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Summaries of Papers in this Issue

The Determination of Bis(tri-n-butyltin) Oxide and Di-n-butyltin Oxide in Preserved Softwood by Atomic-absorption Spectrophotometry and Polarography

Methods are described for the determination of the total organotin compounds by atomic-absorption spectrophotometry and for the specific determination of bis(tri-n-butyltin) oxide and di-n-butyltin oxide by atomic-absorption spectrophotometry and polarography. Bis(tri-n-butyltin) oxide and di-n-butyltin oxide are extracted from the wood with hydrochloric acid - ethanol solution and separated from each other and from wood extractives, fungicides and insecticides by adsorption on to Amberlite CG-120 cation-exchange resin followed by elution with solutions containing different concentrations of hydrochloric acid in ethanol.

The procedures have been used to determine bis(tri-n-butyltin) oxide and di-n-butyltin oxide in Scots pine, Corsican pine, Western hemlock, Japanese larch, Sitka spruce, Douglas fir and Western red cedar.

(The late) A. I. WILLIAMS
Department of the Environment, Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Buckinghamshire, HP17 9PX.

Analyst, 1973, **98**, 233–242.

An Improved Method for the Determination of Whole Blood Lead by Using an Atomic-absorption Technique

The Delves technique for measuring whole blood lead has been considerably improved by wet ashing with aqua regia instead of simply drying with hydrogen peroxide. Smoke is eliminated, and the reproducibility of the method is improved.

G. ALAN ROSE and ELIZABETH G. WILLDEN
Department of Pathology, St. Peter's Hospitals, Endell Street, London, W.C.2.

Analyst, 1973, **98**, 243–245.

Bis(6-Methyl-2-pyridyl)glyoxal Dihydrazone as a Spectrophotometric Reagent for the Rapid Determination of Copper in Alkalis, Milk and Brine

The synthesis, characteristics and analytical applications of bis(6-methyl-2-pyridyl)glyoxal dihydrazone are described. This compound produces coloured solutions selectively with copper(I) ions ($\lambda_{max.}$ = 440 nm, ϵ = $8{\cdot}7 \times 10^3$ l mol^{-1} cm^{-1}) that can be extracted into various organic solvents; it behaves as a cuproine-type reagent. The 1:1 orange - yellow copper(I) complex has been used for the spectrophotometric determination of trace amounts of copper in saturated brine, alkalis and milk. The most important advantage of using this reagent is the total recovery of copper that is possible from ammoniacal solutions.

M. VALCARCEL and F. PINO
Department of Analytical Chemistry, University of Seville, Seville, Spain.

Analyst, 1973, **98**, 246–250.

An Electrical Conductivity Detector for Paper, Thin-layer and Column Chromatography

An electrical conductivity detector has been developed for application to the paper, thin-layer and column chromatography of ionic substances.

Two arms of an a.c. Wheatstone bridge comprise two pairs of electrodes applied directly on to the separating layer so that a signal ensues from the bridge when a chromatographic spot passes one electrode pair while the other pair is traversed by the eluting agent. The sensitivity is about 1 μg and the precision is about 2 to 3 per cent.

V. DI STEFANO and P. MARINI
Centro Sperimentale Metallurgico, Via di Castel Romano, Rome, Italy.

Analyst, 1973, **98**, 251–256.

THE ANALYST

The Determination of Bis(tri-n-butyltin) Oxide and Di-n-butyltin Oxide in Preserved Softwood by Atomic-absorption Spectrophotometry and Polarography

BY (THE LATE) A. I. WILLIAMS

(Department of the Environment, Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Buckinghamshire, HP17 9PX)

Methods are described for the determination of the total organotin compounds by atomic-absorption spectrophotometry and for the specific determination of bis(tri-n-butyltin) oxide and di-n-butyltin oxide by atomic-absorption spectrophotometry and polarography. Bis(tri-n-butyltin) oxide and di-n-butyltin oxide are extracted from the wood with hydrochloric acid - ethanol solution and separated from each other and from wood extractives, fungicides and insecticides by adsorption on to Amberlite CG-120 cation-exchange resin followed by elution with solutions containing different concentrations of hydrochloric acid in ethanol.

The procedures have been used to determine bis(tri-n-butyltin) oxide and di-n-butyltin oxide in Scots pine, Corsican pine, Western hemlock, Japanese larch, Sitka spruce, Douglas fir and Western red cedar.

THE fungicidal properties of organotin compounds were first described in the literature in 1954 by van der Kerk and Luijten.[1] In more recent years, bis(tri-n-butyltin) oxide has found increasing use for the protection of timber against fungal attack.[2,3] Bis(tri-n-butyltin) oxide is known in the timber preservation industry as tributyltin oxide or TBTO. The usual form of treatment is carried out by impregnating seasoned wood with solutions of the preservative in organic solvents either by the double vacuum process[4] or the Drilon process,[5] or by application to the surface of the timber by brushing, dipping or deluge. Some preservative solutions contain only TBTO, but in others the organotin may be formulated together with other constituents, *e.g.*, pentachlorophenol, gamma-benzene hexachloride, dieldrin, copper naphthenate, zinc naphthenate, polychloronaphthalene, monochloronaphthalene, *o*-phenylphenol, lauryl pentachlorophenate or water-repellent compounds. It is necessary, therefore, to be able to determine TBTO in the presence of these compounds and wood extractives.

TBTO is a reactive compound and readily forms TBTX compounds with acids, where X is an anion. Wood contains natural phenolic and acidic extractive constituents and it is possible that in treated wood the anions of these compounds replace the oxide radical. Therefore, in this work the tributyltin radical is determined and the results are expressed as TBTO. This approach also applies to di-n-butyltin oxide (DBTO).

The chemical determination of TBTO is needed for the study of the loading and distribution of the preservative achieved by treatment with different processes and preservative formulations and to investigate the permanence of the preservative under various service conditions. Also, because bis(tri-n-butyltin) oxide may be converted into di-n-butyltin oxide *in situ* (*e.g.*, this conversion is known to be caused by ultraviolet light), it is important, for research studies on the permanence of the preservative, to be able to determine separately tributyltin and dibutyltin. In laboratory tests, DBTO was shown to be ten times less toxic than TBTO to fungi.[6] Existing methods for the determination of TBTO are mostly based on the determination of inorganic tin after the decomposition of the organometallic compound. Methods involving the use of X-ray fluorescence spectrometry,[7,8] polarography,[9,10] gas - liquid chromatography,[11,12] thin-layer chromatography,[13] radioactivation analysis,[14] atomic-absorption spectrophotometry,[15] colorimetric techniques[16,17] and titration procedures[18] have been described in the literature for the determination of organotin compounds, but most of these methods are not suitable for the determination of TBTO in wood. Some of the methods

are not specific and give inaccurate results and others are insensitive. Also, because it is difficult to decompose TBTO completely, procedures that involve wet-ashing techniques are slow and tedious.

The initial problem in the development of procedures for the determination of TBTO in timber is the extraction of the organotin compound from wood. Recent work has shown that some preservative chemicals can be rapidly leached from thin sections of wood[19–21] or from sawdust.[22] It has now been found that leaching with a 0·05 per cent. *V*/*V* solution of concentrated hydrochloric acid in ethanol followed by an atomic-absorption spectrophotometric finish affords a rapid method for the determination of total tin in preserved wood. This procedure is suitable for the routine determination of organotin compounds in treated wood when a non-specific method is required.

Because atomic-absorption spectrophotometry is non-specific, an alternative technique was sought for the separate determination of TBTO and DBTO. It is known that organotin compounds are reduced directly at the dropping-mercury electrode,[23,24] which offered the possibility of using a polarographic method. Unfortunately, wood extractives, which are also extracted from the wood during leaching, interfere and it is necessary to separate the organotin compounds from wood extractives before polarographic analysis. This separation was achieved by adsorption of the organotin compounds as chlorides (TBTCl and $DBTCl_2$) on Amberlite CG-120 cation-exchange resin, and by using suitable solvents the TBTCl and $DBTCl_2$ could be eluted separately.

Atomic-absorption spectrophotometry is a more common technique for the determination of preservatives and the polarographic procedures were developed principally as a means of checking the ion-exchange eluates to confirm that separation of TBTCl and $DBTCl_2$ had taken place. Also, they were used to check the results obtained by the atomic-absorption spectrophotometric procedures.

Experimental

Preparation of standard samples and sampling—

Standard samples were prepared by impregnating wood with dioxan solutions containing known amounts of technical grade TBTO, and the full cell process[25] and freeze-drying[26] were used so as to prevent re-distribution and loss of the preservative. The sample blocks were freeze-dried at 0 °C to a residual solvent content of about 6 per cent. and no TBTO was detected in the dioxan condensate. From the observed mass of treating solution retained in the blocks after impregnation, the percentage of TBTO, based on the oven-dry mass of wood, was found by calculation to lie in the range 0·023 to 1·34 per cent.

Despite these precautions, the distribution of TBTO in the treated blocks will not be uniform owing to the anatomical structure of wood. Concentration gradients of TBTO can occur across the annual rings, more being present in the spring or early wood, as the void space is greater, than in the summer or late wood. Therefore, for development work on the procedures, it was decided to use radial sections (cut across the annual rings), as they are more representative of the bulk of the wood. Microtome sections 0·1 mm thick were taken at intervals through the dry block and combined to make one sample for analysis. Adjacent thin sections were taken in order to make up replicate samples. The amounts of the samples taken for analysis were in the range 0·3 to 1 g.

Separation of organotin compounds—

Initially, TBTO was extracted from standard samples with ethanol for polarographic analysis and with isobutyl methyl ketone for atomic-absorption spectrophotometry. Although TBTO was completely recovered from freshly treated timber with these solvents, it was not possible to recover all of the organotin compounds from aged samples and up to 50 per cent. remained in the wood. For complete recovery, the organotin compounds were extracted as the chlorides with a 0·05 per cent. *V*/*V* solution of hydrochloric acid in ethanol. This solvent quantitatively removed the organotin compounds from aged samples and was found to be suitable for atomic-absorption spectrophotometry and cation-exchange procedures.*

The direct polarographic determination of TBTCl and $DBTCl_2$ in the above hydrochloric acid in ethanol leach solutions was not possible owing to interference of the reduction wave

* The ethanol used throughout this work was of 97·5 per cent. concentration.

by wood extractives. In order to separate the TBTCl and $DBTCl_2$ from wood extractives, the organotin compounds were adsorbed on Amberlite CG-120 cation-exchange resin (chromatographic grade, 200 mesh). Direct application of TBTCl and $DBTCl_2$ in a 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol to columns of resin resulted in only 20 per cent. of the organotin compounds being adsorbed. However, on diluting the leach solution with water (10 ml of water to 20 ml of leach solution), more than 99 per cent. of the organotin compounds was retained. The wood extractives passed through in the initial eluate.

The possibility of separating TBTCl and $DBTCl_2$ on the resin column was also investigated. The elution of the organotin compounds was monitored by polarographic analysis of fractions of the eluate. Separation could not be effected by using different concentrations of hydrochloric acid in ethanol, but it was achieved by varying the water content of the eluting solution. Under the conditions described in the method, it was possible to remove the TBTCl from the resin in less than 10 ml of a solution containing 10 per cent. *V/V* of water and 0·3 per cent. *V/V* of hydrochloric acid in ethanol. In solutions that contained 10 per cent. or more of water, only TBTCl was eluted. $DBTCl_2$ was subsequently eluted with 5 per cent. *V/V* of hydrochloric acid in ethanol. In a solution of this concentration it was possible to remove the $DBTCl_2$ from the resin in less than 10 ml of eluting agent. If a solution containing less hydrochloric acid in ethanol is used, much greater volumes of eluting agent are required in order to elute $DBTCl_2$ from the resin. It is important that the volume of eluate obtained is kept to a minimum so as to avoid loss in sensitivity during atomic-absorption studies with these solutions. The use of this cation-exchange procedure also provides a means of concentrating TBTCl and $DBTCl_2$ from dilute extracts. The separation of wood extractives, TBTCl (expressed as TBTO) and $DBTCl_2$ (expressed as DBTO) is shown in Fig. 1.

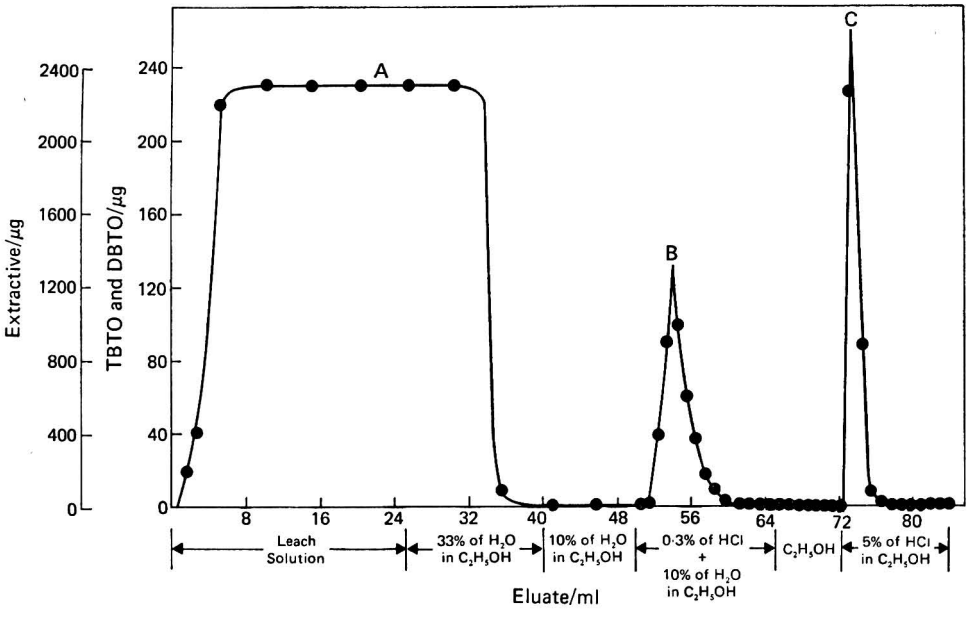

Fig. 1. Elution of (A) wood extractives, (B) TBTCl (expressed as TBTO) and (C) $DBTCl_2$ (expressed as DBTO)

ATOMIC-ABSORPTION SPECTROPHOTOMETRY—

The use of hydrochloric acid in leaching procedures and in ion-exchange separations made it necessary to examine the effect of different concentrations of hydrochloric acid in ethanol in the use of these solutions as media for atomic-absorption spectrophotometry. The absorbance was recorded for solutions containing 20 $\mu g\ ml^{-1}$ of TBTO and increasing amounts of hydrochloric acid in ethanol. The results showed that maximum absorbance occurred with solutions containing less than 2 per cent. *V/V* of hydrochloric acid. Similar

results were obtained for DBTO. Hence the leach solutions consisting of 0·05 per cent. *V*/*V* of hydrochloric acid in ethanol used for the extraction of total organotin give approximately maximum sensitivity. A solution of 5 per cent. *V*/*V* of hydrochloric acid in ethanol was used to elute $DBTCl_2$ from the cation-exchange column. With a solution with this acid concentration a small but acceptable loss in sensitivity occurs.

The use of water in the ion-exchange procedure required that the effect of increasing concentrations of water in a 0·3 per cent. *V*/*V* solution of hydrochloric acid in ethanol on the absorbance signal for tin in TBTO be studied. The results showed that the absorbance signal for tin decreased with increasing concentration of water, but the sensitivity obtained by using a solution of 10 per cent. *V*/*V* of water and 0·3 per cent. *V*/*V* of hydrochloric acid in ethanol was adequate for the levels of TBTO encountered in wood.

Wood contains calcium, potassium, sodium and strontium and some samples of certain species contain lithium. These elements enhance the tin absorbance signal, lithium much more so than the others. This interference is usually observed with Western red cedar, but it occasionally occurs with pines. The interference was effectively overcome by the addition of an excess of lithium (1000 μg ml^{-1}) to the test and calibration solutions in the direct method. The presence of calcium, potassium, sodium and strontium in solutions containing an excess of lithium ions did not affect the tin absorbance signal. The interfering elements are separated from TBTCl and $DBTCl_2$ in the ion-exchange procedure and, therefore, do not influence the equilibrium in the flame between atoms and ions of tin during the specific determination of TBTCl and $DBTCl_2$.

Polarography—

A 2-ml volume of the TBTCl eluate was diluted to 10 ml with a support electrolyte, consisting of 0·94 per cent. *V*/*V* of hydrochloric acid and 2·5 per cent. *V*/*V* of ethanol in 1 M aqueous potassium chloride solution, for polarographic analysis. A similar volume of the eluate containing $DBTCl_2$ was diluted with 1 M aqueous potassium chloride solution for analysis. This dilution ensured that in both test solutions the concentrations of ethanol, hydrochloric acid and potassium chloride were similar. In this electrolyte, $DBTCl_2$ gave two reduction waves at peak potentials of −0·64 and −0·75 V, and TBTCl gave one reduction wave at a peak potential of −0·85 V. Owing to interference to the −0·75 V $DBTCl_2$ reduction wave by the TBTCl reduction wave, it was not possible to use the TBTCl wave for the determination of TBTCl in the presence of $DBTCl_2$. It was possible to determine $DBTCl_2$ in the presence of TBTCl by using the −0·64 V peak.

Effect of other fungicides and insecticides—

Commercial formulations of TBTO wood preservative solutions may also contain other constituents. The effect of the presence of such compounds on the determination of TBTO by atomic-absorption spectrophotometry and polarography was examined. Solutions containing 20 μg ml^{-1} of TBTO and 400 μg ml^{-1} each of pentachlorophenol, lauryl pentachlorophenate, copper naphthenate, zinc naphthenate, *o*-phenylphenol, monochloronaphthalene, polychloronaphthalene and water-repellent waxes, or 40 μg ml^{-1} each of lindane and dieldrin, were examined by the proposed procedures. In the atomic-absorption and polarographic procedures, after cation-exchange separation no interference occurred and complete recovery of TBTO was achieved. Only copper and zinc, from copper and zinc naphthenates, were adsorbed on the resin during cation-exchange separation of the organotin compounds. The ions of these two elements had a more negative reduction potential than TBTCl and caused no interference to the polarographic waves of $DBTCl_2$ or TBTCl. The presence of copper and zinc ions also caused no interference during atomic-absorption spectrophotometry.

Copper and zinc naphthenates caused interference during the direct determination (without ion exchange) of total organotin compounds in leach solutions by the atomic-absorption procedure by enhancing the absorbance signal of tin. The extent of the interference was investigated by preparing two series of solutions, one series containing 20 μg ml^{-1} of TBTO *plus* increasing amounts of copper naphthenate and the other containing 30 μg ml^{-1} of TBTO *plus* increasing amounts of zinc naphthenate. All of the solutions were made up with 0·05 per cent. *V*/*V* of hydrochloric acid in ethanol. The solutions were aspirated and the recorded absorbances plotted against concentrations of either copper naphthenate or

zinc naphthenate (Fig. 2). In both instances the tin signal was enhanced and reached a plateau; similar results were obtained with $DBTCl_2$. This interference can be overcome if an excess of lithium ions is added to both the test and calibration solutions.

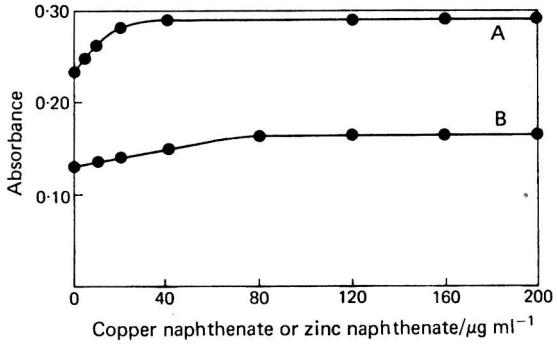

Fig. 2. Interference to tin absorbance signal. Curve A, interference by zinc naphthenate; and curve B, interference by copper naphthenate

ANALYSIS OF TECHNICAL TBTO—

Technical TBTO may contain tri-n-butyltin chloride, di-n-butyltin compounds and solvent. The purity of the technical TBTO used in this work was checked by a polarographic procedure that differed from that previously described. Approximately 0·1 g of TBTO, accurately weighed, was dissolved in ethanol and the solution diluted to 100 ml with ethanol. A 10-ml aliquot of this solution was diluted to 100 ml with a support electrolyte consisting of 6·7 ml of ammonia solution (sp. gr. 0·880), 5·7 ml of glacial acetic acid and 2 ml of 1 per cent. m/V Triton X-100 solution, diluted to 1 litre with water. Polarograms were recorded with a Southern Analytical K1000 cathode-ray polarograph with the following instrument settings—

	Start potential/V	Peak potential/V
Bis(tri-n-butyltin) oxide	−1·05	−1·35
Tri-n-butyltin chloride	−0·70	−0·97
Di-n-butyltin oxide (insoluble) ..	—	—
Di-n-butyltin dichloride	−0·80	−1·07
n-Butyltin trichloride	−0·45	−0·71

No $DBTCl_2$ and only trace amounts of TBTCl were detected in the test solution. No reduction wave was observed for DBTO, probably owing to its high insolubility.

In order to detect DBTO, another sample was dissolved in a 5 per cent. V/V solution of hydrochloric acid in ethanol and the solution examined for $DBTCl_2$ by the polarographic procedure described later, but only a trace amount of DBTO was found. Therefore, it was concluded that the TBTO used in the preparation of the standard samples was pure and did not introduce any errors in the calculated content of the standards.

RESULTS

The procedures outlined above were used to determine the loading of organotin compounds in standard samples of treated wood. All of the results were based on the oven-dry mass of wood and were expressed as TBTO or DBTO content. The results, given in Table I, were in good agreement with each other and with the calculated TBTO content. Some of the standard samples were examined immediately after they had been freeze-dried. DBTO was not detected in these samples. The remaining samples were examined 6 months after impregnation, and these samples contained both TBTO and DBTO.

The standard deviation, based on seven determinations at the 0·20 per cent. level, was ±0·0039 per cent. for the direct determination of organotin compounds in leach solutions. The standard deviations for TBTO and DBTO, based on seven determinations at the 0·07 per cent. level for TBTO and the 0·0035 per cent. level for DBTO, for the atomic-absorption

TABLE I

LOADING OF ORGANOTIN COMPOUNDS IN STANDARD SAMPLES COMPARED WITH LOADING CALCULATED FROM SOLUTION RETENTIONS

Species	Time of examination	Calculated TBTO content, per cent.	Organotin content by atomic-absorption spectrophotometry, expressed as TBTO, per cent.	After cation-exchange separation: Atomic-absorption spectrophotometry: TBTO content, per cent.	DBTO content, per cent.	DBTO expressed as TBTO equivalent, per cent.	After cation-exchange separation: Polarography: TBTO content, per cent.	DBTO content, per cent.	DBTO expressed as TBTO equivalent, per cent.
Scots pine	Directly after treatment	0·78	—	0·76	Not detected	—	0·77	Not detected	—
Corsican pine		0·39	—	0·39		—	0·39		—
Western hemlock		0·60	—	0·61		—	0·62		—
Sitka spruce		1·12	—	1·11		—	1·10		—
Scots pine		0·038	—	0·040		—	0·039		—
Western hemlock		0·030	—	0·027		—	0·028		—
Japanese larch		0·024	—	0·026		—	0·026		—
Douglas fir		0·026	—	0·028		—	0·027		—
Western red cedar	After storage	1·34	1·35	0·92	0·32	0·38	0·92	0·32	0·38
Scots pine		0·75	0·74	0·72	0·021	0·025	0·72	0·021	0·025
Corsican pine		0·36	0·36	0·35	0·0091	0·011	0·35	0·0088	0·011
Western hemlock		0·62	0·61	0·57	0·036	0·043	0·57	0·036	0·043
Sitka spruce		1·18	1·15	1·10	0·033	0·040	1·10	0·031	0·037
Western red cedar		0·040	0·043	0·029	0·097	0·012	0·030	0·010	0·012
Scots pine		0·038	0·039	0·035	0·0046	0·0055	0·034	0·0047	0·0056
Western hemlock		0·028	0·026	0·024	0·0024	0·0029	0·025	0·0024	0·0029
Japanese larch		0·023	0·024	0·017	0·0050	0·0060	0·018	0·0050	0·0060
Douglas fir		0·027	0·028	0·025	0·0029	0·0035	0·025	0·0029	0·0035

determination after cation-exchange separation were ±0·0010 per cent. for TBTO and ±0·00010 per cent. for DBTO. The standard deviations, based on seven determinations at the 0·20 per cent. level for TBTO and the 0·06 per cent. level for DBTO, for the polarographic technique were ±0·0036 per cent. for TBTO and ±0·00025 per cent. for DBTO.

The atomic-absorption sensitivities were 2 μg ml^{-1} for TBTO in 0·05 per cent. V/V of hydrochloric acid in ethanol, 2·5 μg ml^{-1} for DBTO in 5 per cent. V/V of hydrochloric acid in ethanol and 3 μg ml^{-1} for TBTO in 0·3 per cent. V/V of hydrochloric acid and 10 per cent. V/V of water in ethanol.

To demonstrate the potential value of the proposed atomic-absorption procedure, the distribution of TBTO was investigated in double vacuum treated Scots pine sapwood and in Corsican pine sapwood that had been dip-treated for 3 minutes. Specimens, with surface dimensions of 25 × 25 mm and depth 20 mm, were sawn from the bulk of the treated wood and sections were taken through the radial face in the tangential direction. For the double vacuum treated specimen, starting at the surface, ten thin sections 0·1 mm in thickness were cut on a microtome to form one sample for analysis. The sampling process was repeated to a depth of 12 mm from the surface of the specimen. For the Corsican pine, the first three samples were made up of five thin sections, 0·1 mm in thickness, and the next three samples of ten thin sections, 0·1 mm in thickness. The total organotin content, expressed as TBTO, was plotted against depth of sample. The curves, given in Fig. 3, showed that it is possible to evaluate the distribution of preservative over small areas.

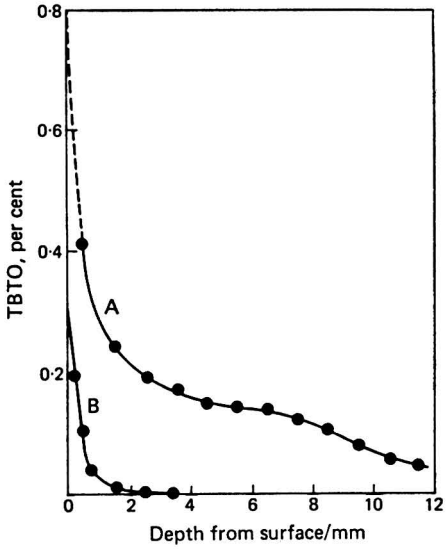

Fig. 3. Distribution of organotin, expressed as TBTO, in (A) double vacuum treated Scots pine sapwood and (B) dip-treated Corsican pine sapwood

The amounts involved would permit the analysis of each 0·1-mm section of the double vacuum treated wood and the first millimetre of dip-treated timber separately so as to obtain a more close distribution pattern. It is also possible to evaluate the conversion of TBTO and DBTO and the distribution of these compounds by using the cation-exchange procedures.

METHODS

Apparatus—

Atomic-absorption spectrophotometry—The atomic-absorption equipment consisted of a Pye Unicam, Model SP90A, Series 2, single-beam spectrophotometer fitted with an EMI No. 9662A photomultiplier and a Pye Unicam, Model AR25, linear recorder. A Cathodeon tin hollow-cathode lamp for use at a wavelength of 224·4 nm was used.

Polarography—Polarograms were recorded with a Southern Analytical Instruments K1000 cathode-ray polarograph and a mercury-pool reference electrode. Solutions were de-oxygenated with oxygen-free nitrogen prior to measurement at 25 ± 0·25 °C.

