 2.4 cm; volumetric flow-rate, 40 ml min^{-1}.

No.	Compound	Concentration/ mg l^{-1}	Limiting current/ μA	Concentration determined by the voltammetric method/mg l^{-1}	Error, %
1	Promethazine hydrochloride	0.50	0.79	0.4918	−1.64
		5.00	8.14	5.0677	+1.35
		10.00	15.96	9.9362	−0.64
		15.00	24.22	15.0786	+0.52
		30.00	48.40	30.1323	+0.44
		60.00	95.60	59.5175	−0.80
2	Promazine hydrochloride	0.50	0.80	0.5079	+1.58
		5.00	7.80	4.9524	−0.95
		10.00	15.80	10.0317	+0.32
		15.00	24.00	15.2381	+1.58
		30.00	46.60	29.5873	−1.37
		60.00	96.40	61.2063	+2.01
3	Sulphanilamide	0.50	1.52	0.4978	−0.44
		5.00	15.40	5.0443	+0.88
		10.00	30.62	10.0296	+0.29
		15.00	45.70	14.9691	−0.20
		20.00	61.20	20.0462	+0.23
		30.00	92.20	30.2003	+0.66
4	Adenine	0.50	1.90	0.5009	+0.18
		5.00	19.20	5.0624	+1.25
		10.00	38.10	10.0458	+0.45
		15.00	56.70	14.9502	−0.33
		20.00	76.00	20.0390	+0.19
		25.00	95.20	25.1015	+0.40
5	Uric acid	0.50	1.59	0.5021	+0.42
		5.00	15.90	5.0210	+0.42
		10.00	31.40	9.9157	−0.84
		15.00	47.50	15.0000	0.00
		20.00	63.40	20.0210	+0.10
		25.00	78.70	24.8526	−0.98
6	Hydroxytyrosine (DOPA)	0.50	1.36	0.5064	+1.28
		5.00	13.42	4.9901	−0.19
		10.00	27.00	10.0548	+0.54
		15.00	40.10	14.9332	−0.44
		20.00	53.80	20.0351	+0.17
		30.00	80.70	30.0527	+0.17
7	Amethocaine hydrochloride	0.50	0.88	0.4942	−1.16
		5.00	9.00	5.0542	+1.08
		10.00	17.64	9.9064	−0.93
		15.00	26.90	15.1067	+0.71
		30.00	53.00	29.8764	−0.41
		60.00	105.40	59.1913	−1.34
8	3-Morpholino-4-hydroxy-diphenyl	0.50	0.98	0.4959	−0.82
		5.00	9.88	5.0000	0.00
		10.00	19.88	10.0607	+0.60
		15.00	29.48	14.9190	−0.54
		30.00	59.00	29.8582	−0.47
		50.00	98.80	50.0000	0.00

all instances were found to be remarkably reproducible, indicating that the electrode surface remains completely unaffected. Because of these observations, it was considered desirable to determine the phenothiazines in either 0.01 M sulphuric acid solution or in a buffer of pH 2 or above as the supporting electrolyte.

A minimum of five test solutions of each phenothiazine in the concentration range 10^{-6} to 2×10^{-4} M were prepared using Britton - Robinson buffer of pH 2 as the supporting electrolyte. Standard solutions of exactly 10^{-4} M in each phenothiazine were also prepared.

Standard solutions (10^{-4} M) of each phenothiazine were passed through the electrode at a constant flow-rate. In order to increase the sensitivity, the electrode length was doubled and the flow-rate increased 4-fold after ensuring that the flow remained laminar. The electrode was exposed to a potential of 0.70 V *versus* S.C.E., which was found, from the current - voltage graphs, to be sufficiently anodic to cause electro-oxidation of all the phenothiazines in a buffer of pH 2. The limiting current was recorded after allowing sufficient time (2 min) for a steady diffusion state to be attained. Under identical conditions, the limiting currents for the various test solutions of each phenothiazine were recorded. Assuming a linear current - concentration relationship, the concentrations of the test solutions were calculated from the values of the limiting currents obtained for the standard and test solutions.

Altogether more than 50 test solutions of nine different phenothiazines were analysed. The results obtained for two of the phenothiazines are given in Table II. Similar results were obtained for the other phenothiazines.

TABLE III

VOLTAMMETRIC DETERMINATION OF VARIOUS PHARMACEUTICALS THAT CAUSE ELECTRODE FILMING*

Supporting electrolyte, electrode length and volumetric flow-rate as in Table II.

No.	Component	Concentration/ mg l^{-1}	Limiting current/μA: Standard solution (10^{-4} M)	Limiting current/μA: Test solution	Concentration determined by the voltammetric method/mg l^{-1}	Error, %
9	Sulphapyridine	0.50	52.40	1.04	0.4961	−0.78
		5.00	52.20	10.34	4.9521	−0.95
		10.00	52.00	20.60	9.9038	−0.96
		15.00	51.80	30.70	14.8166	−1.22
		30.00	51.60	61.00	29.5542	−1.48
		40.00	51.40	80.90	39.3482	−1.62
10	Ferulic acid	0.50	49.90	1.28	0.4976	−0.48
		5.00	49.80	12.76	4.9707	−0.58
		10.00	49.60	25.40	9.9346	−0.65
		15.00	49.40	37.80	14.8445	−1.03
		20.00	49.20	50.30	19.8337	−0.83
		30.00	49.00	74.80	29.6146	−1.28

* Nos. continued from Table II.

Determination of Sulpha Drugs

The current - voltage graphs recorded for electro-oxidations of various sulpha drugs indicated electrode filming in four of the eight samples (samples 14–17, Table I). In view of this complication, the experimental procedure was modified so that the standard solution of the sample was passed before each passing of the test solution through the electrode. Following this procedure it was observed that although the limiting current for the standard solution (10^{-4} M) as well as for a test solution of a particular concentration gradually decreased (apparently because of electrode filming) the ratio of the two currents remained almost exactly the same. More than 40 test solutions with various concentrations of eight different sulpha drugs were then analysed by the appropriate procedure. The results obtained for two representative samples (one of which caused electrode filming while the other did not) are shown in Tables II and III.

Determination of Purines, Phenolic Acids, Local Anaesthetics and Antifertility Compounds

Following the appropriate procedure, based on the reproducibility or otherwise of the relevant current - voltage graph obtained for electro-oxidation of the analyte, about 150 test solutions with various concentrations of the above pharmaceuticals were analysed voltammetrically. The results obtained for representative members of the various series of compounds are given in Table II.

It should be noted that the amounts of the various substances determined voltammetrically were found to be fortuitously close to the amounts actually present in the corresponding test solutions. The percentage error was small, lying well within the permissible range for analytical work (see also Table II). These observations establish the reliability of the voltammetric technique for the determination of the pharmaceuticals tested. Concentrations as low as 10^{-6} M can be determined with great accuracy. The amount of analyte (5–10 mg) required for determination to be made by the voltammetric method is very small compared with that required (400–600 mg) when the standard chemical or electrochemical methods are used. Further, the standard chemical and electrochemical methods are generally laborious and time consuming whereas the voltammetric method is both rapid and convenient.

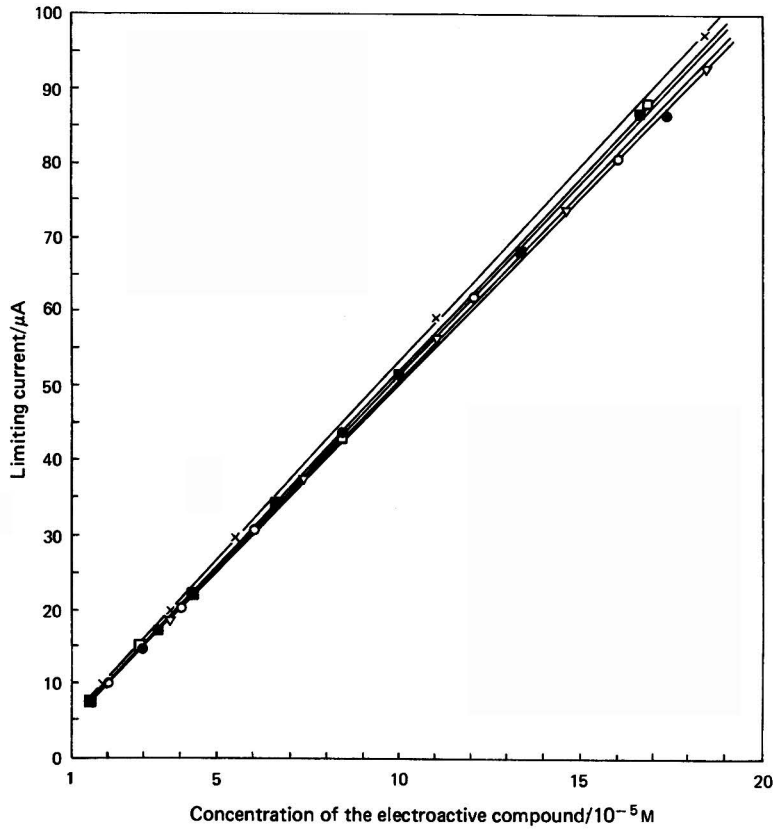

Fig. 2. Current - concentration graphs for various pharmaceuticals. ●, Ethopropazine hydrochloride; □, chlorpromazine hydrochloride; ○, sulphadiazine; ×, procaine hydrochloride; ■, guanine; and ▽, hypoxanthine.

The current - concentration data obtained for further analytes are presented graphically in Fig. 2; good straight lines are obtained, many of which are almost superimposed on one another.

Determination of Compounds in Pharmaceutical Preparations

Between five and ten tablets of a commercial pharmaceutical preparation of a particular compound were finely powdered to give a homogeneous sample. A suitable, accurately weighed amount of the sample was then dissolved in ethanol and diluted with the appropriate supporting electrolyte to give a solution that was approximately 10^{-4} M in the analyte (based on the label claim). This solution was then analysed to determine the amount of analyte present in the tablet. Following this procedure, a number of preparations were analysed by the voltammetric method and by standard chemical and electrochemical methods. The results obtained are shown in Table IV; good agreement between the various values is apparent.

TABLE IV

DETERMINATION OF SOME PHARMACEUTICALS IN COMMERCIAL PREPARATIONS

Supporting electrolyte, Britton - Robinson buffer of pH 2 for phenothiazines and 0.1 M sulphuric acid for sulpha drugs; electrode length, 1.2 cm; volumetric flow-rate, 10 ml min^{-1}; label claim, 50.00 mg of analyte per tablet.

No.	Commercial preparation	Compound determined	Average tablet content of the analyte, determined voltammetrically/mg	Average tablet content of the analyte, determined by alternative methods/mg
1	Largactil (May and Baker)	Chlorpromazine	50.40	50.80
2	Thorazine (Smith, Kline and French)	Chlorpromazine hydrochloride	50.60	50.50
3	Phenargen (May and Baker)	Promethazine hydrochloride	49.50	49.70
4	Tamarial (Smith, Kline and French)	Trimeprazine tartrate	49.70	50.30
5	Siquil Injection (Sarabhai Chemicals)	Triflupromazine hydrochloride	50.40	50.60
6	Sulphadiazine (May and Baker)	Sulphadiazine	49.70	49.30
7	Adiazine (Dolpham)	Sulphadiazine	49.20	49.50
8	Sulphaguanidine (May and Baker)	Sulphaguanidine	49.30	49.10
9	Sulphathiazole (May and Baker)	Sulphathiazole	49.10	48.70
10	Elkosin (Ciba-Geigy)	Sulphasomidine	50.70	50.60

Rapid Voltammetric Determination of Organic Compounds

The data in Table I show that the limiting currents for electro-oxidation of all the substances tested are similar, varying only between 19 and 21 μA. The number of electrons involved in each of these oxidations is reported to be two.[17,18] Consideration of the Levich equation would therefore suggest that the diffusion coefficients of all of these substances, irrespective of their nature, should be similar. The diffusion coefficients were calculated from the Levich equation. The effective length of the electrode used in the equation was determined by the procedure described earlier.[12,18] The values of the diffusion coefficients obtained were found to lie between 0.550×10^{-5} and 0.640×10^{-5} cm^2 s^{-1}. These values are very similar to those reported earlier by Sharma and Kalia[12] for various aromatic amino and phenolic compounds. These observations suggest that the diffusion coefficients of most aromatic organic compounds lie in the range quoted above, irrespective of their nature.

Assuming a typical value of 0.600×10^{-5} cm^2 s^{-1} for D, with an electrode of effective length 1 cm and a flow-rate of 1 ml s^{-1} of the electrolyte solution, the Levich equation simplifies to

$$I_l = 1.729 \times 10^2 nC \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where I_l A is the limiting current and C mol ml^{-1} is the concentration.

For anodic oxidations of a large variety of aromatic amino and phenolic compounds and for all of the pharmaceuticals listed in Table I, the value of n has been shown to be 2.[8,9,18] It can therefore be concluded that for anodic oxidation of aromatic compounds in general, the value of n may be assumed to be 2. Equation (3) may then be simplified to

$$I_l = 3.458 \times 10^2 C \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Equation (4) permits the concentration of any unknown electrochemically oxidisable organic substance to be determined directly in one step without using a reference solution. The method is of particular importance when pure substances for preparing standard reference solutions are not readily available.

In order to investigate the accuracy of equation (4) about 50 test samples of a variety of organic compounds, including aromatic amines, phenols, antibiotics, vitamins and phenothiazines, at a concentration of 10^{-4} M, were analysed by the voltammetric method and the results calculated from equation (4). In about 60% of the samples tested the results were within $\pm 2\%$ of the actual values. In other instances the variation was somewhat greater but in no instance did it exceed $\pm 5\%$.

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Determination of Dinitrotoluene Isomers in Sea Water and Industrial Effluent by High-resolution Electron-capture Gas Chromatography with a Glass Capillary Column

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A method for the rapid determination, with base-line separation, of dinitrotoluene isomers in sea water and industrial effluent using gas chromatography with glass capillary columns and electron-capture detection is described. The samples were extracted with benzene and the extract was injected into the gas chromatograph using a support-coated open-tubular (SCOT) glass capillary column coated with Apiezon L grease. The chromatogram was completed in 8 min. Sea water control samples were spiked at the levels of 0.8 and 8 $\mu g\ l^{-1}$ of 2,6-, 2,3- and 2,5-dinitrotoluene, at 3.2 and 32 $\mu g\ l^{-1}$ of 2,4- and 3,5-dinitrotoluene and at 1.6 and 16 $\mu g\ l^{-1}$ of 3,4-dinitrotoluene. Recoveries of 91–102% ($n = 5$) were obtained for each isomer.

The limits of detection were 0.059, 0.045, 0.031, 0.13, 0.12 and 0.17 $\mu g\ l^{-1}$ for 2,6-, 2,3-, 2,5-, 2,4-, 3,4- and 3,5-dinitrotoluene, respectively.

The method described in this work was applied successfully to the determination of dinitrotoluenes in sea water and industrial effluent without requiring further clean-up procedures.

A Silar 10C column also gave base-line separation of the dinitrotoluenes.

Keywords: Dinitrotoluene isomer determination; gas chromatography; electron-capture detection; sea water analysis; industrial effluent analysis

Dinitrotoluenes, mainly 2,4-dinitrotoluene, have been widely used for the industrial production of 2,4,6-trinitrotoluene (TNT) explosive, dyestuffs and urethane products. The dinitrotoluenes are readily absorbed by skin contact with these materials or their solutions, and can cause anaemia, methaemoglobinaemia, cyanosis and liver damage.[1] The threshold limit value for 2,4-dinitrotoluene set by the American Conference of Governmental Industrial Hygienists is 1.5 mg m^{-3} in air.[2] The aquatic toxicity rating of dinitrotoluenes was reported to be TLm 96 (96 h median tolerance limit) 100–10 p.p.m.,[3] but no regulations could be found concerning the permissible concentrations of dinitrotoluenes in water. However, dinitrotoluenes are major pollutants and appear in the toxic pollutant list published by the Environmental Pollution Agency of the United States.[4] It was recently reported that bacterial reduction of the nitro group in nitro compounds, including 2,4-dinitrotoluene, might be responsible for the mutagenicity of these compounds.[5]

Several methods have been reported for the gas-chromatographic separation and detection of dinitrotoluene isomers using various kinds of packed columns.[6–12] However, these methods did not give a complete separation of each dinitrotoluene isomer. This paper describes the complete separation of six isomers using a support-coated open-tubular (SCOT) glass capillary column equipped with an electron-capture detector and the applications of the method to qualitative and quantitative analyses of trace amounts of dinitrotoluene isomers in industrial effluent and sea water.

Experimental

Reagents and Stock Solutions

Acetone. Nano-grade material for pesticide residue analysis was obtained from Hayashi Pure Chemical Industries Ltd., Osaka, Japan.

Benzene. Nano-grade for pesticide residue analysis.

Anhydrous sodium sulphate. Heated at 625 °C for 5 h to remove organic impurities.

Sodium hydroxide solution, 1 M.

Acetic acid solution, 1 + 1.

Dinitrotoluene isomers. 2,6-, 2,4-, 2,3- and 3,4-dinitrotoluene (Tokyo Kasei Industries Ltd., Tokyo, Japan) and 2,5-dinitrotoluene (ICN, K and K Laboratories Inc., Plainview, N.Y., USA) were purchased commercially at a purity of 99% or better. 3,5-Dinitrotoluene was donated by M. S. Hara (Kyushu Institute of Technology, Kitakyushu, Japan). These materials were used without further purification.

Standard stock solutions of dinitrotoluene isomers. A 10.0-mg amount of 2,6-, 2,5- or 2,3-dinitrotoluene was dissolved in acetone and diluted to 100 ml in a calibrated flask. A 10.0-mg amount of 2,4- or 3,5-dinitrotoluene was dissolved in acetone and diluted to 25 ml in a calibrated flask. A 10.0-mg amount of 3,4-dinitrotoluene was dissolved in acetone and diluted to 50 ml in a calibrated flask.

Working standard solutions. Dilute the stock solutions with acetone for the study of recovery or with benzene for gas chromatography, so as to obtain standard solutions containing 0.01–0.10 μg ml^{-1} of 2,6-, 2,5- and 2,3-dinitrotoluene, 0.04–0.4 μg ml^{-1} of 2,4- and 3,5-dinitrotoluene and 0.02–0.2 μg ml^{-1} of 3,4-dinitrotoluene.

Apparatus

A Shimadzu GC 5AP$_3$FE gas chromatograph (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) equipped with a nickel-63 electron-capture detector was used. Coiled SCOT glass capillary columns coated with Apiezon L grease, Silar 10C, OV-101 silicone and Free Fatty Acid Polyester (FFAP), 20 m long $\times$ 0.28 mm i.d., were obtained from Gaskuro Kogyo Co. Ltd., Tokyo, Japan. A 3385A Automation System integrator (Yokokawa Hewlett-Packard Ltd., Tokyo, Japan) was used for accurate retention-time and peak-area measurements.

The following gas-chromatographic conditions were used: the column temperatures were 140 °C for SCOT glass capillary columns coated with Apiezon L or OV-101 and 160 °C for Silar 10C or FFAP; the injector and detector temperatures for all columns were 180 °C and 275 °C, respectively; the carrier gas (nitrogen) flow-rate was 15 ml min^{-1}; the splitting ratio was 1:11.

The effluent from the glass capillary column was scavenged at 80 ml min^{-1} and passed into the electron-capture detector. All columns were well conditioned before use.

Sampling

Sea water samples were taken monthly at sampling sites A and B, located at Dokai Bay, Japan, from April 4th, 1979, to August 6th, 1979, and sampling site C, located at the Japan Sea, Japan, was taken as the control for sea water. Industrial effluent was sampled on January 25th, 1979.

These samples were brought to the laboratory immediately and extracted with benzene as described in the text within 5 h of collection.

Procedure

The pH of 100 ml of the sample was adjusted to about neutral with sodium hydroxide solution (1 M) or acetic acid solution (1 + 1) using phenolphthalein indicator. The solution was then extracted once with 30 ml of benzene in a 150-ml separating funnel. The extract and washings were dried over anhydrous sodium sulphate. The extract was then concentrated to approximately 8 ml under a gentle flow of dry nitrogen and was diluted to 10 ml in a calibrated flask. A 5-μl sample of extract was injected into the gas chromatograph.

Calibration Graph

Aliquots of 5 μl of the working standard solutions were injected on to the chromatograph. The peak area and retention time for each dinitrotoluene isomer were measured using the 3385A Automation System. A calibration graph of peak area against concentration (micrograms per millilitre) for each dinitrotoluene isomer was then constructed.

Results and Discussion

Selection of Column for the Separation of Dinitrotoluene Isomers

Four different SCOT glass capillary columns were investigated for the separation of dinitrotoluene isomers. On the Apiezon L column, dinitrotoluene isomers were eluted in

the order 2,6-, 2,3-, 2,5-, 2,4-, 3,4- and finally 3,5-dinitrotoluene within 8 min. All of the isomers were separated completely, as shown in Fig. 1. The Silar 10C column also gave base-line separation and eluted the isomers in the order 2,6-, 2,5-, 2,4-, 3,5- and finally 3,4-dinitrotoluene within 15 min, as shown in Fig. 2. The OV-101 column eluted the isomers in the order 2,6-, 2,5-, 2,3- and/or 2,4-, 3,5- and finally 3,4-dinitrotoluene within 8.5 min. The 2,3- and 2,4-dinitrotoluenes were not separated. The FFAP column eluted the isomers in the order 2,6-, 2,5-, 2,4- and/or 3,5-, 2,3- and finally 3,4-dinitrotoluene within 14 min. The 2,4- and 3,5-dinitrotoluenes were not separated. The relative retention times of the other dinitrotoluene isomers compared with 2,4-dinitrotoluene on each SCOT glass capillary column are summarised in Table I; Table I was useful for the qualitative analysis of dinitrotoluenes. The Apiezon L column was selected for the routine determination of dinitrotoluenes because of its base-line separation and rapid analysis time (8 min).

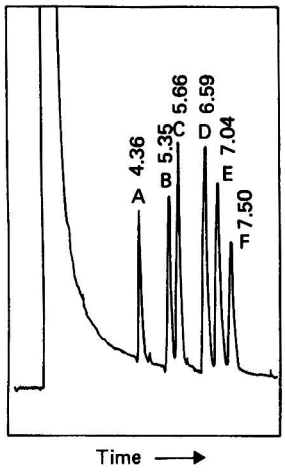

Fig. 1. Typical chromatogram of dinitrotoluenes with Apiezon L grease SCOT glass capillary column. Chromatographic peaks are: A, 2,6-DNT; B, 2,3-DNT; C, 2,5-DNT; D, 2,4-DNT; E, 3,4-DNT; and F, 3,5-DNT. Chromatographic conditions: detector, ^{63}Ni electron capture; carrier gas N_2, flow-rate, 15 ml min^{-1}, splitting ratio 1:11, scavenger N_2 gas flow-rate, 80 ml min^{-1}; column temperature 140 °C; injection temperature 180 °C; and detector temperature 275 °C. The numbers on the peaks are the retention times in minutes.

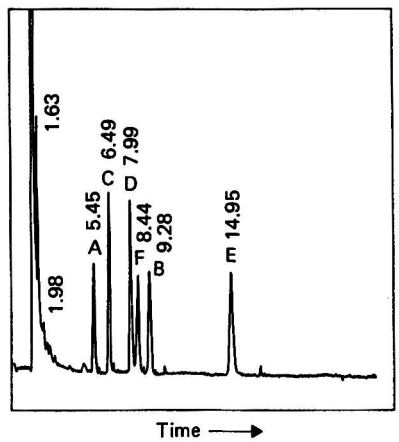

Fig. 2. Typical chromatogram of dinitrotoluenes with Silar 10C SCOT glass capillary column. Chromatographic peaks and conditions as in Fig. 1, except for column temperature, which was 160 °C.

Recovery of Dinitrotoluene Isomers from Sea Water

Typical chromatograms, obtained using SCOT glass capillary columns coated with Apiezon L, for a sea water sample, a sea water blank and a spiked sea water blank are shown in Fig. 3. The spiked samples contained 2,6-, 2,5- and 2,3-dinitrotoluenes at concentrations of 0.08 and 0.8 μg per 100 ml of sample, 2,4- and 3,5-dinitrotoluenes at concentrations of 0.32 and 3.2 μg per 100 ml of sample, and 3,4-dinitrotoluene at concentrations of 0.16 and 1.6 μg per 100 ml of sample.

The isomers were well recovered (91–102% recovery with coefficients of variation under 10%, calculated from a series of five replicate determinations) the results are shown in Table

II. The limits of detection, when the integrator was set to reject the readout of peaks with areas of less than 50 area units (this was approximately four times the noise level) were 0.059, 0.045, 0.031, 0.13, 0.12 and 0.17 $\mu g\ l^{-1}$ for 2,6-, 2,3-, 2,5-, 2,4-, 3,4- and 3,5-dinitrotoluene, respectively. The calibration graphs obtained by plotting the peak area against the concentration of each isomer for 5-μl injections were linear from 0 to 0.4 ng for 2,6-, 2,3- and 2,5-dinitrotoluene, from 0 to 0.8 ng for 3,4-dinitrotoluene and from 0 to 1.6 ng for 2,4- and 3,5-dinitrotoluene.

TABLE I

RELATIVE RETENTION TIMES OF DINITROTOLUENES COMPARED WITH 2,4-DINITROTOLUENE

Dinitrotoluene isomer	Retention time relative to the 2,4-isomer on various columns			
	Apiezon L	OV-101	Silar 10C	FFAP
2,6-	0.671	0.772	0.682	0.770
2,3-	0.810	1.00	1.16	1.24
2,5-	0.866	0.879	0.812	0.876
2,4-	1.00	1.00	1.00	1.00
	(6.96)*	(6.67)*	(7.99)*	(7.84)*
3,4-	1.05	1.22	1.87	1.76
3,5-	1.13	1.07	1.06	1.00

* Absolute retention time in minutes.

Applications

The method has been applied satisfactorily to qualitative and quantitative analyses of dinitrotoluene isomers in sea water and industrial effluent without the need for further clean-up procedures.

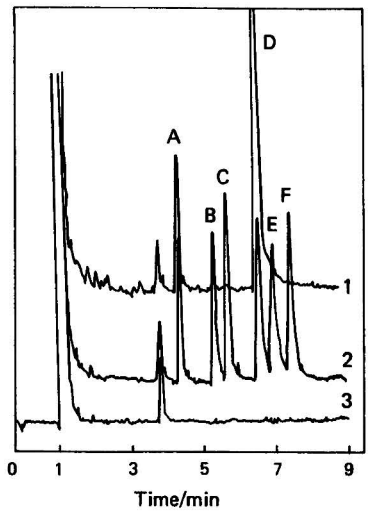

Fig. 3. Typical chromatograms of dinitrotoluenes with SCOT capillary column coated with Apiezon L grease. Peak numbers are identical with Fig. 1. Concentrations of peaks were A, B and C 0.008 $\mu g\ ml^{-1}$, D and F 0.04 $\mu g\ ml^{-1}$ and E 0.02 $\mu g\ ml^{-1}$. 1, Sea water sample; 2, standard solution, spiked to sea water control; and 3, sea water control.

TABLE II

RECOVERY OF DINITROTOLUENE ISOMERS FROM SEA WATER USING A SCOT GLASS CAPILLARY COLUMN COATED WITH APIEZON L

Isomer	Spiked concentration/ μg per 100 ml	Number of determinations, *n*	Recovery, %	Coefficient of variation, %
2,6-	0.08	5	91.1	3.57
	0.8	5	92.1	3.88
2,3-	0.08	5	94.3	4.32
	0.8	5	96.7	1.19
2,5-	0.08	5	97.7	6.08
	0.8	5	96.9	2.62
2,4-	0.32	5	92.4	3.19
	3.2	5	91.9	1.65
3,4-	0.16	5	91.9	5.38
	1.6	5	95.2	1.05
3,5-	0.32	5	96.5	9.25
	3.2	5	102	1.56

Analysis of industrial effluent

An industrial waste was analysed using three SCOT glass capillary columns coated with Apiezon L, OV-101 and Silar 10C. The chromatograms obtained are shown in Fig. 4. In the chromatogram obtained from the SCOT glass capillary column coated with Apiezon L, 2,6-, 2,3-, 2,5- and 2,4-dinitrotoluene were easily recognised by comparison of their retention times with those of the standards and of the relative retention times compared with 2,4-dinitrotoluene (*i.e.*, 0.672, 0.812, 0.866 and 1.00, respectively). However, the shoulder of the 2,4-dinitrotoluene peak in the chromatogram was not counted by the integrator because it was affected by the adjacent large peak. The chromatogram obtained with the SCOT glass

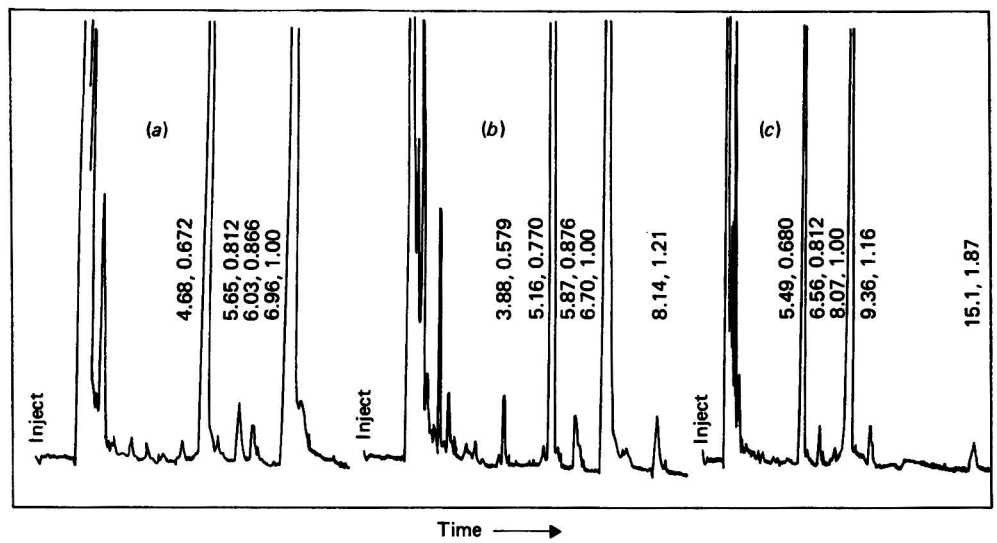

Fig. 4. Chromatograms of dinitrotoluene isomers in industrial effluent using SCOT glass capillary columns coated with (*a*) Apiezon L grease, (*b*) OV-101 and (*c*) Silar 10C. The pairs of numbers on the peaks are absolute retention times (minutes) and retention times relative to 2,4-dinitrotoluene, respectively.

capillary column coated with Silar 10C revealed that 2,6-, 2,5-, 2,4-, 2,3- and 3,4-dinitrotoluene were present in the industrial effluent sample; this was shown by the retention times of the other peaks relative to the 2,4-dinitrotoluene peak (*i.e.*, 0.68, 0.812, 1.00, 1.16 and 1.87, respectively). The chromatogram from the SCOT glass capillary column coated with

OV-101 also showed the presence of 2,6-, 2,5-, 2,3- and/or 2,4-, and 3,4-dinitrotoluene, shown by the retention times of the other peaks relative to the 2,4-dinitrotoluene peak (*i.e.*, 0.770, 0.876, 1.00 and 1.21, respectively).

The results showed that the column coated with Silar 10C was more suitable than that with Apiezon L in this case, and that the combination of more than two columns was useful for the qualitative analysis of dinitrotoluene. The 2,6-, 2,5-, 2,4-, 2,3- and 3,4-dinitrotoluene concentrations were found to be 375, 7.5, 1550, 12.5 and 19.0 $\mu g\ l^{-1}$, respectively. It was concluded that 3,5-dinitrotoluene was not present in the industrial effluent.

Analysis of sea water

The method was used for routine monitoring of dinitrotoluene concentrations in sea water from Dokai Bay, Japan, for the 5 months from April 4th, 1979 to August 6th, 1979, the results are shown in Table III. Both 2,6- and 2,4-dinitrotoluene were detected. Concentrations of 2,4-dinitrotoluene in surface water samples were higher than those in bottom water samples in 8 out of 10 samples. The ratio of 2,6-dinitrotoluene to 2,4-dinitrotoluene in the samples was not constant. The ratios at the sampling site A were higher than those at the sampling site B in all instances.

TABLE III

CONCENTRATIONS OF 2,6- AND 2,4-DINITROTOLUENE IN SEA WATER FROM DOKAI BAY, JAPAN, FROM APRIL 4TH TO AUGUST 6TH, 1979

Date	Sampling site	Sample*	Dinitrotoluene isomer/ $\mu g\ l^{-1}$ 2,6-	2,4-	2,6- to 2,4-isomer ratio
4.4.79	A	s	ND	1.19	0
		b	0.18	5.40	0.033
	B	s	ND	1.74	0
		b	ND	0.25	0
15.5.79	A	s	1.40	16.0	0.088
		b	0.22	3.58	0.061
	B	s	ND	0.78	0
		b	ND	0.59	0
5.6.79	A	s	0.57	14.1	0.040
		b	0.12	3.08	0.039
	B	s	ND	1.11	0
		b	ND	1.03	0
4.7.79	A	s	2.10	28.3	0.074
		b	0.44	2.66	0.165
	B	s	0.23	3.00	0.077
		b	ND	0.13	0
6.8.79	A	s	0.26	4.80	0.054
		b	0.65	5.91	0.110
	B	s	0.08	2.26	0.035
		b	ND	0.97	0

* s = surface water sample at high tide; b = bottom water sample at high tide.
† ND = not detected.

Conclusions

Complete separation of dinitrotoluene isomers was obtained on a glass capillary column coated with Apiezon L or Silar 10C. Detection was achieved by electron capture. The method was applied successfully to the qualitative and quantitative analysis of dinitrotoluene isomers in sea water and industrial effluent. For qualitative analysis the use of more than two separation column systems such as Apiezon L as a non-polar liquid phase and Silar 10C as a polar one gave more reliable results.

The SCOT glass capillary column coated with Apiezon L was selected for use in the routine monitoring of dinitrotoluenes in sea water because it gave complete separation of the isomers with a rapid analysis time (8 min). The method proved useful in this study, helping to clarify the behaviour of dinitrotoluenes when discharged into the environment.

The authors thank Dr. T. Akiyama, former Research Director of this Institute, for his valuable advice and encouragement, and Dr. M. Sonoda, the present Research Director, for his permission to publish this work.

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Retention of Metabolised Trace Elements in Biological Tissues Following Different Drying Procedures

Part II.* Caesium, Cerium, Manganese, Scandium, Silver and Tin in Rat Tissues

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Losses of silver, cerium, caesium, manganese, scandium and tin during freeze drying and oven drying at 80, 105 and 120 °C were studied in rat tissues and faeces that contained radioactive isotopes. No loss was observed for any of these elements on freeze drying. A significant loss of tin was observed in muscle even at 80 °C, and in kidney and liver at 105 °C. Also, cerium and manganese were lost from heart and muscle at this temperature. An increase in the temperature to 120 °C was found to be safe only for silver and caesium in all of the tissues. For the remaining four elements the following losses were observed: up to 5% for cerium in brain and heart, for scandium in fur and heart and for manganese in liver and muscle; up to 10% for scandium in kidney and muscle and for tin in blood, fur, heart, liver, ovary and uterus; and over 10% for tin in brain, kidney, lung and muscle.

Keywords: *Biological tissues*; *metabolised trace elements*; *drying*; *retention*

The purpose of this series of investigations was outlined in Part I,[1] which dealt with antimony, cobalt, iodine, mercury, selenium and zinc. That investigation showed that there are tissue-specific differences in the retention of various trace elements, in particular the volatile elements. In this part, similar observations have been extended to a further six elements: caesium, cerium, manganese, scandium, silver and tin.