REAGENTS—

Ethanol—97·5 per cent. Use for the preparation of reagents.

Hydrochloric acid in ethanol, 5 per cent. V/V solution—Dilute 50 ml of concentrated hydrochloric acid to 1 litre with ethanol.

Hydrochloric acid in ethanol, 0·05 per cent. V/V solution—Dilute 10 ml of the 5 per cent. *V/V* solution of hydrochloric acid in ethanol to 1 litre with ethanol.

Hydrochloric acid and water in ethanol, solution containing 0·3 per cent. V/V of hydrochloric acid and 10 per cent. V/V of water—Dilute 3 ml of concentrated hydrochloric acid and 100 ml of water to 1 litre with ethanol.

Water in ethanol, 10 per cent. V/V solution—Dilute 100 ml of water to 1 litre with ethanol.

Water in ethanol, 33 per cent. V/V solution—Dilute 333 ml of water to 1 litre with ethanol.

Hydrochloric acid solution, 20 per cent. V/V—Dilute 20 ml of concentrated hydrochloric acid to 100 ml with water.

Lithium chloride solution, 5000 μg ml⁻¹—Dissolve 3·06 g of anhydrous lithium chloride in and dilute to 100 ml with the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol.

Support electrolyte 1—Dissolve 7·4600 g of potassium chloride in water and dilute to 100 ml with water.

Support electrolyte 2—Dissolve 7·4600 g of potassium chloride in water, add 0·94 ml of concentrated hydrochloric acid and 2·5 ml of ethanol, and dilute to 100 ml with water.

Cation-exchange resin—Amberlite CG-120 chromatographic resin, 200 mesh.

Bis(tri-n-butyltin) oxide standard solution 1—Dissolve 0·1000 g of bis(tri-n-butyltin) oxide in ethanol, add 5 ml of the 5 per cent. *V/V* solution of hydrochloric acid in ethanol and dilute to 500 ml with ethanol.

1 ml of solution ≡ 200 μg of TBTO.

Bis(tri-n-butyltin) oxide standard solution 2—Dissolve 0·1000 g of bis(tri-n-butyltin) oxide in ethanol and dilute to 500 ml with ethanol.

1 ml of solution ≡ 200 μg of TBTO.

Di-n-butyltin oxide standard solution—Dissolve 0·0500 g of di-n-butyltin oxide in 50 ml of a warm solution of 5 per cent. *V/V* of hydrochloric acid in ethanol, cool, and dilute to 500 ml with the 5 per cent. *V/V* solution of hydrochloric acid in ethanol.

1 ml of solution ≡ 100 μg of DBTO.

Atomic-absorption method for determining organotin compounds—The instrument operating conditions were as follows—

Wavelength	224·4 nm
Slit width	0·05 mm
Attenuator setting	1
Lamp current	7 mA
Scale expansion	Up to × 10
Burner	Nitrous oxide - acetylene
Burner height	0·7 cm
Aspiration rate	3 to 4 ml min⁻¹
Acetylene flow-rate	3800 ml min⁻¹ at a pressure of 0·7 kg cm⁻²
Nitrous oxide flow-rate	5 l min⁻¹ at a pressure of 2·1 kg cm⁻²

CALIBRATION SOLUTIONS

Transfer by pipette, with suitable precautions, 1, 2, 3, 5, 10, 15, 20 and 25 ml of TBTO standard solution 1 into 100-ml calibrated flasks containing 20 ml of lithium chloride solution, dilute to the mark with the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol and mix. The solutions contain 2, 4, 6, 10, 20, 30, 40 and 50 μg ml⁻¹ of TBTO, respectively.

PROCEDURE—

Transfer the weighed sample into a 50-ml distillation flask. Add 30 ml of the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol and fit a reflux distillation condenser to the flask. Boil the solution for 10 minutes, cool it to room temperature, decant the leach solution from the wood into a flask, fit a stopper and allow any particles of wood to settle.

Transfer 8 ml of the leach solution into a 10-ml calibrated flask, dilute to the mark with lithium solution and mix.

Use the operating conditions given above and aspirate a suitable range of calibration solutions followed by the sample solution. Do not disturb the sediment during aspiration of the sample solution. Check the calibration solutions after the last sample has been run. Aspirate the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol between each test or calibration solution. Plot a calibration graph of the concentration ($\mu g\ ml^{-1}$) of TBTO against absorbance. To determine the TBTO equivalent of the organotin compounds in the sample solution, compare the absorbance reading with the calibration graph.

The volume of the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol for leaching the organotin compounds from the wood can be varied according to the amount of sample taken for analysis and its organotin content.

Cation-exchange separation of wood extractives, TBTO and DBTO

Chromatographic column—

A quick semimicro-scale column, of 10 cm effective length and 1 cm bore, with a tap is used. The reservoirs have a capacity of 50 ml.

Preparation of chromatographic column—

Soak the cation-exchange resin in water for 24 hours. Slurry sufficient resin into the column to form a bed 2·5 cm deep when the solids settle down. Elute the column sequentially with 50 ml of 2 M sodium hydroxide solution, water until the eluate is free from alkali, 50 ml of 2 M hydrochloric acid, water until the eluate is free from acid, and finally 20 ml of ethanol. It is necessary to agitate the resin after eluting it with ethanol so as to remove air bubbles. The resin column is now ready for use.

To regenerate the column after each run, elute it successively with 20 ml of 20 per cent. *V/V* hydrochloric acid solution, water until the eluate is free from acid, and 20 ml of ethanol.

Procedure—

Weigh the sample and transfer it into a 50-ml distillation flask. Add 20 ml of the 0·05 per cent. *V/V* solution of hydrochloric acid in ethanol and fit a reflux distillation condenser to the flask. Boil the solution for 10 minutes, add 10 ml of water and continue to boil the mixture for 2 minutes. Cool the contents of the flask to room temperature, transfer a suitable aliquot (up to 25 ml) into the chromatographic column reservoir and elute at the rate of 1 drop per 2 s. Rinse the reservoir and elute the resin with 2 volumes of 5 ml of the 33 per cent. *V/V* solution of water in ethanol, then 5 ml of the 10 per cent. *V/V* solution of water in ethanol. Elute the TBTCl with the solution of 0·3 per cent. *V/V* of hydrochloric acid and 10 per cent. *V/V* of water in ethanol, discard the first 1 ml of eluate and collect the next 10 ml in a 10-ml calibrated flask. Wash the reservoir and elute the resin with 5 ml of ethanol. Elute the $DBTCl_2$ with the 5 per cent. *V/V* solution of hydrochloric acid in ethanol, discard the first 0·5 ml of eluate and collect the next 10 ml in a 10-ml calibrated flask. The solutions are ready for examination by atomic-absorption spectrophotometry or polarography.

Determination of TBTO by atomic-absorption spectrophotometry

Calibration solutions—

Transfer by pipette, with suitable precautions, 1, 2, 3, 5, 10, 15, 20 and 25 ml of TBTO standard solution 2 into 100-ml calibrated flasks containing 50 ml of ethanol, 10 ml of water and 6 ml of the 5 per cent. *V/V* solution of hydrochloric acid in ethanol, dilute to the mark with ethanol and mix. The solutions contain 2, 4, 6, 10, 20, 30, 40 and 50 $\mu g\ ml^{-1}$ of TBTO, respectively.

Procedure—

Continue as described in the second paragraph of the Procedure (p. 240) for the atomic-absorption method for determining organotin compounds. Aspirate the solution of 0·3 per cent. *V/V* of hydrochloric acid and 10 per cent. *V/V* of water in ethanol between each test or calibration solution.

Determination of DBTO by atomic-absorption spectrophotometry

Calibration solutions—

Transfer by pipette, with suitable precautions, 1, 2, 3, 5, 10, 15, 20 and 25 ml of DBTO standard solution into 100-ml calibrated flasks, dilute to the mark with the 5 per cent. *V/V* solution of hydrochloric acid in ethanol and mix. The solutions contain 1, 2, 3, 5, 10, 15, 20 and 25 μg ml^{-1} of DBTO, respectively.

Procedure—

Continue as described in the second paragraph of the Procedure (p. 240) for the atomic-absorption method for determining organotin compounds. Aspirate the 5 per cent. *V/V* solution of hydrochloric acid in ethanol between each test or calibration solution.

Determination of TBTO by polarography

Transfer 2 ml of the TBTO eluate into a 10-ml calibrated flask, dilute to the mark with support electrolyte 2 and mix. Transfer 5 ml of the test solution into a polarographic cell containing a mercury-pool electrode, de-oxygenate for 10 minutes with oxygen-free nitrogen and record the peak current at −0·85 V with a start potential of −0·65 V. To obtain the TBTO content of the test solution, compare the peak current with a calibration graph. Prepare a calibration graph by using the TBTO atomic-absorption standards and the polarographic technique described above.

Determination of DBTO by polarography

Transfer 2 ml of the DBTO eluate into a 10-ml calibrated flask, dilute to the mark with support electrolyte 1 and mix. Transfer 5 ml of the test solution into a polarographic cell containing a mercury-pool electrode, de-oxygenate for 10 minutes with oxygen-free nitrogen and record the peak current at −0·64 V with a start potential of −0·50 V. To obtain the DBTO content of the test solution, compare the peak current with a calibration graph. Prepare a calibration graph by using the DBTO atomic-absorption standards and the polarographic technique described above.

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An Improved Method for the Determination of Whole Blood Lead by Using an Atomic-absorption Technique

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The Delves technique for measuring whole blood lead has been considerably improved by wet ashing with aqua regia instead of simply drying with hydrogen peroxide. Smoke is eliminated, and the reproducibility of the method is improved.

IN a recent survey, Keppler *et al.*[1] found that less than half of the clinical laboratories that were reported to be undertaking blood lead determinations were doing so with a reasonable degree of accuracy. This situation was improved by Delves,[2] who used atomic-absorption techniques in conjunction with what is now called the "Delves technique." In this method whole blood was placed in a cup together with 100-volume hydrogen peroxide and, after drying the mixture, the cup was introduced directly into an air - acetylene flame with an absorption tube assembly attached. Unfortunately, the recorded smoke trace produced when using Delves' method can be very misleading and difficulties in the interpretation of the results can lead to inaccuracies.

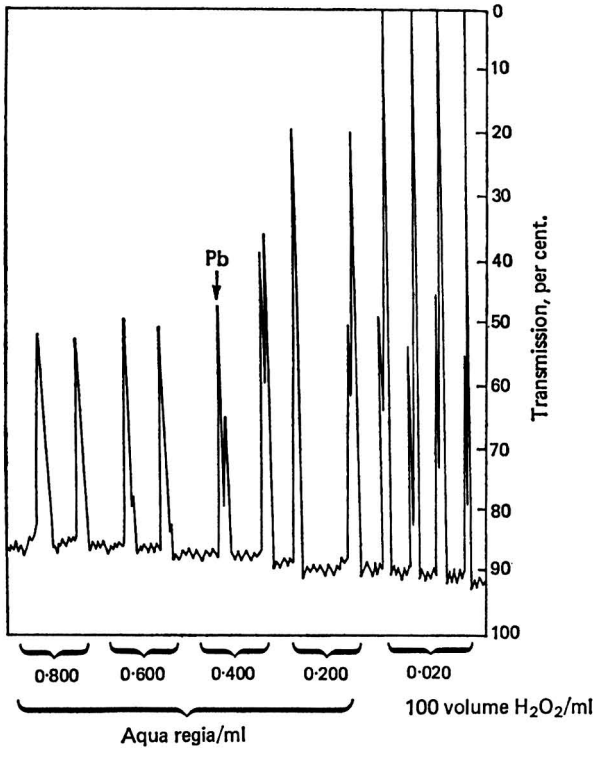

Fig. 1. Comparison of replicate blood samples containing 23 μg of lead per 100 ml by using Delves' method with various amounts of aqua regia

We have found considerable advantages in using aqua regia instead of hydrogen peroxide. Firstly, the smoke produced on introduction of the sample can be eliminated, and secondly, the reproducibility can be greatly increased. The original tube and cups, which were made of nickel, had to be replaced by similar apparatus made of fused quartz (obtainable from Thermal Syndicate Limited, Wallsend-on-Tyne).

Different volumes of aqua regia were tried. In each instance, 0·010 ml of blood was subjected to wet ashing by adding the aqua regia and evaporating to dryness on a hot-plate, care being taken to avoid spluttering. Measurements were carried out on a Perkin-Elmer 290B spectrophotometer with the modified Delves' absorption tube attachment.

Results obtained by use of the Delves technique, in which whole blood (0·010 ml) and 100-volume hydrogen peroxide (0·020 ml) were mixed together in the cup, have been compared with our results, which were obtained by using aqua regia instead of hydrogen peroxide. As can be seen from Fig. 1, the two methods give comparable results, but the interpretation of the results was facilitated, and their reproducibility considerably enhanced, by using our improved method. It will be noted that when 0·600 ml of aqua regia is added to the sample, the smoke traces are very small, and are negligible with 0·800 ml.

TABLE I

COMPARISON OF RESULTS OBTAINED BY USING THE TWO METHODS ON REPLICATE BLOOD SAMPLES

Method	Number of assays	Mean/ μg per 100 ml	Range/ μg per 100 ml	Standard deviation
Delves' method(with the original matched cups)	15	69·26	59 to 79	4·32
Delves' method (with quartz cups)	15	67·13	47 to 79	8·96
Aqua regia method (with unmatched cups) ..	20	68·60	60 to 77·5	4·93
Aqua regia method (with matched cups) ..	20	68·25	64 to 72	2·49

A blood sample with a high lead content was examined by the two methods and the results subjected to statistical analysis. The comparison is shown in Table I, from which it can be seen that oxidation of the blood with hydrogen peroxide is unsatisfactory when the quartz cups are used. The aqua regia method with unmatched quartz cups is almost as accurate as the original Delves method with matched nickel cups, but is considerably improved when the quartz cups are matched in terms of length of time in use.

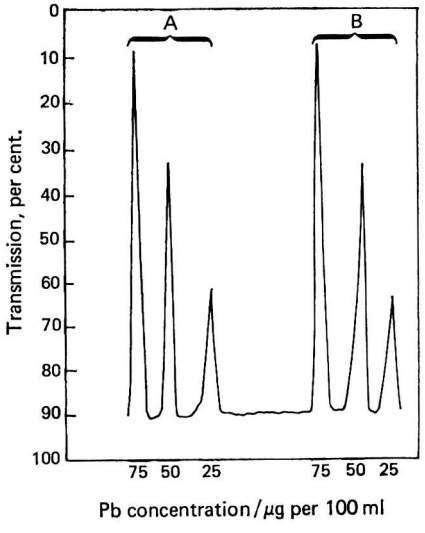

Fig. 2. Comparison of pure lead standards, with (peaks A) and without (peaks B) added electrolytes

Fig. 2 shows typical peaks for pure lead standards over the range required for determinations on whole blood samples, both with and without addition of electrolytes at levels comparable with those found in whole blood. The differences between the two sets of results are very small and can be attributed to normal experimental errors.

Calibration graphs of aqueous standards and of whole blood containing increasing amounts of lead are shown in Fig. 3. This method of addition, involving the use of whole blood of known lead content spiked with various amounts of lead solution, gave very similar results to those obtained with the aqueous solutions. Thus, the two lines are almost parallel and the error introduced by the deviation from absolute parallelism was 3 per cent., *i.e.*, recoveries of added lead were 100 $\pm$ 3 per cent.

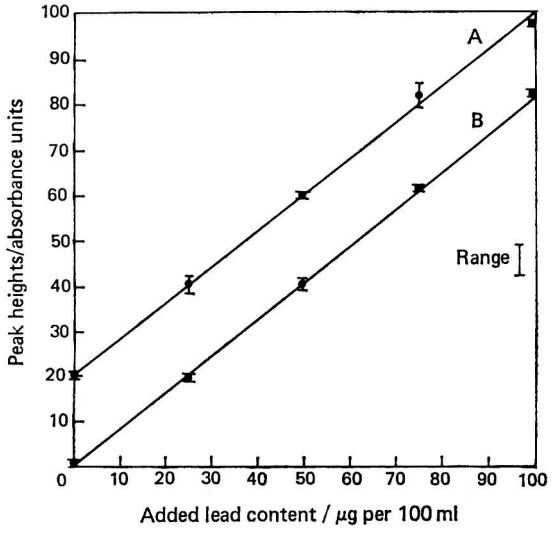

Fig. 3. Comparison of whole blood and aqueous lead standards. A, whole blood with added lead standards; and B, aqueous standards

In order to establish that the smoke was being removed satisfactorily, measurements were taken on a nearby non-resonant wavelength by using blood with a lead content of 50 μg per 100 ml. An absorption signal equivalent to 3 to 5 μg per 100 ml was obtained. Thus, the non-specific absorption accounts for up to 10 per cent. of the whole blood lead. This value compares favourably with that found by Delves' original method.[2]

The method has proved to be reliable for whole blood lead values both in the normal and the abnormally high ranges.

The equipment was donated by the Institute of Urology, London.

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Bis(6-methyl-2-pyridyl)glyoxal Dihydrazone as a Spectrophotometric Reagent for the Rapid Determination of Copper in Alkalis, Milk and Brine

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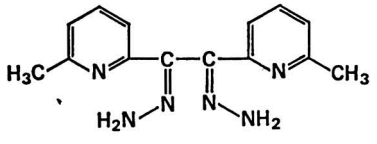
The synthesis, characteristics and analytical applications of bis(6-methyl-2-pyridyl)glyoxal dihydrazone are described. This compound produces coloured solutions selectively with copper(I) ions ($\lambda_{max.}$ = 440 nm, ϵ = 8·7 × 10^3 l mol^{-1} cm^{-1}) that can be extracted into various organic solvents; it behaves as a cuproine-type reagent. The 1:1 orange-yellow copper(I) complex has been used for the spectrophotometric determination of trace amounts of copper in saturated brine, alkalis and milk. The most important advantage of using this reagent is the total recovery of copper that is possible from ammoniacal solutions.

THE work described in this paper forms part of an investigation into the use of hydrazones for trace-metal analysis. The ferroin-type reagents, 2,2′-bipyridylglyoxal dihydrazone, diacetyl dihydrazone and phenyl 2-pyridyl ketone hydrazone, have been applied[1] to the spectrophotometric determination of trace amounts of iron while 6-methylpicolinaldehyde hydrazone was used[2] as a selective cuproine-type reagent for the determination of copper.

In the present work, bis(6-methyl-2-pyridyl)glyoxal dihydrazone was used for the selective determination of trace amounts of copper in different materials.



EXPERIMENTAL

SYNTHESIS OF REAGENT—

Five millilitres of 99·9 per cent. *m/m* hydrazine hydrate were added to 6 g of bis(6-methyl-2-pyridyl)glyoxal (Fluka) dissolved in 150 ml of hot absolute ethanol and the mixture was refluxed for 24 hours. On cooling to 0 °C, the crystals that separated out were filtered off and recrystallised twice from hot ethanol. The crystals finally obtained, dried at 60 °C under pressure, were white and melted at 154 to 155 °C. On analysis their elemental content was found to be: C 62·5, H 5·9 and N 31·5 per cent.; the content calculated for $C_{14}H_{16}N_6$ was: C 62·67, H 5·96 and N 31·34 per cent.

APPARATUS—

Spectrophotometers—Unicam SP800, Unicam SP600 and Beckman DU spectrophotometers, equipped with 1·0-cm glass or quartz cells.

Digital pH meter—Philips, PW9408, with glass - calomel electrodes.

SOLUTIONS—

All solvents and reagents were of analytical-reagent grade.

Bis(6-methyl-2-pyridyl)glyoxal dihydrazone reagent solutions—Solutions of concentration 0·1 per cent. *m/V* in ethanol and 0·05 per cent. *m/V* in nitrobenzene.

Standardised solutions of copper(II).

Ascorbic acid, 2 per cent. m/V *aqueous solution (as reducing agent).*

Acetic acid - sodium acetate buffer solution, pH 4·8.

Extraction solution 1—Dissolve 5 g of sodium perchlorate monohydrate and 3 g of ascorbic acid in 250 ml of the above buffer solution. This solution remains stable for 1 week.

Extraction solution 2—Dissolve 5 g of sodium perchlorate monohydrate and 3 g of ascorbic acid in 250 ml of distilled water. This solution also remains stable for 1 week.

Trichloroacetic acid, 50 per cent. m/V aqueous solution.

Recommended procedures

Alkalis—

To 10 ml of extraction solution 2 in a separating funnel add 50 ml of alkali, followed by 10 ml of 0·05 per cent. *m/V* reagent solution in nitrobenzene. Shake the mixture vigorously for 2 minutes, allow the phases to separate and transfer the lower, organic, layer into a flask containing anhydrous sodium sulphate. Measure the absorbance of this solution at 440 nm against the reagent solution in nitrobenzene. Prepare a calibration graph in a similar manner by adding appropriate amounts of copper to 2 M sodium hydroxide solution.

Saturated brine—

Method A—To 25 ml of saturated brine in a 50-ml calibrated flask, add 10 ml of acetic acid - sodium acetate buffer solution, 2 ml of 2 per cent. *m/V* ascorbic acid solution and 5 ml of 0·1 per cent. *m/V* ethanolic reagent solution, and dilute to the mark with distilled water. Measure the absorbance of the solution at 440 nm against a reagent blank prepared in a similar manner.

Method B—To 10 ml of saturated brine in a separating funnel add 10 ml of extraction solution 1 and 10 ml of 0·05 per cent. *m/V* reagent solution in nitrobenzene. Shake the funnel vigorously for 1 minute, allow the phases to separate and transfer the lower layer into a flask containing anhydrous sodium sulphate. Measure the absorbance at 440 nm against the reagent solution in nitrobenzene.

Calibration graphs are constructed from standard solutions treated in the same way.

Milk—

To 100 ml of milk in a 300-ml Erlenmeyer flask, add 25 ml of 50 per cent. *m/V* trichloroacetic acid solution, slowly and with constant shaking. Shake the flask vigorously, place it in a boiling water bath for 15 minutes and cool it in ice - water to 10 °C. Transfer 25 ml of the supernatant liquid into a separating funnel. Add 7 ml of 2 M sodium hydroxide solution, 10 ml of extraction solution 1 and 10 ml of 0·05 per cent. *m/V* reagent solution in nitrobenzene. Shake the funnel vigorously for 1 minute, allow the phases to separate and transfer the lower layer into a flask containing anhydrous sodium sulphate. Measure the absorbance of this solution at 440 nm against the reagent solution in nitrobenzene. Obtain the content of copper, in micrograms, from a standard graph prepared by substituting the appropriate copper solutions and distilled water for the milk sample.

Results and discussion

Bis(6-methyl-2-pyridyl)glyoxal dihydrazone reagent—

The ultraviolet spectrum for the reagent shows a bathochromic shift in an acidic medium ($\lambda_{max.}$ 347 and 268 nm) compared with an alkaline medium ($\lambda_{max.}$ 295 nm, with a shoulder at 266 nm), with two isosbestic points at 277 and 313 nm. The Phillips and Merritt[3] method is used for the determination of the ionisation constant; the average p*K* value is 4·82. This behaviour may be caused by protonation of the nitrogen atoms in the pyridine rings; the p*K* value is very similar to that of pyridine and its derivatives.

The reagent is resistant to hydrolysis in a strongly acidic medium (6 M hydrochloric acid). This property is usual with α-diimines, in contrast with the corresponding imines; 6-methylpicolinaldehyde hydrazone[2] hydrolyses in 2 M hydrochloric acid at 20 °C in 30 minutes.

The reaction of the reagent with thirty cations at various pH values was investigated; it reacts only with copper(I) and palladium, and the absorption spectra of solutions of these metals are shown in Fig. 1. It acts as a cuproine-type reagent in that methyl groups adjacent to the nitrogen atom in pyridine produce the well known blocking effect.

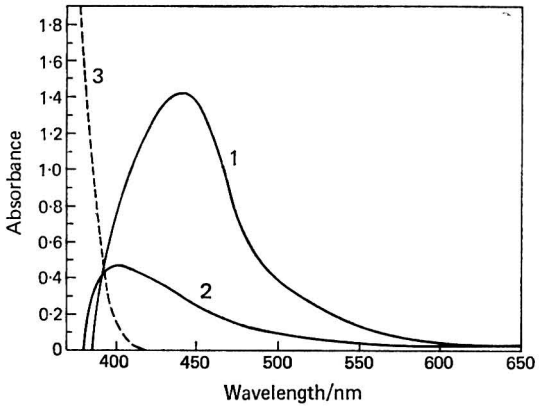


Fig. 1. Absorption spectra of solutions of complexes formed with reagent: 1, 10 p.p.m. of copper (I); 2, 10 p.p.m. of palladium; and 3, reagent alone (water blank)

REACTION WITH COPPER(I)—

Aqueous media—The orange - yellow 1:1 copper(I) complex (ratio found by the Job absorptiometric method, by isolation of the perchlorate complex, $[Cu(C_{14}H_{16}N_6)]ClO_4$, in the solid state and elemental analysis*) of the reagent is formed completely over the pH range from 4·5 to 11·2 in aqueous solution (see Fig. 2). The effect of other experimental variables was determined. The system conforms to Beer's law over this pH range, the molar absorptivity (ϵ) being $8{\cdot}7 \times 10^3$ l mol^{-1} cm^{-1}. The optimum concentration range, evaluated by Ringbom's method, is 2 to 5 p.p.m. of copper. The colour intensity of the solutions of the complex remains constant for several hours. The relative error ($P = 0{\cdot}05$) of the method is $\pm 0{\cdot}13$ per cent.

The colour reaction is selective for copper. Silver, mercury(II), mercury(I), cadmium, zinc, iron(II), cobalt(II), thallium, lead, tin(II), uranium(VI), calcium, strontium and barium do not interfere at the 300 p.p.m. level in pH 4·8 buffer. Nickel does not interfere at the

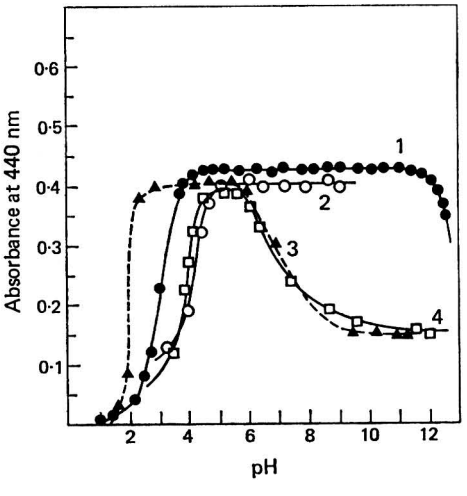


Fig. 2. Effect of pH on the formation of the copper (I) complex: 1, in water; 2, in pentanol; 3, in nitrobenzene; and 4, in chloroform

* The results of the analysis were C 38·7, H 3·5 and N 19·1 per cent.; calculated values, C 38·96, H 3·71 and N 19·48 per cent.

50 p.p.m. level, while iron(III), bismuth, antimony(III) and manganese(II) precipitate at the 100 p.p.m. level. The most serious interferences are from palladium, gold(III), EDTA and oxalate. At pH 8·7 interferences are greater.

If an excess of reagent instead of ascorbic acid is used as reducing agent, the same results are obtained over the pH range 4·5 to 9.

Extraction—The coloured complex formed in aqueous solution can be extracted into various organic solvents, such as pentanol, chloroform and isobutyl methyl ketone, with no perchlorate, but in the pH range from 9 to 12 the absorbance of the organic layer is not stable with time (Fig. 2).