Experimental

Grouping of the Elements

In planning experiments for analysing for various elements simultaneously it is useful to know the relative uptake of the elements by various tissues and body fluids. This helps to establish optimum radioactivity concentration levels for various radionuclides in order to prevent the dominance of the gamma spectrum by the gamma-ray energy lines of any one radioisotope, which creates difficulties in evaluating the peaks for low uptake situations. To give a specific example, because of the chemical similarity between caesium and potassium, the uptake of caesium-134 is relatively high in all samples. This results in the dominance of the gamma spectrum by caesium-134, which has several gamma-ray emissions. Hence a preliminary experiment was conducted with three animals to assess the optimum combinations of the radionuclides in the water for feeding the experimental animals. As a result, it was found to be convenient to prepare the water in three different combinations.

Preparation of the Treated Water

The radioactive tracers used were obtained commercially (Amersham Buchler, NEN Chemicals, Germany). The chemical forms of the elements, their concentrations in the treated water, the nuclear data for the radioisotopes chosen and the specific activities are given in Table I. The labelled water was prepared in one batch by adding the required amounts of the radioactive solutions to the drinking water in order to obtain three different combinations: 1, caesium; 2, manganese + tin; and 3, silver + cerium + scandium.

* For details of Part I of this series, see reference list, p. 801.

TABLE I

CHEMICALS USED FOR PREPARING THE TREATED WATER

Radionuclide	Half-life	γ-Ray energy/keV	Chemical form	Specific activity	Approximate concentration of the elements in treated water due to the addition of radioactive isotopes/μg l^{-1}
Caesium-134 ..	2.1 yr	605	Caesium chloride in water	10 mCi mg^{-1}	100
Cerium-141 ..	32.5 d	145	Cerium chloride in 0.1 M HCl	2 mCi mg^{-1}	500
Manganese-54 ..	312 d	835	Manganese chloride in 0.1 M HCl	100 μCi μg^{-1} (carrier-free)	1
Scandium-46 ..	84.2 d	888 and 1120	As dilute solution in chloride form	3 mCi mg^{-1}	333
Silver-110m ..	255 d	658	As solution in nitrate form	3 mCi mg^{-1}	333
Tin-113	115 d	392 (^{113}In^{m})	Tin(II) chloride in 4 M HCl	13.4 mCi mg^{-1}	75

Procedure

Three groups of adult Wistar rats, including both males and females, were used. Each group contained 10 animals, which were kept in separate cages. Throughout the experimental span of 10 weeks they were maintained under identical conditions, except for the difference in the treated water, which they drank *ad libitum*. Whereas group 1 was fed with water containing caesium-134, groups 2 and 3 received waters mixed with manganese-54 + tin-113 and silver-110m + cerium-141, respectively. At the end of the tenth week, the treated waters were replaced with normal water for a period of 3 d. Following this stage, blood samples were collected from all of the animals by cardiac puncture under ether anaesthesia. The animals were dissected and duplicate sets of samples of brain, faeces, fur, heart, kidney, liver, lung, muscle, ovary, spleen, testis and uterus were collected and prepared for the drying experiments. Blood-rich organs were rinsed with water to minimise the detrital blood. Faeces samples were collected directly from the intestine. The specimen denoted as "fur" was actually a piece of outer skin of the rat with a thick bunch of hair. Urine samples were not collected owing to technical difficulties.

Drying of the Samples and Radioactivity Measurements

The initial radioactivity of all of the samples was measured. The first set of samples was freeze dried and the second batch was subjected to oven drying at 80 °C for 3 d followed by 105 and 120 °C for 1 d each, respectively. Procedural details concerning various phases of the drying process, equipment used and the radioactivity measurements were essentially as described in Part I.[1] However, one change was introduced in the present experiment by measuring the radioactivity of the samples at the end of each stage of drying instead of counting the samples at the end of each day of drying.

Results and Discussion

Freeze Drying

The results for freeze-dried whole blood, brain, faeces, fur, heart, kidney, liver, lung, muscle, ovary, spleen, testis and uterus obtained for the six elements showed recovery values greater than 98%.

Our results for manganese agree with the findings of Fourie and Peisach,[2] who also found no loss of this element from oysters, using experimental conditions comparable to ours. Freeze drying has generally been found to be suitable for a number of elements in different matrices, with the exception of mercury in fish, cerebral pons and sea cucumber, and both mercury and iodine in water.[3,4] A few results from the literature are summarised in Table II for comparison.

TABLE II

LOSS OF ELEMENTS DURING FREEZE DRYING OF BIOLOGICAL SAMPLES

Data collected from references 1–7.

Element	Matrix	Procedure or mode of incorporation	Pressure/ Torr	Time/ h	Loss observed, %
Co	Oyster	Radioisotope, metabolised	?	24	No loss
Cr	Oyster		?	24	No loss
Fe	Oyster		?	24	No loss
Hg	Fish	Chemical analysis	?	?	20
	Fish homogenate	Chemical analysis	?	?	16–39
		Radioisotope, spiking	?	?	No loss
	Butterfish	Chemical analysis	?	?	70
	Human brain (pons)		?	?	18–57
	Plankton		?	?	50–64
	Guinea pig, rat—	Methylmercury (^{203}Hg)	?	?	3
	Muscle		0.05	24	3, 3
	Liver				1, 7
	Kidney				No loss
	Heart				1, 5
	Blood				2, 8
	Faeces				No loss
	Muscle	Phenylmercury (^{203}Hg)	0.05	24	No loss
	Liver				2
	Kidney				No loss
	Blood				No loss
	Faeces				9, 3
	Sea cucumber	Chemical analysis	?	?	59
	Water		0.01–0.05	48–72	39
	Human urine	^{203}Hg (organic), intravenous	0.05	48	2
I	Water	Chemical analysis	0.01–0.05	48	32
				72	
	Human urine	Radioisotope, metabolised	0.05	48	2
Mn	Oyster	Radioisotope, metabolised	?	24	No loss
Pb	Oyster		?	24	No loss
Se	Human urine	^{75}Se (organic), intravenous	0.05	48	3

Oven Drying

The results for oven-dried blood, brain, faeces, fur, heart, kidney, liver, lung, muscle, ovary, spleen, testis and uterus showed certain differences for the six elements. A few details concerning the behaviour of the individual elements are discussed below.

Caesium

Drying at up to 105 °C poses no danger of losing this element from any of the 13 samples studied. At 120 °C, several tissues showed a slight loss of caesium, reflected by the increased standard deviation in the mean recovery values. However, the losses were not statistically significant. It may be mentioned in this connection that caesium belongs to the group of elements that cause problems of loss only on dry ashing,[8] and our findings support this conclusion.

Manganese

Drying at up to 105 °C was found to be safe for all of the samples except muscle, which showed a loss of about 2%, whereas at 120 °C a significant loss of manganese was also observed in liver, but remained below 5%.

There have been a few studies concerning the behaviour of manganese under various dehydrating conditions. For example, Gorsuch[8] reported no loss of this element during dry ashing of biological materials and listed it under "elements causing little difficulty." Similarly, Fourie and Peisach,[2] who experimented with oysters, recorded no loss of metabolised manganese-54 on drying at up to 120 °C. However, on drying molluscs at 110 °C,

Strohal *et al.*[9] observed that 14% of manganese was lost and therefore recommended wet ashing as the method of choice for the determination of radioactive contaminants (manganese-54, cobalt-56, -57 and -58, ruthenium-106, cerium-144 and protactinium-233) in biological material.

Some of the available literature information on the loss of several elements during oven-drying is summarised for comparison for a variety of matrices in Table IV.

Tin

Oven drying of rat tissues containing metabolised tin is generally safe for all of the samples at 80 °C. Although statistically significant losses were found only for kidney, liver and muscle at 105 °C, several samples showed an increased dispersion in the mean recovery values, suggesting that the marrix is no longer stable with respect to tin at this temperature, and that it is apparently at a critical point about to decompose. This is clearly seen with an increase in temperature to 120 °C, at which all of the tissues lost this element to varying extents. The losses were highly significant in several instances (Table III). However, faeces (which is not a tissue) was the only exception that showed a better matrix stability even at 120 °C by retaining tin quantitatively.

TABLE III

SIGNIFICANCE TEST: FREEZE DRYING *versus* OVEN DRYING AT VARIOUS TEMPERATURES

	80 °C		105 °C		120 °C	
Sample	$2P < 0.02$	$2P < 0.002$	$2P < 0.02$	$2P < 0.002$	$2P < 0.02$	$2P < 0.002$
Blood ..						Sn†
Brain ..					Ce*; Sn‡	
Faeces ..						
Fur ..					Sc*; Sn†	
Heart ..			Ce*		Ce*; Sc*; Sn†	
Kidney ..			Sn*		Sc†	Sn‡
Liver ..			Sn*		Mn*	Sn†
Lung ..						Sn‡
Muscle ..	Sn*		Mn*; Sn*		Sc†; Mn*	Sn‡
Ovary ..						Sn†
Spleen ..						
Testis ..						
Uterus ..						Sn†

* Loss up to 5%.
† Loss up to 10%.
‡ Loss up to 15%.

Tin belongs to the group of seven elements (arsenic, gold, germanium, mercury, lead, selenium and tin) that cause problems with losses under several conditions. A few examples from the literature showing the reported losses of these elements from diverse matrices are summarised in Tables IV and V for oven drying and dry ashing, respectively. Tin is an essential trace element required by biological systems for normal physiological processes.[10] It is also recognised that tin is a volatile element, yet systematic studies to investigate its retention under well defined tissue dehydration procedures are still lacking. In this connection it is of interest to consider the tin values from the classic studies of Tipton *et al.*,[19] who analysed a large number of human tissue samples. They analysed ashed samples (>400 °C) by atomic-emission spectroscopy and reported concentrations lower than those known now.[20] Results from the present experiment show that the biological matrix is no longer stable with respect to tin even at 120 °C. This clearly indicates that errors resulting from dehydration steps at high temperatures are serious for tin.

Silver

The retention of silver in different samples following drying showed that both 80 and 105 °C are suitable temperatures for drying tissues containing metabolised silver. The

tendency seen at 120 °C resembles that for caesium, except for kidney, where some loss was detected (recovery 96 ± 3.35%); other samples showed only a slight decline in the recovery values.

TABLE IV

LOSS OF ELEMENTS DURING OVEN DRYING OF BIOLOGICAL SAMPLES

Data collected from references 1, 2, 4, 9 and 11.

Element	Matrix	Procedure or mode of incorporation	Temperature/ °C	Time/ h	Loss observed, %
Cd	Oyster	Radioisotope, metabolised	120	48	No loss
			90	48	No loss
			50	48	No loss
	Rat liver	Radioisotope, intravenous	110	16	1
	Rat kidney		110	16	1
Co	Oyster	Radioisotope, metabolised	120	43	No loss
	Mollusc	Radioisotope, metabolised	110	?	14
	Rat, many tissues	Radioisotope, intravenous	80	72	No loss
			110	24	No loss
			120	24	No loss
Cr	Rat liver	Radioisotope, intravenous	120	48	No loss
	Rat blood		110	16	3
Fe	Oyster	Radioisotope, intravenous	110	16	5
	Rat blood		105	48	No loss
Hg	Human urine	^{203}Hg (organic), intravenous	80	72	3
			105	24	15
			120	24	25
	Plankton	Chemical analysis	60	50	51–60
	Rat liver	Radioisotope, metabolised	80	72	5
			105	24	3–10
			120	24	7–15
	Rat brain	Radioisotope, metabolised	120	24	5–16
	Rat muscle		120	24	5–21
I	Human urine	Radioisotope, metabolised	80	72	2
			105	24	4
			120	24	7
	Rat—				
	Muscle	Radioisotope, metabolised	120	24	<5
	Blood		120	24	7
	Serum		120	24	7
	Erythrocytes		120	24	8
	Brain		120	24	10
	Kidney		120	24	15
	Lung		120	24	7
Mn	Oyster	Radioisotope, metabolised	50–120	48	No loss
	Mollusc		110	?	14
Pb	Oyster	Radioisotope, metabolised	60	48	10
			100	48	17
			120	48	20
Sb	Rat blood	Radioisotope, metabolised	105	24	<5
			120	24	<5
	Rat—				
	Brain		120	24	8
	Kidney		120	24	9
	Lung		120	24	6
	Spleen		120	24	7
Se	Herbage	Chemical analysis	30	12	No loss
			60	12	No loss
			100	12	No loss
	Rat—				
	Blood	Radioisotope, metabolised	120	24	<5
	Brain		120	24	<5
	Lung		120	24	<5
	Muscle		120	24	<5

TABLE IV—continued

Element	Matrix	Procedure or mode of incorporation	Temperature/ °C	Time/ h	Loss observed, %
Se (cont.) ..	Human urine	^{75}Se (organic), intravenous	80	72	12–30
			105	24	30–50
			120	24	50–65
	Oyster	Radioisotope, metabolised	60	48	<5
			100	48	<5
			120	48	>20
Zn	Rat blood	Radioisotope, intravenous	110	16	No loss
	Rat liver		110	16	No loss
	Rat, many tissues	Radioisotope, metabolised	80	72	No loss
			110	24	No loss
			120	24	No loss
	Mollusc	Radioisotope, metabolised	110	?	9

Cerium

Drying at up to 120 °C presented no serious problems for most of the samples. Although significant losses were recorded for this element in heart and brain at 105 and 120 °C, respectively, the losses did not exceed 5%. The recovery of cerium has previously been studied by Strohal *et al.*[9] in marine biological samples. They found no loss on drying at 110 °C, but dry ashing above 250 °C resulted in a loss of up to 13%.

Scandium

Up to 105 °C there is no danger of losing this element from any of the 13 samples. However, drying of rat tissues containing metabolised scandium at 120 °C was found to be unsuitable for many tissues. A number of tissues showed a tendency to lose this element, and the losses observed for fur, heart, kidney and muscle were significant (Table III). Only spleen and testis showed better matrix stability at this temperature.

TABLE V

LOSS OF ELEMENTS DURING DRY ASHING OF BIOLOGICAL SAMPLES

Data collected from references 4, 9 and 12–18.

Element	Matrix	Procedure or mode of incorporation	Temperature/ °C	Time/ h	Loss observed, %
Ag	Animal liver	Chemical analysis	450	?	<5
	Animal kidney		450	?	<20
Al	Animal liver	Chemical analysis	450	?	16
	Animal kidney	Chemical analysis	450	?	12
As	Ox blood (dry)	Radioisotope, spiking	850	16	35
			550	16	29
			450	16	28
	Rat bone	Radioisotope, intravenous	450	16	44
	Rat blood		450	16	86
	Rat kidney		450	16	82
Ba	Animal liver	Chemical analysis	450	?	—
	Animal kidney		450	?	4
Ca	Human rib	Radioisotope, spiking	420	16	<1
			600	16	<1
			710	16	<1
Cd	Animal liver	Chemical analysis	450	?	<0.7
	Animal kidney		450	?	<6
	Rat liver	Platinum dish	600	16	1.6
	Rat liver	Chemical analysis	500	16	2
	Rat kidney		500	16	4.4
Co	Animal liver	Chemical analysis	450	?	<14
	Mollusc	Radioisotope, metabolised	450	?	26
			800	?	22

TABLE V—continued

Element	Matrix	Procedure of mode of incorporation	Temperature/ °C	Time/ h	Loss observed, %
Cr	Sugar, refined	Graphite furnace	450	?	0
	Sugar, brown		450	?	13
	Sugar, unrefined	Chemical analysis	450	?	47
	Mollasses		450	?	52
	Sugar, refined	Muffle furnace	450	?	63
	Sugar, brown	Chemical analysis	450	?	62
	Sugar, unrefined		450	?	86
	Mollasses		450	?	89
	Animal kidney	Chemical analysis	450	?	<25
	Animal liver		450	?	<7
	Rat liver	Radioisotope, platinum dish	700	16	2.2
			500	16	6.1
	Rat blood		700	16	51.3
			500	16	4
Cu	Animal kidney	Chemical analysis	450	?	0.4
	Animal liver		450	?	0.2
Fe	Animal kidney	Chemical analysis	450	?	0.1
	Animal liver		450	?	0.3
	Rat liver	Platinum dish	500	16	No loss
	Rat blood	Chemical analysis	500	16	0.4
Hg	Fish (whole)		110	24	81.4
K	Human rib		420	16	<1
			600	16	55
			710	16	90
Mn	Mollusc	Radioisotope, metabolised	450	?	15
			800	?	21
	Animal kidney	Chemical analysis	450	?	0.4
	Animal liver		450	?	0.3
Mo	Animal kidney	Chemical analysis	450	?	<1.5
	Animal liver		450	?	<0.4
Na	Human rib	Radioisotope, spiking	420	16	<3
			600	16	10
			710	16	20
	Ox blood		450	16	Slight
Ni	Animal kidney	Chemical analysis	450	?	<15
	Animal liver		450	?	3
Pb	Animal kidney	Chemical analysis	450	?	<12
	Animal liver		450	?	<2.4
	Human rib		600	16	<5
			710	16	40
Sn	Animal kidney	Chemical analysis	450	?	<0.3
	Animal liver		450	?	11
Sr	Animal kidney	Chemical analysis	450	?	0.5
	Animal liver		450	?	<2.5
	Ox blood	Radioisotope, spiking	450	16	9
	Rat bone	Radioisotope, intravenous	450	16	Slight
Sr	Rat blood		450	16	16
	Rat kidney		450	16	5
Zn	Mollusc	Radioisotope, metabolised	450	?	33
			800	?	44
	Seaweed	Chemical analysis	500	16	No loss
			1000	16	No loss
	Mussels	Chemical analysis	500	16	No loss
			1000	16	No loss
	Ox blood	Radioisotope, spiking	450	16	No loss
			550	16	No loss
			850	16	No loss
	Rat blood	New porcelain	700	16	1
		Chemical analysis	500	16	No loss
	Animal kidney	Chemical analysis	450	?	<1
	Animal liver		450	?	<1
	Rat liver	Etched porcelain	700	16	1.1
		Chemical analysis	500	16	1.3

The biological matrix

It has been shown in Part I[1] that most biological matrices decompose above 100 °C and that intrinsically volatile elements such as mercury can be easily lost. The losses observed for tin in this experiment confirmed this further. The loss of the elements from the decomposed matter varies, depending on the chemical properties of the individual elements.