Copper(I) ions are completely extracted with either nitrobenzene or chloroform, with perchlorate in the aqueous phase. The optimum pH range is 3·2 to 5·7 (nitrobenzene) and 4·5 to 5·7 (chloroform); from pH 9 to 12 the absorbance remains lower but stable with pH and time (Fig. 2). Beer's law is adhered to at pH 4·8 and 10·9 with both of these solvents. The coloured organic solutions are stable for several hours. The extraction has a high relative error with chloroform but the relative error ($P = 0·05$) of the method with nitrobenzene is $\pm 0·36$ per cent. The optimum concentration range evaluated by Ringbom's method is 2 to 6 p.p.m. of copper. After the extraction the effect of the ions mentioned above can be investigated. Those ions which did not interfere with the colour reaction do not interfere when using this technique at pH 4·8 or 11. Interference from nickel, iron(III), bismuth and manganese(II) is now suppressed up to a level of 300 p.p.m. Palladium, gold(III), EDTA and oxalate also interfere in this method.

Determination of copper—

Typical results for the determination of copper in sodium hydroxide, potassium hydroxide and ammonia solutions are given in Table I. The method is very useful because the recovery

Table I

Determination of copper in alkalis

Each result is the average of three separate analyses

Solution	Copper added/μg g^{-1}	Copper recovered/μg g^{-1}	Recovery, per cent.
Sodium hydroxide.. ..	10·0	10·25	102·5
Potassium hydroxide ..	14·3	14·6	102·1
Ammonia	24·1	24·1	100·0

from ammonia solutions is complete. The present reagent is more suitable than 6-methyl-2-pyridylphenyl ketoxime, proposed by Pemberton and Diehl[4] in 1969 as a reagent for the determination of copper in alkalis, because with the latter the recovery of copper from ammonia solutions is only partial (32 per cent.).

Trace amounts of copper in saturated brine can be determined accurately in homogeneous media (Method A) and with the extraction technique (Method B). Typical results for the determination of copper are given in Table II.

Table II

Determination of copper in saturated brine

Each result is the average of three separate analyses

Copper added/μg ml^{-1}	Copper found/μg ml^{-1}		Recovery, per cent.	
	Method A	Method B	Method A	Method B
2·0	2·0	1·95	100	97·5
4·0	4·0	4·0	100	100
6·3	6·2	6·3	98·5	100
6·8	6·8	6·7	100	99

The reagent is also suitable for the rapid determination of the copper content of milk. The results (Table III) are comparable with those obtained by Smith[5] in 1967 when using the same method with a 0·05 per cent. m/V solution of zinc dibenzyldithiocarbamate in toluene.

Table III

Determination of copper in milk

Commercial sample	Number of determinations	Average concentration of copper/μg per 100 ml	Confidence limits (95 per cent.)
1	6	34·5	±3·6
2	6	8	±2·3
3	6	12	±2·6

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An Electrical Conductivity Detector for Paper, Thin-layer and Column Chromatography*

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An electrical conductivity detector has been developed for application to the paper, thin-layer and column chromatography of ionic substances.

Two arms of an a.c. Wheatstone bridge comprise two pairs of electrodes applied directly on to the separating layer so that a signal ensues from the bridge when a chromatographic spot passes one electrode pair while the other pair is traversed by the eluting agent. The sensitivity is about 1 μg and the precision is about 2 to 3 per cent.

THE problem involved in the quantitative determination of the components of a mixture after a chromatographic separation has been carried out is still difficult and complex. While the identification of the individual components can be simplified to measurement of R_F values, the quantitative analysis is carried out by using techniques and methods that are often exclusively applicable to only one or other of the components of a mixture and great care must be exercised to avoid interference effects from the chromatographic support.

The methods capable of more general use can be classified as direct and indirect methods. The former permit quantitative analysis to be carried out directly on the chromatographic medium without other manipulation, while indirect methods require a series of manipulations after the development of the chromatogram. Examples of the application of such methods can be found in the literature.[1–9]

With indirect methods, quantitative analysis needs to be performed by highly qualified personnel and by use of rather complex instrumentation, while chromatographic separations are carried out simply and often with very simple apparatus. However, there are relatively few direct methods of quantitative analysis of general applicability, especially in paper and thin-layer chromatography. The present work is intended as a contribution towards providing a direct detection method that is useful in many instances. A description with illustrations is given of an electrical conductivity detector applied to paper, thin-layer and column chromatography, which is capable of detecting small amounts of any substance that is a charge carrier and that can be eluted by substances that exhibit a certain electrical conductivity.

CONFIGURATION OF THE SYSTEM—

The simplest form of such a detector as originally assembled consists of two parallel, narrow, thin gold plates between which is enclosed a strip of paper, thus forming a conductivity cell, which is used as an arm of a Wheatstone bridge; the bridge is connected to a differential amplifier whose output is fed into a chart recorder. One end of the paper strip is immersed in the eluting agent and a flow of eluting agent is established along the strip. When this flow becomes constant, the signal from the Wheatstone bridge is also constant and the bridge can be set to zero. A solution of a conducting substance in the eluting agent used is prepared and 2 to 10 μl of this solution are applied with a precision microsyringe close to the immersed end of the paper strip, which results in a spot being formed which travels in the flow of eluting agent towards the conductivity cell. When the spot reaches the electrodes a conductivity peak is recorded.

More elaborate arrangements were subsequently designed and the electrodes were applied to thin layers of silica, alumina and cellulose, as well as to a special type of column. In Fig. 1 is shown the best arrangement attained at present. Four electrodes are now used, forming a working cell and a reference cell. They consist of thin, narrow platinum strips, 1 mm wide × 1 mm thick and 35 mm long, placed 1·5 mm apart, embedded in a PTFE support on which the thin layer is deposited in the usual way. The eluting agent is fed in at the upper end and the sample is applied at a suitable distance from the feeding point

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of the eluting agent, either with a microsyringe or by using a narrow strip of polished gold on which the substances to be determined have been deposited from solution by evaporation of the solvent. The conveniently supported gold strip is brought into contact with the layer and contact maintained until a further sample is required to be applied. One end of the thin layer, which is kept in a horizontal position, is exposed to the air so as to enable the eluting agent to evaporate; the other parts of the chromatographic system are suitably sealed in order to prevent its evaporation; efforts are at present being made to embed the electrodes in a glass plate, glass being a more satisfactory material in this respect than PTFE. The above electrode arrangement yielded the most satisfactory results, but other arrangements were also tried, as shown in Fig. 2. In Fig. 2 (*a*), an arrangement is shown in which one electrode is embedded in the support and the other gently applied over the thin layer. We found that this system produced cells the apparent volume of which extended too far within the layer, thus giving rise to enlarged peaks. In Fig. 2 (*b*), a comb-like type of electrode is shown, which gave rise to a slight perturbation of the flow and to tailed peaks.

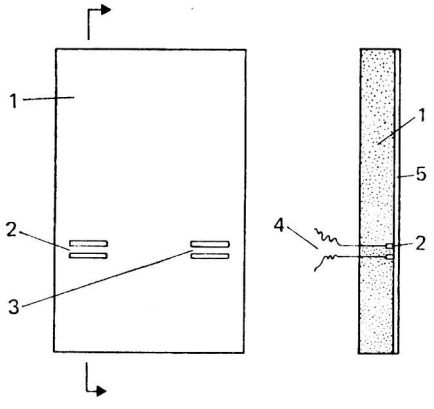

Fig. 1. Detector system: 1, support; 2 and 3, electrodes; 4, leads; and 5, thin layer

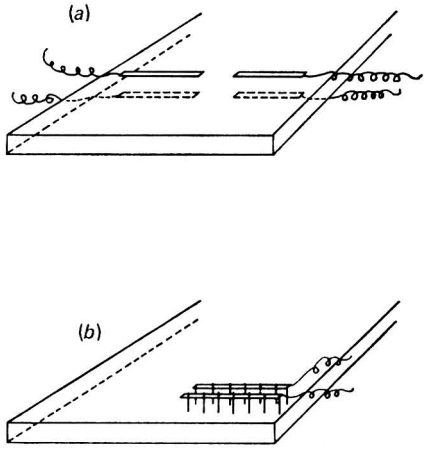

Fig. 2. Different arrangements of electrodes

In Fig. 3 is shown the design of a special column, which was developed in order to allow the conductivity detector to be applied to column systems. A large conventional column could easily accommodate at least one pair of electrodes, but is not suitable for the separation and detection of micro-amounts of substances. A capillary column does not afford enough room for the electrodes, so that the annular space enclosed between two concentric columns, which may be regarded as a thin-layer column, was used. This space is currently 1 mm wide but could be further reduced, possibly to 0·3 to 0·4 mm, thus obtaining a cylindrical layer suitable for micro-analytical purposes.

Filling such a column does not present particular difficulties and can be achieved by carefully pouring the substance chosen as the chromatographic support, in the form of a slurry, from the top of the column while the latter is maintained in a vertical position and in contact with a plate vibrating at about 50 Hz and with moderate amplitude.

The two conductivity cells, applied to the separating layer, constitute two adjacent arms of a Wheatstone bridge to which an oscillator feeds a 2-V, 2000-Hz signal. The output of the bridge is fed into a differential amplifier, which could be replaced by a lock-in amplifier for greater accuracy. This amplified signal is fed both into an integrator and into a chart recorder. If the cells are arranged in such a way that one electrode of one of the cells is short-circuited with one of the electrodes of the other cell, while the signal from the oscillator is applied to the other two electrodes, the zero line is almost independent of variations in the flow of the eluting agent and changes in the environmental conditions.

Electrodes can be set side by side in pairs located symmetrically with respect to the cylinder axis. Samples are applied with a microsyringe through neoprene diaphragms located upstream at convenient distances from the electrodes.

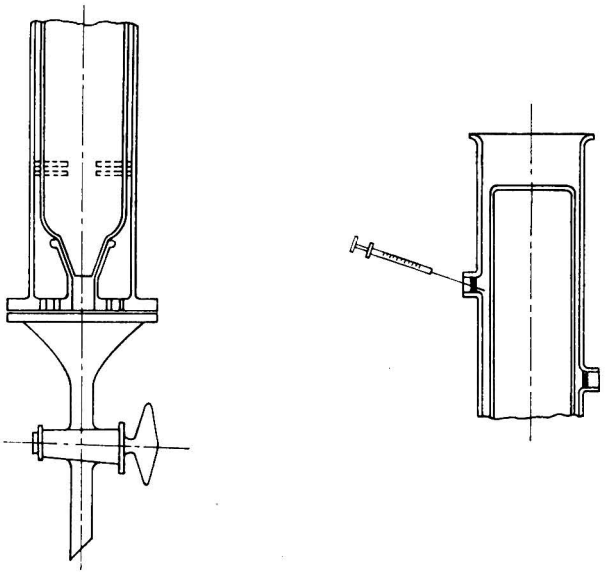

Fig. 3. Chromatographic column

THEORY OF THE RESPONSE—

It can easily be demonstrated that the response of the bridge in terms of the potential across its terminals when a chromatographic spot is flowing through the working cell is—

$$\rho = \frac{V\sigma_1 k\lambda}{(\sigma + \sigma_1)^2}\left(C - \frac{k\lambda}{\sigma + \sigma_1} C^2 + \ldots\right)$$

where ρ is the instantaneous root mean square of the potential across the bridge; V the root mean square of the supply potential; σ_1 the conductivity of the arm adjacent to the working cell; σ the conductivity of the working cell before and after the spot has passed, whose value is usually very close to σ_1; k the constant of the working cell, a function of the geometry of the electrodes and of the electrical path between them; λ the equivalent conductivity of the spot material $\times$ 10^3; and C the instantaneous equivalent concentration of the spot material.

As a first approximation the response is proportional to concentration, which has been confirmed experimentally as will be shown later. The higher the supply potential the higher the response; it was found that, as with most conductimeters, 2 V are sufficient to obtain a good and clean response.

The response is higher if the conductivity of the eluting agent is low, but the conductivity of the latter cannot be too low or the stability of the zero line and linearity of response with varying concentration will be affected. In a series of experiments with a diethyl ether based eluting agent, which gave rise to a resistance across the electrodes of 250 kΩ, it was found that trace amounts of moisture in the sampling device are capable of rendering the entire system unstable for a long period of time.

Thus, if the conductivity of the eluting agent is reduced to very low values, the electronics of the device must be suitably designed and great care should be used in handling the apparatus.

If we set

$$\psi = \frac{(\sigma + \sigma_1)^2}{V\sigma_1 k\lambda}$$

it can be seen that ψ is the constant of the detector and as a first approximation

$$C = \psi\rho$$

The complete response with time, that is, the chromatogram, is a peak, and the amount of the substance under examination is proportional to the area of the peak.

Results and discussion

The logical first application of such a detector is to separation systems for inorganic ions and the work carried out on some of these systems is reported below.

In the course of experiments performed with the various types of electrodes described in this paper it was found that better results were obtained with cell configurations of the type shown in Fig. 1 and interest was accordingly concentrated on these cells.

In Fig. 4 (*a*) a separation of Fe^{3+} from Zn^{2+} ions in a mixture of these ions on a thin layer of silica gel G is reported; the eluting agent used was acetone - 12 N hydrochloric acid -

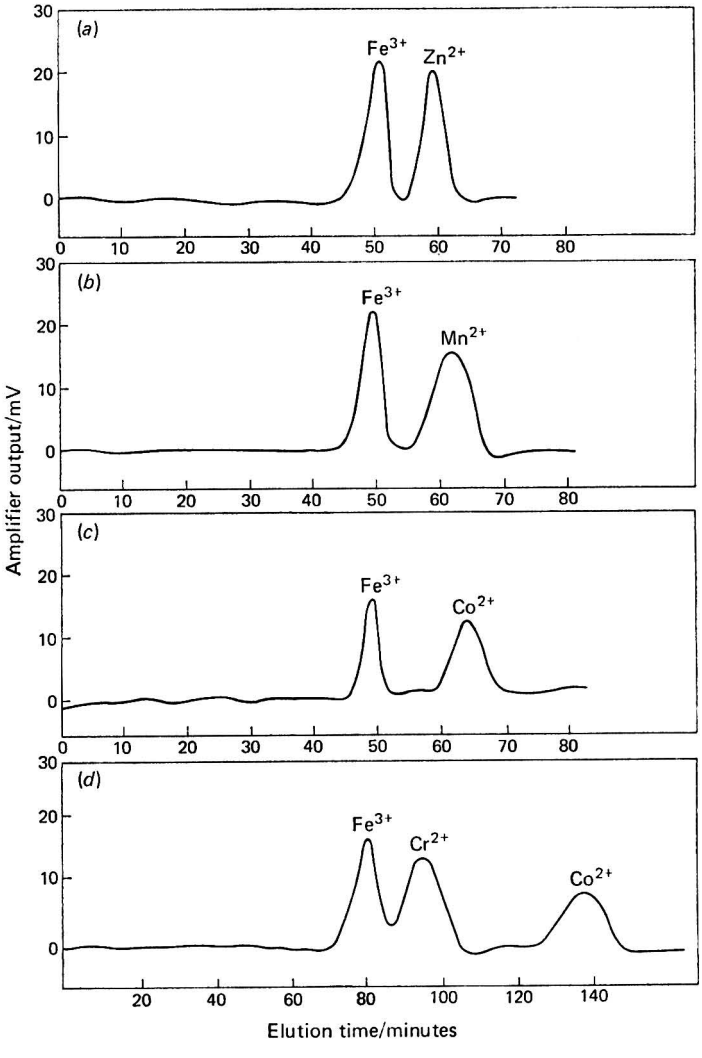

Fig. 4. Separation of inorganic ions on a thin layer of silica gel G: (*a*) separation of Fe^{3+} (2 μg μl^{-1}) and Zn^{2+} (2 μg μl^{-1}) with acetone - 12 N hydrochloric acid - hexane-2,5-dione (100 + 1 + 0·5) as eluting agent; (*b*) separation of Fe^{3+} (2 μg μl^{-1}) and Mn^{2+} (2 μg μl^{-1}) with acetone - 12 N hydrochloric acid (100 + 1) as eluting agent; (*c*) separation of Fe^{3+} (0·75 μg μl^{-1}) and Co^{2+} (1·70 μg μl^{-1}) with acetone - 12 N hydrochloric acid - hexane-2,5-dione (100 + 1 + 0·5) as eluting agent; and (*d*) separation of Fe^{3+} (1·5 μg μl^{-1}), Cr^{2+} (2 μg μl^{-1}) and Co^{2+} (2 μg μl^{-1}) with acetone - pentyl acetate - pentane-2,4-dione - 6 N hydrochloric acid (150 + 10 + 1·5 + 1·5) as eluting agent

hexane-2,5-dione (100 + 1 + 0·5). The sample analysed consisted of 10 μl of a solution containing 0·2 μg μl^{-1} of both iron and zinc ions. On the y-axis of the graph the output of the amplifier is given in millivolts. The true resistance of each cell was about 400 Ω.

In Fig. 4 (*b*) the chromatogram for the mixture of Fe^{3+} and Mn^{2+} ions, which was obtained under the same conditions as for the previous chromatogram, shows that the Fe^{3+} peak appears after almost exactly the same time as the Fe^{3+} peak in Fig. 4 (*a*). The same is true of Fig. 4 (*c*), which relates to the separation of a mixture of Fe^{3+} and Co^{2+} ions carried out under the same conditions as before. With the mixture of Mn^{2+}, Co^{2+} and Zn^{2+} ions, separations were not attempted as efforts were devoted more to testing the properties of the detector than to studying separation techniques.

The sensitivity of the entire system depends largely on the characteristics of the electronics used and the level obtained with the prototype is shown in Fig. 4 (*c*), for which the sample taken amounted to only 0·75 μg of Co^{2+}.

The chromatogram shown in Fig. 4 (*d*) is the result of an attempted separation of a mixture of Fe^{3+}, Cr^{2+} and Co^{2+} ions, by using as eluting agent acetone - pentyl acetate - pentane-2,4-dione - 6 N hydrochloric acid (150 + 10 + 1·5 + 1·5).

For every substance it is necessary to plot a calibration graph and consequently care should be taken to maintain constant experimental conditions. No difficulties arose and thermostatic control of the system was found to be unnecessary. The only precautions necessary were the maintenance of a sufficient supply of eluting agent to the layer and prevention of evaporation of the standard solutions prepared with the same eluting agent.

The calibration graph for iron is plotted in Fig. 5. On the abscissa is the area originally obtained in millivolts per minute, but it has been plotted in square centimetres, with an approximation of 10 per cent., in order to demonstrate the extension of the peak. Standard solutions with different concentrations were used in order that samples should be at constant volume, but it was noticed that the volume of the eluting agent had no effect and all of the straight lines obtained could be extrapolated to zero, which was further confirmed with a sample of the pure eluting agent. Nevertheless, we found that a higher dispersion was obtained with samples at constant concentration and with variable volume, which was caused by the graduation and calibration of the microsyringe, and we therefore finally resorted to the use of samples of constant volume.

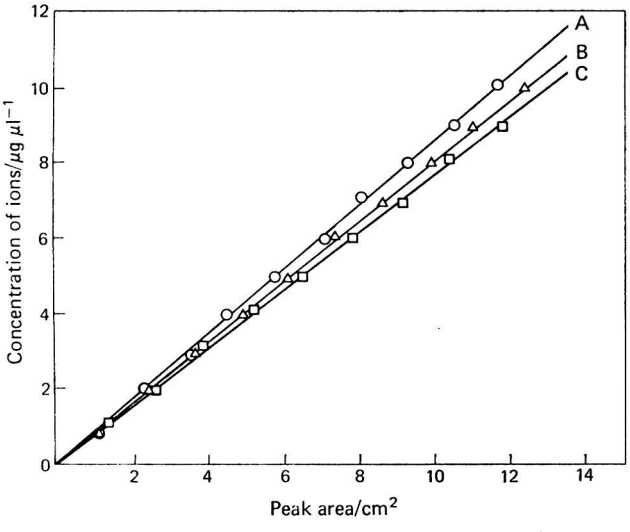

Fig. 5. Calibration graphs for different ions: A, Co^{2+}; B, Fe^{2+}; and C, Zn^{2+}

The calibration graphs for Zn^{2+} and for Co^{2+} ions are also shown in Fig. 5. From day to day, or after any interruption of the flow of eluting agent through the chromatograph, we

noticed some variations in the angular coefficients of the calibration graphs, so that for analytical purposes it is advisable to introduce a standard sample at the beginning of each analytical cycle. In order to observe the effect of time upon the angular coefficient of the calibration line, we examined, once every hour for 7 hours, a standard volume of 4 μl of a solution containing about 1 μg μl^{-1} of iron. The result of this experiment is shown in Table I. The mean value was 4·11 μg, the standard deviation 0·25 μg and the coefficient of variation 6·2 per cent.

TABLE I

EFFECT OF TIME ON ANGULAR COEFFICIENT OF CALIBRATION LINE FOR IRON

Time/hours ..	0	1	2	3	4	5	6	7
Amount of iron/μg ..	4·2	4·0	4·2	3·9	4·1	4·3	4·0	4·2

CONCLUSION

A conductivity detector consisting of one or two electrode pairs applied to a chromatographic layer appears to be a simple device that is capable of minimising difficulties and manipulations associated with quantitative liquid chromatography. For paper and thin-layer chromatography, the best results were obtained by applying, in contact with the layer, narrow strip electrodes embedded in the supporting material. With column chromatography, good results were obtained by filling the annular space between concentric columns with the solid phase, while the electrodes were supported by the glass of the column. Samples from a solution in the eluting agent of the substances to be analysed are applied directly to the layer with a precision microsyringe. In order to obtain accurate results, analyses should be made and calibration graphs obtained by using sample solutions with a constant volume of eluting agent.

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The Identification and Semi-quantitative Assay of Some Fat-soluble Vitamins and Antioxidants in Pharmaceutical Products and Animal Feeds by Thin-layer Chromatography

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A thin-layer chromatographic method for the identification and semi-quantitative assay of vitamin A (alcohol), its acetate and palmitate, vitamin D, α-tocopherol, α-tocopheryl acetate, BHA (butylated hydroxyanisole; 2-t-butyl-4-methoxyphenol), BHT (butylated hydroxytoluene; 2,6-di-t-butyl-4-methylphenol) and ethoxyquin in vitamin preparations is described. The sample solutions are applied to thin layers of silica gel and the vitamins and antioxidants are separated by using n-hexane - ethyl methyl ketone - di-n-butyl ether (34 + 7 + 6) as the developing solvent. Decomposition of vitamins A and D when applied to the adsorbent layer is inhibited by the presence of triethylamine in the spotting solvent. The compounds are identified by means of their R_F values, their appearance in ultraviolet radiation of wavelengths 254 and 360 nm and their response to iron(III) chloride - bipyridyl and iron(III) chloride - potassium hexacyanoferrate(III) spray reagents; they are assayed by visual comparison with standards. The method has been applied to gelatin-protected vitamin beads, animal feed additives, multi-vitamin tablets, oily vitamin concentrates and halibut-liver oil samples.

A simple colour test for distinguishing vitamin D_2 from vitamin D_3 after removal of vitamin A and its esters is also described.

VITAMIN A (retinol) and its esters, vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol) and vitamin E (α-tocopherol) are usually added to vitamin preparations either in the form of gelatin-protected beads, in which the vitamins are dispersed in a starch-coated matrix of gelatin and sugar, or in the form of concentrated solutions in oil. In both of these concentrates, the vitamins are stabilised by the presence of antioxidants, such as α-tocopherol, 2-t-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), 2,6-di-t-butyl-4-methylphenol (butylated hydroxytoluene, BHT) and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin). Rapid and reliable methods for the examination of the vitamin concentrates and their formulated products were required, and the use of thin-layer chromatography for this purpose was investigated.

Many thin-layer chromatographic methods for the separation, identification and assay of vitamins A, D and E have been reported; these methods have been reviewed by Bolliger[1] and Strohecker and Henning.[2]

The main difficulty in the assay of vitamins A and D by thin-layer chromatography arises from the instability of the vitamins on dry chromatographic adsorbents. Bolliger and König[3] described a method in which the decomposition of vitamin D on silica gel layers was minimised by developing the chromatogram immediately after applying the vitamin extract (*i.e.*, while the adsorbent was still saturated with the spotting solvent). Hanewald, Mulder and Keuning[4] reduced the extent of decomposition of vitamin D by adding squalane to the vitamin solution and BHT to the developing solvent, while Ponchon and Fellers[5] used a method in which the vitamin D solution was applied to the adsorbent layer in an atmosphere of nitrogen.

Attempts to apply the published methods were often unsuccessful. None of the chromatographic systems gave a satisfactory separation of all the vitamins and antioxidants, and some decomposition of the vitamin A took place when it was applied to the adsorbent layers, even though the recommended precautions had been taken. The procedures described in this paper enable the vitamins and antioxidants to be applied to silica gel layers without decomposition and to be separated, identified and assayed in a single chromatographic system.

When administered to chicks,[6] vitamin D_2 has only 1 or 2 per cent. of the antirachitic activity of vitamin D_3 and it is therefore essential to ensure that the vitamin D beads used in poultry feed supplements contain vitamin D_3 and not vitamin D_2. The two vitamins have been differentiated by infrared and nuclear magnetic resonance spectroscopy,[2,7] by thin-layer partition chromatography[1] and by the difference in colour of their spots on silica gel plates after spraying with concentrated sulphuric acid.[1] The instrumental methods require the use of large amounts of the vitamins in a pure form and are therefore unsuitable for the examination of formulations. The partition chromatographic and colour-test procedures require less sample and are less sensitive to the presence of impurities, but in our hands they did not prove very reliable. A simple colour test, which can be applied in the presence of many of the compounds likely to occur in formulations, has therefore been developed.

Experimental

Apparatus and reagents—

Chromatographic plates—Prepare Kieselgel (Merck) HF_{254} plates, 20 × 20 cm, with a 0·25-mm adsorbent layer and activate the plates by heating them at 110 °C for 1 hour.

Diethyl ether—Anaesthetic ether B.P.

Chloroform—Re-distil chloroform B.P., collecting the fraction that distils between 60 and 62 °C.

Methanol—Analytical-reagent grade.

Di-n-butyl ether—Shake 2 litres of di-n-butyl ether with 100 ml of 10 per cent. aqueous sodium metabisulphite solution and allow it to stand for 24 hours. Run off the aqueous layer and wash the di-n-butyl ether with two 50-ml volumes of 1 N sodium hydroxide solution and then with water until the washings are neutral to litmus. Dry the di-n-butyl ether with anhydrous sodium sulphate and apply the peroxide test described for anaesthetic ether in the British Pharmacopoeia 1968. If no peroxides are detected, distil the di-n-butyl ether until its volume has been reduced to about 250 ml, collecting the fraction that distils between 138 and 141 °C. Store the di-n-butyl ether under nitrogen in a cool dark place and check that it is peroxide-free immediately before use.

Vitamin and antioxidant standards—Use pure crystalline samples of vitamin A and D with the following activities: vitamins D_2 and D_3, 40 000 000 i.u. g^{-1}; vitamin A (alcohol), 3 300 000 i.u. g^{-1}; vitamin A acetate, 2 900 000 i.u. g^{-1}; and vitamin A palmitate, 1 818 000 i.u. g^{-1}. The BHA and BHT used should comply with the requirements of the British Pharmacopoeia 1968. The other compounds used as standards should be of the highest commercially available quality.

Standard solution of vitamins and antioxidants—Dissolve 5 mg each of vitamin D_3, vitamin A (alcohol), vitamin A acetate, BHA and ethoxyquin, and 10 mg each of α-tocopherol, BHT, vitamin A palmitate and α-tocopheryl acetate, in cyclohexane - triethylamine (9 + 1) and dilute the solution to 20 ml with the same solvent mixture. Store the solution in a refrigerator.