Conclusions

Freeze drying for up to 72 h under an operating pressure of 0.05 Torr yields satisfactory retention for all six elements.

Oven drying at 80 °C is generally safe, except for tin in muscle samples.

Oven drying at 105 °C results in a loss of up to 5% for cerium in heart, manganese in muscle and tin in kidney, liver and muscle samples.

Elevating the temperature to 120 °C causes no loss of silver or caesium. However, cerium, manganese, scandium and tin are lost from several tissues to various extents. Tin is the element most affected, with losses exceeding 20% in some tissues.

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

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Automatic Titration by Stepwise Addition of Equal Volumes of Titrant

Part V.* Extension of the Gran I Method for Calculation of the Equivalence Volume in Acid - Base Titrations

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A very simple method for numerical calculation of the equivalence volume (V_e) from titration data is presented. It is based on the method of stepwise addition of equal volumes of titrant combined with an extended version of the Gran I method.

The expressions derived may be summarised in a general equation:

$$V_e = V_j + \frac{V_j - V_i}{a_{ij} - 1}$$

where V_i and V_j are two additions of titrant (V_i is iV_p, where V_p is the volume of each addition). The term a_{ij} is different for strong acids and weak acids and for conditional titrations, but in all instances only the ratio between hydrogen ion concentrations is required. *E.g.*, for a weak acid

$$a_{ij} = \frac{V_i[H]_i}{V_j[H]_j}$$

The titration method can also be used for the determination of the conditional normal potential E_0' and the constant j_H in the junction potential $j_H[H]$ for an electrode couple in a given medium.

Keywords: *Gran I method*; *acid - base titration*; *automatic titration*; *equivalence volume calculation*; *potentiometric titration*

Nearly 30 years ago one of the present authors published two papers treating the determination of the equivalence volume in potentiometric titrations. The methods are usually called Gran I[1] and Gran II.[2] The formulae presented at that time were approximate; this was necessary in order to prevent the numerical evaluation becoming too time consuming. The original equations therefore have a rather limited field of application. For the Gran II method this has been investigated in detail by Pehrsson *et al.*[3]

The development and application of electronic computers has completely changed the situation. Nowadays it is often unnecessary to use the approximate formulae, and complete titration equations can be applied instead. These are mainly extended Gran II expressions for acid - base titrations.[4] In this connection calculation programs for numerical evaluation of data from titrations of one or several acids in a mixture have been described (*e.g.*, refs. 5–8). The Gran I method has rarely been used. However, Still[9] recently used a modification of this method to test the electrode response of, *e.g.*, an ion-selective electrode.

For the reasons given we have found it of interest to investigate the circumstances under which the Gran I method can be usefully applied.

The Gran I method is characterised by $\Delta V/\Delta$pH or $\Delta V/\Delta E$ being plotted against a function of V, where V denotes the volume of titrant added and pH or E the corresponding pH or e.m.f. value. The function of V against which the differential quotient is plotted is different for strong and weak acids. It can therefore be assumed that the equations cannot be applied to acids of intermediate strength. Problems of this kind will be treated in two papers. In this paper the equations presented are simple so that a non-programmable calculator is sufficient for evaluations to be made. The equations in the second paper are more sophisticated and their evaluation requires a programmable calculator to perform iterations. The latter equations can be used for acids with a wider range of stability constants.

* For details of Part IV of this series, see reference list on p. 810.

Theory

Consider the case where V_0 ml of a monoprotic acid HA is titrated with a strong base, *e.g.*, a sodium hydroxide solution of concentration C_B. After addition of V ml of the titrant solution the following relationship applies [equation (3) in Part III[7]]:

$$V + K\left(V[\mathrm{H}] + \frac{V_0 + V}{C_B}\cdot[\mathrm{H}]^2 - \frac{V_0 + V}{C_B}\cdot K_w\right) + \frac{V_0 + V}{C_B}\left([\mathrm{H}] - \frac{K_w}{[\mathrm{H}]}\right) - V_e = 0 \quad (1)$$

where K is the stability constant of the acid, K_w is the ionic product of water and V_e is the equivalence volume. K and K_w are concentration constants, *i.e.*, the electrode couple is calibrated by using known concentrations instead of activities in a certain medium. [H] is therefore the hydrogen ion concentration, not the activity.

All terms in equation (1) represent volumes and their contributions can be of very different magnitudes. Terms of very small size can be neglected, *e.g.*, in acidic solutions the term $(V_0 + V)K_w/[\mathrm{H}]C_B$, which represents the contribution from hydroxide ions, can be neglected. The term $K(V_0 + V)K_w/C_B$ is also very small except for very weak acids.

Assuming that these two terms can be neglected, equation (1) can be written in the following way for two additions of titrant (V_i and V_j):

$$C_B\,(V_e - V_i) = \{KC_BV_i + K[\mathrm{H}]_i\,(V_0 + V_i) + V_0 + V_i\}[\mathrm{H}]_i \quad .. \quad (2)$$

$$C_B\,(V_e - V_j) = \{KC_BV_j + K[\mathrm{H}]_j\,(V_0 + V_j) + V_0 + V_j\}\,[\mathrm{H}]_j \quad .. \quad (3)$$

Dividing equation (2) by equation (3) and subtracting 1 from each of the two quotients, one obtains

$$\left(\frac{V_e - V_i}{V_e - V_j}\right) - 1 = \frac{V_j - V_i}{V_e - V_j} = \left\{\frac{KC_B\,V_i + K[\mathrm{H}]_i\,(V_0 + V_i) + V_0 + V_i}{KC_B\,V_j + K[\mathrm{H}]_j\,(V_0 + V_j) + V_0 + V_j}\cdot\frac{[\mathrm{H}]_i}{[\mathrm{H}]_j}\right\} - 1 \quad (4)$$

Rearrangement of equation (4) gives

$$V_e = V_j + \frac{V_j - V_i}{\left\{\dfrac{KC_B\,V_i + K[\mathrm{H}]_i\,(V_0 + V_i) + V_0 + V_i}{KC_B\,V_j + K[\mathrm{H}]_j\,(V_0 + V_j) + V_0 + V_j}\cdot\dfrac{[\mathrm{H}]_i}{[\mathrm{H}]_j}\right\} - 1} \quad .. \quad (5)$$

This equation can be simplified for very strong acids and some weak acids.

For strong acids $K = 0$ and equation (5) becomes

$$V_e = V_j + \frac{V_j - V_i}{\left(\dfrac{V_0 + V_i}{V_0 + V_j}\cdot\dfrac{[\mathrm{H}]_i}{[\mathrm{H}]_j}\right) - 1} \quad .. \quad .. \quad .. \quad (6)$$

For weak acids, if $(V_0 + V)$ is much less than $\{KC_BV + K[\mathrm{H}]\,(V_0 + V)\}$ which, under normal titration conditions, requires that $K \geqslant 10^4$, then equation (5) can be written as

$$V_e = V_j + \frac{V_j - V_i}{\left\{\dfrac{C_B\,V_i + [\mathrm{H}]_i\,(V_0 + V_i)}{C_B\,V_j + [\mathrm{H}]_j\,(V_0 + V_j)}\cdot\dfrac{[\mathrm{H}]_i}{[\mathrm{H}]_j}\right\} - 1} \quad .. \quad .. \quad (7)$$

Except for small values of V, the term $[\mathrm{H}]\,(V_0 + V)$ can be neglected in comparison with C_BV and equation (7) then simplifies to

$$V_e = V_j + \frac{V_j - V_i}{\left(\dfrac{V_i}{V_j}\cdot\dfrac{[\mathrm{H}]_i}{[\mathrm{H}]_j}\right) - 1} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

Acids of Intermediate Strength

For acids of intermediate strength equation (5) has to be used. There is the difficulty that the value of the stability constant K may be unknown; the calculations can, however,

be performed in the following way. A preliminary value for V_e is calculated from equation (6), by assuming that $K = 0$. Using this value of V_e a preliminary value for K is calculated from the equation

$$K = \frac{C_B (V_e - V) - [H] (V_o + V)}{C_B [H] V + [H]^2 (V_o + V)} = \frac{C_B V_e - \{C_B V + [H] (V_o + V)\}}{[H] \{C_B V + [H] (V_o + V)\}} \quad .. \quad (9)$$

From these approximate values for V_e and K more exact values are obtained by iteration. This procedure will be dealt with in more detail in the next part of this series.

Conditionally Strong Acids

Acids with $\log K < 5$ can be studied by the conditional titration method.[10] In this method the sample, acid HB, is added to an excess of a solution of a salt, NaA, of a weaker acid, HA. This means that an amount of the acid HA is liberated equivalent to the amount of acid HB added.

The mixture is then titrated with a standard solution of a strong base. If the concentration of NaA is more than 100 times higher than the total concentration of the acid HB multiplied by the ratio of K_{HB} to K_{HA}, then the titration can be treated as that of a strong acid, though with a conditional value for the ionic product of water (K_{HA} and K_{HB} are the stability constants of the acids).

In some cases it may not be practical to use very high concentrations of NaA. However, if the total concentration of the salt is known a correction factor can be calculated allowing much lower salt concentrations to be used. Sodium acetate can be used in this type of titration, if the stability constant of the sample acid is less than about 10^5. For weaker acids sodium sulphite may be used, but in this case the concentration of the hydroxide ion cannot be neglected. This case is also treated in the next part of this series.

Assume as above that V_o ml of solution is titrated with V ml of strong base of concentration C_B. The sample solution contains the acid HB and a salt NaA with a total concentration C_{AO}. Part of this salt has been converted into the acid HA due to reaction with the acid HB. (*N.B.*, C_{AO} is not identical with C_A^o in refs. 7 and 10. C_A^o is the concentration of the anion A^- at the start of the titration, while C_{AO} is the sum of $[A^-]$ and [HA]). The following expressions are valid:

$$K_{HA} = \frac{[HA]}{[H][A]} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$

$$[HA] = \frac{C_B (V_e - V)}{(V_o + V)} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

$$[A] = \frac{[C_{AO} V_o - C_B (V_e - V)]}{(V_o + V)} \quad .. \quad .. \quad .. \quad .. \quad (12)$$

where V_e is the equivalence volume. The stability constant is a concentration constant. From these expressions the following equation is obtained:

$$C_B (V_e - V) = K_{HA}[H] \{C_{AO} V_o - C_B (V_e - V)\} \quad .. \quad .. \quad (13)$$

The division of such expressions for two additions V_i and V_j gives

$$\frac{V_e - V_i}{V_e - V_j} = \frac{C_{AO} V_o - C_B (V_e - V_i)}{C_{AO} V_o - C_B (V_e - V_j)} \cdot \frac{[H]_i}{[H]_j} \quad .. \quad .. \quad .. \quad (14)$$

Subtraction of 1 from each quotient and rearrangement gives

$$V_e = V_j + \frac{V_j - V_i}{\left\{\frac{(C_{AO} V_o/C_B) - (V_e - V_i)}{(C_{AO} V_o/C_B) - (V_e - V_j)} \cdot \frac{[H]_i}{[H]_j}\right\} - 1} \quad .. \quad .. \quad (15)$$

The volume V_e in the denominator is not known, but by choosing even a very approximate value V'_e, for the equivalence volume, iteration of equation (15) twice or perhaps three times gives the value for V_e, even if the initial value of V'_e is taken to be zero.

Comparison with the Gran I Method

The derivation of the equations given above (*cf.*, ref. 9) is slightly different from that given in ref. 1. In that paper equations for $\Delta V/\Delta pH$ and dV/dpH were set up (*e.g.*, for a strong acid). At a certain volume V', the differential quotient and the derivative are equal. The value of V′ can be calculated, but an approximate value can be obtained from

$$V' = V_i + 0.5\,(V_j - V_i)C \quad .. \quad .. \quad .. \quad .. \quad (16)$$

where C is a factor, usually about 1, which is tabulated in ref. 1. The equation finally derived was

$$\frac{\Delta V}{\Delta pH} = \left(\frac{V_o + V'}{V_o + V_e}\right)(V_e - V')\ln 10 \quad .. \quad .. \quad .. \quad (17)$$

The equivalence volume was found by plotting $(\Delta V/\Delta pH)/(V_o + V')$ against V', when a straight line was obtained, which intersected the V' axis at V_e.

In order to obtain a similar straight line by using the equations derived in this paper, $V_e - V_i$ has to be plotted against V_i. This line will intersect the V_i axis at $V_i = V_e$.

Determination of $[H]_i/[H]_j$

When equation (6), (8) or (15) is used for the calculation of V_e, the value of $[H]_i/[H]_j$ is obtained from measured values by the expression

$$\frac{[H]_i}{[H]_j} = 10^{(pH_j - pH_i)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18)$$

or

$$\frac{[H]_i}{[H]_j} = 10^{\left(\frac{E_i - E_j}{Q}\right)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (19)$$

where $Q = (RT\ln 10)/F$. As only the difference between two pH or E values is required, it is not necessary for the meter to be calibrated very accurately.

If the titrations are carried out using a glass electrode and a reference electrode the following expressions are valid after addition of V_i and V_j ml of titrant:

$$E_i = E'_{o(i)} + Q_i\log[H]_i + j_H[H]_i \quad .. \quad .. \quad .. \quad (20)$$

$$E_j = E'_{o(j)} + Q_j\log[H]_j + j_H[H]_j \quad .. \quad .. \quad .. \quad (21)$$

where E_i is the e.m.f. measured after the addition of V_i ml of titrant, E_j the corresponding value after addition of V_j ml. E'_o and j_H are the conditional constants defined in Part III of this series.[7] Q has a constant value if the temperature is kept constant during the titration and therefore

$$\log\frac{[H]_i}{[H]_j} = \frac{(E_i - E_j) - [E'_{o(i)} - E'_{o(j)}] - j_H\,([H]_i - [H]_j)}{Q} \quad .. \quad (22)$$

The term $E'_{o(i)} - E'_{o(j)}$ disappears if E'_o can be kept constant during the titration, which requires a constant ionic strength. For most glass electrodes E'_o is constant up to a pH of 5 and also in the pH range 8.5–10.5. When the pH is between 5 and 8.5 E'_o may change by several millivolts because the protective layer of hydrous silica formed in acidic solutions on the surface of the glass electrode will start to dissolve.[11] This source of error can be largely avoided if the titrations are performed as rapidly as possible. In order to indicate the magnitude of the quantities in equation (22) the following example may be considered.

If 100 ml of 0.01 M hydrochloric acid are titrated with nine 1-ml portions of 0.1 M sodium hydroxide solution, the hydrogen ion concentrations will be about 0.01, 0.009, 0.008 M, etc., with $E_i - E_{(i+1)}$ increasing from about 3 mV to about 20 mV or by about 60 mV altogether.

The e.m.f. can be measured to within 0.1 mV without difficulty, *i.e.*, the error will be about ± 0.05 mV, equivalent to a few tenths of a per cent. in the value of $[H]_i/[H]_{(i+1)}$.

For a good glass electrode j_H is about -25 mV l mol^{-1} if the ionic strength of the solution is 0.5 M, and about -70 mV l mol^{-1} if the ionic strength is 0.1 M. In the actual example the term $j_H\{[H]_i - [H]_{(i+1)}\}$ has the values 0.025 mV or 0.23 mV for the whole range.

Summary of Equations

For strong acids (log K less than about 2),

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{V_0 + V_i}{V_0 + V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad (23)$$

All acids, strong or weak, for which log K is less than about 5 can be titrated in a sodium acetate solution when

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{b + V_i}{b + V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad (24)$$

where

$$b = \left(\frac{C_{A0}\, V_e}{C_B}\right) - V_e \quad .. \quad .. \quad .. \quad .. \quad (25)$$

For weak acids with log K between 4.2 and 7,

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{V_i}{V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad .. \quad (26)$$

If all additions of titrant are made using a pipette of volume V_p, equations (23)–(26) may be simplified. For example, equation (26) becomes

$$V_e = V_p \left\{ j + \frac{j - i}{\left(\frac{i[H]_i}{j[H]_j}\right) - 1} \right\} .. \quad .. \quad .. \quad .. \quad (26a)$$

as $V_i = i\, V_p$, etc., where i and j are whole numbers.

In all of the above instances $[H]_i/[H]_j$ can be calculated from equation (18) or (19). From now on, however, ph is used instead of pH in order to signify that the meter is calibrated by means of solutions with known hydrogen ion concentration and not hydrogen ion activity.

In acidic solutions with ph less than about 2.5 at ionic strength 0.1 M, or ph less than about 2 at ionic strength 0.5 M, a correction should be made in the ph and E values for changes in the liquid junction potential, *i.e.*, consideration should be given to the j_H term in equation (20) in accurate titrations.

Formulae for strong and weak acids similar to equations (23) and (26), respectively, have also been deduced by Cörmös and Marasciac.[12]

Calculation Procedure

The necessary calculations can be performed on a simple pocket calculator. It should be able to calculate 10^x and it is advantageous if it is equipped with memories. A programmable calculator will of course speed up the calculations. The ability to perform a linear regression analysis is a further advantage.

The titration may be conveniently carried out by adding the titrant with an accurate automatic pipette. After each addition the ph or the e.m.f. is measured. The titration is continued until the equivalence point has been passed. All values after the equivalence point are discarded. This can be done either by calculating a preliminary value V'_e for the equivalence volume from two points definitely situated before the equivalence point or by means of an approximate value of ph at the equivalence point.

The value of V_e is calculated from a number of combinations of V_i and V_j according to equations (23)–(26). With equations (24) and (25) the preliminary value V'_e is used in the calculation of b and then a more accurate value of V_e is calculated by iteration.

As $V_e = V_j +$ quotient, it is obvious that the value of the quotient will be lower the greater the value of V_j. The determination of V_j can be made very accurately and it is therefore best to use as high a value as possible for V_j and to then combine this value with a number of values for V_i. As the ph or the E values are usually less accurate than the volumes, the value of the quotient calculated from different V_i values will differ slightly. The most accurate value of V_e will then normally be obtained if the different V_e values are plotted against V_i and a line through them is extrapolated until it intersects the line $V_e = V_i$. This can be done either graphically or numerically. As an alternative the method described under Comparison with the Gran I Method can be used.

Example 1

Standardisation of a sodium hydroxide solution against hydrochloric acid of known concentration

Titration of 100.0 ml of 0.01000 M hydrochloric acid (ionic strength adjusted to 0.1 M by the addition of sodium chloride) at 25.0 °C with approximately 0.1 M sodium hydroxide solution.

Additions were made with a 1.0011-ml automatic pipette. A combined glass, silver - silver chloride electrode was used, with a digital voltmeter for the e.m.f. measurements.

In Table I columns 1 and 2 give the experimental values of V and E. Columns 3 and 4 were calculated from equation (23) without any correction for the change in the liquid junction potential due to the change in the acidity, *i.e.*, the term $j_H([H]_i - [H]_j)$ in equation (22) has been neglected. In columns 5 and 6 this term was included with $j_H = -82$ mV l mol^{-1}. This value was calculated using the method described in example 2. Columns 3–6 were calculated using $j = 8$ and $i = 0, 1, 2, \ldots, 7$.

TABLE I

STANDARDISATION OF A SODIUM HYDROXIDE SOLUTION

100.0 ml of 0.01000 M hydrochloric acid were titrated with approximately 0.1 M sodium hydroxide solution. The ionic strength of the acid was increased to 0.1 M with sodium chloride. The temperature was 25.0 °C.

	Experimental values		Calculated from equation (23) neglecting $j_H([H]_i - [H]_j)$*		Calculated from equation (23) including $j_H([H]_i - [H]_j)$*	
Value No.	V/ml	E/mV	$V_e - V_j$/ml	V_e†/ml	$V_e - V_j$/ml	V_e†/ml
0	0	276.4	1.3212	9.3300	1.2788	9.2876
1	1.0011	273.3	1.3168	9.3256	1.2796	9.2884
2	2.0022	269.8	1.3139	9.3227	1.2814	9.2902
3	3.0033	265.8	1.3121	9.3209	1.2841	9.2929
4	4.0044	261.2	1.3066	9.3154	1.2832	9.2920
5	5.0055	255.7	1.2984	9.3072	1.2798	9.2886
6	6.0066	248.7	1.2943	9.3031	1.2803	9.2891
7	7.0077	239.2	1.2896	9.2984	1.2793	9.2881
8	8.0088	224.2				

* See text under Example 1.

† Linear regression analysis of the values in column 4 gives $V_e = 9.3315 - 0.004582\ V_i$. For $V_i = V_e$ the value obtained is $V_e = 9.2889$ ml; $C_B = 0.10766$ M. A corresponding calculation on column 6 gives $V_e = 9.2897$ ml; $C_B = 0.10765$ M.

Linear regression analysis of column 6 gives V_e = 9.2897 ml (C_B = 0.10765 M), and column 4 gives V_e = 9.2889 ml (C_B = 0.10766 M) while the arithmetic mean is 9.3154 (C_B = 0.10735 M). Linear regression therefore gives the same values for V_e and C_B as those calculated with correction for the change in the liquid junction potential.

As a check another V_j value can be chosen. With j = 7 and i = 0, 1, 2, . . ., 6 and 8 the following values are obtained: with a correction for the change in the liquid junction potential, arithmetic mean V_e = 9.2917 ml and C_B = 0.10762 M, linear regression analysis gave V_e = 9.2919 ml and C_B = 0.10762 M; without correction, linear regression analysis gave V_e = 9.2899 ml and C_B = 0.10764 M, arithmetic mean V_e = 9.3364 ml and C_B = 0.10711 M. Only the last pair of values differ appreciably from the other values.

It may therefore be concluded that linear regression gives correct values while the arithmetic mean gives correct values only when j_H is considered. Linear regression analysis is also recommended in those cases when equation (23) is used for other than very strong acids, *i.e.*, moderately strong acids with log$K$ up to 2–2.5. For example, sulphuric and picric acids can be titrated with great accuracy by this method.

Example 2

Determination of j_H and E'_0

The titration data in Table I can also be used for calculation of the value of the constants j_H and E'_0. To obtain a more accurate value for j_H, hydrochloric acid of higher concentration should be titrated. Table II illustrates such a titration of 100.04 ml of 0.05002 M hydrochloric acid with an approximately 0.4 M sodium hydroxide solution at 25.0 °C. (The ionic strength of both solutions was adjusted to 0.5 M by the addition of barium chloride.)

TABLE II

DETERMINATION OF j_H AND E'_0

100.04 ml of 0.05002 M hydrochloric acid were titrated with approximately 0.4 M sodium hydroxide solution.
Linear regression analysis gives V_e = 12.939; C_B = 0.3867; E'_0 = 416.96 ± 0.02; j_H = − 30 ± 1; standard deviation = 1.

Value No.	V/ml	E/mV	[H]/M	E'_0
0	0	338.5	0.05002	416.96
1	1.0063	336.3	0.04568	416.96
2	2.0126	333.9	0.04140	416.96
3	3.0189	331.3	0.03722	416.97
4	4.0252	328.4	0.03312	416.94
5	5.0315	325.2	0.02910	416.95
6	6.0378	321.6	0.02516	416.97
7	7.0441	317.4	0.02129	416.94
8	8.0504	312.5	0.01749	416.98
9	9.0567	306.4	0.01376	416.93
10	10.063	298.6	0.01010	416.96
11	11.0693	287.5	0.00651	417.04
12	12.0756	267.5	0.00298	417.01

First V_e and C_B are calculated as described in example 1 (without correction for j_H). The values obtained were 12.939 and 0.3867 M. The hydrogen ion concentrations can be calculated from

$$[H] = \frac{(V_e - V)C_B}{(V_0 + V)} \quad .. \quad .. \quad .. \quad .. \quad (27)$$

These values can be used to calculate j_H using equation (22) after re-arrangement to

$$j_H = \frac{E_i - E_j - Q\log\frac{[H]_i}{[H]_j}}{[H]_i - [H]_j} \quad .. \quad .. \quad .. \quad .. \quad (28)$$

With i = 0, 1, 2, . . ., 7 and $j = i + 3, i + 4, \ldots, 10$, a j_H value of −30 ± 1 was obtained.

The calculation procedure was repeated using this j_H value. No significant differences were obtained; $V_e = 12.941$ ml and $C_B = 0.3867$ M.

E'_0 can be calculated as the mean of all $E'_{0(i)}$ values from equation (20), which gives $E'_0 = 416.96$.

Example 3

Conditional titration of oxalic acid

The solution contains 59.48 mg of oxalic acid dissolved in a 0.50 M sodium acetate solution with $V_0 = 50.0$ ml, temperature 21.8–22.0 °C, pipette volume 0.4997 ml and titrant 0.1 M sodium hydroxide solution.

The titrant solution was initially standardised against 0.5 mmol of hydrochloric acid in 0.5 M sodium acetate solution. Two titrations gave $V_e = 5.1345$ and 5.1358 ml and $C_B = 0.09738$ and 0.09736 M. The mean value 0.09737 for C_B was used in the titration of oxalic acid.

The results of the titrations are given in Table III. With $i = 0, 2, 4, \ldots, 18$ and $j = 19$, the following values are obtained: by linear regression analysis $V_e = 9.5617$ ml, yield 98.67%; arithmetic mean $V_e = 9.5619$ ml, yield 98.67%. With $i = 0, 2, 4, \ldots, 16$ and $j = 18$ one obtains by linear regression analysis $V_e = 9.5567$ ml, yield 98.62%; arithmetic mean $V_e = 9.5631$ ml, yield 98.68%.

In a similar titration of 54.88 mg of oxalic acid a yield of 98.75% was obtained.

TABLE III

TITRATION OF A CONDITIONALLY STRONG ACID

59.48 mg of oxalic acid dissolved in 50 ml of 0.5 M sodium acetate solution. Titrant, 0.09737 M sodium hydroxide solution.
Linear regression analysis gives $V_e = 9.5617$ and yield 98.7%.

Value No.	V/ml	E/mV	$\lvert V_e - V_i \rvert$/ml	V_e/ml
0	0	45.4	0.0678	9.5621
2	0.9994	42.3	0.0683	9.5626
4	1.9988	39.1	0.0682	9.5625
6	2.9982	35.4	0.0681	9.5624
8	3.9976	31.1	0.0681	9.5624
10	4.9970	26.0	0.0680	9.5623
12	5.9964	19.8	0.0675	9.5618
14	6.9958	11.4	0.0674	9.5617
16	7.9952	−1.2	0.0672	9.5615
18	8.9946	−27.3	0.0677	9.5620
19	9.4943	−81.4		

Example 4

Titration of a weak acid

The sample used was 1.0823 mmol of *p*-nitrophenol with $V_0 = 100.0$ ml, temperature 25.0 °C, ionic strength 0.5 M, pipette volume 1.0011 ml and $C_B = 0.0992$ M.

For *p*-nitrophenol log K is about 7 and thus equation (26) should be used. As e.m.f. values have been measured the equation takes the form

$$V_e = V_j + \frac{V_j - V_i}{\frac{V_i}{V_j} \cdot 10^{(E_i - E_j)/Q} - 1} \quad \ldots\ldots\ (29)$$

An inspection of the E values or a preliminary V_e value calculated from, *e.g.*, values 3 and 4, shows that V_e is between 10 and 11. Thus values 11 and 12 are discarded. Values 0 cannot be used as $V_i = 0$ and values 1 are also left out as the V_e value differs too much from the other values [see the discussion by equation (7)].

With $i = 2, 3, 4, \ldots, 9$ and $j = 10$ the following values are calculated: by linear regression analysis $V_e = 10.8632$ ml ($\equiv 1.0775$ mmol, yield 99.56%); arithmetic mean $V_e = 10.8608$ ml ($\equiv 1.0774$ mmol, yield 99.55%). If other combinations of data from Table IV are used, *e.g.*, $i = 2, 3, 4, \ldots, 8, 10$ and $j = 9$, very similar results are obtained.

TABLE IV

TITRATION OF A WEAK ACID

Titration of 1.0823 mmol of *p*-nitrophenol with 0.0992 M sodium hydroxide solution. V_0 = 100.0 ml. Ionic strength, 0.5 M. Temperature, 25 °C.

Linear regression analysis gives V_e = 10.8623 and yield 99.6%.

Value No.	V/ml	E/mV	$V_e - V_i$/ml	V_e/ml
0	0	126.3	[−10.0110]*	[0]*
1	1.0011	46.8	[0.8583]*	[10.8693]*
2	2.0022	26.5	0.8489	10.8599
3	3.0033	13.0	0.8490	10.8600
4	4.0044	2.1	0.8492	10.8602
5	5.0055	−7.7	0.8497	10.8607
6	6.0066	−17.2	0.8496	10.8606
7	7.0077	−27.1	0.8498	10.8608
8	8.0088	−38.3	0.8517	10.8627
9	9.0099	−52.4	0.8502	10.8612
10	10.0110	−75.1		
11	11.0121	−180.2		
12	12.0132	−233.3		

* These pairs of values have been omitted from consideration; see text under Example 4.

Discussion

The method described for the determination of equivalence volumes requires only simple calculations. Equations (23)–(26) may be summarised in the general equation

$$V_e = V_j + \frac{V_j - V_i}{a_{ij} - 1} \quad .. \quad .. \quad .. \quad .. \quad (30)$$

The calculation of a_{ij} differs for titrations of strong acids and weak acids and for conditional titrations, but in all three cases only the ratio between hydrogen ion concentrations is required.

If a maximum error in V_e of 0.5% is allowed it is sufficient to choose two pairs of measurements and calculate V_e using equation (30).

If a very accurate result is required, a V_j value near the equivalence point is combined with several V_i values and a linear regression analysis is performed. In this case there is another advantage that it is not necessary to keep the temperature constant during the titration, and a uniform temperature rise may be allowed.

The method has some limitations, however. Titrations of acids with log K between 2 and 4.2 give incorrect results, if they are evaluated using the equations for either strong or weak acids. Titrations of very weak acids (with log $K > 7$) cannot be evaluated using this simple method. Another limitation is that the components in a mixture of acids cannot be determined.

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

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SHORT PAPERS

Determination of a Volatile Phenol in Serum by High-performance Liquid Chromatography

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Keywords: Volatile phenols determination; o-chlorophenol determination; serum analysis; micro-extraction; high-performance liquid chromatography

Recently, a railroad tank car carrying a phenolic mixture overturned and its contents were spilled. The principal components were *o*-chlorophenol (71%) and phenol (24%). Several days after this spill, a young child living in the immediate area suffered respiratory arrest, and it was not known whether this incident was related to the infant's exposure to these phenols. Shortly thereafter, the Center for Disease Control (CDC) was asked to determine the presence of *o*-chlorophenol in the infant's serum and in the serum of seven workers involved in clean-up operations after the accident.

Most techniques for the determination of contaminants in serum involve extraction with an organic solvent, evaporation of the organic solvent to leave a residue containing the contaminant and then reconstitution of the residue.[1] This procedure is adequate for relatively involatile compounds, but it cannot be used with volatile compounds such as *o*-chlorophenol (boiling-point 175–176 °C; vapour pressure 2.4 mm at 25 °C),[2] as they will be lost during the evaporation step. An alternative procedure for the determination of a volatile phenol in serum is described in this paper.

Experimental*

Apparatus

A Waters liquid chromatographic system, consisting of a U6K injector, an M6000A pump and 440 ultraviolet detector monitoring at 254 and 280 nm, was used with a Waters μBondapak C_{18} column (300 × 3.9 mm i.d.).

Procedure

To 1 ml of serum were added 810 ng of *m*-cresol (internal standard) in microlitre amounts of acetonitrile. After 200 mg of ammonium sulphate had been added, the serum was extracted with 2 ml of benzene. The sample was shaken by hand for 1 min, swirled with a vortex mixer and centrifuged. The benzene layer was transferred into a conical centrifuge tube and 100 μl of 1 N sodium hydroxide solution and 400 μl of water were added. The sample was shaken by hand, swirled with a vortex mixer and centrifuged. The benzene was removed by pipette and discarded. After six drops of 6 N hydrochloric acid and 50 μl of chloroform had been added to the remaining aqueous layer, the mixture was shaken, swirled with a vortex mixer and centrifuged. A 15-μl aliquot from the chloroform "bead" in the bottom of the tube was injected into the chromatograph. The mobile phase was acetonitrile - water - acetic acid (25 + 74.5 + 0.5) with a flow-rate of 1.5 ml min^{-1}.

Results and Discussion

To demonstrate the loss of the *o*-chlorophenol during evaporation of the extraction solvent, we prepared five samples containing 210 μg of *o*-chlorophenol in chloroform - propan-2-ol

*The use of trade-names is for identification purposes only and does not constitute endorsement by the Public Health Service or by the US Department of Health, Education, and Welfare.

(10 + 1). Under mild conditions of evaporation, which consisted of blowing a gentle stream of nitrogen into each of the tubes, in a 35–40 °C water-bath, two of the five samples showed complete loss of *o*-chlorophenol. Other experiments gave similar results.

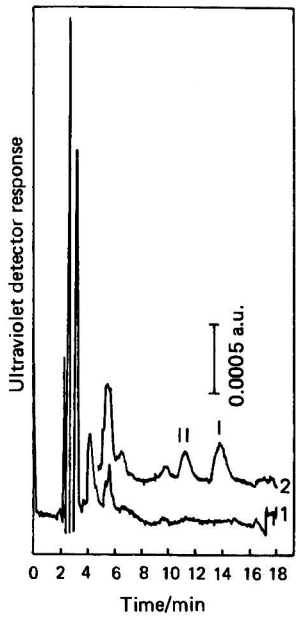

Fig. 1. Chromatograms of 1-ml serum samples; 15-μl aliquots were injected under conditions described in text, detection was at 280 nm: 1, the serum blank; and 2, fortified with *o*-chlorophenol (I, 210 ng) and *m*-cresol (II, 810 ng).

As the evaporation step had to be eliminated and the sensitivity had to remain adequate, we reasoned that a micro-extraction procedure might be appropriate because it would allow the sample to be concentrated into a small volume. The procedure developed involved pre-extraction of the phenol into an organic solvent, extraction of the phenol into an alkaline aqueous mixture and finally acidification and re-extraction of the phenol into 50 μl of chloroform. Of the various solvents used to extract the *o*-chlorophenol from serum, benzene gave the highest recoveries with no interference (Fig. 1 and Table I). Hexane gave a fair recovery (61%) with no interferences, but three extractions were required. Chloroform - propan-2-ol (10 + 1), chloroform, toluene and toluene - ethyl acetate (8 + 2) were also evaluated, but these systems gave either poor recoveries or interfering peaks.

TABLE I

ESTIMATE OF PRECISION OF ANALYSIS AND RECOVERY OF *o*-CHLOROPHENOL FROM 1-ml SERUM SAMPLES USING THE PROCEDURE DESCRIBED

Amount of *o*-chlorophenol/ng*	Recovery, %†	Peak-height ratio†	Relative standard deviation, %
210	87 ± 13	1.54 ± 0.08	4.0
840	80 ± 7.8	5.28 ± 0.55	8.4

* Amount of *m*-cresol (internal standard) added, 810 ng.
† Mean ± $ts(n)^{-\frac{1}{2}}$ (95% confidence limits); $n = 5$.

In the micro-extraction step, chloroform was used as the solvent as it formed an immiscible phase in the bottom of the conical tube. Aliquots were easily drawn from this layer for chromatography. Chloroform was immiscible with the reversed-phase chromatography system, but when it was used good reproducibility was still achieved (Table I). The calibration graph from 210 to 1850 ng per sample was linear, with a regression line of $y = 0.007x + 0.61$. The detection limit of the procedure with the 280-nm detector was about 50 ng for a 1-ml sample (signal to noise ratio ≈ 2).

The infant's serum sample, collected 6 days after respiratory arrest, and the serum samples from the clean-up workers gave negative results for *o*-chlorophenol.

The procedure described offers an alternative to the usual procedure of sample concentration and may be particularly useful in analyses for volatile compounds.