Standard solutions of vitamin A acetate, vitamin A palmitate, vitamin D, BHA, BHT and ethoxyquin—Dissolve 18·75 mg of vitamin D_3 (≡ 750 000 i.u.), 41·2 mg of vitamin A palmitate (≡ 75 000 i.u. of vitamin A), 25·8 mg of vitamin A acetate (≡ 75 000 i.u. of vitamin A), 25 mg of BHA, 25 mg of ethoxyquin and 50 mg of BHT in 50 ml of cyclohexane - triethylamine (9 + 1), and dilute 2, 3, 4, 5 and 6 ml of the solution to 10 ml with the same solvent mixture. Store the solutions in a refrigerator.

Standard solutions of α-tocopherol and α-tocopheryl acetate—Dissolve 25 mg of α-tocopherol and 25 mg of α-tocopheryl acetate in 25 ml of cyclohexane - triethylamine (9 + 1) and dilute 2, 3, 4, 5 and 6 ml of the solution to 10 ml with the same solvent mixture. Store the solutions in a refrigerator.

Iron(III) chloride - potassium hexacyanoferrate(III) reagent—Dissolve 1·3 g of iron(III) chloride ($FeCl_3.6H_2O$) in 100 ml of 2 N hydrochloric acid. Dissolve 0·7 g of potassium hexacyanoferrate(III) in 100 ml of water. Mix equal volumes of the freshly prepared solutions immediately before use.

Iron(III) chloride - potassium hexacyanoferrate(III) reagent (strongly acidic)—Mix two volumes of freshly prepared iron(III) chloride - potassium hexacyanoferrate(III) reagent with one volume of concentrated hydrochloric acid immediately before use.

Iron(III) chloride - bipyridyl reagent—Dissolve 0·5 g of 2,2′-bipyridyl in 100 ml of ethanol. Dissolve 0·2 g of iron(III) chloride ($FeCl_3.6H_2O$) in 100 ml of ethanol. Mix equal volumes of the freshly prepared solutions immediately before use.

Standard vitamin D_2 solution—Dissolve 5 mg of vitamin D_2 (≡ 200 000 i.u.) in 5 ml of chloroform immediately before use.

Standard vitamin D_3 solution—Dissolve 5 mg of vitamin D_3 (≡ 200 000 i.u.) in 5 ml of chloroform immediately before use.

Developing solvent—n-Hexane - ethyl methyl ketone - di-n-butyl ether (34 + 7 + 6).

PROCEDURE FOR THE IDENTIFICATION AND ASSAY OF VITAMINS AND ANTIOXIDANTS (SEE NOTE 1)—

Extraction of vitamins and antioxidants from formulations—For the examination of low-potency vitamin formulations, transfer an amount of sample equivalent to 30 000 i.u. of vitamin D (see Note 2) to a low-actinic glass separator and disperse it in 120 ml of water at a temperature of 70 °C. Cool the mixture to ambient temperature, add 20 ml of methanol and extract the vitamins and antioxidants with 100 ml of diethyl ether, by gently shaking the separator. Allow the mixture to stand until the aqueous and diethyl ether layers have separated, run off the aqueous phase into a second separator and decant the diethyl ether layer, leaving any emulsified material in the first separator. To the residual emulsified material, add 20 ml of methanol, mix and add 100 ml of diethyl ether. Add the mixture to the aqueous phase in the second separator and shake the mixture gently so as to extract the vitamins and antioxidants. Again allow the phases to separate, run off the aqueous layer and decant the diethyl ether phase, adding it to that obtained from the first extraction. Repeat the extraction procedure until no vitamins or antioxidants are detected when the extracts are evaporated to dryness and examined by thin-layer chromatography (three or four extractions should suffice). Wash the bulked diethyl ether extracts by gently shaking them with two successive 20-ml volumes of water. Discard the washings, filter the diethyl ether solution through a cotton-wool plug and evaporate it to dryness under vacuum in a rotary film evaporator with the water-bath at a temperature of 50 °C. Dry the residue by dissolving it in successive 10-ml volumes of acetone and re-evaporating the solutions.

If the volume of the residue from the extraction is negligible, dissolve it in exactly 4 ml of cyclohexane - triethylamine (9 + 1). Otherwise, dissolve the residue in a small volume of cyclohexane and transfer the solution to a 10-ml measuring cylinder, washing out the container with further small volumes of cyclohexane. Evaporate the cyclohexane at 50 °C in a stream of nitrogen, until the volume has been reduced to about 3 ml, then add 0·4 ml of triethylamine and dilute the mixture to 4 ml with cyclohexane.

For the examination of vitamin beads and high-potency formulations, carry out a similar procedure, but reduce the volumes of water, diethyl ether and methanol used in the extraction to one quarter of those specified for low-potency vitamin formulations.

For the examination of solutions of vitamins in oil, dilute the sample with cyclohexane - triethylamine (9 + 1) until the solution contains 7500 i.u. ml^{-1} of vitamin D and apply it directly to a thin-layer plate.

NOTES—

1. The vitamins should be protected from light at all times. Excessive heating and exposure to atmospheric oxidation should also be avoided.

2. The amount of sample to be taken for the assay can be calculated by reference to any of the vitamins or antioxidants, depending on the relative amounts of each present. For most of the samples examined, it has been found best to calculate the amount of sample by reference to the vitamin D content.

Identification of the extracted vitamins and antioxidants—Line a chromatographic tank with filter-paper, pour the developing solvent into the tank, saturating the filter-paper lining, and allow the tank to equilibrate for 30 minutes. Apply 2 μl of the sample solution and 2 μl of the standard solution of vitamins and antioxidants to each of two chromatographic plates and score the surfaces of the plates 15 cm from the spots. Develop the chromatograms in the dark until the solvent fronts reach the scored lines, remove the plates from the tank and allow them to stand in a stream of cold air until most of the solvent has evaporated.

Without delay, spray one of the plates with the strongly acidic iron(III) chloride - potassium hexacyanoferrate(III) reagent and heat it in an oven at 40 °C for 15 minutes.

Inspect the second plate successively in ultraviolet radiation of wavelengths 254 and 360 nm; then spray the plate with iron(III) chloride - bipyridyl reagent and allow it to stand in the dark until the spots reach maximum intensity. Identify the vitamins and antioxidants by comparing the sample and standard chromatograms.

Semi-quantitative assay of vitamins and antioxidants—By diluting suitable volumes of the sample solution with cyclohexane - triethylamine (9 + 1), prepare solutions containing 7500 i.u. ml^{-1} of vitamin D, 750 i.u. ml^{-1} of vitamin A as the acetate or palmitate, 0·5 mg ml^{-1} of BHT, tocopherol and tocopheryl acetate, and 0·25 mg ml^{-1} of BHA and ethoxyquin. Apply 2 μl of the appropriate sample solutions and 2 μl of each of the standard solutions of vitamin A acetate, vitamin A palmitate, vitamin D, BHA, BHT and ethoxyquin to a chromatographic plate and score the surface of the plate 15 cm from the line of spots. Develop the chromatogram, in the dark, in a tank that has been equilibrated with the developing solvent for 30 minutes.

When the solvent front reaches the scored line, remove the plate from the tank and allow it to stand in a stream of cold air until most of the solvent has evaporated. Then, without delay, spray the plate with iron(III) chloride - potassium hexacyanoferrate(III) reagent and assay the vitamins and antioxidants in the sample by comparing the sizes and intensities of the sample spots with the standards, which correspond to 0·6, 0·9, 1·2, 1·5 and 1·8 i.u. of vitamin A as the acetate, 0·6, 0·9, 1·2, 1·5 and 1·8 i.u. of vitamin A as the palmitate, 6, 9, 12, 15 and 18 i.u. of vitamin D, 0·2, 0·3, 0·4, 0·5 and 0·6 μg each of BHA and ethoxyquin, and 0·4, 0·6, 0·8, 1·0 and 1·2 μg of BHT.

For the assay of tocopherol and tocopheryl acetate, apply 2 μl of the appropriate sample solutions and 2 μl of each of the standard solutions of α-tocopherol and α-tocopheryl acetate to a second chromatographic plate and develop the chromatogram as previously described. If tocopherol alone is present, use iron(III) chloride - potassium hexacyanoferrate(III) as the spray reagent; if tocopheryl acetate is present, use the strongly acidic iron(III) chloride - potassium hexacyanoferrate(III) reagent. Assay the tocopherol and tocopheryl acetate in the sample by comparing the sizes and intensities of the sample spots with the standards, which correspond to 0·4, 0·6, 0·8, 1·0 and 1·2 μg each of α-tocopherol and α-tocopheryl acetate.

PROCEDURE FOR DIFFERENTIATING VITAMIN D_2 FROM VITAMIN D_3–

Extract the vitamins and antioxidants from the sample by the method previously described. If the sample contains no vitamin A, dissolve the residue from the extraction in sufficient chloroform to give a theoretical vitamin D content of 40 000 i.u. ml^{-1} and transfer 0·1 ml of this solution and 0·1 ml of each of the standard vitamin D solutions to three test-tubes. Evaporate the solutions to dryness in a stream of nitrogen at room temperature and dissolve the residues in 0·1 ml of glacial acetic acid. To each tube, add 2 ml of 72 per cent. *m/m* perchloric acid solution (see Note 3), mix the solutions well and immediately heat the tubes in a water-bath at 70 °C, with constant agitation, until the colour of the vitamin D_2 standard reaches a maximum (heating for about 60 s will usually suffice). Cool the solutions rapidly, add 1 ml of chloroform to each tube and shake the tubes vigorously. The presence of vitamin D_2 is revealed by the red or purple colour of the chloroform layer; if vitamin D_3 alone is present, the chloroform layer is coloured greenish yellow.

NOTE 3—Perchloric acid should be handled with care. It is a powerful oxidising agent, which may react violently if allowed to come into contact with strong reducing agents.

If the sample contains vitamin A, dissolve the residue from the extraction in sufficient cyclohexane - triethylamine (9 + 1) to give a theoretical vitamin D content of 40 000 i.u. ml^{-1} and apply 0·1 ml of this solution, in the form of a narrow band, to a chromatographic plate. At one end of the band, apply 5 μl of standard vitamin D_2 solution to act as a marker, and score the surface of the plate 15 cm from the band. Develop the chromatogram in the n-hexane - ethyl methyl ketone - di-n-butyl ether (34 + 7 + 6) developing solvent until the solvent front reaches the scored line, remove the plate from the tank and inspect it in ultraviolet radiation of wavelength 254 nm. Score the surface of the plate around the edge of the vitamin D band and, without delay, scrape the enclosed area of silica gel from the plate and transfer it to a small chromatographic column containing 10 ml of chloroform. Elute the vitamin D with chloroform (about 60 ml) and evaporate the eluate to dryness under vacuum in a rotary-film evaporator with the water-bath at a temperature of 50 °C. Dissolve

the residue in a small volume of chloroform and transfer it to a test-tube. Place 0·1 ml of standard vitamin D_2 solution and 0·1 ml of standard vitamin D_3 solution in two other test-tubes and continue by the method previously described, starting from "Evaporate the solutions to dryness in a stream of nitrogen at room temperature. . . ."

EXTRACTION OF VITAMINS AND ANTIOXIDANTS—

Attempts to extract the vitamins and antioxidants from solid formulations by shaking aqueous dispersions of the samples with water-immiscible solvents usually resulted in the formation of emulsions. A preliminary digestion of the samples with trypsin reduced the extent of emulsification caused by gelatin, but did not affect that produced by other constituents, such as wheat meal or maize meal. The most efficient extraction was obtained by using diethyl ether as the extracting solvent and adding small volumes of methanol during the extraction so as to break down emulsified material. Methanol was preferred to ethanol for this purpose as it caused smaller amounts of water and water-soluble impurities to be carried through with the diethyl ether to the final evaporation stage.

The small amount of hydroquinone or propyl gallate present in anaesthetic ether B.P. to prevent peroxide formation does not interfere, either in the chromatographic separation (when it remains at the origin), or in the extraction procedure. It can be removed, if necessary, by passing the solvent through a column of activated alumina.

STABILITY OF VITAMINS A AND D DURING THE CHROMATOGRAPHIC EXAMINATION—

When solutions of vitamin A, vitamin A esters and vitamin D are applied to thin layers of silica gel, rapid decomposition of the vitamins takes place, leading to a reduction in the size and intensity of the vitamin spots and to the appearance of spots corresponding to the decomposition products on the subsequent chromatogram. The addition of a small amount of triethylamine to the spotting solvent suppresses this decomposition and if the vitamins are applied in a cyclohexane - triethylamine (9 + 1) solution, they can remain in contact with the silica gel for up to 10 minutes before development, without any detectable decomposition taking place. The triethylamine remains at the origin during the chromatographic run and does not interfere in the separation.

Although no evidence of decomposition of vitamins A and D during the chromatographic separation has been observed, decomposition can occur when the plates are dried, leading to a reduction in the size and intensity of the vitamin spots. This decomposition is inhibited by ethyl methyl ketone and di-n-butyl ether, and if the plates are allowed to remain moist with developing solvent and the chromatograms are examined as soon as possible after chromatography, no loss of vitamins or antioxidants can be detected.

Bolliger and König's quantitative assay procedure for vitamin D,[3] in which the vitamin is eluted from the silica gel adsorbent after chromatography and assayed colorimetrically by its reaction with antimony trichloride, has been carried out on solutions of pure vitamin D with cyclohexane - triethylamine (9 + 1) as the spotting solvent and hexane - ethyl methyl ketone - di-n-butyl ether (34 + 7 + 6) as the developing solvent. In duplicate determinations, recoveries of 98·5 and 99·5 per cent. of vitamin D were obtained.

IDENTIFICATION AND ASSAY OF VITAMINS AND ANTIOXIDANTS ON THE CHROMATOGRAM—

As a general reagent for the detection of vitamins and antioxidants, the strongly acidic iron(III) chloride - potassium hexacyanoferrate(III) spray reagent is preferred. It reacts with all the compounds under investigation and it is one of the few reagents that will detect tocopheryl acetate without the need for a preliminary hydrolysis. For the assay of the vitamins and antioxidants (except tocopheryl acetate), the less acidic iron(III) chloride - potassium hexacyanoferrate(III) spray reagent is used, because it gives a lower level of background colour on the chromatogram. Plates that have been sprayed with either of the two reagents should be protected from excessive heat and light, which produce dark-coloured backgrounds.

Although the various compounds can usually be identified by their R_F values (see Table I), it may be necessary to confirm the identification by use of the more selective methods of detection and identification indicated in Table II. The identification of vitamin A and its esters can be confirmed by the characteristic greenish yellow fluorescent spots observed when the chromatogram is viewed in ultraviolet radiation of wavelength 360 nm; under these

TABLE I

R_F VALUES OF VITAMINS AND ANTIOXIDANTS

Compound	R_F value	Compound	R_F value
Propyl, octyl and dodecyl gallates	0	Ethoxyquin	0·42
Hydroquinone	0·03	α-Tocopherol	0·51
Vitamin A (alcohol)	0·17	Vitamin A acetate	0·58
Vitamin D_2	0·22	α-Tocopheryl acetate	0·66
Vitamin D_3	0·22	BHT	0·75
Pre-vitamin D_2	0·30	Anhydrovitamin A	0·81
Pre-vitamin D_3	0·30	Vitamin A palmitate	0·85
BHA	0·32	β-Carotene	0·88

conditions, ethoxyquin and anhydrovitamin A give bluish white and orange - brown spots, respectively. The antioxidants and tocopherol are distinguished by the red-coloured spots produced when the chromatogram is sprayed with the iron(III) chloride - bipyridyl reagent. The D vitamins and their thermal isomerisation products, the pre-vitamins (Velluz, Amiard and Petit[8]) are identified by spraying the chromatogram with trichloroacetic acid or antimony trichloride reagent and viewing it in ultraviolet radiation of wavelength 360 nm; all the compounds give greenish yellow fluorescent spots with the trichloroacetic acid reagent and pink fluorescent spots with the antimony trichloride reagent.

If necessary, the selective methods of detection can be used for assay purposes. Because of the possibility of decomposition of the vitamins (particularly vitamin A) in ultraviolet radiation, chromatograms that have been examined by one of the ultraviolet procedures should not subsequently be examined by another method.

TABLE II

APPROXIMATE LIMITS OF DETECTION OF VITAMINS AND ANTIOXIDANTS*

	Method of detection used							
	A	B	C	D	E	F	G	H
Vitamin A (alcohol)	0·05	0·1	0·05	0·05	—	0·05	0·1	0·05
Vitamin D_2	—	0·1	0·05	0·05	—	0·05	0·1	0·05
Vitamin D_3	—	0·1	0·05	0·05	—	0·05	0·1	0·05
BHA	—	—	0·1	0·1	0·25	1·0	—	1·0
Ethoxyquin	0·1	0·25	0·25	0·1	0·5	0·1	—	0·05
Tocopherol	—	—	0·2	0·2	0·4	—	—	—
Vitamin A acetate	0·05	0·25	0·2	0·1	—	0·1	0·25	0·05
Tocopheryl acetate	—	—	—	0·2	—	—	—	—
BHT	—	—	0·2	0·2	0·2	—	—	—
Vitamin A palmitate	0·1	0·25	0·2	0·2	—	0·2	0·5	0·2

A = Inspection in ultraviolet radiation of wavelength 360 nm.
B = Inspection in ultraviolet radiation of wavelength 254 nm.
C = Iron(III) chloride - potassium hexacyanoferrate(III) reagent.
D = Iron(III) chloride - potassium hexacyanoferrate(III) reagent, strongly acidic. Heat at 40 °C for 15 minutes.
E = Iron(III) chloride - bipyridyl reagent.
F = 25 per cent. solution of trichloroacetic acid in chloroform. Heat at 120 °C for 10 minutes and inspect in ultraviolet radiation of wavelength 360 nm.
G = 20 per cent. solution of antimony trichloride in chloroform. Heat at 120 °C for 10 minutes.
H = As for G, but inspect in ultraviolet radiation of wavelength 360 nm.

* The figures show the lowest loading of the compound, expressed in micrograms, that can be detected on the chromatogram. Where no figure is given, the compound is not detected at a level of 2·0 μg.

DIFFERENTIATION OF VITAMIN D_2 FROM VITAMIN D_3—

During attempts to distinguish between vitamins D_2 and D_3 by means of the coloured products obtained when vitamin D reacts with aromatic aldehydes in perchloric acid solution (Schaltegger[9]), the formation of a transient red colour on warming vitamin D_2 with perchloric acid solution was observed. By choosing experimental conditions that favoured this reaction and stabilising the coloured product by extracting it into an organic solvent, a method of distinguishing vitamin D_2 from vitamin D_3 was developed. Tocopherol, tocopheryl

acetate, BHA, BHT, hydroquinone, propyl gallate and ethoxyquin do not interfere, but vitamin A and its esters give an immediate blue - purple colour that rapidly fades to an intense golden yellow colour; any vitamin A (alcohol or ester) present must therefore be separated from the vitamin D by thin-layer chromatography before the colour test is applied. A thin-layer chromatographic separation of the vitamin D may also be necessary if the sample extract contains organic matter that chars badly on heating with perchloric acid. Pre-vitamin D_2 gives the same response as vitamin D_2. Vitamin D_3 and pre-vitamin D_3 give greenish yellow colours.

Results and discussion

Recovery experiments were carried out by submitting a mixture of vitamins and antioxidants to the assay procedure, alone and in the presence of the appropriate amounts of gelatin, arachis oil and a mixture of gelatin, limestone, wheat meal, maize meal and B-group vitamins. A loading on the chromatographic plate of 15 i.u. of vitamin D, 1·5 i.u. of vitamin A as the acetate, 1·5 i.u. of vitamin A as the palmitate, 1 μg each of BHT, tocopherol and tocopheryl acetate, and 0·5 μg each of BHA and ethoxyquin was used and the results were assessed independently by four analysts. Mean recoveries for the individual compounds varied between 94 and 99 per cent. of those required by theory, depending on the compound and the type of formulation examined, with results from individual operators falling within ± 10 per cent. of the means. No difference in response between vitamin D_2 and vitamin D_3 could be detected and the same standard solutions (which contain vitamin D_3) can be used for the assay of both compounds.

The recommended procedures were applied to manufactured batches of several different vitamin preparations; the results obtained are shown in Tables III and IV. Except in one instance (the assay of vitamin D in a multivitamin syrup), no significant interference from other constituents of the formulations was encountered.

In the samples examined, BHA and BHT were the antioxidants most generally used. Ethoxyquin was detected only in preparations destined for use in animal feeds; with minor exceptions, its use in foodstuffs is prohibited.[10] Propyl, octyl and dodecyl gallates were not detected in any of the samples. In some vitamin A concentrates, BHT was detected at levels that were too low for it to have any significant antioxidant effect; in such samples, the BHT is probably derived from the vitamin A, which may contain small amounts introduced during the manufacturing process.

Samples that had been stored for long periods were found to contain small amounts of unidentified impurities, apparently produced by decomposition of the vitamins and antioxidants. In samples containing vitamin A esters, compounds with R_F values of 0·43 (from vitamin A acetate) and 0·92 (from vitamin A palmitate) were detected. A compound with an R_F value of 0·65 was detected in samples containing ethoxyquin, and pre-vitamin D was found in some samples that contained vitamin D. Fortunately, the decomposition products either do not interfere in the assay, or the interference can be eliminated by the use of a selective method of detection. The impurities were shown to be decomposition products by dissolving the individual vitamins and antioxidants in hexane, exposing the solutions to ultraviolet radiation and examining the degradation products; the same impurities were detected in the artificially degraded vitamin A acetate, vitamin A palmitate, vitamin D and ethoxyquin as had been detected in the stored samples.

The recommended assay procedure was applied to the determination of vitamin A in halibut-liver oil with synthetic vitamin A palmitate as the standard. Although the vitamin A in fish-liver oils is present as a mixture of long-chain fatty esters of the all-*trans*-, 13-*cis*-, 9-*cis*- and 9,13-di-*cis*-isomers of vitamin A,[11] no separation of the isomers occurred and the single spot of vitamin A ester obtained from the halibut-liver oil was similar in appearance to and had the same R_F value as that obtained from the vitamin A palmitate. The quantitative results obtained by using iron(III) chloride - potassium hexacyanoferrate(III) reagent and ultraviolet radiation as the methods of detection, were comparable with those obtained by the official method of assay of the British Pharmacopoeia.[12] Attempts to ascertain the vitamin A content of halibut-liver oil by hydrolysing the sample and determining the liberated vitamin A (alcohol) by thin-layer chromatography were unsuccessful, because two hydrolysis products, with R_F values of 0·17 and 0·21, were obtained, which may correspond to mixtures of different isomeric forms of the vitamin.

TABLE III

VITAMIN AND ANTIOXIDANT CONTENTS OF COMMERCIAL SAMPLES (SOLIDS)

Sample*	Vitamin D/ i.u. g^{-1}	Vitamin A (as acetate)/i.u. g^{-1}	Vitamin A (as palmitate)/i.u. g^{-1}	BHA, per cent.	BHT, per cent.	Ethoxyquin, per cent.	Tocopherol, per cent.	Tocopheryl acetate, per cent.
Gelatin-protected beads								
Vitamin A beads—								
Vitamin A, 325 000 i.u. g^{-1}		340 000			9			
		340 000	17 000		9			
		338 000			0·4	0·75		
Vitamin A, 500 000 i.u. g^{-1}		545 000			Trace	6		
		465 000			0·65			
		400 000		0·65	1·3			
Vitamin D_2 beads—								
Vitamin D, 850 000 i.u. g^{-1}	1 150 000			1·6	1·4			
	450 000						0·35	
Vitamin D_3 beads—								
Vitamin D, 400 000 i.u. g^{-1}	430 000			0·38				
	410 000	5400		0·32				
Vitamin E acetate beads—								
Tocopheryl acetate, 25 per cent. ..								24
Vitamin A and D_3 beads—								
Vitamin A, 325 000 i.u. g^{-1}	105 000	330 000	13 000		9			
Vitamin D, 108 000 i.u. g^{-1}	102 000	340 000			0·6	0·37		
Vitamin A, 500 000 i.u. g^{-1}	144 000	630 000				6		
Vitamin D, 125 000 i.u. g^{-1}	125 000	580 000			3·2			
	130 000	320 000	200 000		0·5	9		
Animal feed additives								
Formula 1—								
Vitamin A, 9640 i.u. g^{-1}								
Vitamin D, 2372 i.u. g^{-1}								
Vitamin E, 0·43 per cent. with B-group vitamins and acetomenaphthone in a wheat meal and limestone base	2600	9800	450		0·25			0·43
	2300	10 000	450		0·25			0·42
	2600	9900	450		0·25			0·46
Formula 2—								
Vitamin A, 15 120 i.u. g^{-1}								
Vitamin D, 3770 i.u. g^{-1} with B-group vitamins in a limestone base	4300	15 700	680		0·21			
	4400	15 700	730		0·35			

TABLE III—*continued*

Sample*	Vitamin D/ i.u. g^{-1}	Vitamin A (as acetate)/i.u. g^{-1}	Vitamin A (as palmitate)/i.u. g^{-1}	BHA, per cent.	BHT, per cent.	Ethoxyquin, per cent.	Tocopherol, per cent.	Tocopheryl acetate, per cent.
Formula 3—								
Vitamin A, 9600 i.u. g^{-1}	2550	7200	2900			0·12		
Vitamin D, 2350 i.u. g^{-1} with riboflavine in a maize meal base	2600	7400	2900			0·13		
Formula 4—								
As for formula 3, but in a limestone base	2300	9400				0·09		
	2500	9100				0·10		
Vitamin tablets								
Calciferol Tablets, Strong, B.P.—								
Vitamin D, 152 000 i.u. g^{-1}	150 000							
	150 000							
Calcium with Vitamin D Tablets, B.P.C.—								
Vitamin D, 1000 i.u. g^{-1}	990			Trace	Trace			
	1050			Trace	Trace			
Vitamin A, D and C pellets—								
Vitamin A, 11 100 i.u. g^{-1}	1350		10 900	Trace	Trace			
Vitamin D, 1460 i.u. g^{-1} with vitamin C in a sugar base	1380		11 600	Trace	Trace			
	1410		11 100	Trace	Trace			
Multivitamin pellets—								
Vitamin A, 7300 i.u. g^{-1}								
Vitamin D, 590 i.u. g^{-1} with B-group vitamins, vitamin C and trace metals in a sugar-coated chocolate base	570		7950	Trace	Trace			
	625		7350	Trace	Trace			
	600		7200	Trace	Trace			
Syrup								
Multivitamin syrup†—								
Vitamin A, 750 i.u. g^{-1}								
Vitamin D, 75 i.u. g^{-1} with B-group vitamins and vitamin C in a syrup base	40		750	Trace	Trace			
	67		690	Trace	Trace			

* The figures quoted for vitamin concentrates are those declared by the manufacturer; the figures quoted for formulations are based on the declared vitamin content of the concentrate used in their preparation. Vitamin concentrates usually contain an excess of the vitamins to allow for decomposition on storage.

† Because of solubility difficulties, acetone - triethylamine (9 + 1) was used as the spotting solvent for this formulation. The vitamin D was assayed quantitatively by Bolliger and König's procedure,[3] but with acetone - triethylamine (9 + 1) as the spotting solvent and hexane - ethyl methyl ketone - di-n-butyl ether (15 + 5 + 3) as the developing solvent; the vitamin D could not be assayed by visual comparison with standards because of distortion of the vitamin D spot.

TABLE IV

VITAMIN AND ANTIOXIDANT CONTENTS OF COMMERCIAL SAMPLES (OILS)

Sample*	Vitamin A (as palmitate)/ i.u. g^{-1}	Vitamin A (B.P. assay[12])/ i.u. g^{-1}	Vitamin D/ i.u. g^{-1}	BHA, per cent.	BHT, per cent.	Tocopherol, per cent.
Vitamin A palmitate solution—						
Vitamin A, 1 000 000 i.u. g^{-1}	1 030 000	1 015 000		0·48	Trace	
	1 120 000	1 068 000		0·51	Trace	
	980 000	1 010 000				0·7
Vitamin A and D concentrate—						
Vitamin A, 500 000 i.u. g^{-1} }	540 000	498 000	57 000	0·32	Trace	
Vitamin D, 50 000 i.u. g^{-1} }	525 000	507 000	57 000	0·30	Trace	
Halibut-liver oil†	24 800	22 800	Not determined			0·17
	29 700	29 500	Not determined			0·18
	26 400	25 200	Not determined			0·20
	22 500	22 400	Not determined			0·17

* As for Table III.

† Vitamin D is present in this material at too low a level to be assayed by the recommended procedure. The tocopherol was assayed by applying an increased loading (5 μl) of sample and standard solutions to the chromatographic plate and using iron(III) chloride - bipyridyl reagent for detection.

The procedures described in this paper have been successfully applied to the analytical control of vitamins and antioxidants in vitamin concentrates and formulated products. Because of their speed and simplicity, they enable a comprehensive control of manufacturing procedures to be maintained at minimum cost.

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Gas - Liquid Chromatographic Determination of Vitamin D in Cod-liver Oil

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A gas - liquid chromatographic method for the determination of vitamin D (cholecalciferol) in cod-liver oil is described. It involves saponification of the oil, extraction of the unsaponifiable matter, removal of interferences such as cholesterol and retinol (vitamin A) by precipitation, and column chromatography on Sephadex LH-20 and Florisil; the final determination of cholecalciferol is carried out by gas - liquid chromatography. A determination can be completed in less than 2 days.

EXISTING methods for the determination of vitamin D in foodstuffs and natural products are not entirely satisfactory. Biological assays with rats and chickens are time consuming, expensive and lack precision; chemical methods are insufficiently sensitive and may be seriously affected by the presence of vitamin A and cholesterol, both of which frequently occur with vitamin D and often in considerable excess. The well known reaction between vitamin D and antimony trichloride suffers also from the disadvantages that the resultant coloured product is highly sensitive to trace amounts of moisture and its intensity varies with time.

Of the physicochemical methods, ultraviolet spectroscopy can detect as little as 2 μg of calciferol by absorption measurement at wavelength 265 nm, but many other substances absorb in this region of the spectrum and effectively restrict the value of the technique to the determination of vitamin D in concentrates. Infrared spectrophotometry has been used to distinguish ergocalciferol from cholecalciferol on the basis of the additional double bond present in the former, but for quantitative work the technique lacks sensitivity, 250 μg of the vitamin being the minimum amount required. In 1960, the application of gas - liquid chromatography to vitamin D determinations gave the first indication that the use of more sensitive methods was possible[1] and, in 1966, Murray, Day and Kodicek[2] made a major advance by converting ergocalciferol and cholecalciferol into their isovitamins by treatment with antimony trichloride reagent in order to obtain single separate peaks that could be distinguished from those of small amounts of cholesterol. About this time, a need arose in our laboratory for a chemical method of determining vitamin D in products such as cod-liver oil and pharmaceutical preparations. This paper describes the development of a gas - liquid chromatographic method for determining vitamin D in cod-liver oil by saponification and extraction of unsaponifiable matter; removal of vitamin A and other interferences in two stages by partition and adsorption chromatography; precipitation of cholesterol; formation of trimethylsilyl derivatives of the isovitamins; and gas - liquid chromatography. Ergocalciferol is used as an internal standard in the method as vitamin D is present as cholecalciferol in cod-liver oil.

EXPERIMENTAL

REAGENTS AND MATERIALS—

All reagents should be of analytical-reagent quality unless otherwise stated.

Digitonin B.P.—The material supplied by Koch-Light has been found to be suitable.

Sephadex LH-20—Supplied by Pharmacia, Uppsala, Sweden.

Florisil, 60 to 100 *mesh.*

Antimony trichloride solution, 20 *per cent.* m/V *in chloroform.*

Tartaric acid solution, 40 *per cent.* m/V.

Bis(trimethylsilyl)acetamide—Supplied by Phase Separations, Queensferry, Flintshire.

Ergocalciferol (*vitamin* D_2), *pure.*

Cholecalciferol (*vitamin* D_3), *pure.*

5α-*Cholestane solution*—Dissolve 1 mg of pure 5α-cholestane in 100 ml of chloroform.

APPARATUS—

Glass columns for chromatography—These were 1·5 and 2 cm in diameter, and of minimum length 40 cm, each fitted with a sintered-glass disc of porosity 2, Quickfit socket and PTFE key.

Gas chromatograph—Pye, Model 104, with flame-ionisation detector and 2·7 m × 3 mm i.d. glass columns packed with 3 per cent. OV-17 on Gas-Chrom Q, 100 to 120 mesh.

Recorder—Honeywell Electronik 19, 1-mV input, fitted with a disc integrator.

PREPARATION OF CHROMATOGRAPHIC COLUMNS—

Sephadex LH-20 partition column—A 90 + 10 methanol - water mixture was used as the stationary phase and 2,2,4-trimethylpentane as the mobile phase. Saturate each solvent with the other by shaking equal volumes together for 1 minute and then store them separately. Allow about 100 g of Sephadex LH-20 to swell in 90 per cent. methanol for at least 24 hours before use. During this period decant the supernatant liquor at least twice, re-shaking with fresh 90 per cent. methanol each time. Fit an extension column to the top of a 40 × 2-cm glass column and add the Sephadex slurry to a height of twice that of the column required, *e.g.*, to 70 cm for a final column height of 35 cm. Open the tap at the bottom of the column and allow the Sephadex to pack by settling. When the flow from the column drops to the rate of 1 ml min^{-1}, close the tap, wash the sides of the extension column with 90 per cent. methanol and allow the Sephadex to settle by gravity until the supernatant liquid is completely clear. After opening the tap, apply pressure to the top of the column extension by means of a blowball and gently compress the Sephadex until no noticeable further reduction in column height is observed. Adjustments to the height of the column can be made by adding or withdrawing slurry but at all times the Sephadex must be kept covered with liquid. Pass at least one bed volume of 2,2,4-trimethylpentane through the Sephadex, after which the column is ready for use. Remove the extension tube for sample addition and replace it for elution.

Florisil column—Heat 210 to 220 g of Florisil for 3 hours at 160 °C. Cool and weigh 200 g into a clean, dry, screw-capped bottle, add 20 ml of water and shake the mixture vigorously until it is thoroughly mixed and the powder is free flowing. Place the bottle on a roller mixer for 2 to 3 hours and allow the bottle to stand overnight. Meanwhile, prepare the eluting agent, a 1 + 4 mixture of diethyl ether and 2,2,4-trimethylpentane, add anhydrous sodium sulphate, shake the mixture and allow it to stand overnight in a stoppered flask. Fill a column, 40 × 1·5 cm, about half-full with 2,2,4-trimethylpentane. Weigh 30 g of the prepared Florisil into a 100-ml separating funnel that has been fitted with a cone and connected to the top of the column. Open the tap of the separating funnel and allow the Florisil to run smoothly into the solvent in the column, tapping the side of the column occasionally to ensure even packing of the Florisil and the release of any entrained air bubbles. As the packing proceeds it may be necessary to run off some of the 2,2,4-trimethylpentane from the column so as to prevent the liquid level from reaching the stem of the separating funnel. When all of the Florisil has been added, remove the separating funnel and add a 2-cm layer of anhydrous sodium sulphate to the top of the column. Lower the liquid level in the column until it just covers the solid material. The column is now ready for use.

CALIBRATION OF COLUMNS—

Determine the eluting characteristics of both the Sephadex and Florisil columns before use by transferring approximately 100 μg of vitamin D in the appropriate solvent to the top of each column and collecting fractions of the eluate. For the Sephadex column, collect 5-ml fractions after the appearance of the solvent front by using a flow-rate of approximately 30 to 40 ml h^{-1}; for the Florisil column collect 20-ml fractions at a flow-rate of approximately 60 to 80 ml h^{-1}. All the fractions are scanned on an ultraviolet spectrophotometer at 265 nm to locate the vitamin D peak and thus obtain the eluting position of vitamin D for each column. On Sephadex, vitamin D is normally found in the 20 to 50-ml fraction; on Florisil, the 140 to 210-ml fraction usually contains the vitamin D.

PROCEDURE

Carry out all operations in artificial light.

SAPONIFICATION AND EXTRACTION—

Weigh 20 g of potassium hydroxide pellets into a 250-ml twin-necked round-bottomed flask. Add about 10 ml of water followed by 140 ml of absolute ethanol, shake the mixture and warm it until the solid is completely dissolved. Prepare a solution of ergocalciferol in absolute ethanol, 1 ml of which is equivalent to 50 μg of the vitamin. Add 2 ml of this solution to the flask as an internal standard followed immediately by 50 g of cod-liver oil. Reflux the mixture for 15 minutes while bubbling a slow stream of nitrogen through the liquid, then add 25 ml of water and cool to room temperature as quickly as possible. Transfer the contents of the flask to a 1000-ml separating funnel, dilute to 600 ml with water and add immediately 400 ml of a mixture of equal volumes of diethyl ether and light petroleum (boiling range 40 to 60 °C). Shake the mixture thoroughly for 2 minutes. Allow the phases to separate completely, approximately 10 minutes being required, and run the aqueous layer into a second 1000-ml separating funnel. Repeat the extraction with a further 200 ml of the solvent mixture, discarding the aqueous layer and adding the solvent layer to the first separating funnel. Wash the combined solvent extracts twice by spraying with 100 ml of water from a wash-bottle. Do not shake the separating funnel but when the phases have separated discard the aqueous layer. Add 250 ml of water to the separating funnel, shake it gently, allow the phases to separate and discard the washings.

Repeat this procedure twice more, shaking the funnel vigorously on the last occasion. At this stage the layers should be clear and completely separated. If not, repeat with a further washing. Run the solvent layer into a large rotary evaporating flask, add 20 ml of absolute ethanol and proceed to remove the solvent under low pressure. At the first sign of cloudiness or precipitation add more absolute ethanol (25 to 50 ml) and evaporate to a volume of about 10 ml, then transfer the solution to a smaller flask and remove all of the solvent.

PRECIPITATION OF STEROLS—

Dissolve the residue in the flask with small amounts of warm methanol and transfer the liquid to a 10-ml graduated centrifuge tube. Cool it at 0 °C for 30 minutes, centrifuge it at 1000 to 2000 r.p.m. for 5 minutes and return the supernatant liquor to the evaporating flask. Remove the solvent, add a few millilitres of 2,2,4-trimethylpentane and evaporate again. Dissolve the residue in 2 ml of 2,2,4-trimethylpentane.

SEPHADEX COLUMN CHROMATOGRAPHY—

With a pipette, transfer the extract of vitamin D on to the prepared Sephadex column, rinsing the flask with 2 ml of 2,2,4-trimethylpentane, and elute with solvent at the rate of approximately 20 to 40 ml h^{-1}, collecting the fraction that contains vitamin D. Add a few millilitres of ethanol and evaporate to dryness on a rotary evaporator.

REMOVAL OF STEROLS AS DIGITONIDES—

Dissolve the residue in methanol, transfer the solution to a 10-ml centrifuge tube and dilute it to 9 ml. Add 1 ml of water, shake the mixture vigorously, cool it to 0 °C and allow it to stand for at least 30 minutes. Centrifuge it and pour the supernatant liquor into a 30-ml centrifuge tube containing a digitonin solution prepared by dissolving 0·4 g of digitonin in 10 ml of 90 + 10 methanol - water mixture. Stopper the centrifuge tube, shake it and allow it to stand overnight in a refrigerator. Centrifuge and decant the supernatant liquor through a Whatman No. 541 filter-paper into a 50-ml separating funnel. Shake the precipitate of digitonides with 10 ml of the methanol - water mixture, centrifuge the mixture and use the supernatant liquor to wash the filter-paper. Extract the liquid in the separating funnel with two 15-ml portions of carbon tetrachloride, collecting the extract in an evaporating flask. Remove the solvent by rotary evaporation and repeat the evaporation after the addition of a few millilitres of 2,2,4-trimethylpentane.

FLORISIL COLUMN CHROMATOGRAPHY—

Dissolve the residue in 2 ml of 1 + 4 diethyl ether - 2,2,4-trimethylpentane and transfer the extract to the prepared Florisil column. Elute with the solvent mixture at a flow-rate of 60 to 80 ml h^{-1} and collect the fraction containing vitamin D.

PREPARATION OF THE ISOVITAMINS AND FORMATION OF THE TRIMETHYLSILYL ETHERS—

Remove the solvent on a rotary evaporator and dissolve the residue in 1 ml of the 5α-cholestane solution. Add 4 ml of the antimony trichloride solution, shake the mixture and allow it to stand for exactly 1 minute, then add 6 ml of tartaric acid solution, shake the mixture vigorously and transfer it to a 25-ml separating funnel. Rinse the flask with 10 ml of light petroleum (boiling range 40 to 60 °C), add it to the separating funnel and shake the funnel for about 15 s. Discard the lower layer and wash the light petroleum layer three times with an equal volume of water. Filter the light petroleum through a Whatman No. 541 filter-paper into a small flask, wash the paper with a small volume of solvent and evaporate to dryness. Add 0·1 ml of bis(trimethylsilyl)acetamide to the residue and allow it to stand for 10 to 15 minutes. The sample is now ready for injection on to the gas chromatograph as described below. The operating conditions of the gas chromatograph were: column oven temperature, 235 °C; detector oven temperature, 250 °C; injection block temperature, 300 °C; carrier gas, nitrogen at the rate of 50 ml min^{-1}, hydrogen at the rate of 50 ml min^{-1} and air at the rate of 500 ml min^{-1}. The recorder chart speed was 2 min cm^{-1}.

CALIBRATION OF THE GAS CHROMATOGRAPH—

Prepare a standard mixture containing 100 μg of ergocalciferol, 100 μg of cholecalciferol and 10 μg of 5α-cholestane, isomerise the vitamins and prepare teh trimethylsilyl ethers as previously described. Inject between 1 and 2 μl of this solution on to the gas chromatograph and adjust the sensitivity of the instrument so that responses of the vitamin are between half and full-scale deflection on the recorder. Calculate the areas under the ergocalciferol and cholecalciferol peaks and the retention times of the vitamins relative to 5α-cholestane. The approximate retention times for these conditions are 21 minutes for 5α-cholestane and 46, 61 and 68 minutes for the trimethylsilyl ethers of cholesterol, cholecalciferol isovitamin and ergocalciferol isovitamin, respectively.

Inject the sample in the same way, measure the areas under the ergocalciferol and cholecalciferol peaks and calculate the amount of cholecalciferol present from the known addition of ergocalciferol and the ratio of the areas obtained from equal masses of the two forms of the vitamin.

CALCULATION—

$$\text{Cholecalciferol (vitamin } D_3)/\mu g\ g^{-1} = \frac{\text{Peak area of cholecalciferol} \times \text{ergocalciferol added } (\mu g)}{\text{Peak area of ergocalciferol} \times R \times \text{mass of sample (g)}}$$

where R is the ratio of the peak areas of cholecalciferol to ergocalciferol for equal masses of these substances.

RESULTS AND DISCUSSION

Saponification losses of vitamin D are minimised by heating the oil with a fairly concentrated ethanolic solution of potassium hydroxide for a short period and by bubbling nitrogen gently through the liquid. Extraction of the unsaponifiable matter with a 1 + 1 mixture of diethyl ether and light petroleum gives a cleaner separation than with diethyl ether alone.

Most procedures for the removal of interfering substances involve the use of one or more adsorption columns of alumina, magnesium oxide or silicic acid. Celite impregnated with poly(ethylene glycol)[3] and, more recently, Fluoropak 80[4] have also been used. Other systems rely on the conversion of retinol (vitamin A) into a derivative that is more readily separated from vitamin D than the parent compound.[5,6] In the proposed method, vitamin D is well separated from retinol and some other interfering substances on a partition column of Sephadex LH-20.[7] This technique has the advantage that such columns are easy to handle and can. be readily regenerated. Separation of vitamin D from cholesterol is, however, incomplete and the residual sterol is removed by precipitation with digitonin before a final treatment on a column of Florisil to remove anhydrovitamin A and other unknown interfering compounds. By this means cholesterol present in cod-liver oil can be reduced to manageable proportions but, as shown in the chromatogram of a typical cod-liver oil in Fig. 1, a small amount still remains.

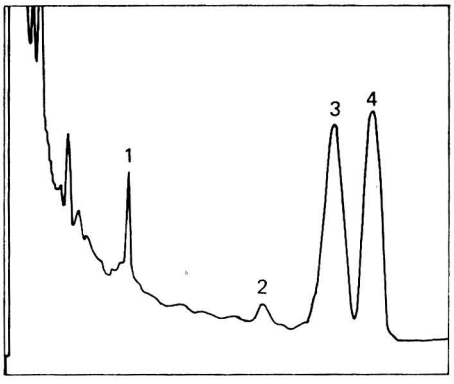

Fig. 1. Gas - liquid chromatogram of cholecalciferol in cod-liver oil: peak 1, added 5α-cholestane; peak 2, cholesterol trimethylsilyl ether; peak 3, cholecalciferol isovitamin trimethylsilyl ether; and peak 4, added ergocalciferol isovitamin trimethylsilyl ether

In our early work, a sensitivity of 1 μg of vitamin D was obtained on the gas chromatograph when using glass columns containing Chromosorb G coated with 2·5 per cent. silicone elastomer 30. Later, increased sensitivity and superior separations were obtained with column supports and stationary phases specially designed for steroid analysis, such as 3 per cent. OV-17 on 100 to 120-mesh Gas-Chrom Q. Under these conditions, less than 0·1 μg of vitamin D can easily be determined by using a flame-ionisation detector.

TABLE I

RESULTS OF GAS - LIQUID CHROMATOGRAPHIC DETERMINATION OF CHOLECALCIFEROL IN REFINED COD-LIVER OILS

Sample designation	A	B	C	D	E	F	G	H	I	J
Cholecalciferol/μg g^{-1}	2·8, 3·2	2·5, 2·8	2·9, 3·5	2·7, 3·3	3·5, 3·5	3·3, 3·5	3·3, 3·5	3·9, 3·9	3·7, 3·5	3·4, 3·8

Tables I, II and III show that the proposed method gives repeatable results that are in reasonable agreement with those given by the standard bioassay method. The procedure, which can be completed in less than 2 days, is suitable for the quality control of the vitamin D content of cod-liver oil.

TABLE II

CHOLECALCIFEROL CONTENT OF SOME COD-LIVER OILS: COMPARISON OF RESULTS BY THE BIOLOGICAL RAT ASSAY AND GAS - LIQUID CHROMATOGRAPHIC METHODS

		Cholecalciferol content				
		Bioassay		Gas - liquid chromatography		
Type of oil	Fiducial range/ i.u. g^{-1}	μg g^{-1}	i.u. g^{-1}	μg g^{-1}	i.u. g^{-1}	Mean difference, per cent.
High potency	309 to 466	9·6	382	9·8	393	+6
				10·2	408	
				10·2	409	
				10·2	410	
Low potency	40 to 68	1·3	52	1·2	49	−6
Veterinary*	56 to 99	1·9	76	1·8	73	0
	62 to 95	1·9	77	2·0	80	
	64 to 93	2·0	78	2·1	83	
	66 to 102	2·1	83	—	—	
Medicinal	72 to 100	2·1	85	2·3	92	+8

*Prepared by adding to the low-potency oil 0·75 μg (30 i.u.) of cholecalciferol per gram.

TABLE III

CHOLECALCIFEROL CONTENT OF REFINED COD-LIVER OILS: COMPARISON OF RESULTS BY THE BIOLOGICAL RAT ASSAY AND GAS - LIQUID CHROMATOGRAPHIC METHODS

	Cholecalciferol content					
		Bioassay		Gas - liquid chromatography		
Code number	Fiducial range/ i.u. per fl. oz.	$\mu g\ g^{-1}$	i.u. per fl. oz.	$\mu g\ g^{-1}$	i.u. per fl. oz.	Difference, per cent.
91684	2229 to 3170	2·6	2733	2·9	3067	+12
92485	3232 to 4322	3·6	3758	3·0	3090	−18
94787	2377 to 5250	3·4	3548	3·4	3523	−1
94787	2377 to 5250	3·4	3548	3·4	3500	−1
93687	2377 to 5250	3·4	3548	3·6	3772	+6
00588	2212 to 4931	3·5	3626	3·5	3681	+2
00788	2212 to 4931	3·5	3626	3·6	3726	+3
00788	2212 to 4931	3·5	3626	3·3	3480	−4
01889	2207 to 3394	2·6	2759	3·1	3249	+17
01889	2207 to 3394	2·6	2759	2·9	2999	+9

Our thanks are due to the Marfleet Refining Company for the provision of samples of cod-liver oil and for determining their vitamin D content by the bioassay procedure of the British Pharmacopoeia. This paper is published with the permission of the Government Chemist.

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The Determination of Trace Amounts of Cobalt and Other Metals in High-purity Water by Using Ion-exchange Membranes

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Microgram amounts of cobalt, chromium, copper, iron, nickel and zinc are concentrated on ion-exchange resin impregnated membranes from large volumes of reactor cooling waters. Atomic-absorption measurement on the acid-extracted membranes has permitted the determination of cobalt down to 0·01 μg l^{-1} in water samples, with an analytical precision of $\pm$12 per cent. at the 96 per cent. confidence limit, based on twelve replicate observations. Gamma-spectrometric measurement of the nuclide cobalt-60 present in the reactor cooling waters has enabled membrane efficiency to be determined; at cobalt levels of 0·01 and 0·1 μg l^{-1}, the efficiencies are shown to be 85 per cent. and greater than 99 per cent., respectively.

The determination of trace metals, nuclides and insoluble material in cooling waters from the Steam Generating Heavy Water Reactor (SGHWR) is required in order to make an assessment of corrosion, erosion and re-deposition rates in the cooling circuits. Continuous methods of analysis currently in use for the determination of iron[1] and copper[2] are capable of achieving a limit of detection of approximately 1 μg l^{-1} (p.p.b.). Determinations of cobalt, nickel and zinc are, however, required at levels considerably lower than this concentration. The presence of trace amounts of cobalt in the cooling circuits is of particular interest as it is a source of the long-lived nuclide cobalt-60, which contributes significantly to radiation doses received during reactor maintenance.

Various solvent-extraction methods applied to samples of relatively small volume, and semi-automated on-line techniques, were found to be incapable of determining the low trace amounts of metals in some reactor cooling waters. Trials with small columns packed with powdered cation-exchange resins and chelating resins showed a high retention of metals at the 100 μg l^{-1} level. Schulek, Remport-Horváth, Lásztity and Körös[3] described the use of specially prepared carboxycellulose as a collector for nanogram amounts of metals in water. The use of columns of ion-exchange resins or carboxycellulose on reactor cooling water sampling points, however, proved difficult to operate and maintain. Blaedel and Haupert[4] reviewed the analytical potentialities of ion-exchange membranes for the extraction and concentration of ionic species; by using radioactive cationic tracers they showed that 100-fold enrichment in concentration could be achieved. Campbell, Spano and Green[5] studied the characteristics of cation and anion-exchange resin loaded paper discs by X-ray fluorescence. They showed that greater than 99 per cent. retention of cations such as Ca^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+} and Zn^{2+} was attained at the 100-μg level in 50-ml volumes of solution. A method for the determination of cobalt down to 0·05 μg l^{-1} has been described by Batley.[6] This method is based on the oxidation of alizarin red S with hydrogen peroxide, which is catalysed by trace amounts of cobalt, and was developed specifically for the determination of cobalt in reactor cooling water circuits. However, from experimental data provided on the levels of iron throughout SGHWR cooling water circuits, and from examinations of the amount of cobalt present in associated insoluble materials, it was calculated that the levels of cobalt in some circuits would be considerably less than the detection limit of 0·05 μg l^{-1} of cobalt given by the catalytic method. From initial trials it was considered that ion-exchange membranes would provide the most convenient method of sample concentration. This paper describes the investigations and development of the use of ion-exchange membranes for the quantitative collection and subsequent analysis of microgram amounts of cationic impurities obtained from large volume-integrated samples.

Cation-exchange and anion-exchange resin impregnated membranes (trade name Acropor) were obtained from Gelman-Hawksley Limited. The Acropor membranes, 47 mm in diameter, incorporate finely divided ion-exchange resin into a structure of acrylonitrile - poly(vinyl chloride) copolymer reinforced with nylon. Dispersion of the finely divided resin through the membrane produced a more effective surface area per unit mass of resin than is available with a column of powdered ion-exchange resin. Acropor membranes have a mass of approximately 100 mg per disc, of which the ion-exchange resin constitutes about 20 per cent. *m/m*. Ion-exchange capacity is claimed to be from 0·05 to 0·1 mg-equiv per membrane, and the membranes are effective up to a working temperature of 100 °C, or 125 °C for short periods. Two types of cation-exchange membrane are available at present: Acropor, Type SA, is a strongly acidic cation-exchange membrane containing sulphonic acid groups; and Acropor, Type CH, is impregnated with chelating resin containing iminodiacetate groups with a high preference for iron, copper and other heavy metals. In early laboratory trials it was found that a greater pressure was necessary to obtain a flow through the chelating membrane than for the strongly acidic cation-exchange membrane; also, the inherent cobalt content was greater in the chelating membrane. For these reasons further investigations were conducted by using the Acropor, Type SA, cation-exchange membranes.

Anion-exchange membranes, Acropor, Type SB, are impregnated with strongly basic anion-exchange resin containing quaternary ammonium groups. Attempts to regenerate these membranes to the hydroxide form for use as collectors of trace amounts of chloride have shown little success. However, they have proved useful for the collection and determination of trace amounts of sulphate and nitrate in water.

Because of the extremely fine filtration properties of Acropor membranes, it is necessary to introduce a pre-filter so as to prevent blockage of the membrane. The filtration of insoluble species in SGHWR cooling waters has been standardised by retention of the species on microporous filters of 0·45-μm pore size. It was found to be essential to incorporate this type of pre-filter immediately before the ion-exchange membrane. The retentive capacity of the microporous pre-filter for soluble cationic species has not been investigated, but is expected to be very low.

Experimental

Preliminary tests—

Flow-rates of the capillary sampling lines on the reactor cooling water circuits vary from 10 to 50 ml min^{-1}. For simulation purposes, a mock solution containing 100 μg each of cobalt, copper, iron, nickel and zinc in a total volume of 3 litres of distilled de-ionised water was passed at the rate of 17 ml min^{-1} through a single Acropor cation-exchange membrane protected by a microporous pre-filter. The mock solution (pH 5) of the mixed metal ions was prepared by dilution of weakly acidic standard solutions of the individual metal ions. A peristaltic pump was used to deliver the 3 litres of solution to the pre-filter membrane held in a 47 mm diameter filter-funnel. Liquid was drawn through the membrane assembly under gentle suction. The pre-filter and Acropor membrane were together digested with 5 ml of analytical-reagent grade concentrated hydrochloric acid. It was found that the nylon support fabric of the Acropor membrane was unaffected by this treatment; the copolymer and ion-exchange resin had been disrupted, giving a finely divided suspension in solution. Filtration of the solution through a small (25 mm diameter) microporous filter removed the nylon disc and most of the suspended material; successive washings with hot 2 M hydrochloric acid removed soluble metals from the filtered residues. The filtered solution *plus* washings were diluted to 25 ml for the determination of cobalt, copper, iron, nickel and zinc by atomic-absorption spectrophotometry. A reagent - apparatus blank was run with the mock solution; as a check on membrane efficiency, the 3 litres of eluate were concentrated to 50 ml by distillation in silica apparatus, and the concentrate was also analysed by atomic-absorption spectrophotometry. The results obtained are shown in Table I. It was assumed that at the nearly neutral pH of reactor cooling waters, membrane efficiencies would be greater than the values obtained for the slightly acidic mock solution. The high percentages of metals retained by the pre-filter and Acropor membrane were encouraging, especially for cobalt, copper and nickel.