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Direct Spectrofluorimetric Determination of the Free Amino Group of Cephalexin in its Lysine Salt

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Keywords: *Cephalexin determination*; *cephalexin - lysine salt*; *spectrofluorimetry*

The β-lactam moiety of cephalosporins and penicillins is subject to nucleophillic attack under a variety of conditions,[1] leading to biologically inactive products. With cephalexin and other α-aminoarylcephalosporins and penicillins, the free amino groups can also take part in intra- and intermolecular reactions.[2–4] Specifically with cephalexin lysinate,[5] a method for the measurement of the free amino group of cephalexin may provide additional information about the degradation of this product. Such a method would also be of interest in studying reactions between cephalexin and lysine, as reaction of cephalexin with the ϵ-amino groups of lysine residues in proteins may be involved in allergic reactions of this compound.[6] Several methods have been described for the determination of cephalexin,[7] but nearly all measure the intact β-lactam group. The free amino group of cephalexin was measured by Bundgaard[2] by a modification of the trinitrobenzene - sulphuric acid technique of Satake *et al.*[8] However, we were unable to differentiate between the amino groups of cephalexin and lysine by this method.

A sensitive method for the determination of the free amino group of cephalexin in the presence of lysine is described in this paper.

Experimental

Reagents and Solutions

Fluorescamine solutions. A 0.1-g amount of fluorescamine (Fluram, Hoffman-LaRoche) was dissolved in analytical-reagent grade acetone (100 ml) and aged for 24 h before use.[9]

Buffer solutions. pH 2: mix 0.2 M potassium chloride solution (25 ml) with 0.1 N hydrochloric acid (13 ml) and dilute to 100 ml with water. pH 3–7: prepare from 0.5 M disodium

hydrogen orthophosphate solutions by addition of hydrochloric acid. pH 8–10: prepare from 0.05 M potassium chloride in 0.05 M sodium borate solution by appropriate adjustment with acid or base.

Drug substances. Cephalexin of pharmacopoeial grade (Lilly), lysine hydrochloride of reagent grade (Merck) and cephalexin lysinate (Laboratorios Almirall) were used.

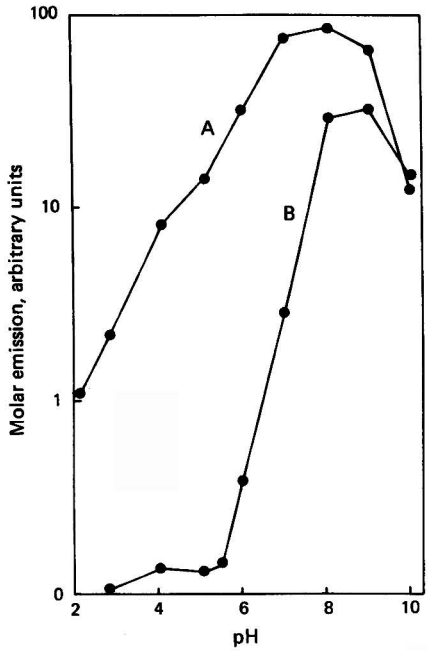

Fig. 1. Ratio of fluorescence of cephalexin and lysine at different pH (excitation at 390 nm and emission at 475 nm): A, cephalexin; and B, lysine.

Instrument

Fluorescence measurements were made with an Aminco-Bowman spectrofluorimeter with a double monochromator. The 1-mm slit was used in all positions.

Procedure

To a mixture of the sample (1 ml, containing about 20 μg ml^{-1} of cephalexin) and buffer solution of pH 5.5 (5 ml) in a 10-ml calibrated flask was added fluorescamine solution (1 ml) and the contents were mixed and made up to volume with water. The solution was scanned in the spectrofluorimeter with an excitation wavelength of 390 nm and an emission wavelength of 475 nm. The fluorescence measurements were uncorrected for energy output and instrument artefacts. The free amino group of the cephalexin in the sample was read off from a calibration graph prepared by carrying out the same procedure with a series of cephalexin standard solutions (10–30 μg ml^{-1}).

Results and Discussion

Both cephalexin and lysine react with fluorescamine under appropriate conditions to give fluorescent products with a high fluorescence yield and having similar excitation - emission characteristics (maximum excitation at 387 and 395 nm and maximum emission at 476 and 472 nm for cephalexin and lysine, respectively).

The emission fluorescence of the cephalexin and lysine derivatives at the same molar concentration plotted against the pH at which the reaction was carried out is shown in Fig. 1.

The ratio of the fluorescence of the cephalexin and lysine derivatives is a maximum when the reaction is carried out at pH 5.5 and is sufficient for the sensitive determination of cephalexin (1 μg ml^{-1}) to be carried out in the presence of lysine with minimum interference by the latter (approximately 0.7%).

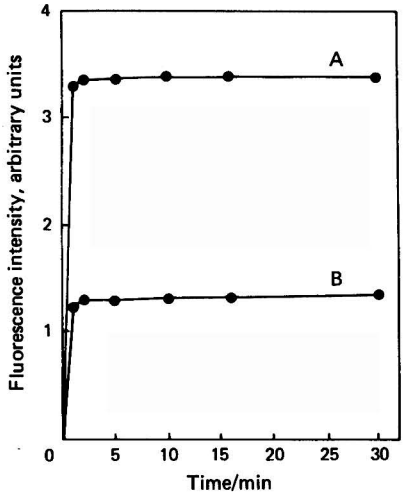

Fig. 2. Effect of time on fluorescence at pH 5.5: A, cephalexin (0.1 mM); and B, lysine (5 mM).

The fluorescence does not vary with reaction time (5–30 min, Fig. 2). There is a linear correlation between fluorescence and concentration within the range 0–100 μg ml^{-1}, with good reproducibility (Table I), and the amount of lysine does not affect the determination of cephalexin, the error between the theoretical and found values being less than 3% (Table II).

TABLE I

COEFFICIENTS OF CALIBRATION GRAPHS FOR CEPHALEXIN AT pH 5.5

Range of concentration/ μg ml^{-1}	No. of determinations	Slope	Correlation coefficient	Standard error of determination, %
11–45	6	1.14	0.9999	0.54
10–41	9	1.10	0.9995	1.09
9–40	9	0.91	0.9992	1.40
20–102	10	0.97	0.9962	2.04

TABLE II

FLUORIMETRIC DETERMINATION OF CEPHALEXIN AT pH 5.5 IN THE PRESENCE OF DIFFERENT LYSINE CONCENTRATIONS

Cephalexin/μg ml^{-1}					
True value	Found (a)*	Found (b)*	True value	Found (c)*	Found (d)*
9.52	9.24	9.74	20.40	20.80	20.81
9.77	9.84	9.84	40.70	39.80	41.80
19.00	18.70	18.70	61.10	59.62	61.89
19.50	19.60	19.50	81.51	80.59	79.51
38.10	38.50	38.60	101.91	101.51	103.02

* Lysine concentration: a, 15; b, 20; c, 30; and d, 60 μg ml^{-1}.

The cephalexin content of samples of cephalexin lysinate which had spontaneously degraded on storage at room temperature in the solid state was determined by iodimetric assay (β-lactam nucleus)[10] and by the fluorimetric method described above (free side-chain amino group), and the results are shown in Table III. Both methods confirm that degradation has occurred but the poor correlation between the results obtained by the two methods indicates that degradation in the solid state occurs by intra- and intermolecular rupture of the β-lactam nucleus.

TABLE III

CEPHALEXIN DETERMINATION IN CEPHALEXIN - LYSINE SALT BY OFFICIAL IODIMETRIC METHOD (BP) AND FLUORIMETRIC METHOD AT pH 5.5

	Cephalexin concentration, %*		
Sample	Iodimetric method	Fluorimetric method	Difference
1	66.9	67.1	0.3
2	66.9	68.2	1.9
3	64.9	63.6	−2.2
4	63.9	66.5	3.9
5	61.2	64.7	5.4
6	59.2	62.5	4.3

* Percentage of non-degraded cephalexin, means of the three determinations.

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Automated Ion-exchange System for the Radiochemical Separation of the Noble Metals

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Keywords: Automation; ion exchange; radiochemical separation; noble metals; neutron-activation analysis

Automated radiochemical separation speeds the processing, reduces the handling of radioactive material and improves the reproducibility of the method. Ion-exchange separation is particularly suitable for mechanisation and automated ion exchange has been applied to the activation analysis of biological and environmental samples.[1–3] In this work a system

has been designed for experimental studies, which can be adapted for different modes of operation. The equipment is based on a large-volume sampler for the automatic presentation of 500 ml of liquid to a sampling probe. The sample is delivered to the ion-exchange column by means of a peristaltic pump.

The purpose of this work was to automate a procedure for separating the noble metals from irradiated geological samples, for neutron-activation analysis. The process of digesting the rock sample is carried out manually in 30 min and is not suited to unattended operation. The volume of the resulting liquid sample may be 100 ml and so the manual separation step may take as long as 1.25 h per sample. The reason for automating this part of the procedure is to reduce the separation time for a group of five samples and consequently to improve the sensitivity of the analysis for radionuclides with short half-lives.

This paper describes the automatic ion-exchange system and the ways in which it can be used. The mode of operation for the separation of the noble metals is given in detail. The reproducibility of the system has been assessed by repeated measurements on a standard reference matte.

Experimental and Results

Apparatus

Large-volume sampler

The sampler (Camlab, LVS 500) consists of a turn-table with stations for ten wide-mouthed polythene bottles. The sampling probe, which is connected to a suitable pump, is lowered automatically into each bottle in turn. A sampling period of up to 35 min can be set on the clock timer, which regulates the time spent by the probe at each position. At the end of the pre-set time the probe is lowered into the next bottle and the clock timer is re-set. When all of the bottles have been sampled the equipment switches off automatically.

An additional timer unit was supplied by the manufacturer, to our specifications. It has ten clock timers, one for each station, which can be set independently for up to 72 min. When the unit is connected to the sampler, the appropriate timer is selected by means of a push-button. A neon light indicates which timer is in operation. When the timer reaches its pre-set period, it initiates the next one and re-sets itself automatically.

Manual operation of the sampler, such as switching it on and off, overriding the timer, reversing the motor or running it continuously, are controlled by illuminated push-buttons. Auxiliary power supply sockets are used for peripheral equipment, such as the pump or a fraction collector. These will be switched off automatically with the sampler at the end of the cycle. There is an audible alarm to signal "end of run" if required. The turntable also acts as a water-bath, with inlet and outlet pipes, if temperature control is needed.

Peristaltic pump

Liquid is transferred from the sampler into the ion-exchange column in polythene tubing (2 mm i.d.), by means of a 14-channel peristaltic pump (Quickfit Instrumentation). The speed of the pump is adjustable, giving flow-rates of up to 20 ml min^{-1}, and the direction of flow is reversible. Silicone rubber tubing is used in the pump and the variation in flow-rate is quoted as 2.5% for any single channel and 7.5% between channels.

Ion-exchange columns

Plastic disposable pipettes, of the appropriate size, make suitable ion-exchange columns. They are connected to the polythene tubing from the pump by means of PTFE adaptors (Omnifit Products, Biolab Ltd.). For experimental studies the outlets from the columns can be positioned above a fraction collector (Quickfit Instrumentation) with its own automatic timer control.

Mode of Operation

The system is used in its simplest form to deliver a series of eluting agents to one ion-exchange column, assuming that the sample is already on the resin. If a distributor, such as a 7-way PTFE valve (Omnifit Products, Biolab Ltd.), is introduced before the peristaltic pump, up to seven columns can be eluted simultaneously. Several resins are used in series

by connecting the outlet from one column to the inlet of the next. If the sample has a large volume it may be delivered to the column from the sampler. Column size, flow-rate and the volumes of the samples and eluting agents are variable, so the system can be adapted to the requirements of the experiment.

The system is applied to the separation of the noble metals from geological samples. The procedure followed is that of Nadkarni and Morrison.[4] The irradiated rock sample is digested by means of an oxidising fusion and dissolved in acid. The liquid sample is passed through the ion exchanger (Srafion NMRR, Ayalon Water Conditioning Co. Ltd.) at 2 ml min^{-1} and the noble metals are selectively adsorbed on to the resin. The column is washed with acid (0.05 N hydrochloric acid) before the resin is transferred into a counting vial for analysis.

The configuration of the system is shown in Fig. 1. Reservoir bottles (R_1–R_5) have polythene tubing fixed permanently in them to supply the respective ion-exchange columns (C_1–C_5) with liquid. The reservoirs contain 0.05 N hydrochloric acid to condition the resin and keep the columns wet. The remaining bottles (S_1–S_5) contain the liquid samples. The probe transfers each of the samples, in turn, into its respective reservoir, in the adjacent position. The reservoirs are topped up with acid from the acid reservoir outlet, fixed above the station that is opposite the sampling position. In this way the ion-exchange columns receive the appropriate sample between deliveries of acid solution, without any air entering the system. The flow-rate in all the tubing is 2 ml min^{-1}, except for the sampling probe, which delivers the sample to the reservoir at 4 ml min^{-1}. The whole system is surrounded by a lead wall for radiological protection.

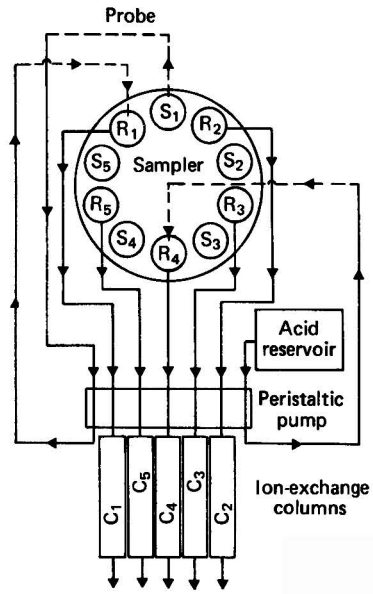

Fig. 1. Flow diagram for automated separation of the noble metals.

Preparation

Pack the ion-exchange resin into five columns and connect them to the polythene tubing from the pump. Lower the tips of the columns into acid solution (0.05 N hydrochloric acid). Switch on the pump in reverse action, drawing acid up into the columns to remove any air bubbles in the resin. Continue to fill the system back to where the tubing enters the reservoirs, then stop the pump. Fill the reservoirs R_1, R_2, R_3, R_4 and R_5 with 50, 130, 200, 210 and 280 ml of acid, respectively. Set the sampling periods on the timer unit to 30 min for stations containing samples and 5 min for stations containing reservoirs.

Digest the first rock sample and make the volume of the solution up to 100 ml with water. Transfer the sample into S_1 and start the system in the continuous mode with the pump in forward action at a flow-rate of 2 ml min^{-1}. Digest the remaining samples and put them in the sampler at 35-min intervals. As the resins become available for counting, remove them from the columns and when the fifth resin is ready switch off the system. The operation of the system, described below, is completely unattended, leaving the operator free to produce the digested rock samples as they are required.

Operation

When the system is started, the probe is lowered into S_1, so sample 1 is transferred into R_1 at 4 ml min^{-1}. Meanwhile the columns are supplied with acid solution at 2 ml min^{-1} from the reservoirs. The acid in R_4 is replenished by the acid reservoir. After 30 min, when S_1 is empty, a signal from the first timer initiates the sampler motor and starts the second timer. The probe is raised, the turntable moves the bottles one station and the probe is lowered into R_2. Acid solution in R_2 is transferred into the empty bottle S_1 to wash the tubing free of any contamination from sample 1. After 5 min the sampler moves to S_2 and the procedure is repeated for sample 2. Every 35 min a sample is transferred into the respective reservoir and on to the ion-exchange resin. By the time a reservoir bottle reaches the outlet from the acid reservoir it contains only 10 ml of solution. After washing with acid for 30 min the resin is unpacked and analysed. The first resin sample is counted 1.67 h after the system was initiated and samples 2–5 are ready after 2.25, 2.83, 3.42 and 4.00 h, respectively.

Performance

The reproducibility of the method was tested by repeated analyses of the noble metals in a standard reference matte. Table I confirms that the method gives accurate and reproducible results. Some variations in the results are to be expected because of inhomogeneities in the distribution of the metals, which become apparent with the small sample size (0.5 g) used for these measurements.

TABLE I

DETERMINATIONS OF NOBLE METALS IN A STANDARD REFERENCE MATTE (PTM)

	Concentration/$\mu g\ g^{-1}$			
Sample No.	Palladium	Platinum	Iridium	Gold
1	6.31	5.61	0.40	1.37
2	6.73	5.89	0.31	1.97
3	6.74	5.89	0.44	1.40
4	8.43	5.73	0.39	2.20
5	8.91	6.12	0.51	1.60
6	9.37	4.89	0.28	1.69
7	9.43	5.74	0.36	1.49
8	9.76	6.43	0.36	1.96
9	10.20	5.87	0.31	1.65
10	10.49	6.69	0.36	1.95
11	10.53	6.76	0.49	2.35
Mean	8.81	5.97	0.38	1.78
Standard deviation	1.56	0.53	0.07	0.32
Recommended value[5]	8.1	5.8	0.34*	1.8
95% confidence level	7.4–8.8	5.5–6.2	0–1.20*	1.6–1.9

* Values for information, not recommended.

Discussion

The automated ion-exchange system is a simple device, constructed from equipment that is commercially available. All parts of the system that come into contact with the samples can withstand corrosion and the polythene tubing is replaced to avoid cross-contamination. The system has a number of different modes of operation.

The system is particularly suited to large liquid samples that cannot be loaded on to the ion-exchange column manually. In the application of the system to the noble metals, the separation time is reduced from 8.5 to 4.5 h by mechanisation. Analysis of five samples is completed within 24 h of the end of irradiation, consequently improving the sensitivity of the technique. The dose rate to the operator is reduced by a factor of 10 with the automated system.

Repeated analyses of the standard reference matte give results that are in good agreement with recommended values for palladium, platinum and gold. The results confirm the reproducibility of the separation procedure.

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Simple Direct Concentration Readout Method for the Standard Additions Technique in Atomic-absorption Spectrophotometry

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Keywords: *Atomic-absorption spectrophotometry*; *direct concentration readout*; *standard additions technique*

The conventional method of standard additions requires a graph to be plotted of absorbance *versus* the standard addition concentration in the sample solution. The graph is then extrapolated back to the concentration axis and the negative intercept gives the concentration of the unknown. Fuller[1] has demonstrated that, using a bipolar digital readout, this can be simplified by nebulising the unknown solution and setting the output to read zero. The sample plus a suitable addition of standard is then nebulised and scale expansion is used to give a readout corresponding to the magnitude of the standard addition. A blank solution is then nebulised and a negative readout will be obtained, which corresponds to the concentration of the element in the sample solution. The range is limited by the maximum negative range available on the digital readout, typically 0.15 A. This method eliminates the need to draw a calibration graph, but it is still necessary to make standard additions to each sample. A method that would eliminate this step, after the first sample, would have obvious advantages.

Different samples containing variable amounts of analyte but identical concentrations of the same interfering matrix elements will produce standard addition calibration graphs that are parallel. It should therefore be possible to devise a technique in which, once the slope has been established, and set to pass through zero, subsequent samples can be determined directly without further additions of standard. The readout should be positive instead of negative, as this would expand the working range and allow curve correction facilities to be used. This paper describes such a technique.

Experimental

Equipment

The instrument used was a Baird A5100 atomic-absorption spectrophotometer.

Operating Procedure

The linearity of the calibration graph, over the range to be used, is established using aqueous standards. Curve correction procedures can be applied where necessary. The sample is aspirated into the flame and the reading for the sample is set to zero by means of the gain control and/or auto-zero facility. A 10-ml aliquot of sample, spiked with 10 or 20 μl of a concentrated standard, is then aspirated into the flame, and the scale expansion control is adjusted so that a readout equal to the concentration of the added standard is obtained. The solvent blank is aspirated into the flame and the readout is then re-set to zero by means of the gain control and/or auto-zero facility. The sample is then aspirated into the flame and the concentration of the element present will be displayed. The concentration of the element of interest in the sample is now known, and therefore the calibration accuracy can now be checked by aspirating an aliquot of sample that contains a smaller standard addition than used previously. The sum of the sample and added standard values should be displayed. Subsequent samples are then aspirated into the flame, without the need to add standards, and a readout in direct concentration is obtained.

The following requirements should be noted. The sample and sample plus standard additions must fall on a linear or linearised portion of the calibration graph. This can be checked by aspirating aqueous standards that cover the same calibration range. There should be negligible non-specific absorption by the matrix unless simultaneous background correction is applied. The matrix variation between samples must be negligible.

The applicability of the method was checked using as examples (1) the depressive effect of iron(III) on chromium(III) signals in an air - acetylene flame and (2) the enhancing effect of barium on calcium in an air - acetylene flame.

Example 1

The following conditions were used: element, chromium(III); wavelength, 357.9 nm; matrix, 500 μg ml^{-1} of iron(III); scale expansion, $\times 1$; slit width, 1 (0.025 mm); and flame condition, fuel-rich.

The spectrophotometer was arranged to determine chromium in an air - acetylene flame in the conventional manner. Aqueous calibration standards were prepared containing 2.0, 4.0 and 8.0 μg ml^{-1} of chromium(III), and the calibration was linearised using the curve correction facility.

A sample containing 1.0 μg ml^{-1} of chromium(III), in a matrix of 500 μg ml^{-1} of iron(III), was prepared. To an aliquot of this sample were added 2.5 μg ml^{-1} of chromium(III). A second sample containing 2.5 μg ml^{-1} of chromium(III), in the same matrix, was prepared and to an aliquot of this sample were added 2.5 μg ml^{-1} of chromium(III).

A blank solution containing 500 μg ml^{-1} of iron was prepared and the sample, sample plus addition and blank solutions were aspirated sequentially into the flame using the method given above.

Example 2

The following conditions were used: element, calcium; wavelength, 422.7 nm; matrix, 100 μg ml^{-1} of barium; scale expansion, approximately $\times 1.5$; slit width, 1 (0.025 mm); and flame condition, fuel-rich.

The spectrophotometer was set up to determine calcium in a fuel-rich air - acetylene flame. Aqueous calibration standards containing 1.0, 2.5 and 5.0 μg ml^{-1} of calcium were prepared, and the calibration was linearised using the curve correction facility.

A sample containing 2.5 μg ml^{-1} of calcium, in a matrix of 100 μg ml^{-1} of barium, was prepared. An aliquot of this sample was taken, and an addition equivalent to 2.5 μg ml^{-1} of calcium was made. A second aliquot of sample was taken, and an addition equivalent to 1.0 μg ml^{-1} of calcium was made. A blank solution containing 100 μg ml^{-1} of barium was also prepared. The sample, sample plus addition and blank solution were aspirated sequentially using the method given.

It was observed that the calibration obtained with the aqueous standards was curved, and required the use of maximum curve correction. However, the calibration obtained over the same range (1–5 μg ml^{-1} of calcium) with the addition of 100 μg ml^{-1} of barium was almost linear and required only a small amount of correction. In order to obtain the enhancement figure the calibration was linearised with aqueous standards, and the reading given by a standard containing 5.0 μg ml^{-1} of calcium was compared with that given by a standard containing 5.0 μg ml^{-1} of calcium plus 100 μg ml^{-1} of barium. In view of the difference in the calibration curvature characteristics, the percentage enhancement value may not be accurate. However, this difference does not affect the principle of the method, and the correct result was obtained.

Results and Discussion

Example 1

Table I shows a typical set of results obtained. The amount of depression caused by the matrix was 71%.

TABLE I

EFFECT OF IRON(III) ON CHROMIUM(III) RESULTS

Concentration of iron(III) = 500 μg ml^{-1}.

1.0 μg ml^{-1} of Cr(III)	2.5 μg ml^{-1} of Cr(III)
1.0	2.50
0.99	2.51
0.95	2.48
0.94	2.49
0.96	2.49
0.95	2.50
0.96	2.50
0.95	2.48
0.95	2.47
0.95	2.48

Example 2

Table II shows a typical set of results obtained for this analysis. The amount of enhancement caused by the matrix was 15% (at a level of 5.0 μg ml^{-1} of calcium).

TABLE II

EFFECT OF BARIUM ON CALCIUM RESULTS

Concentration of barium(II) = 100 μg ml^{-1}.

2.5 μg ml^{-1} of Ca	2.5 μg ml^{-1} of Ca
2.51	2.51
2.50	2.51
2.50	2.49
2.51	2.50
2.50	2.51

There is no reason to suppose that this method could not be used for any combination of matrix and element, provided that complete suppression does not occur.

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High-performance Liquid Chromatographic Determination of Trifluprom azine Hydrochloride in Tablets and Injections

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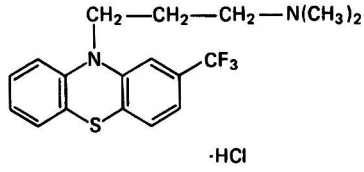
Keywords: Trifluprom azine hydrochloride determination; stability assay; high-performance liquid chromatography

Trifluprom azine hydrochloride [10-(3-dimethylaminopropyl)-2-trifluoromethylphenothiazine hydrochloride, I] belongs to the phenothiazine class of drugs, which are widely used for the treatment of patients with emotional or mental disorders.



I

The major known degradation products of phenothiazines are the sulphoxides[1,2] and *N*-oxides[3] resulting from aerial oxidation or photo-oxidation. Several methods, such as non-aqueous titrimetry,[4] UV spectrophotometry,[4] colorimetry,[5] redox titrimetry[6] and coulometry,[7] have been employed to assay trifluprom azine. However, these procedures are either non-specific for trifluprom azine or will not indicate the presence of the major oxidative degradation products. Consequently, for stability studies a method is required that is specific for trifluprom azine in the presence of its major degradation products, and this is probably best achieved by a chromatographic separation utilising either gas - liquid chromatography (GLC) or high-performance liquid chromatography (HPLC).

The GLC separations of trifluprom azine and its sulphoxide have been reported by several workers.[8–10] However, the separation of the *N*-oxide has not been reported. Also, because of the high temperatures involved, there may be a problem with sample decomposition. Therefore, the favoured method was HPLC.

The HPLC procedure described here is rapid and specific for the assay of trifluprom azine in the presence of the sulphoxide and *N*-oxide.

Experimental

Apparatus

The HPLC equipment was assembled from the following commercially available parts: an Altex 110A reciprocating pump, a Cecil 212A variable-wavelength UV monitor, a Perkin-Elmer 56 recorder and a 20 cm × 4.6 mm i.d. column. It is recommended that a loop injection device be used. In this work, an automatic sample processor (WISP Model 710, Waters Associates) was used.

The HPLC column was packed with Hypersil ODS (5 μm) according to the instructions supplied by the manufacturer (Shandon Products, Runcorn, Cheshire).

The operating conditions for the HPLC equipment were as follows: column temperature, ambient; mobile phase flow-rate, 1.0 ml min^{-1}; detector range, 0.1 absorbance unit full-scale deflection; wavelength, 275 nm; and injection volume, 8 μl.

Reagents

Acetonitrile. HPLC grade.

Methanol. Analytical-reagent grade.

Dilute ammonia solution. Dilute 10 ml of analytical-reagent grade ammonia solution (sp. gr. 0.88) to 100 ml with distilled water.

Mobile phase. A mixture of 65 volumes of methanol, 25 volumes of acetonitrile and 10 volumes of dilute ammonia solution. De-gas the methanol - acetonitrile mixture by vacuum application prior to the addition of the dilute ammonia solution.

Procedure

Protect the standard and sample solutions from light.

Standard solution

Prepare a solution of triflupromazine hydrochloride in methanol having a known concentration of approximately 0.4 mg ml^{-1}.

Sample solutions

Tablets. Extract one tablet with 10.0 ml of 0.05 N hydrochloric acid in a stoppered centrifuge tube by shaking on a reciprocating shaker for 40 min. Centrifuge the contents of the tube at 5000 rev min^{-1} for 5 min and dilute 4.0 ml of the supernatant liquid with methanol to 25 ml (25-mg tablet) or 50 ml (50-mg tablet) to give an expected concentration of 0.4 mg ml^{-1}.

Injectable product. Dilute an aliquot of the injectable product with methanol to give an expected concentration of 0.4 mg ml^{-1}.

Fig. 1. Chromatogram of a tablet sample extract.

Fig. 2. Separation of mixture of 1, Triflupromazine *N*-oxide; 2, Triflupromazine sulphoxide; and 3, Triflupromazine.

Chromatography

Establish a stable base line and make replicate injections of the standard solution on to the column until reproducible peak heights are obtained. Then, inject the sample and standard solutions in duplicate, the standard solution being injected after every two samples.

Calculate the amount of triflupromazine hydrochloride present in the sample from the mean peak height for the sample and the mean peak height for the standard eluted immediately before and after the sample. A typical sample chromatogram is shown in Fig. 1.

Results and Discussion

Trifluprazine hydrochloride in the concentration range 0.1–1.0 mg ml^{-1} gave a linear response with a regression coefficient of 0.999. The accuracy of the assay method was determined by measuring recoveries of trifluprazine hydrochloride from spiked tablet and injectable formulations. Recoveries from tablet formulations were measured by spiking excipient blends of the tablet formulations with trifluprazine hydrochloride. The average recovery (10 trials) and precision results are given in Table I. The colouring agents, diluents and fillers employed in the formulations did not interfere.

TABLE I

ASSAY RESULTS FOR TRIFLUPROMAZINE HYDROCHLORIDE IN INDIVIDUAL TABLETS

	Amount of trifluprazine hydrochloride/mg	
Sample No.	25-mg tablets	50-mg tablets
1	25.2	51.1
2	24.7	50.0
3	25.1	49.9
4	25.4	50.8
5	24.4	49.2
6	24.9	50.0
7	26.0	49.9
8	24.7	49.2
9	24.3	50.8
10	26.2	49.5

Owing to the absence of an internal standard it is recommended that a loop injector be used for injecting samples.

The stability-indicating nature of the assay procedure was verified by chromatographing authentic samples of two degradation products, the sulphoxide and *N*-oxide. A chromatogram showing the separation obtained is shown in Fig. 2. The sulphoxide was prepared by oxidation of the trifluprazine hydrochloride with cerium(IV) sulphate.[6] The product obtained by refluxing an ethanolic solution of trifluprazine with 2 mol of 30% hydrogen peroxide was identified by mass spectral analysis as the *N*-oxide.

TABLE II

REPLICATE ASSAY RESULTS FOR TRIFLUPROMAZINE HYDROCHLORIDE IN INJECTABLE PRODUCTS

	Concentration of trifluprazine hydrochloride/mg ml^{-1}	
Sample No.	10.2 mg ml^{-1} injectable product	20.4 mg ml^{-1} injectable product
1	10.2	20.1
2	10.3	19.8
3	10.1	20.5
4	10.1	20.7
5	10.2	20.7
6	10.3	20.7
7	10.3	20.5
8	10.1	20.0
9	10.2	20.3
10	10.1	20.5

Results obtained for individual tablets and intramuscular injections are given in Tables II and III and are in good agreement with the label claims. No traces of degradation products were observed on the chromatograms of the samples assayed.

TABLE III

RECOVERY AND PRECISION RESULTS (10 TRIALS) FOR THE ASSAY OF TRIFLUPROMAZINE HYDROCHLORIDE IN SPIKED TABLET AND INJECTABLE FORMULATIONS

Sample	Mean recovery, %	Standard deviation, %	Coefficient of variation, %
25-mg tablets	101.1	0.79	0.78
50-mg tablets	98.4	0.75	0.76
10.2 mg ml^{-1} injectable product	99.6	0.72	0.72

Conclusion

The results obtained indicate that the HPLC method described is capable of good accuracy and precision. The method can be used to monitor the stability of trifluprom azine formulations and to check the content uniformity of tablets and the purity of trifluprom azine hydrochloride raw material. It is possible to develop the method further in order to quantitate the separated trifluprom azine degradation products.

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Utilisation of the Mercury(II) Chloride Complex for the Long-term Storage of Samples Containing Part per 10^9 Levels of Mercury

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Keywords: *Mercury determination*; *water analysis*; *water sample storage*; *mercury(II) chloride complex*

In the determination of mercury in water samples at the parts per billion (10^9) level there are major difficulties associated with storage before analysis. Matsunaga *et al.*[1] have recently published a detailed paper on the errors caused prior to measurement of mercury in natural waters. The loss of mercury from samples during storage has been recognised by many workers[2,3] and probably occurs by sorption on container walls, association with particulate matter and vaporisation. The simplest stabilisation method is acidification with mineral acids.[4] The use of acids and oxidants (*e.g.*, sulphuric acid in combination with potassium permanganate or potassium dichromate) has been reported by several investigators.[5–8] The use of complex-forming agents such as iodide, cyanide,[9] thiocyanate[10] and cysteine[11] has also been reported.

The effects of ligands that form stable mercury(II) complexes are of interest for the prevention of mercury losses. This paper describes the preservation of mercury solutions stored in glass and polyethylene in the presence of sodium chloride and nitric acid. This technique has proved to be very effective in the prevention of mercury losses. Other workers[4,10] have found this approach to be very satisfactory.

The method of sample preservation presented was developed for composite samples collected from an experimental system set up to study the effect on ground water of the application of sewage sludge to agricultural soils. The proposed method of sample preparation was effective when the composite sampling time was as long as 3 months.

Experimental

Apparatus

All glassware used was rinsed with concentrated nitric acid followed by distilled water.

Mercury measurements were made using a Fisher Scientific Hg-3 mercury meter equipped with a Hewlett-Packard recorder (Model 7101B-24).

Reagents

The chemicals used for sample preservation and mercury analysis met ACS specifications.

Tin(II) chloride solution, 10% *m/V*. Dilute a 40% *m/V* solution of tin(II) in concentrated hydrochloric acid with distilled water. Store both the 10% and 40% solutions in dark bottles.

Mercury standard solution, 100 p.p.m. Dissolve 1.354 g of mercury(II) chloride in distilled water, add 10 ml of concentrated nitric acid and dilute the solution to 1 l with distilled water. Dilute 10 ml of the 1 000 p.p.m. standard to 1 l with distilled water, then dilute 100 ml of this 10 p.p.m. solution to 1 l with distilled water. This solution was stored in a pre-conditioned glass container and remained stable for several months. The glass container was pre-conditioned by allowing a 1 p.p.m. mercury standard solution to stand in the container for 5 d. This solution was discarded and fresh 1 p.p.m. mercury solution was placed in the container.

Procedures

Determination of mercury

Standards were analysed by cold-vapour atomic-absorption spectrometry after treatment with nitric acid and potassium permanganate and reduction with hydroxylammonium chloride and tin(II) chloride. The elemental mercury was purged into the absorption cell of the mercury meter using compressed air at 1 800 cm^3 min^{-1}.[12]

Samples were pre-treated using the digestion procedure of Bothner and Robertson,[4] followed by cold-vapour atomic-absorption spectrometric measurement as described above.

Method of preservation

Dilute mercury standards and samples were stabilised by making the solutions 3% *m/V* in sodium chloride and 0.1 M nitric acid.

A 250-ml bottle fitted with an aspirator was used to generate atomic mercury vapour for measurement.[12] Mercury-laden vapour was dried using a concentrated sulphuric acid drying trap.[13] At the outlet of the absorption cell, an activated carbon filter and saturated acidic potassium permanganate solution were used to remove the mercury vapour.

Flint-glass bottles (16 oz) were used to conduct the storage study on mercury standards in glass. For storage studies on samples, glass Winchester acid bottles (capacity 2 200 ml) and 1-gal plastic bottles were used.

Results and Discussion

Blank Level of Sodium Chloride

Initially there was some concern over the strong complexes that mercury forms with chloride. It was felt that the 3% sodium chloride - 0.1 M nitric acid reagent could contribute substantial blank mercury readings. Representative blank levels were of the order of

0.08 to 0.10 p.p.b. of mercury. These results were obtained using analytical-reagent grade sodium chloride that was not heat-treated for removal of mercury.

Storage of Inorganic Mercury Standards

Standards containing up to 4 p.p.b. of mercury prepared from mercury(II) chloride and stabilised as above with sodium chloride and nitric acid were analysed regularly over a period of several months. The results (Table I) show that standards at the parts per billion of mercury level can be stored for 3 months using the proposed method of sample preservation.