TABLE I

ACROPOR ION-EXCHANGE MEMBRANE EFFICIENCY

	Metal (100 μg per 3 litres of solution)				
	Cobalt	Copper	Iron	Nickel	Zinc
Retained on pre-filter and Acropor membrane (blank deducted), per cent.	99	96	95	98	93
Found in concentrated eluate, per cent. ..	<2	<2	5	<2	~5

In order to determine the lower levels of impurities, such as cobalt, it was found necessary to concentrate the metals collected by the filter - membrane system into a reasonably small volume. Two methods of extraction were investigated: digestion with concentrated acid and elution of the membrane with dilute acid. Ignition of the filter and membrane with dissolution of the ash in acidic solution was considered, but it was thought that the volatile losses incurred would probably be large and erratic, in addition to which airborne radioactivity would be a hazard. Complete wet oxidation of the membrane was not considered to be necessary as all that is required, apart from dissolution of insoluble solids on the pre-filter, is the removal of the absorbed cations from the ion-exchange resin. The use of additional reagents on complete dissolution by wet oxidation would also increase blank values. Insoluble impurities present in the samples of cooling water, which had collected on the microporous pre-filter, were found to be soluble in hot concentrated hydrochloric acid. Oxidation with a few drops of concentrated nitric acid also served to dissolve the filter material. Although experience has shown that the insoluble iron found in SGHWR cooling waters is generally readily soluble in concentrated hydrochloric acid, highly intractable magnetite could, if present, be made soluble by fusion with a small amount of sodium fluoroborate or potassium hydrogen sulphate.

A solution containing 20 μg ml^{-1} of copper was passed at a flow-rate of 5 ml min^{-1} through a pre-filter and Acropor membrane, to give a 10 per cent. breakthrough of copper. Analysis of the filtrate was carried out continuously on a Technicon AutoAnalyzer by using the zincon method.[2] The copper content of the filtrate up to first breakthrough was less than 0·4 μg ml^{-1}, indicating a membrane retaining efficiency of greater than 98 per cent. By integration, the amount of copper collected on the filter system verified the capacity of 0·05 mequiv per membrane. The pre-filter and Acropor membrane were eluted with five successive 10-ml portions of 2 M hydrochloric acid and the eluate fractions (1 to 5) were then analysed to determine copper by atomic-absorption spectrophotometry.

In a similar experiment, the pre-filter and Acropor membrane, loaded to 10 per cent. breakthrough with copper, were digested with 5 ml of concentrated hydrochloric acid for 15 minutes. On dilution of the solution to 10 ml, a small amount of insoluble material precipitated from solution; this material was believed to consist of ion-exchange resin fines or breakdown products from the copolymer matrix. The Acropor nylon support disc and suspended solids were removed by filtration through a 0·45-μm pore-size filter, which was washed with three successive 5-ml volumes of water, making the total volume of filtrate 25 ml. The 0·45-μm filter was re-treated with 5 ml of hot concentrated hydrochloric acid and washed with a total of 20 ml of water. Copper was determined on the two filtrates by atomic-absorption spectrophotometry. Results from the two methods of cation extraction, acid elution and acid digestion, are given in Table II.

The results given in Table II show that both extraction methods gave approximately the same recovery of copper. Acid digestion was preferred, however, as this method would be more appropriate than the elution method for the treatment of samples containing filterable insoluble solids. Also, the extractant acid can be controlled to a low volume in the digestion method, which is essential for the determination of impurities below 1 μg ml^{-1}.

IN-LINE SAMPLING APPARATUS—

Samples taken from the SGHWR cooling water circuit were delivered isokinetically via stainless-steel capillary lines to a central sampling cabinet. The pre-filters and Acropor membranes were housed in stainless-steel (or aluminium) pressure filter holders supplied by Gelman-Hawksley Limited. Membranes were placed on a stainless-steel support mesh in

the holder base, a thrust ring allowing the top of the filter holder to be screwed on to the base without causing twist distortion of the membranes. The filter holders are designed to permit filtration at pressures of up to 1·38 MN m^{-2} (200 p.s.i.); however, there is no restriction of flow apart from that caused by the membranes, and samples at pressures up to 5·52 MN m^{-2} (800 p.s.i.) were taken regularly. Restriction of flow caused by blockage of the pre-filter by solids has been investigated. Capillary flow-rates during integrated-volume sampling were not affected by up to 5 mg of insoluble solids; as a control on gamma-activity levels, this amount of material represented the maximum normally collected.

There was no evidence to indicate that the membranes were contaminated by pick-up of iron, nickel or chromium from the inner surfaces of the filter holder. However, in order to eliminate any possible metal contaminants, all inner surfaces of the holder were sprayed occasionally with a PTFE aerosol. A diagram of the filter holder is shown in Fig. 1. It was essential to measure accurately the volume of sample that passed through the membrane in order to calculate the concentrations of metals collected. To facilitate this measurement, an integrated-volume meter was designed.

The filtrate received continuously from the membrane filter holder was passed into a siphon, made of copper tubing, of about 125-ml capacity. The filtrate from the intermittently emptying siphon was directed into a counterbalanced receiver connected through a magnetic Reed switch to a 1000-Ω coil Sodeco counting meter, operating from 48 V d.c. A magnet was used to counterbalance the siphon receiver. As the balanced beam tilted during an emptying cycle of the siphon, the magnet operated the Reed switch and actuated the counter so as to record each siphonful delivered. Ordinary metal contacts can be used in place of the magnetic Reed switch and magnet system. Integrated-volume counters were made so as to provide one for each sample point on the cooling water circuits in order to complete a full survey over the same period of time. Flow-rates of the capillary sample lines were observed to be between 0·6 and 2·5 l h^{-1}, depending on their position in the circuit. The precision of the volumes measured by using integrated-volume counters at flow-rates between 0·5 and 3 l h^{-1} for volumes up to 20 litres was within ±3 per cent. The precision is expected to apply to sample volumes greatly in excess of 20 litres. A diagram of the integrated-volume meter is shown in Fig. 2.

TABLE II

COMPARISON OF ACID ELUTION AND ACID DIGESTION

Technique	Fraction		Copper removed/mg	Cumulative, per cent. removed
Acid elution*	1		1·90	64·0
	2		0·60	84·2
	3		0·32	94·9
	4		0·12	99·0
	5		0·03	100·0
		Total	2·97	
Acid digestion†	(*i*)		2·85	99·0
	(*ii*)		0·03	100·0
		Total	2·88	

* Elution with five successive 10-ml portions of 2 M hydrochloric acid.

† (*i*) Filtrate (*plus* washings) from digest of pre-filter and Acropor membrane; and (*ii*) filtrate (*plus* washings) from re-digest of 0·45-μm filter.

METHOD

APPARATUS—

Gamma spectrometry—A Technical Measurement Corporation (TMC, Model 401D) 400-channel pulse-height analyser coupled to a resolver - integrator (Model 522A), printer (Model 500A), and equipped with an 8-cm^3 lithium-drifted germanium crystal (20th Century Electronics Ltd.) coupled to the TMC analyser through "Harwell 2000 Series" units, was used for all gamma-spectrometric measurements. Counter geometry was standardised by

measurement of 15·0-ml volumes contained in screw-capped 60-cm³ polyethylene bottles. Standard nuclide solutions were obtained from the Radiochemical Centre, Amersham, Bucks., and all sample measurements were calculated to t_0. When long-duration samples were taken, for example, 7-day periods, t_0 was calculated to the middle of the sampling period. The peak energies of the major nuclides sought are as follows—

Nuclide	^{60}Co	^{59}Fe	^{65}Zn	^{51}Cr
Gamma-spectrometric peak energy/keV	1173	1095	1115	320
	1332	1295		

Atomic absorption—A Varian Techtron AA5 instrument was used for all atomic-absorption measurements, readings being displayed on a 200-mm scale slave recorder. The burner compartment was fitted with a chimney and ducting to take radioactive gases derived from reactor cooling water samples into a series of filters for purification.

Standard solutions of chromium, cobalt, copper, iron, nickel and zinc were prepared by dissolving accurately weighed amounts of high-purity metals (obtained from Koch-Light Laboratories Ltd.) in Aristar grade hydrochloric and nitric acids. Stock standard solutions containing 1 mg ml^{-1} of metal were diluted to the required levels with Acropor-filtered demineralised water. Blank values were found to be very low; for example, the apparatus blank reading obtained by aspiration of air measured at the peak absorption sensitivity for cobalt was not affected by acidic reagent *plus* water blanks. Atomic-absorption calibrations for the six elements sought were checked daily by direct standardisation with the particular element, and checked regularly by the method of mixed standard additions. Wavelengths of maximum sensitivity were used for measurements of chromium, cobalt, nickel and zinc; concentrations of copper and iron in the Acropor digest solutions were measured at less sensitive wavelengths so as to obviate the need for dilution.

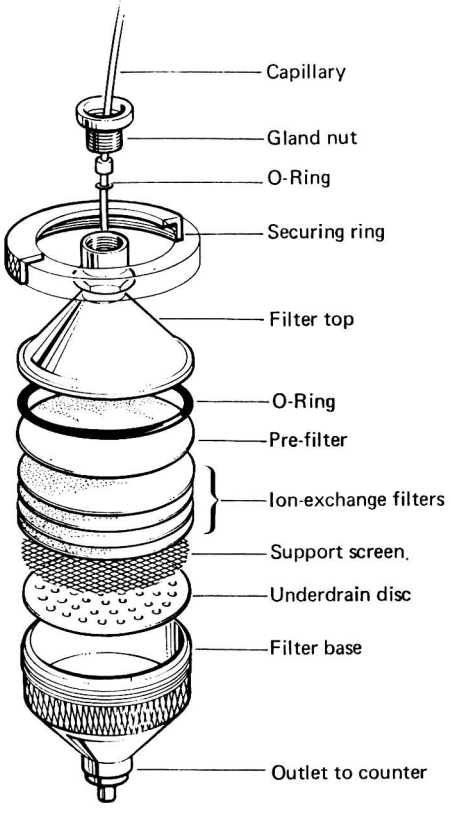

Fig. 1. In-line filter holder

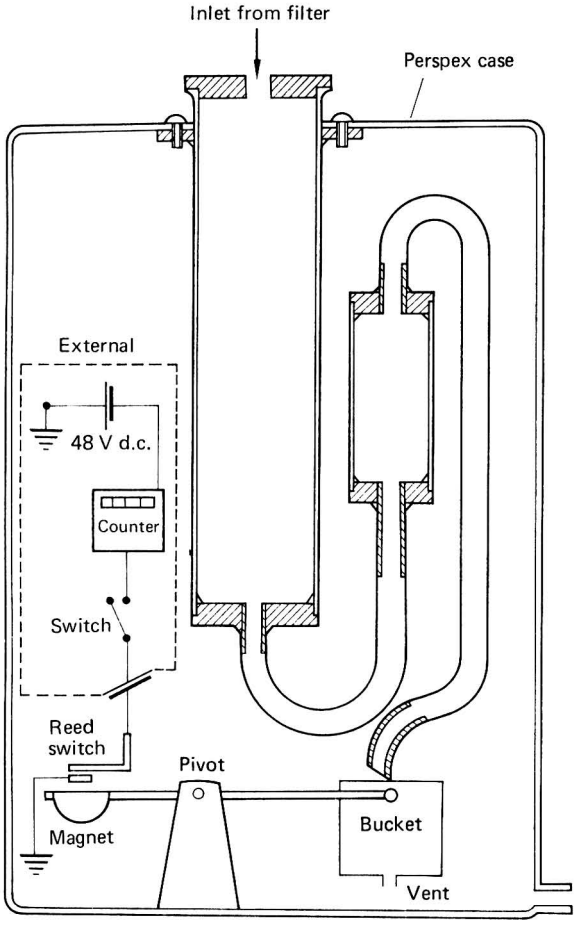

Fig. 2. Integrated volume counter (scale reduction 2·4:1)

Optimum wavelengths, range and limits of detection applicable to air - acetylene gas mixtures are shown in Table III.

TABLE III

EXAMINATION OF ACROPOR DIGEST SOLUTIONS WITH VARIAN TECHTRON AA5

Element	Wavelength/nm	Concentration range/μg ml^{-1}	Limit of detection/μg ml^{-1}
Chromium	357·8	0 to 10	0·02
Cobalt	240·7	0 to 5	0·015
Copper	324·7	0 to 10	0·01
	217·9	5 to 25	
Iron	248·3	0 to 5	0·02
	372·0	5 to 100	
Nickel	232·0	0 to 5	0·02
Zinc	213·9	0 to 2	0·005

For sampling, 47 mm diameter microporous filters, 0·45-μm pore-size [available from Millipore (U.K.) Ltd.], were used.

Also used were types SA and SB 47 mm diameter cation- and anion-exchange Acropor membranes and 47 mm diameter stainless-steel or aluminium filter holders (available from

Gelman-Hawksley Ltd.), and a gland nut and O-ring for the union between the capillary line and the filter holder (available from North Hants Engineering Co. Ltd.).

For sample preparation, 25 mm diameter microporous filters, 0·8-μm pore-size, and a 25 mm diameter micro-filtration flask and funnel [Millipore (U.K.) Ltd.] were used.

REAGENTS—

The concentrated acids used were Aristar grade reagents.

Hydrochloric acid, concentrated, sp. gr. 1·18.

Nitric acid, concentrated, sp. gr. 1·42.

De-mineralised water used for the dilution and preparation of samples and standard solutions was purified by passing it through a mixed stack of two cation- and two anion-exchange Acropor membranes protected by a microporous pre-filter.

PROCEDURE—

Capillary line sampling—The radioactive impurities present in reactor water circuits were efficiently concentrated by the Acropor membranes and gave high levels of activity. Gloves, safety spectacles and protective clothing were worn during sampling, preparation and analysis. Special shielding was necessary for some samples.

Place one anion-exchange membrane on the photo-etched screen in the filter holder base, followed by three cation-exchange membranes. Finally, place one millipore pre-filter (0·45 μm) on top of the membranes in order to complete the stack. Ensuring that the large O-ring in the filter holder top is in place, screw down the securing ring (hand-tight) to complete the filter holder assembly. Fit the gland nut, washer and small O-ring to the filter holder top and pass the capillary sample line through the filter holder top to within 1 cm of the pre-filter disc. Tighten the gland nut so as to make a leak-free seal with the filter holder and capillary line. When sufficient sample has been collected, slacken off the gland nut to allow the capillary line to be withdrawn from the holder. Read the integrated-volume meter counter for subsequent calculation of total volume passed. Keeping the filter holder upright, apply gentle suction to the filter holder outlet so as to remove surplus liquid through the membrane stack. This prevents loosely held solids from being washed off the pre-filter on to the lower Acropor membranes when the holder is dismantled.

Acropor sample preparation—If filterable "insoluble" and filtrate "soluble" species are required, separate the pre-filter from the membrane stack by using chisel-edged plastic tweezers. Lay the membrane discs flat on the bottom of Pyrex beakers with an internal diameter slightly greater than the discs. With a pipette, add 5·0 ml of Aristar grade concentrated hydrochloric acid to each beaker and cover it with a watch-glass. Digest the contents of the beaker with the acid nearly at its boiling-point on a hot-plate for about 15 minutes. Add five drops of Aristar grade concentrated nitric acid at this stage and continue the digestion for a further 10 minutes or until all traces of insoluble solids have dissolved. (The pre-filter is completely dissolved by this treatment.) Dilute the "insoluble" solution to 25 ml in a calibrated flask.

Diluted solutions prepared from the acid digest of the Acropor membranes were found to contain a small amount of very finely divided insoluble material derived from the membrane matrices in addition to the intractable nylon support discs.

Dilute the acid extract solution from each Acropor membrane stack so that the concentration of acid is less than 6 M and filter it slowly through a single 25-mm diameter 0·8-μm pore-size Millipore filter-pad held in a microfiltration apparatus. Pulp the nylon discs and transfer the pulp from the beaker to the Millipore pad, pressing it with a PTFE rod while applying gentle suction to the filtration flask. Wash the digestion beaker, PTFE rod and pulped nylon pad with successive 3-ml portions of hot 1 M Aristar grade hydrochloric acid. Continue the rinsing and washing so as to give a total filtrate volume of 25 ml. It was found that occasionally a small amount of the white organic material precipitated from the filtrates on standing. No changes in atomic-absorption measurements were observed, however, with addition of standard solutions of the cations cobalt, copper, iron, nickel and zinc to blank Acropor digest filtrates containing relatively large suspensions of organic material.

Prepare blank solutions by digesting unused Millipore filters, and Acropor membranes, with the same amounts of reagents as those used for sample solutions. Filter and wash the Acropor blank solutions in the same manner as that used for the Acropor sample solutions

and finally dilute the solution to 25 ml. If separation of "insoluble" and "soluble" species is not required, digest the whole stack of pre-filter and membranes together, filter and wash them to give a total volume of 25 ml; the blank containing one pre-filter and the requisite number of membranes is prepared accordingly.

Results

Cation-exchange membrane efficiency—

Initial laboratory trials showed that the retention of a single cation-exchange membrane was greater than 95 per cent. for cobalt, copper, iron and nickel at the 30 $\mu g\ l^{-1}$ level. It was considered, therefore, that a stack of three such membranes would be sufficient to remove almost 100 per cent. of the cations, assuming an equal efficiency for the second and third membranes in collecting decreasing concentrations of cations.

In consideration of handling radioactivity levels concentrated by the Acropor technique, samples were taken over periods from 24 hours for the highly active samples, to 7 days for the low-activity samples. Generally, the integrated sample volumes varied accordingly between 50 and 350 litres; samples of large volume were taken for the determination of small trace amounts of impurities such as cobalt. Samples of cooling water were taken at points showing the widest variations in trace-metal content and flow-rate. Acid digest solutions were prepared from the separated pre-filters and individual Acropor membranes for analysis by atomic-absorption spectrophotometry and gamma spectrometry.

Table IV

Collection of cations on Acropor membranes

Atomic-absorption results are given as total microgram amounts collected on the pre-filter and separated Acropor membranes; results for gamma spectrometry are given in microcuries. All results have been corrected for blank values and background counts

Sample A (flow-rate 35 ml min^{-1}; total volume collected 80 litres)

			"Insoluble" (0·45-μm pre-filter)		"Soluble" First Acropor membrane		Second Acropor membrane		Third Acropor membrane	
Method	Cation	Nuclide	μg	μCi	μg	μCi	μg	μCi	μg	μCi
Atomic absorption	Co		1·5		10		0·5		<0·5	
	Cu		7		750		110		<2	
	Fe		860		12		5		<5	
	Ni		11		16		5		<1	
	Zn		<1		30		7		<5	
Gamma spectrometry		^{60}Co		2·9		11·3		0·5		<0·01
		^{59}Fe		0·95		<0·1		<0·1		<0·1
		^{65}Zn		<0·1		0·75		0·2		<0·1

Sample B (flow-rate 300 ml min^{-1}; total volume collected 20 litres)

			"Insoluble" (0·45-μm pre-filter)		"Soluble" First Acropor membrane		Second and Third Acropor membranes		Fourth and Fifth Acropor membranes	
Method	Cation	Nuclide	μg	μCi	μg	μCi	μg	μCi	μg	μCi
Atomic absorption	Co		2·5		1·5		<0·5		<0·5	
	Cu		10		380		130		4	
	Fe		1300		140		30		10	
	Ni		23		12		10		2	
	Zn		2·5		20		10		5	
Gamma spectrometry		^{60}Co		5·0		1·6		0·4		<0·02
		^{59}Fe		3·0		<0·1		<0·1		<0·1
		^{65}Zn		0·1		0·5		0·2		<0·1

Five Acropor membranes were used for the collection of cations in sample B taken from a 0·8-mm bore line that delivered at a flow-rate of 300 ml min^{-1}. An integrated-volume counter was not used for volume measurement at this high flow-rate and regular readings of timed flow-rates were taken instead. Some typical results of these investigations are shown in Table IV.

An assessment of the ratio of "insoluble" to "soluble" metals can be made from the results obtained. For example, iron is shown to be a largely filterable particulate species, whereas copper and zinc are mainly present in "soluble" ionic form. Although the figures for cobalt obtained by atomic-absorption spectrophotometry show values of "less than 0·5 μg" in each instance after the first Acropor membrane, the very sensitive gamma-spectrometric results on the same solutions define a diminishing level of cobalt-60 in the lower membranes. The sum of the particulate and cationic forms of cobalt collected, in terms of the integrated volume passed, indicate a measured level of 0·14 $\mu g\ l^{-1}$ of cobalt for sample A and 0·20 $\mu g\ l^{-1}$ of cobalt for sample B. The two samples were taken from different parts of the water circuit.

BLANK VALUES—

It is essential to use reagents and water of the highest purity, especially for the determination of impurities below the parts per billion level, even though they have been concentrated approximately 1000-fold by the Acropor technique. Blank values for the microporous pre-filter, three Acropor cation-exchange membranes and reagents were determined by atomic-absorption measurements of the 25 ml of extraction solutions of pre-filters and membranes. The results shown in Table V have been calculated as total microgram amounts in extraction solutions; the results are the mean values of thirty determinations.

TABLE V

ACID-EXTRACTION BLANKS

	Microporous pre-filter in 25 ml	Three Acropor cation-exchange membranes in 25 ml	Reagent blank (25 ml)
Cobalt/μg	<0·4	<0·4	<0·4
Copper/μg	1·5	1	0·5
Iron/μg	5	5	1
Nickel/μg	1·5	1	0·8
Zinc/μg	5	5	2

Hence, the blank levels of combined pre-filter and three Acropor membranes, applied to a 100-litre integrated sample, would be equivalent to 0·1 $\mu g\ l^{-1}$ for iron and zinc, 0·03 $\mu g\ l^{-1}$ for copper and nickel and $<0{\cdot}004\ \mu g\ l^{-1}$ for cobalt.

CATION COLLECTION LOSSES—

It was considered that cations could be displaced from membrane to membrane down the stack during sampling with subsequent loss of cations in the membrane eluate. In order to determine the magnitude of possible losses, membrane eluates were sampled and concentrated by distillation. Atomic-absorption measurements on 100-fold concentrations were found to be at the limits of detection for cobalt, copper, iron and nickel. Consequently, for an assessment of membrane stack efficiency, the long-lived isotopes cobalt-60 and iron-59 present in the eluate concentrates were compared with cobalt-60 and iron-59 found in the membrane digest solutions. Eluates were collected in 2·5-litre glass bottles containing 0·5 ml of analytical-reagent grade concentrated hydrochloric acid; eluate fractions were taken just after the start of a sampling period and just before the end of the period. The eluates were concentrated to 15 ml for gamma-spectrometric measurement. The results summarised in Table VI show the percentages of cobalt-60 and iron-59 retained on one microporous pre-filter and three Acropor cation-exchange membranes.

In a similar experiment with the short-lived copper-64 isotope in the cooling water sample as a tracer, the retention efficiency of an Acropor stack was shown to be 98 per cent. at the 1 $\mu g\ l^{-1}$ of copper level; eluate samples were taken at the beginning and end of a 7-day sampling period, 340 litres being passed.

The cobalt content of sample C shown in Table VI at 0·010 $\mu g\ l^{-1}$ concentration is retained by the Acropor stack at 85 per cent. efficiency. Only a slight improvement in

retention efficiency at this level of cobalt was achieved by doubling the number of cation-exchange membranes used. Sample D, which contained almost ten times the cobalt content of sample C, is shown to be 99 per cent. retained. Cobalt-60 activities in the final eluates of samples C and D are similar to the activities in the initial eluates, indicating that no washing out or breakthrough of cobalt collected occurred. It is assumed that membrane retention efficiencies will decrease below 85 per cent. for cobalt levels lower than 0·01 $\mu g\ l^{-1}$ and a correction is made for any samples at this level. However, it is possible that at these very low levels, the membrane stack efficiency would increase for flow-rates of less than the 2 l h^{-1} delivered at sample point C.

TABLE VI

CATION LOSSES IN MEMBRANE ELUATE

Sample point	C	D	D	E
Element sought	Cobalt	Cobalt	Iron	Chromium
Integrated volume/litres	250	60	60	65
Flow-rate/l h^{-1}	2	2·5	2·5	2·5
Retained on stack	0·010 $\mu g\ l^{-1}$ of Co	0·095 $\mu g\ l^{-1}$ of Co	45·8 $\mu g\ l^{-1}$ of Fe	2·0 $\mu g\ l^{-1}$ of Cr
Activity on stack	1·70 nCi l^{-1} of ^{60}Co	140 nCi l^{-1} of ^{60}Co	92 nCi l^{-1} of ^{59}Fe	730 nCi l^{-1} of ^{51}Cr
Initial activity in eluate	0·29 nCi l^{-1} of ^{60}Co	0·9 nCi l^{-1} of ^{60}Co	<0·3 nCi l^{-1} of ^{59}Fe	500 nCi l^{-1} of ^{51}Cr
Final activity in eluate	0·31 nCi l^{-1} of ^{60}Co	1·1 nCi l^{-1} of ^{60}Co	<0·3 nCi l^{-1} of ^{59}Fe	950 nCi l^{-1} of ^{51}Cr
Activity retained on stack, per cent.	85	99·3	>99·6	~50

In gamma-spectrometric examinations the efficiency of the lithium-drifted germanium crystal used for the detection of iron-59 is low; also, the abundance of iron-59 in SGHWR cooling waters is small. Therefore, evaluation of losses in the eluate by measurement of iron-59 has been limited to sample D, which contained the relatively high iron content of 45 $\mu g\ l^{-1}$. Concentration of eluates to a greater extent than 100-fold would be necessary to detect iron-59 in samples containing less than 45 $\mu g\ l^{-1}$ of iron. Generally, iron in the cooling waters has been found to be material that can be removed by filtration; even at the lower levels of about 10 $\mu g\ l^{-1}$ of iron and less, 90 per cent. of the total iron collected is removed by the microporous pre-filter.

The poor retention of chromium-51 shown in Table VI was investigated; it was believed that the chromium may not have been present in a cationic form in sample E. Laboratory trials showed that soluble chromate was quantitatively collected on Acropor anion-exchange membranes with the resin in the chloride form. It was also shown that the chromate collected was completely removed from anion-exchange membranes by elution with 2 N sodium hydroxide solution or by digestion with hot concentrated hydrochloric acid solution. An integrated sample of cooling water was collected on a stack of membranes comprising, in order, a microporous pre-filter, three cation-exchange membranes and two anion-exchange membranes at the bottom of the stack.