TABLE I

STORAGE OF INORGANIC MERCURY STANDARDS

Mercury concentrations in parts per billion (10^9).

Standard, p.p.b.	Time of storage/months				
	0	1	2	3	6
0.2	0.21	0.20	0.22	0.21	0.18
0.4	0.41	0.42	0.40	0.42	0.40
1.0	1.0	1.0	1.1	1.0	0.8
2.0	2.0	2.1	1.9	1.8	1.7
4.0	4.0	3.7	3.8	3.8	3.5

Storage of Samples

The composite samples referred to earlier were similarly analysed over a 3-month period using the proposed method of sample preservation in both glass and plastic bottles. The results (Table II) show clearly that the composite samples can be stored for 3 months using the proposed preservation method without any apparent loss of mercury.

TABLE II

STORAGE OF SAMPLES IN GLASS AND PLASTIC BOTTLES

Mercury concentrations in parts per billion (10^9).

Time of storage	Sample 1		Sample 2	
	Glass	Plastic	Glass	Plastic
Start	1.4	1.4	2.0	2.0
1 week	1.4	1.5	2.2	1.8
2 months	1.4	1.4	2.2	2.0
2½ months	1.3	1.4	1.9	1.9
3 months	1.4	1.4	2.0	1.8

Effect of Freezing

The conditions under which the composite samples under consideration are collected could include a "freeze - thaw" situation. Therefore, it was desirable to know the effect that the freezing of a sample would have on the results obtained when the proposed method of sample preparation was used. Composite samples were preserved using the proposed method, then frozen for various periods of time. The results are shown in Table III and indicate that freezing for up to 3 months does not change the mercury content of samples preserved using the proposed method.

Use of Plastic Sample Containers

It has been reported[1,4] that the use of plastic bottles for the storage of preserved mercury samples can lead to erroneously high results. Possible reasons that have been suggested include the leaching of mercury from container surfaces and the passage of mercury vapour from ambient air through the container walls into the solution.

The results obtained in this work indicate that preserved samples can be stored in either plastic or glass containers (Tables II and III). The plastic bottles used in this instance were much thicker than the usual sample bottles.

TABLE III

EFFECT OF FREEZING AND THAWING OF PRESERVED SAMPLES IN GLASS AND PLASTIC BOTTLES

Mercury concentrations in parts per billion (10^9).

Time frozen	Sample 1		Sample 2	
	Glass	Plastic	Glass	Plastic
Start	1.3	1.3	2.0	2.0
3 days	1.3	1.3	2.0	2.0
3 months	1.4	1.4	2.0	2.0

Applicability of Method

The proposed method for the preservation of mercury solutions (standards and samples) is particularly suitable when a reliable procedure is required for the preservation of very dilute solutions (parts per billion of mercury levels). The method can be applied when samples are collected over an extended time period and also when there is a likelihood of sample freezing and thawing during the collection period.

All of the experimental work was carried out at the Wastewater Technology Centre, Burlington, Ontario, Canada. The author acknowledges the assistance of Ken Conn, Wastewater Technology Centre, for his helpful comments on the manuscript.

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Book Reviews

CHARACTERISATION OF POROUS SOLIDS. PROCEEDINGS OF A SYMPOSIUM HELD AT THE UNIVERSITÉ DE NEUCHÂTEL, SWITZERLAND, FROM 9 TO 12 JULY 1978. Edited by S. J. GREGG, K. S. W. SING and H. F. STOECKLI. Pp. xiv + 392. Society of Chemical Industry. 1979. Price £21; $45. ISBN 0 90100 157 0.

The "Characterisation of Porous Solids," edited by Gregg, Sing and Stoeckli, contains the edited proceedings of a symposium of the same name held at the Université de Neuchâtel, Switzerland in July 1978. This paper-backed book contains the up-to-date methodology, opinions and current philosophy of some of the most active and authoritarian workers, throughout the world, in the field of porous-powder characterisation and material structure analysis by adsorption.

The book is divided into five sections (sessions), rather than chapters, which deal with microporosity, diffusion, capillary condensation and finally the application of adsorption to industrial products.

Sections 1 and 2, which are concerned with the micro-structure of materials, are preceded by a plenary lecture, an article given by Academician Dubinin of the USSR Academy of Science, Moscow. In this article a new category of pore size, the slit-like super- or ultra-micropore, is introduced into the IUPAC classification of pore sizes. The mechanism or theory of volume filling of these super-micro pores (TVFM) is based on what was in the past the Dubinin - Kaganer - Radushkevich equation, but which is now simply known as the Dubinin - Radushkevich (DR) equation. The limiting dimensions within porous material is not, however, solely dependent upon the adsorbent but is dependent upon the interaction between both adsorbent and adsorptive.

Section 3 is concerned with Knudsen diffusional flow of gases through porous media and was introduced by a review by Professor Barrer, Imperial College, London, which illustrated sorption rates through porous media with special reference to zeolites. Section 4 is devoted to the techniques of capillary condensation, gas and dye adsorption and mercury porosimetry to elucidate the structure of porous solids in the non-microporous size range.

The fourth section, introduced as in Sections 1 and 3 by an invited plenary lecturer, was preceeded by an article from Professor Everett, University of Bristol, on the validity of capillary condensation for the characterisation of the structure of porous bodies. The final section (Section 5) consists of short contributions from accepted but non-invited papers on the application of adsorption studies in the characterisation of a wide field of industrial and extra-terrestial powders. The whole book contains three plenary lectures, 31 invited papers and 7 short contributions. Inclusion of the discussions at the end of the paper sessions informs, enhances and clarifies the understanding of these erudite articles.

"The Characterisation of Porous Solids" is thus highly recommended to all personnel in academic research and industrial development as a comprehensive collection of current ideas and ideals for the characterisation of porous materials.

N. G. STANLEY-WOOD

QUANTITATIVE ANALYSIS. By CHARLES T. KENNER and KENNETH W. BUSCH. Pp. xxiv + 623. Macmillan. 1979. Price £14.95; $27.45.

A new text-book of quantitative analysis is always a matter of particular interest to a reviewer who has the responsibility for teaching the subject and recommending a suitable book to impecunious students. Any new book must stand up to certain criteria, not the least being value for money. This book differs little in content from the several other similar texts that have emanated from the USA in recent years. It covers the now customary wide range of analytical methodology, and could well find use in those courses where limited time is devoted to the theory and practice of analytical chemistry, but where the student may be required to have more than a cursory knowledge of the more widely used techniques.

Almost half of the book (the first 15 chapters) deals with gravimetric and titrimetric analysis, statistical treatment of data, potentiometric and kinetic methods. Four chapters are devoted to spectroscopic methods, one (brief) to electrolytic methods and four to separation methods (solvent extraction and the various chromatographic techniques). There follow single chapters on auto-

mation, miscellaneous methods (nuclear magnetic resonance, mass spectrometry and radiochemical methods) and solid-state microelectronics. The text is concluded with a chapter on laboratory techniques (mainly associated with gravimetry and titrimetry). There are no procedures given for laboratory exercises, but the theoretical background to many of the more commonly encountered determinations is given in some detail in the text. There are worked examples throughout the text and problems at the end of most chapters.

Viewed as a whole, there is little to distinguish this book from others like it. Obviously, the treatments differ and it becomes a matter of personal choice which book one prefers. This reviewer was unhappy with the discussion of redox processes, the Nernst equation and formal potentials (no mention of these at all) and redox indicators, whereas the treatment of precipitation processes is unusually good. The chapters on spectroscopic methods give well balanced treatments of the various techniques, but the same cannot be said for the chapters on chromatographic methods, which make difficult reading. It would be all too easy to reject this book as one that does not provide anything new in the way of a student text. Probably, it will not appeal widely to students at a price of almost £15. But it does have some commendable features and those interested in this subject must decide for themselves whether it is worthwhile to possess a copy of this book. Even after a thorough perusal of the book, this reviewer remains ambivalent in his recommendation.

W. I. STEPHEN

PROCEEDINGS OF THE 5TH EUROPEAN SYMPOSIUM ON POLYMER SPECTROSCOPY (ESOPS 5) IN COLOGNE, SEPTEMBER 1978. Edited by D. O. HUMMEL. Pp. xii + 332. Verlag Chemie. 1979. Price DM88 (softback).

The Editor and authors of the papers contained in this book are to be congratulated on presenting the Proceedings of the 5th European Symposium on Polymer Spectroscopy in a form that, for the spectroscopist, is indeed as readable as it is informative. The symposium sought to cover the rapid advances currently being made in polymer science and in the spectroscopies by which so much of the science is investigated and understood.

The Proceedings are presented in nine complete chapters and one summary note. Each chapter is complete with its own reference list and a reasonable subject and author index is given at the end of the book. The print is rather small for easy reading but this is not unusual for this form of presentation. The subject matter is grouped to give the more general presentations first and to follow these by descriptions of more specific spectroscopic techniques and applications.

Thus, the first chapter by H. F. Mark gives a history of macromolecular structural studies, and shows how the increasing complexity in this field demands new basic and practical approaches. H. A. Willis then reviews a range of spectroscopic methods and their application in combination to studies of the physical and chemical structures of a number of polymers. This paper also covers such important applications as the study of contamination, the examination of polymer surfaces and the identification of multi-component systems.

At a time when computers and microprocessors are being used in all types of analytical instrumentation, both for instrument control and data processing, it is appropriate that the third chapter, by M. M. Colemann and P. C. Painter, reviews the advantages gained by computer assisted vibrational spectroscopy in the characterisation of complex polymers. Practical examples are given, particularly in the field of Fourier-transform infrared (FTIR) and laser Raman spectroscopy. This leads on conveniently to a paper by K. Holland-Moritz, which deals with many aspects of the determination of the state of order in polymers by infrared and Raman spectroscopy including dependence on the temperature and the orientation of the polymer. Some selected problems are described.

A summary treatment of the basic theoretical and instrumental principles of FTIR, presented by H. W. Siesler, will be found most useful by the newcomer to the technique. The review demonstrates the advantages to be gained by the rapid-scan capability for the study of fast reactions and phase transitions. Studies in the orientation of isotactic polypropylene, the segmental orientation of polyesterurethane and phase transitions in polybutylene terephthalate are included as examples. Chapter 6 by F. Ciardelli and his co-authors underlines the use made of the high sensitivity of optical rotatory dispersion and circular dichroism in investigating the structure of macromolecules and in Chapter 7 I. Luderwald describes some recent results and new techniques in the pyrolysis electron impact mass spectroscopic study of polymers. This chapter is particularly valuable to

those concerned with thermal degradation studies and the use of such studies in structural assignments. Much can be learned from the fragmentation behaviour of oligomers that are thermally produced in high vacuum for immediate mass spectroscopic analysis.

The concluding chapters in this compendium deal with recent applications of nuclear magnetic resonance (NMR) spectroscopy. K. J. Ivin describes the use of carbon-13 NMR spectroscopy for microstructural studies of several hydrocarbon polymers and saturated and unsaturated acyclic and cyclic model compounds. Features studied include alignments, *cis/trans* isomerism, configurational isomerism, copolymer composition and sequence distribution. W. Gronski and R. Peter then provide an account of recent developments in the study of the dynamics of homo- and copolymers by high-resolution NMR spectroscopy. Relaxation mechanisms and information on the nature of local chain and side group motions both in solution and in the polymer melt are obtained by carbon-13 NMR.

This book is not for those without a working knowledge of the techniques and spectroscopies described, but for spectroscopists entering the polymer field it contains much valuable information and methodology and provides a very useful reference list.

D. C. M. SQUIRRELL

HANDBOOK OF ANALYTICAL DERIVATIZATION REACTIONS. By DANIEL R. KNAPP. Pp. xx + 741. John Wiley. 1979. Price £21.50.

Much of the work in the examination of organic compoounds, particularly those involving biological samples, by gas chromatography, mass spectrometry, combined gas chromatography - mass spectrometry and more recently by high-performance chromatography, involves derivatisation prior to instrumental analysis. Numerous and diverse derivatisation methods have been published; the present text is an attempt to consolidate this methodology into a single reference work in a manner that has proved extremely useful in the area of synthetic organic chemistry.

The first section of the book deals with uses of analytical derivatisation, derivative types and reagents, general considerations and finally apparatus used in the various derivatisation techniques.

In contrast to much of the previous discussion of analytical derivatisation the material is organised by sample type rather than by derivative or reagent type. Methods for each major compound compound type are reviewed, followed by sub-sections of compound types and within each subsection according to derivative type. Each method description follows a standard format: Derivative type, Reagent, Sample type, Reaction (equations given), Typical Procedure (often includes isolation from matrix), Comments and References. The methods included have been restricted to those involving covalent derivatives of organic compounds formed prior to the analysis; hence, ion pairs, derivatives of inorganic ions and post-column derivatives are excluded. The main compound types have been divided into 15 groups and include the main organic functional groups and compound types such as steroids, fatty lipids and carbohydrates. The separation of optical isomers and drugs are interesting individual chapters, however. Abbreviations are extensively used to conserve space, most are well known, only a few confuse and all are listed.

Two useful appendices are composition of Brand Name Reagent and Suppliers of Reagents and Apparatus. A comprehensive system of indices are given, namely by Derivative and Reagent, Author and finally General Subject, to facilitate information retrieval.

This is a well concurred and organised text, written in a concise yet pleasant style and represents truly excellent value for money on the basis of the wealth of readily accessible information it contains. Despite the bioanalytical emphasis the book will be of value to almost all laboratories dealing with any aspect of organic analysis.

D. THORBURN BURNS

Retention of Metabolised Trace Elements in Biological Tissues Following Different Drying Procedures
Part II. Caesium, Cerium, Manganese, Scandium, Silver and Tin in Rat Tissues

Losses of silver, cerium, caesium, manganese, scandium and tin during freeze drying and oven drying at 80, 105 and 120 °C were studied in rat tissues and faeces that contained radioactive isotopes. No loss was observed for any of these elements on freeze drying. A significant loss of tin was observed in muscle even at 80 °C, and in kidney and liver at 105 °C. Also, cerium and manganese were lost from heart and muscle at this temperature. An increase in the temperature to 120 °C was found to be safe only for silver and caesium in all of the tissues. For the remaining four elements the following losses were observed: up to 5% for cerium in brain and heart, for scandium in fur and heart and for manganese in liver and muscle; up to 10% for scandium in kidney and muscle and for tin in blood, fur, heart, liver, ovary and uterus; and over 10% for tin in brain, kidney, lung and muscle.

Keywords: *Biological tissues*; *metabolised trace elements*; *drying*; *retention*

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Analyst, 1980, **105**, 794–801.

Automatic Titration by Stepwise Addition of Equal Volumes of Titrant
Part V. Extension of the Gran I Method for Calculation of the Equivalence Volume in Acid - Base Titrations

A very simple method for numerical calculation of the equivalence volume (V_e) from titration data is presented. It is based on the method of stepwise addition of equal volumes of titrant combined with an extended version of the Gran I method.

The expressions derived may be summarised in a general equation:

$$V_e = V_j + \frac{V_j - V_i}{a_{ij} - 1}$$

where V_i and V_j are two additions of titrant (V_i is iV_p, where V_p is the volume of each addition). The term a_{ij} is different for strong acids and weak acids and for conditional titrations, but in all instances only the ratio between hydrogen ion concentrations is required. *E.g.*, for a weak acid is

$$a_{ij} = \frac{V_i[H]_i}{V_j[H]_j}$$

The titration method can also be used for the determination of the conditional normal potential E_0' and the constant j_H in the junction potential $j_H[H]$ for an electrode couple in a given medium.

Keywords: *Gran I method*; *acid - base titration*; *automatic titration*; *equivalence volume calculation*; *potentiometric titration*

AXEL JOHANSSON and GUNNAR GRAN
Department of Analytical Chemistry, The Royal Institute of Technology, S-10044 Stockholm 70, Sweden.

Analyst, 1980, **105**, 802–810.

Determination of a Volatile Phenol in Serum by High-performance Liquid Chromatography

Short Paper

Keywords: *Volatile phenols determination*; *o-chlorophenol determination*; *serum analysis*; *micro-extraction*; *high-performance liquid chromatography*

LARRY L. NEEDHAM, ROBERT H. HILL, JR. and SANDRA L. SIRMANS
Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, Ga. 30333, USA.

Analyst, 1980, **105**, 811–813.

Direct Spectrofluorimetric Determination of the Free Amino Group of Cephalexin in its Lysine Salt

Short Paper

Keywords: *Cephalexin determination*; *cephalexin - lysine salt*; *spectrofluorimetry*

J. L. FABREGAS and J. E. BENEYTO
Analytical Division, Instituto de Investigación, Laboratorios Almirall, S.A., C/Cardoner 68–74, Barcelona (24), Spain.

Analyst, 1980, **105**, 813–816.

Automated Ion-exchange System for the Radiochemical Separation of the Noble Metals

Short Paper

Keywords: *Automation*; *ion exchange*; *radiochemical separation*; *noble metals*; *neutron-activation analysis*

SUSAN J. PARRY
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Analyst, 1980, **105**, 816–820.

Simple Direct Concentration Readout Method for the Standard Additions Technique in Atomic-absorption Spectrophotometry

Short Paper

Keywords: *Atomic-absorption spectrophotometry*; *direct concentration readout*; *standard additions technique*

J. R. HALL, R. G. GODDEN and D. R. THOMERSON
Baird Atomic Ltd., Warner Drive, Springwood Industrial Estate, Braintree, Essex, CM7 7YL.

Analyst, 1980, **105**, 820–822.

High-performance Liquid Chromatographic Determination of Triflupromazine Hydrochloride in Tablets and Injections

Short Paper

Keywords: *Triflupromazine hydrochloride determination*; *stability assay*; *high-performance liquid chromatography*

P. H. SHIRSAT and G. CLARKE

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Analyst, 1980, **105**, 823–826.

Utilisation of the Mercury(II) Chloride Complex for the Long-term Storage of Samples Containing Parts per 10^9 Levels of Mercury

Short Paper

Keywords: *Mercury determination*; *water analysis*; *water sample storage*; *mercury(II) chloride complex*

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Analyst, 1980, **105**, 826–829.

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THE ANALYST **August, 1980**

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