A sample of eluate taken at the end of a 24-hour sampling period and concentrated by distillation from 2·5 litres to 15 ml was examined for chromium-51 and zinc-65 content by gamma spectrometry. The pre-filter, cation- and anion-exchange membranes were separated for analysis in order to determine the proportions of "insoluble," cationic and anionic species of chromium and zinc in the circuit. The results given in Table VII show a high retention of chromium and zinc on a mixed stack of Acropor membranes. The concentration of chromium collected on the anion-exchange membranes is in close agreement

TABLE VII

COLLECTION OF CHROMIUM AND ZINC ON CATION- AND ANION-EXCHANGE MEMBRANES

	Sample F: integrated volume 58 litres					
	Chromium/ $\mu g\ l^{-1}$	^{51}Cr/ nCi l^{-1}	^{51}Cr, per cent.	Zinc/ $\mu g\ l^{-1}$	^{65}Zn/ nCi l^{-1}	^{65}Zn, per cent.
Retained on pre-filter	0·3	440	26·1	0·15	5·2	9·7
Retained on cation-exchange membranes	0·9	310	18·4	0·9	43·0	80·7
Retained on anion-exchange membranes	2·6	930	55·2	0·1	4·1	7·7
In final eluate	0·04	6	0·3	<0·1	1·0	1·9

with the over-all losses incurred on a stack consisting totally of cation-exchange membranes, as shown in Table VI, which indicates that the chromium lost from a stack of cation-exchange membranes is anionic and not a colloidal or non-ionic species.

It is obvious from the elemental and gamma-spectrometric results shown in Table VII that the particulate and soluble ionic forms of chromium possess different specific activities; this conclusion can also be deduced for cobalt, iron and zinc from the results given in Table IV. The differences in the specific activities of soluble and insoluble species is due to their individual points of origin in the cooling water circuits and life-cycle through the reactor core.

SAMPLE PREPARATION LOSSES—

Use of the gamma-spectrometric measurement of long-lived radioisotopes present in the water samples was extended to investigate the loss of cations during sample preparation. The acid digestion of membranes was used in preference to the method involving elution with acid; however, this procedure involved removal of the membrane support material by filtration, which may have entrained cationic species. Membranes from an integrated-volume sample taken from the cooling circuit were treated in the normal manner by digestion with 5 ml of concentrated hydrochloric acid and oxidised with nitric acid. The nylon support disc and insoluble resin-dispersion polymer were removed by filtration and washed with hot dilute hydrochloric acid to give a total volume of filtrate of 25 ml. Filtered solids were made soluble by wet oxidation with concentrated sulphuric and nitric acids, and examined for cobalt-60, iron-59 and zinc-65 by gamma spectrometry.

The results given in Table VIII show slight losses on the filter. However, the losses are very low in comparison with the levels of cations sought. For example, a loss on the filter of 1 per cent. of cobalt at the 0·1 $\mu g\ l^{-1}$ level, *plus* the 1 per cent. loss in sample eluate (Table V) is within the expected precision limits of the method, including collection, extraction and analysis.

TABLE VIII

FILTRATION LOSSES BY MEMBRANE EXTRACTION OF NUCLIDES

Nuclide	^{60}Co	^{59}Fe	^{65}Zn
Total activity in extract filtrate/nCi l^{-1}	$4{\cdot}05 \times 10^3$	$1{\cdot}76 \times 10^3$	$0{\cdot}92 \times 10^3$
Total activity on extract filter/nCi l^{-1} ..	50	6	18
Activity retained on filter, per cent. ..	1·2	0·3	1·9

The results shown in Table VIII were obtained for a sample taken from a high-temperature region of the cooling water circuit. From the low iron-59 activity retained on the filter it was concluded that the iron, which from this sample point was generally difficult to dissolve, had effectively been made soluble by the acid-digestion procedure.

DETERMINATION OF IRON—

The total iron content determined by the Acropor technique on numerous samples containing insoluble solids was found to be invariably higher than the total iron results obtained by the automated 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) method.[1] In the automated method particulate iron is dissolved by treatment with 1 per cent. *V/V* thioglycollic acid solution in a time-delay coil at 95 °C; digestion time in the delay coil is about 20 minutes. Dissolved iron is then determined by continuous absorptiometric measurement of the coloured complex formed with TPTZ.

A number of 200-ml "grab" samples were taken from two capillary lines (sampling time about 6 minutes) into bottles containing thioglycollic acid. The acidified samples were divided into two equal portions; one series of samples was introduced directly into a Technicon AutoAnalyzer for determination of iron and the other series was digested in a boiling water bath for 1 hour prior to analysis in the AutoAnalyzer. Therefore, the second series had combined periods of digestion of 1 hour at 100 °C and 20 minutes at 95 °C.

The results for iron summarised in Table IX show the effect of increasing the digestion time with thioglycollic acid in the automated procedure. Also included in Table IX are the Acropor results obtained on daily integrated samples covering the short-period "grab" samples. The mean results for iron shown in the top half of the table were obtained from determinations carried out at steady power and during plant operations for water clean-up

(Powdex re-coats.) The eight individual results are shown in Fig. 3. Also shown in Fig. 3, for comparison, are nine daily results for iron obtained by the TPTZ and Acropor methods during a typical reactor start-up period. Incomplete dissolution of insoluble iron by the normal TPTZ method is indicated by the values shown in Table IX, especially for the high iron content of sample Y, and is confirmed by the single determinations shown in the lower half of the table; the portions of "grab" sample receiving additional digestion with thioglycollic acid gave results in close agreement with the value for total iron obtained by Acropor collection.

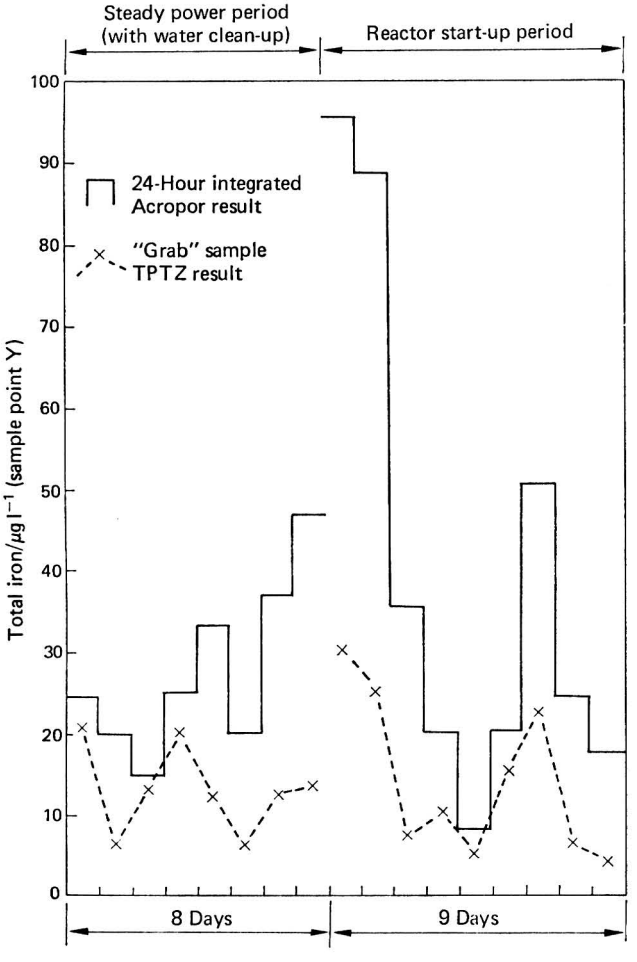

Fig. 3. Daily determination of iron: comparision of results obtained by TPTZ and Acropor methods

Perturbations in the cooling water circuits cause short-term fluctuations in the amount of solids in circulation. It is possible that during such a release of solids the "grab" sample of small volume may miss the resultant high iron content; however, it was found that continuous in-line analysis by the normal TPTZ method also gave lower iron results than the Acropor system. In addition, a direct comparison of the two methods has been obtained by determining the iron by the TPTZ "grab" method and the Acropor technique on twin capillary lines at sample point Y. Identical volumes were collected over a period of 2 hours; the normal TPTZ method gave 28 $\mu g\ l^{-1}$ of iron compared with the Acropor result of 50 $\mu g\ l^{-1}$.

TABLE IX

COMPARISON OF RESULTS FOR IRON BY THE TPTZ AND ACROPOR METHODS

Sample point	Iron in "grab" sample by TPTZ method*/ $\mu g\ l^{-1}$	Iron in "grab" sample by TPTZ method (additional digestion)*/ $\mu g\ l^{-1}$	Iron on Acropors ("soluble")/ $\mu g\ l^{-1}$	Iron in integrated sample on pre-filter ("insoluble")/ $\mu g\ l^{-1}$	Total iron/ $\mu g\ l^{-1}$
X (mean of 13 determinations)	4·9	—	0·6	5·8	6·4
Y (mean of 8 determinations)	13·0	—	1·6	26	27·6
X (single determination)	<2	6	—	—	7
X (single determination)	<2	4	—	—	5
Y (single determination)	15	20	1·4	18·4	19·8
Y (single determination)	22	61	1·9	58	59·9

* Iron results obtained by Mr. R. E. H. Rolfe, SGHWR Chemistry Laboratory.

DETERMINATION OF COPPER—

Routine copper determinations on "grab" samples by using the automated zincon method[2] have shown close agreement with the copper results obtained by the Acropor-integration technique, the limit of detection for copper by the former method being 1 $\mu g\ l^{-1}$. Of the fifteen results obtained for sample X, seven indicated 2 $\mu g\ l^{-1}$ of copper, three 1 $\mu g\ l^{-1}$ and five less than 1 $\mu g\ l^{-1}$. The "less than 1 $\mu g\ l^{-1}$" values were taken as 0·5 $\mu g\ l^{-1}$ of copper for calculation of the mean value. Copper determined by the Acropor-integration technique is shown in Table X to be present mainly as a "soluble" cation. There is no difficulty in dissolving it for the continuous method as there is for the iron determination.

TABLE X

COMPARISON OF RESULTS FOR COPPER CONTENT BY THE ZINCON AND ACROPOR METHODS

Sample point	Copper in "grab" sample by zincon method/ $\mu g\ l^{-1}$	Copper in integrated sample: On Acropors ("soluble")/ $\mu g\ l^{-1}$	Copper in integrated sample: On pre-filter ("insoluble")/ $\mu g\ l^{-1}$	Total copper/ $\mu g\ l^{-1}$
X (mean of 15 determinations)	1·3	1·0	0·1	1·1
Y (mean of 9 determinations)	17	17·5	0·5	18·0

PRECISION—

An assessment of analytical precision was achieved by atomic-absorption determination of known amounts of cations added to Acropor membranes. Twelve separate determinations were performed by using a mixture of standard solutions containing 1 μg of cobalt, 90 μg of copper and 950 μg of iron per 100 ml added to each single cation-exchange membrane. Following a water wash, the membranes were digested with concentrated hydrochloric acid, insoluble organic material was filtered off and the solutions were diluted to 25-ml volumes for analysis by atomic-absorption spectrophotometry. The varied levels of added cations were chosen to simulate approximate ratios of cobalt, copper and iron found in SGHWR cooling waters. The results are summarised in Table XI as the concentrations of cations in the digest extract solution.

TABLE XI

PRECISION OF ATOMIC-ABSORPTION ANALYSES

	Cobalt (Co^{2+})	Copper (Cu^{2+})	Iron (Fe^{3+})
Concentration in extract solution (25 ml)/ $\mu g\ ml^{-1}$	0·041	3·6	38
Precision at 96 per cent. confidence limit (12 determinations)/$\mu g\ ml^{-1}$	±0·005 (±12%)	±0·055 (±1·5%)	±0·95 (±2·5%)

Calculations of the precisions in terms of a volume of cooling water sample of 100 litres are: cobalt 0·01 ± 0·001 $\mu g\ l^{-1}$, copper 0·9 ± 0·02 $\mu g\ l^{-1}$ and iron 9·5 ± 0·25 $\mu g\ l^{-1}$.

With standard solutions of cobalt, copper, iron, nickel and zinc, there does not appear to be any interference in the determination of any one element caused by the presence of the other four in the ratios generally found in SGHWR cooling waters. These ratios for the metals in the order given are 1:100:1000:10:10.

The mean value of the total cobalt content of thirteen integrated samples, taken from one capillary sample point over a period of 15 weeks, was found to be 0·014 $\mu g\ l^{-1}$ with a standard deviation of $\pm 0{\cdot}005\ \mu g\ l^{-1}$. The variation included a number of short-term circuit disturbances that caused slight fluctuations in the concentration of solids, and hence cobalt content, in addition to the analytical errors. Reproducibility of results has also been shown by analysis of ten duplicate 300-litre integrated samples to determine cobalt. These samples were taken on a weekly basis from two capillary lines fed from one multi-headed sample point. Results of the duplicate samples shown in Table XII are, on average, within $\pm 0{\cdot}002\ \mu g\ l^{-1}$ of cobalt at the 0·01 to 0·02 $\mu g\ l^{-1}$ level.

TABLE XII

COMPARISON OF COBALT CONTENTS FROM TWIN CAPILLARY LINES

Results expressed as $\mu g\ l^{-1}$ of cobalt

	Run 1	Run 2	Run 3	Run 4	Run 5
Capillary XI	0·012	0·016	0·016	0·012	0·019
Capillary XII	0·012	0·017	0·015	0·010	0·020
Difference	Nil	0·001	0·001	0·002	0·001

DISCUSSION

The method of collection on Acropor cation-exchange membranes has been in use for over 1 year for the determination of trace-metal impurities in SGHWR cooling water circuits. During that time the method has been developed to enable cobalt to be determined down to the 0·01 $\mu g\ l^{-1}$ level. At this level, the three-membrane stack system has been shown by gamma-spectrometric measurement of cobalt-60 to be approximately 85 per cent. efficient. Further investigation would be required to establish membrane efficiency and analytical precision at levels lower than 0·01 p.p.b.

A major advantage of the Acropor collection system over the determination of impurities in "grab" samples, or in-line continuous methods, is the relative reduction of blank levels. Reagent and apparatus blank values arising from the preparation of samples by the Acropor technique are very low in comparison with the amounts of metals collected from an integrated-volume sample. On a routine basis, sampling time, and hence frequency, depends on the concentration of metals in the water at a particular sampling point. For example, if only the major impurity, such as iron, is to be determined, and it is present in relatively high concentration with respect to blank levels, then a small integrated volume should suffice. On the other hand, if the interest lies in the determination of very small trace amounts of impurities such as cobalt, then a long-duration integrated sample would be necessary.

The separation of "insoluble" filterable impurities by means of a single microporous pre-filter of nominal pore size 0·45 μm is an arbitrary measure. No work has been conducted on the investigation of possible retention of "soluble" cations on a bed of solids collected on the pre-filter during the sampling period. It is considered, however, that any retention would be extremely small; the "insoluble" material consists mainly of oxides of iron, and the amount collected does not generally exceed 0·3 $mg\ cm^{-2}$ of filter area for any sample. The microporous pre-filter also allows the sample to be spread from a narrow jet issuing from the capillary line into a relatively slow-moving "column" of water through the ion-exchange membrane stack. Dissolution of oxides of iron by treatment with thioglycollic acid in a mixing coil at 95 °C has been shown to be incomplete for some SGHWR cooling waters that probably contain magnetite. The results obtained for iron by the normal continuous TPTZ method on samples containing relatively high concentrations of insoluble solids (greater than 20 $\mu g\ l^{-1}$ of iron) are approximately half the values obtained by the Acropor method with concentrated acid digestion.

Gamma-spectrometric measurements of the long-lived isotopes cobalt-60, iron-59 and zinc-65, coupled with atomic-absorption determinations of cobalt, iron and zinc on both "insoluble" and "soluble" species, has provided useful information on the specific activities of these isotopes and their fate in the reactor cooling water circuits.

Anion-exchange resin impregnated membranes have been used to demonstrate the presence of anionic zinc and chromium, probably as zincate and chromate, in SGHWR waters. Analytical applications of the anion-exchange membranes have not yet been fully investigated; however, preliminary determinations of sulphate and nitrate at concentrations below the parts per million level have shown promise.

Conclusion

The method described provides a simple and efficient concentration system for the determination of extremely small trace amounts of cations in water. The final determination of extracted metals by atomic-absorption measurements greatly reduces the relatively high blank levels inherent in solvent-extraction and colorimetric methods. In comparison with in-line continuous methods of analysis, the Acropor integrated system does not provide early information for plant control. However, the integrated sample enables positive determinations of metals in water to be carried out at levels considerably lower than those which could be detected by current chemical methods. Much detailed information on plant efficiencies, steam carry-over and corrosion rates has been obtained from the atomic-absorption and gamma-spectrometric measurements of the filterable "insoluble" and "soluble" species collected by the Acropor system.

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Indirect Complexometric Titration of Barium and Strontium after Stepwise Precipitation as Sulphate from Homogeneous Solution

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An indirect method has been developed for the stepwise titration of barium and strontium with EDTA, based on precipitation of the elements as sulphate from a homogeneous solution. Barium, strontium and other multivalent cations are complexed by titration with EDTA at pH 10·0 by using a mixed indicator comprising Eriochrome black T, Titan yellow and Naphthol green B. Barium is then selectively replaced from its EDTA complex and precipitated as the sulphate with an excess of magnesium sulphate solution. The excess of magnesium is determined by titration with EDTA and the equivalent concentration of barium is calculated. Strontium is determined in the same solution in the same way, with an excess of zinc sulphate solution.

The optimum conditions for precipitation and the interferences due to various anions and cations have also been studied. Copper, nickel, cobalt and iron interfere and a second method for the determination of barium alone has been derived wherein the interfering elements are complexed with triethanolamine, potassium cyanide and ascorbic acid. Phosphate and chromate interfere and require to be separated.

Various methods based on direct and back-titrations of barium and strontium with EDTA (disodium salt) are reported in the literature.[1–4] In all of the methods, these elements, and other alkaline earth elements, if present, are co-titrated and therefore prior separation of the individual elements is essential for their determination. Belcher, Gibbons and West[5] reported a method for the determination of barium sulphate that entailed dissolving it in an excess of a strongly ammoniacal (9 M) solution of EDTA and then back-titrating the excess with magnesium ions from magnesium chloride solution. Precipitation of barium as sulphate from a homogeneous solution of its EDTA complex by the replacement reaction with magnesium and nickel in the presence of sulphate ions has been reported for the gravimetric determination of barium.[6,7] However, precipitation from a homogeneous solution has not yet been utilised in complexometric titration of the element in the presence of other alkaline earth elements. This paper describes a method in which barium, strontium and other alkaline earth elements are complexed by titration with EDTA at pH 10·0 with the use of a mixed indicator. Barium and strontium are then precipitated stepwise as their sulphates at the same pH from a homogeneous solution by displacement reactions with a known (excess) volume of magnesium sulphate and zinc sulphate, respectively, according to the following equations—

$$\underset{(\log k = 7{\cdot}76)}{Ba[EDTA]} + MgSO_4 = BaSO_4 + \underset{(\log k = 8{\cdot}69)}{Mg[EDTA]}$$

$$\underset{(\log k = 8{\cdot}63)}{Sr[EDTA]} + ZnSO_4 = SrSO_4 + \underset{(\log k = 16{\cdot}26)}{Zn[EDTA]}$$

The excess amounts of magnesium and zinc ions are then titrated stepwise with EDTA solution. Barium and strontium are calculated from the equivalent amounts of magnesium and zinc consumed during their respective displacement reactions.

Experimental

Reagents and solutions—

All reagents were of analytical-reagent grade quality unless otherwise stated.

Calcium chloride solution, 2·0 *mg ml*$^{-1}$.

Barium chloride solution, 2·0 *mg ml*$^{-1}$.

Strontium chloride solution, 2·0 *mg ml*$^{-1}$.

Magnesium sulphate solution, 0·05 M.

Zinc sulphate solution, 0·05 M.

Standard magnesium solution, 0·05 M—Dissolve 1·2160 g of pure magnesium turnings in 1 + 1 hydrochloric acid, almost neutralise the solution with 1 M sodium hydroxide solution and dilute to 1 litre with water.

Mixed indicator solution—Dissolve Eriochrome black T (0·03 g), Titan yellow (0·04 g) and Naphthol green B (0·01 g) in 25 ml of triethanolamine.

EDTA solution, 0·05 M—Dissolve 18·6125 g of the disodium salt of EDTA in water and dilute the solution to 1 litre. Standardise the EDTA solution thus obtained with the magnesium sulphate solution at a pH of 10·0 by using mixed indicator and calculate the equivalent amounts of barium oxide and strontium oxide per millilitre of EDTA solution.

Buffer solution, pH 10·0—Dissolve 67·5 g of ammonium chloride in 250 ml of water and 570 ml of ammonia solution (sp. gr. 0·88), dilute to 1 litre and mix thoroughly.

PROCEDURE I. STEPWISE DETERMINATION OF BARIUM AND STRONTIUM OXIDES IN THE PRESENCE OF CALCIUM AND MAGNESIUM—

Transfer a portion of the solution containing barium, strontium, calcium and magnesium into a 250-ml conical flask. Add 2 to 3 g of ammonium chloride, followed by dilute ammonia solution (1 + 4) dropwise until the contents of the flask smell of ammonia. Add 10 to 15 ml of buffer solution and titrate the mixture with 0·05 M EDTA solution in the presence of 10 to 12 drops of mixed indicator until a sharp green colour is obtained.

DETERMINATION OF BARIUM OXIDE—

Dilute the complexed solution to 100 ml, heat it to 50 to 60 °C on a hot-plate and add an excess (3 to 5 ml) over the theoretical requirement of sulphate ions of 0·05 M magnesium sulphate solution (1 ml of 0·05 M magnesium sulphate solution for every 7·67 mg of barium oxide content) slowly from a pipette, keeping the mixture on the hot-plate for 10 minutes. Cool the mixture, add 5 ml of buffer solution and 10 ml of absolute ethanol and titrate the excess of magnesium in the solution with 0·05 M EDTA solution to the sharp green end-point. Reserve the solution for the determination of strontium oxide.

Then,

$$\text{BaO (mg)} = (A - a) \times f$$

where A ml is the volume of EDTA equivalent to the volume of magnesium sulphate solution added, a ml is the volume of EDTA solution required to titrate the excess of magnesium sulphate and f is the equivalent of barium oxide in milligrams per millilitre of EDTA solution.

DETERMINATION OF STRONTIUM OXIDE—

To the solution reserved after the determination of barium, add 0·05 M zinc sulphate solution (5 ml for every 10 mg of strontium oxide) and 5 g of potassium sulphate. Boil the mixture for 10 minutes in order to precipitate strontium sulphate, then cool it, add 10 ml of buffer and 15 to 20 ml of absolute ethanol. Titrate the excess of zinc with 0·05 M EDTA solution to the sharp green end-point.

Then,

$$\text{SrO (mg)} = (B - b) \times g$$

where B ml is the volume of EDTA equivalent to the volume of zinc sulphate solution added, b ml is the volume of EDTA solution required to titrate the excess of zinc sulphate, and g is the equivalent of strontium oxide in milligrams per millilitre of EDTA solution.

PROCEDURE II. DETERMINATION OF BARIUM OXIDES IN THE PRESENCE OF VARIOUS CATIONS—

To a portion of the solution in a 250-ml conical flask add 5 ml of 0·05 M magnesium sulphate solution. Dilute the mixture to 50 ml, add 2 to 3 g of ammonium chloride and then ammonia solution (1 + 4) dropwise until the contents of the flask smell of ammonia. Acidify the solution to pH 3 to 4 by adding 10 per cent. V/V hydrochloric acid and then add a 1-ml excess of the acid. Add 10 ml of 30 per cent. V/V triethanolamine while shaking the flask, followed by 10 ml of buffer solution, 5 to 10 ml of 10 per cent. m/V potassium cyanide solution and 1 g of ascorbic acid. Heat the solution to 70 to 80 °C to remove the iron colour, cool it and titrate it with 0·05 M EDTA solution to the sharp green end-point in the presence of 10 to 12 drops of mixed indicator solution.

Proceed in the same way as described in procedure I for the determination of barium oxide and calculate the amount of barium oxide from the same equation.

Results and discussion

The sulphate ion plays an important rôle in the stepwise precipitation of barium and strontium from homogeneous solution because of its replacement reactions with magnesium and zinc sulphates, respectively. Firsching[7] studied the co-precipitation of alkaline earths during the homogeneous precipitation of barium sulphate from an equimolar solution of these elements in a 1·5 mM sulphate-ion concentration and reported the co-precipitation of strontium to be less than 4 per cent. In the present study, it was observed that the co-precipitation of strontium increased with the increase of excess of sulphate-ion concentration. An excess of 1·5 to 2·5 mM magnesium sulphate concentration over the theoretical requirement of sulphate ions calculated as magnesium sulphate (1 ml of 0·05 M magnesium sulphate solution for every 7·67 mg of barium oxide content) was found to be satisfactory. Addition of magnesium sulphate in the procedure instead of magnesium chloride and ammonium sulphate[7] improved the granular nature of the barium sulphate precipitate. For precipitation of strontium sulphate, however, a large excess of sulphate ions (more than 0·25 M) is preferable.

Temperature is also an important factor in the procedure. Quantitative precipitation of barium sulphate at room temperature takes too long, while with boiling solutions, co-precipitation of strontium sulphate (in an equimolar solution) increases. A temperature of 50 to 60 °C is found to be a suitable compromise for barium sulphate precipitation. However, the precipitation of strontium sulphate requires the solution to be boiling.

The ratio of strontium to barium is also very important in the precipitation of barium sulphate. The determination is possible in solutions containing a molar ratio of up to 1·0, or slightly greater (1·481) when the concentration of strontium oxide is not higher than 20·0 mg per 100 ml of solution (Table I). With increase in strontium-ion concentration, the co-precipitation of strontium sulphate also increased.

Table I

Stepwise determination of barium and strontium in the presence of calcium (20 mg) and magnesium (20 mg)

SrO taken/mg	BaO taken/mg	Molar ratio, SrO : BaO	BaO found*/mg	SrO found*/mg	Difference (BaO)/mg	Difference (SrO)/mg
5	10	0·740	10·02	5·19	+0·02	+0·19
10	10	1·48	10·02	10·07	+0·02	+0·07
20	10	2·96	Drifting of end-point	Drifting of end-point	—	—
5	20	0·37	20·20	5·14	+0·20	+0·14
10	20	0·74	20·12	10·07	+0·12	+0·07
20	20	1·48	20·20	20·10	+0·20	+0·10
30	20	2·22	Drifting of end-point	Drifting of end-point	—	—
10	40	0·37	40·02	10·18	+0·02	+0·18
20	40	0·74	40·10	20·25	+0·02	+0·25
20	60	0·49	59·68	20·35	−0·32	+0·35
20	80	0·37	79·73	20·25	−0·27	+0·25

* Mean of three titrations.

The metallochromic indicator Eriochrome black T is often used in the direct titration of barium and strontium with EDTA solution in the presence of calcium and magnesium at pH 10·0. However, the end-point, from red to blue, is not very sharp and is sometimes confusing. In an attempt to improve the end-point by producing a change from red to green, additions of yellow dyes such as methyl orange,[8] methyl yellow,[8,9] Tropaeoline OO[9,10] and methyl red[11–13] have been reported. In the present work, we used Titan yellow in conjunction with Eriochrome black T and Naphthol green B and this mixed indicator gave a very sharp change from red to green in the titration of barium with EDTA solution. The end-point during the titration of excess of zinc with EDTA solution (procedure I) in the absence of absolute ethanol, however, reverted from green to pink within a few seconds, probably as a result of the greater solubility of strontium sulphate. The addition of 10 to

15 ml of absolute ethanol so as to reduce the solubility of strontium sulphate was necessary in order to eliminate this effect.

The stepwise determination of barium and strontium is possible in the presence of calcium, magnesium and lead. However, iron and manganese, even at a concentration of 1 p.p.m., affected the end-point by causing the colour of the indicator to fade. Copper, nickel and cobalt interfered by inactivating the indicator. In the presence of any of these interfering elements, the procedure for the stepwise determination of barium and strontium fails. Procedure II, in which the interferences due to iron (5·45 mg), aluminium (11·0 mg), titanium (2·0 mg), nickel (31·8 mg), cobalt (20·0 mg), manganese (9·0 mg) and copper (32·0 mg) were eliminated (see Table II) by complexing with triethanolamine and potassium cyanide, and the iron and manganese complexes were subsequently reduced with ascorbic acid, was found to facilitate the determination of barium in the presence of these ions. Higher concentrations of aluminium caused co-precipitation of magnesium during the heating stage of the procedure.

TABLE II

DETERMINATION OF BARIUM IN THE PRESENCE OF VARIOUS CATIONS

BaO taken = 20·0 mg

Ion	Added/mg	BaO found*/mg	Difference/mg
Sr	8·46	19·97	−0·03
Fe	2·62	19·89	−0·11
Fe	5·24	20·04	+0·04
Al	5·50	20·12	+0·12
Al	22·00	21·10	+1·10
Ti	0·30	20·04	+0·04
Ti	1·50	20·20	+0·20
Mn	3·00	19·89	−0·11
Mn	8·95	20·04	+0·04
Ni	12·59	20·20	+0·20
Ni	31·80	20·20	+0·20
Cu	16·00	20·20	+0·20
Cu	32·00	20·04	+0·04
Co	20·00	20·04	+0·04

*Mean of three titrations.

Manganese, titanium and zirconium, when present in higher concentrations than those mentioned above, were also precipitated during heating. In such instances, the removal of iron, aluminium, titanium and zirconium by precipitating them as hydroxides with ammonia solution was found to be satisfactory. Phosphate and chromate ions, even in small amounts, precipitated the alkaline earth elements and thus interfered in both procedures. Chromate could be removed as hydroxide together with R_2O_3-type elements after reduction with ethanol. Phosphate, when present, is required to be removed as zirconium phosphate.

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Molecular Interaction Errors in Phase-solubility Analysis

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Results are presented for the assay by phase-solubility analysis of ibufenac (4-isobutylphenylacetic acid) and of saccharin in the presence of various impurities. The results for impurities obtained for ibufenac mixtures were almost twice those expected, while those for saccharin mixtures were normal. From a consideration of these results, the effect of compound formation by molecular interaction is discussed.

THE theoretical and practical aspects of phase-solubility analysis have been reviewed by Mader,[1] Higuchi and Connors[2] and by Outch[3] and its use has been suggested for purity determination. The technique has recently been applied by the National Bureau of Standards to the determination of the purity of cholesterol, urea and creatinine, which were to be used as clinical reference materials.[4] Only Higuchi and Connors, in discussing the limitations of the technique, emphasised that the solubility of each component must be unaffected by the presence of the other components although this aspect has been mentioned earlier.[5–8] Wilkinson and Wragg[9] obtained anomalous results by phase-solubility analysis for the impurity content of an insecticide (butacarb) when compared with those obtained by the use of gas - liquid chromatography, infrared spectroscopy and isotope dilution analysis. They suggested that compound formation by interaction between components of a mixture was a serious limitation to the application of the procedure. To investigate further the possibilities of molecular-interaction compound formation and its effect on the results obtained, the anti-inflammatory, antirheumatic compound 4-isobutylphenylacetic acid (ibufenac), and the artificial sweetener *o*-sulphobenzoic imide (saccharin), were chosen for phase-solubility studies in model systems.

Experimental and results

Apparatus and technique—

The apparatus used was that described by Garratt, Johnson and King[10] and the procedure was based on that outlined by Mader.[11] Solvents were selected to give solubilities in the range 0·1 to 7·5 per cent., as suggested by Mader, and the results obtained were calculated by using the least-squares method. The compounds were examined by using various equilibration times from 2 to 14 days. The marked scattering of points in the phase-solubility diagrams after equilibration for 2 days indicated that equilibrium conditions were not obtained in this period. However, results obtained after equilibration for 4 days were more consistent and were indistinguishable from those obtained by using longer equilibration periods.

The reliability of the techniques used in this investigation was checked by using the insecticide (butacarb) previously examined in detail by Wilkinson and Wragg. Crude butacarb was purified by recrystallisation from light petroleum (boiling range 40 to 60 °C). A mixture containing 3,5-di-t-butylphenol (5 per cent. *m/m*) and purified butacarb (95 per cent. *m/m*) was prepared. This mixture and the purified butacarb were assayed by phase-solubility analysis by using n-hexane as solvent and an equilibration period of 7 days at 31 °C. Solution compositions were determined gravimetrically by evaporating off the solvent at room temperature under a stream of nitrogen and finally drying the residue to constant mass at 75 °C. The impurity content of the purified butacarb was found to be 0 per cent. [confidence limits ($P = 0{\cdot}95$) $\pm$ 0·14 per cent.] and that of the mixture 11·7 per cent. [confidence limits ($P = 0{\cdot}95$) $\pm$ 0·72 per cent.]. The latter result was rather more than twice the value expected in the absence of interaction, and was in agreement with results obtained by Wilkinson and Wragg.

Phase-solubility determination of ibufenac—A commercial sample of ibufenac was purified by recrystallisation from light petroleum (boiling range 40 to 60 °C) and the resulting crystals were ground in a glass mortar. The drug formed a liquid phase when equilibrated with solvent mixtures of water and methanol or water and acetone, but remained crystalline and had the desired solubility in the non-polar solvents n-hexane, n-heptane, n-octane and 2,2,4-trimethylpentane.

Mixtures of known composition were prepared by adding known amounts of benzoic acid (impurity I), phenylacetic acid (impurity II) and 2-(4-isobutylphenyl)propionic acid (ibuprofen) (impurity III) to the purified ibufenac. Each of the added impurities was previously purified, impurity I by vacuum sublimation and II and III by recrystallisation from light petroleum (boiling range 40 to 60 °C). Homogeneity of the mixtures (Table I) was ensured by dissolving the weighed components in the minimum volume of methanol and evaporating the solution to dryness at 55 °C with the aid of a stream of oxygen-free nitrogen. The residues were dried to constant mass over silica gel under partial vacuum and then ground in a mortar.

TABLE I

COMPOSITION (PER CENT. *m/m*) OF PREPARED IBUFENAC MIXTURES

No.	Recrystallised ibufenac	Impurity I	Impurity II	Impurity III
1	90·0	10·0	—	—
2	95·0	5·0	—	—
3	98·0	2·0	—	—
4	95·0	—	5·0	—
5	98·0	—	2·0	—
6	95·0	—	—	5·0
7	98·0	—	—	2·0
8	90·0	7·0	3·0	—

The purified ibufenac and each of the mixtures were assayed by phase-solubility analysis, with an equilibration temperature of 28 °C and an equilibration period of 4 days. To determine the solution composition, the solvent was removed at 55 °C under a stream of oxygen-free nitrogen and the residue dried over silica gel under partial vacuum.

The above procedure was used to assay a sample of commercial ibufenac in 2,2,4-trimethylpentane and the recrystallised drug in n-hexane, n-heptane and n-octane. In order to test for solid-solution formation in the mixtures Nos. 2 to 7, the solid phases from tubes in the saturated segment of the phase diagrams were separated from the solution phases, washed with a small amount of pure solvent and dried. The solid phases were combined and then assayed by the phase-solubility method. The results for the phase-solubility analysis are summarised in Table II. The confidence limits ($P = 0.95$) for the percentage impurity results were in the range ± 0.4 to ± 1.0 per cent. The solubility of pure ibufenac was found to be 39·4 mg g^{-1}.

TABLE II

RESULTS OF THE ASSAY OF IBUFENAC BY PHASE-SOLUBILITY ANALYSIS

Sample	Equilibration period/days	Solvent	Impurity, per cent. *m/m* Added	Found
Mixture 1	4	2,2,4-Trimethylpentane	10·0	25·9
2	4		5·0	12·3
2	7		5·0	11·6
3	4		2·0	5·3
4	4		5·0	15·5
5	4		2·0	3·1
6	4		5·0	14·5
7	4		2·0	3·3
8	4		10·0	22·6
Recrystallised ibufenac ..	14		—	0·4
	4		—	0·3
	4	n-Hexane	—	0·5
	4	n-Heptane	—	0·3
	4	n-Octane	—	0·5
Commercial ibufenac ..	4	2,2,4-Trimethylpentane	—	3·7
Solid phases	4		—	0·1

Phase-solubility determination of saccharin—Saccharin was purified by recrystallisation from methanol. Mixtures of known composition were prepared by adding known amounts of *o*-toluenesulphonamide (impurity IV) and *p*-sulphonamidobenzoic acid (impurity V), both of which had been previously purified by recrystallisation from water, to the purified saccharin.

The procedures for phase-solubility analysis and preparation of mixtures were the same as those adopted with ibufenac except that all residues were dried to constant mass at 105 °C. Samples of recrystallised saccharin, commercial saccharin and the mixtures given in Table III were assayed by using as solvents acetone - chloroform (3 + 7 *V*/*V*) and pure chloroform; the equilibrium temperature was 34 °C and the period of equilibration was either 4 or 14 days.

TABLE III

COMPOSITION (PER CENT. *m*/*m*) OF PREPARED SACCHARIN MIXTURES

Mixture	Saccharin	Impurity IV	Impurity V
A	90·0	10·0	—
B	95·0	5·0	—
C	95·0	—	5·0
D	90·0	7·0	3·0

To determine the solution composition, the solvent was removed by evaporation under a stream of oxygen-free nitrogen and the residue dried at 105 °C. The samples were also analysed by argentimetric titration based on the procedure of Parikh and Mukherji.[12] The results for the phase-solubility analysis are summarised in Table IV. The confidence limits ($P = 0{\cdot}95$) for the percentage impurity results were in the range $\pm 0{\cdot}2$ to $\pm 0{\cdot}8$ per cent. The solubility of pure saccharin was found to be 19·4 mg g^{-1}.

TABLE IV

RESULTS OF THE ASSAY OF SACCHARIN BY PHASE-SOLUBILITY ANALYSIS

Sample	Equilibration period/days	Solvent	Percentage of total compounds other than saccharin: Added	Phase-solubility analysis	Titration method
Mixture A	4	Acetone - chloroform (3 + 7)	10·0	10·4	8·8
A	14		10·0	10·4	—
B	4		5·0	5·4	5·9
C	4		5·0	4·3	2·2
D	4		10·0	9·7	8·3
Recrystallised saccharin ..	4		—	0·02	0·8
	14		—	0·06	—
Commercial saccharin ..	4		—	1·8	1·3

DISCUSSION AND CONCLUSIONS

Phase-solubility analysis of ibufenac mixtures consistently gave impurity contents of approximately twice the expected values. This behaviour is similar to that which gave rise to the high results obtained by Wilkinson and Wragg[9] with butacarb. Of the possible sources of error, procedural errors, optical isomerism, solid-solution formation and unique ratio can be ruled out. Polymorphism has been suggested as an explanation for high assay results,[1] but, unlike the behaviour in the present instance, such a phenomenon would cause erratic results and could be overcome by increasing the period of equilibration. It is therefore likely that a soluble compound is formed between the major components and their chemically similar impurities.

The interactions responsible for such compound formation would not be covalent bonds but much weaker forces such as those due to van der Waals' interactions or to hydrogen bonding. Both the carboxylic acids of the ibufenac mixtures and the carbamate and phenolic species of the butacarb mixtures fulfil the requirements for weak electrostatic interaction, and it is therefore probable that hydrogen bonding is the most important type of interaction in these systems.

For the ibufenac systems studied the situation is not clear; even if an error of 10 per cent. in the nominal composition of the mixture is accepted, the results do not conform to those anticipated for simple 1:1 compound formation. It is therefore probable that dispersive intermolecular forces, in addition to hydrogen bonding, are involved and that compound formation in these systems is the result of competition between several reactions. Such interactions are sensitive to the nature of the solvent, hydrogen bonding being favoured by non-polar solvents such as those used for the butacarb and ibufenac assays, while dispersive interaction is favoured when non-polar materials are dissolved in polar solvents. It can be seen that the criteria for low solubility in phase-solubility analysis will demand the use of such systems and the compound formation is likely to be a common occurrence. The interactions will be particularly apparent with several molecules the association of which is not hindered by structural factors.

Phase-solubility analysis of saccharin mixtures in acetone - chloroform (3 + 7) gave results close to the expected values. The results obtained with chloroform alone as solvent were less satisfactory, possibly because the equilibration periods of up to 14 days were too short for equilibrium to be fully established. Use of the argentimetric method confirmed the results for mixtures containing *o*-toluenesulphonamide(IV) but gave low results for those mixtures which contained *p*-sulphonamidobenzoic acid (V), probably because of the formation of the silver salt of the latter. An explanation for the satisfactory analysis of saccharin mixtures can also be given on the basis of solvent effects. In the acetone - chloroform solvent the forces of solute - solvent, particularly of solute - acetone, interactions are presumably sufficiently strong to eliminate the possibility of solute - solute interactions. The related extraction - solubility procedure[13] has recently been used[14] to examine compounds of relatively high purity possessing a variety of functional groups, which were prepared as analytical standards. Several difficulties were noted, including those concerned with the choice of solvent, and, in particular, instances of unsatisfactory results when non-polar solvents were used.

From the present results and those reported earlier,[9] we conclude that non-polar solvents should be avoided when assaying substances that are prone to hydrogen bonding, and, further, that the results of any phase-solubility analysis should not be considered to be reliable unless recovery experiments on appropriate mixtures of known composition indicate the absence of complex formation by molecular interaction. The carboxyl group is a common feature of an appreciable number of therapeutically active substances, and, unless suitable precautions are taken, anomalous results might be expected to arise with some frequency as such substances are subjected to phase-solubility analysis.

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Analytical Methods Committee

REPORT PREPARED BY THE FLUORINE SUB-COMMITTEE

A Critical Examination of Procedures for the Assay of Sodium Fluoride

THE Analytical Methods Committee has received the following Report from its Fluorine Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee was: Dr. E. J. Newman* (Chairman since October, 1969), Mr. R. W. Fennell* (Chairman until October, 1969), Dr. A. W. Davison, Mr. J. K. Foreman, Mr. G. S. Goff,* Mr. R. J. Hall, Dr. R. F. Milton, Mr. J. W. Ogleby,* Mr. B. T. Saunderson, Dr. J. M. Skinner* and Mr. C. A. Watson,* with Mr. P. W. Shallis as Secretary. The members marked with an asterisk took part in the work of the Milligram Group of the Sub-Committee, which was responsible for the preparation of this Report. In addition, the following were corresponding members of the Sub-Committee, although those marked with an asterisk took part in the early stages of the work: Professor R. Belcher, Mr. H. A. Foner,* Mr. H. Green,* Mr. R. Waspe* and Dr. J. E. Whitley.

INTRODUCTION

The Milligram Group of the Fluorine Sub-Committee set up by the Analytical Methods Committee in 1965 concentrated its efforts initially on trying to establish a suitable direct method for the assay of sodium fluoride. The main objective was that material thus characterised could then be used in work on methods that required calibration, such as titration with thorium nitrate, colorimetry or potentiometric measurement by means of the fluoride-selective electrode. A survey of the literature and the experiences of the members of the Sub-Committee with various procedures indicated that the precipitation of lead chlorofluoride, followed either by a final weighing or by a titration procedure, might prove to be satisfactory, although it was recognised from the outset that the procedure was lengthy and more open to operator errors than was ideally desirable. Finally, a favoured, although not entirely satisfactory, lead chlorofluoride precipitation procedure was compared with an indirect ion-exchange method.

All the members of the Group had had some experience of at least one of the variants of the method described by Hoffman and Lundell.[1] After some preliminary trials, an agreed procedure was established and a collaborative trial undertaken.

The results summarised in Table I were reported for both gravimetric determination, and, after the precipitate in the filter crucible had been dissolved in dilute nitric acid, for titrimetric determination. Common sources of solid sodium fluoride and of silver nitrate solution were used; each determination was made on a solution containing 25 mg of fluorine, as sodium fluoride.

TABLE I

FIRST COLLABORATIVE TRIAL—LEAD CHLOROFLUORIDE METHOD

	Gravimetric determination			Titrimetric determination		
Laboratory	Mean recovery, per cent.	Standard deviation	No. of results	Mean recovery, per cent.	Standard deviation	No. of results
A	101·19	0·34	10	101·21	0·61	10
B	98·45	0·95	6	98·08	0·82	5
C	100·33	0·87	6	100·05	0·91	6
D	102·10	0·63	6	101·50	0·60	6
E	103·62	0·84	6	104·39	3·79	6

In view of the wide discrepancies between the results obtained in the different laboratories, a detailed examination of the individual manipulative techniques used in each laboratory was made and the following aspects were noted—

(*i*) different percentage mass losses were observed between laboratories and between different amounts of sample on drying the sodium fluoride; and

(*ii*) some operators took the mass of lead chlorofluoride as the difference between the mass of the crucible *plus* precipitate and the original mass of the crucible, whereas others took the difference between the mass of the crucible *plus* precipitate and the final mass of the crucible, *i.e.*, after the precipitate had been dissolved in nitric acid. Most, but not all, operators recorded a loss in mass of the crucible after the lead chlorofluoride precipitate had been dissolved in nitric acid.

A second collaborative trial was carried out, for which the details of the procedure were more strictly laid down. Only the gravimetric technique was used, as the titrimetric procedure did not appear to offer any advantage. Again in each test a solution containing 25 mg of fluorine, as sodium fluoride, was used; the results are summarised in Table II.

TABLE II

SECOND COLLABORATIVE TRIAL—LEAD CHLOROFLUORIDE METHOD

Laboratory	Mean recovery, per cent.	Standard deviation	No. of results
A	101·30	0·48	6
B	97·95	1·05	4
C	101·62	0·19	6
D	103·05	0·34	6
F	99·23	0·32	6

These results are similar to those in Table I for laboratories A to D, although results for laboratories C and D both show an improved precision but a higher mean recovery than those obtained previously. The behaviour of the sodium fluoride on drying indicated that this particular batch was suspect, although not sufficiently variable to invalidate the general picture of the results obtained.

In the procedure used so far the lead had been added to the fluoride-containing solution as solid lead nitrate. Other methods of precipitating lead chlorofluoride that might have offered advantages in terms of speed, accuracy and precision were available. In order to make a preliminary assessment of the merits of the various procedures, two analysts in one laboratory each carried out six determinations by the five methods listed below.

I Gravimetric, solid lead nitrate.
II Titrimetric, solid lead nitrate; precipitate filtered on to, and then dissolved from, a paper-pulp pad instead of a filter crucible.
III Gravimetric, lead chloronitrate solution.[2]
IV Gravimetric, lead acetate solution.[3]
V Gravimetric, lead chloride solution.[4]

A common stock fluoride solution was used by the analysts who took part in this work and for each determination a solution containing 25 mg of fluorine, as sodium fluoride, was used. The results obtained are shown in Table III, from which it can be seen that no single method is outstandingly better than the others, although method IV is significantly inferior in terms of precision. The percentage recovery figures are for comparison only; the

TABLE III

FIRST COMPARISON OF METHODS FOR FLUORIDE DETERMINATION

	Analyst 1		Analyst 2			
Method	Mean recovery, per cent.	Standard deviation	Mean recovery, per cent.	Standard deviation	Grand mean, per cent.	Pooled standard deviation
I	101·45	0·79	100·55	0·84	101·00	0·81
II	101·08	0·61	99·93	0·74	100·43	0·68
III	98·53	0·50	98·13	0·78	98·33	0·66
IV	101·26	1·76	100·72	0·93	100·99	1·41
V	98·19	0·52	98·59	0·78	98·47	0·66

purity of the sodium fluoride used to make up the stock solution was not known although it was believed to be high.

As no definite conclusion could be reached from this test, a standardised solution of sodium fluoride was circulated, and members of the Group were requested to determine its fluoride content by as many of the above methods as they could apply and also by a method based on the indirect ion-exchange method used for the assay of commercial sodium fluoride.[5] For the lead chlorofluoride procedure, each determination was carried out on a solution containing 25 mg of fluorine, as sodium fluoride, and for the ion-exchange method solutions containing 50 mg of fluorine, as sodium fluoride, were used except in laboratory A, which used solutions containing 40 mg of fluorine. The results obtained are summarised in Table IV.

TABLE IV

SECOND COMPARISON OF METHODS FOR FLUORIDE DETERMINATION

Method ..	I			II			III		
Laboratory	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
A	101·35	0·34	5	—	—	—	98·27	0·03*	2
B	101·23	0·73	6	100·79	0·86	6	—	—	—
C	99·60	0·21	6	—	—	—	—	—	—
D	98·06	0·68	6	—	—	—	—	—	—
E	101·45	0·48*	3	—	—	—	99·74	0·18	5
F	—	—	—	—	—	—	—	—	—
G	98·43	0·16	6	98·47	0·21	5	99·07	0·14	6
	99·67	0·68	7	—	—	—	—	—	—

Method ..	IV			V			Ion exchange		
Laboratory	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
A	—	—	—	—	—	—	99·68	0·09	4
B	—	—	—	—	—	—	—	—	—
C	—	—	—	—	—	—	100·16	0·07	6
D	—	—	—	—	—	—	98·31	0·27	6
E	—	—	—	—	—	—	100·15	0·05	4
F	—	—	—	—	—	—	100·00	0·00*	2
G	97·68	0·42	5	100·33	0·12	5	100·34	0·23	6
	—	—	—	—	—	—	—	—	—

a represents mean recovery, per cent.; *b*, standard deviation; and *c*, number of results.
* Range.

Despite the heterogeneity of the results in Tables III and IV, it was possible to derive some general conclusions—

(*i*) method IV could be discounted on grounds of poor precision;

(*ii*) better precision could be expected with the ion-exchange method than with any of the other methods tried;

(*iii*) there was a strong indication that the ion-exchange method was without bias; and

(*iv*) although a negative bias could be expected, method III might have better precision than method I, which tended to have a positive bias.

Those members of the Group who had tried method III agreed that the procedure was simpler than those for methods I, II or V. A collaborative trial was arranged in which three determinations by both method III and the ion-exchange method were made on three separate days, when possible by two analysts. Four laboratories were able to complete the full programme, a fifth could spare only one analyst. The full results, given as percentage recovery, are shown in Table V. A new common supply of sodium fluoride, shown not to have variable characteristics on drying, was used for these tests. The amounts of fluoride taken for determination were as before, *i.e.*, 25 mg for method III and 50 mg for the ion-exchange procedure, except for laboratory A in which 40 mg were taken.

An analysis of variance was applied to the results, starting with those reported by individual laboratories. Highly significant differences between the two methods were found for each laboratory, and significant differences between analysts occurred at some laboratories for method III. Variations from day to day were much larger than would be expected

TABLE V

COLLABORATIVE TRIAL—LEAD CHLORONITRATE (METHOD III) AND ION-EXCHANGE METHODS

Results are percentage recoveries

		Method III		Ion exchange	
Laboratory	Day	Analyst I	Analyst II	Analyst I	Analyst II
A	1	98·27	98·03	99·61	99·48
		98·08	97·82	99·66	99·62
		98·50	97·92	99·52	99·57
	2	98·45	98·08	99·58	99·47
		98·59	97·83	99·48	99·56
		98·27	97·99	99·39	99·52
	3	98·55	98·54	99·48	99·56
		98·50	98·52	99·44	99·47
		98·49	98·54	99·48	99·56
C	1	98·74	99·00	100·40	100·21
		98·76	98·75	100·21	100·21
		99·08	98·97	100·40	100·02
	2	98·70	99·05	100·02	100·21
		99·38	98·99	100·02	100·02
		99·11	98·95	100·02	100·02
	3	99·67	99·45	99·83	99·83
		99·43	99·32	99·83	100·02
		99·32	99·40	100·02	99·83
D	1	99·07	—	—	99·52
		99·32	—	—	99·45
		100·04	—	—	99·63
	2	100·67	—	—	99·81
		100·77	—	—	99·99
		100·86	—	—	99·45
	3	99·33	—	—	99·63
		99·71	—	—	99·99
		99·38	—	—	100·17
F	1	99·58	99·52		
		99·42	99·52		
		99·44	99·44		
	2	99·42	99·90		
		99·22	99·30	All results 99·7 to 99·8*	
		99·30	98·99		
	3	99·38	99·06		
		99·44	99·10		
		99·56	98·92		
G	1	96·33	97·86	99·88	100·26
		96·75	98·58	100·26	100·26
		96·80	96·96	100·26	100·26
	2	97·75	97·38	100·64	100·26
		97·35	97·16	100·26	100·46
		96·33	97·12	100·08	100·08
	3	95·80	98·33	100·26	100·26
		96·40	98·21	100·64	99·88
		95·13	98·55	100·46	100·46

* Results rounded to 0·05 ml of titrant.

from variability within sets of three readings. However, these day-to-day variations did not affect the two methods in the same way; a high result by the gravimetric procedure could occur with a low result by the ion-exchange method.

The methods were then examined separately over all laboratories. Results, expressed as standard deviations in percentage recovery, were—

	Method III	Ion exchange
Between laboratories	0·94	0·33
Between analysts in a laboratory	0·41	—*
Between days for one analyst	0·35	0·08
Within sets of readings	0·31	0·13

* Only three sets of results were available.

It is apparent that, as might be expected, laboratory variability is a dominant factor. That this variability is not caused by the common source of sodium fluoride that was used in each laboratory for making up the solutions is illustrated in Table VI, which is a summary of the results given in Table V. It can be seen, for example, that low results by method III do not coincide with low results by the ion-exchange method. It can also be seen that the ion-exchange method is substantially without bias, whereas the gravimetric method III has a negative bias of at least 1 per cent.

Conclusions

None of the methods tried was entirely satisfactory as an assay (or referee) method for sodium fluoride. The ion-exchange procedure provided the nearest approach on grounds of both accuracy and precision. It has, however, the following disadvantages—

(*i*) it provides a measure of the total anions present in the solution under test and is therefore completely unspecific;

(*ii*) it depends on the accurate standardisation of the alkaline titrant; and

(*iii*) it depends on the efficient functioning of the ion-exchange resin column.

On the other hand, it is simpler to manipulate and considerably more rapid than any of the precipitation procedures.

Table VI

Summary of results in Table V

		Method III		Ion-exchange method	
Laboratory	Analyst	Mean recovery, per cent.	Standard deviation	Mean recovery per cent.	Standard deviation
A	I	98·41	0·17	99·52	0·09
	II	98·14	0·31	99·53	0·05
C	I	99·13	0·35	100·11	0·21
	II	99·10	0·24	100·06	0·15
D	I	99·91	0·70	—	—
	II	—	—	99·74	0·26
F	I	99·42	0·11	$99{\cdot}7_5$	—
	II	99·31	0·32	$99{\cdot}7_5$	—
G	I	96·52	0·78	100·30	0·25
	II	97·79	0·69	100·24	0·18

The collaborative work on lead chlorofluoride precipitation confirmed the impression held in individual laboratories that the method was subject to operator variability, whichever particular procedure was used. The most complete trial of a precipitation procedure was that reported last, wherein lead chloronitrate was used as precipitant (method III), the results of which are summarised in Table VI. The variability in the results, both means and standard deviations, achieved by different analysts is clearly demonstrated. It is of interest to note the consistency between the pairs of means obtained by the analysts in laboratories A, C and F, although the laboratory means vary by about 1 per cent. Another interesting feature, illustrating the variability possible between operators in the same laboratory, is given by laboratory G's results for method III in Tables IV and VI; the results in Table IV are much more in line with those of the other laboratories reported in Table VI, where laboratory G's results can be seen to be outstandingly poor. Laboratory A, on the other hand, reported remarkably consistent mean results on different occasions for this procedure (see Tables III, IV and VI), although these were lower than those reported by most other analysts.

Similar observations can be made on the earlier work on the "lead nitrate" (method I) procedure although the figures obtained were much more variable.

It can be concluded, therefore, that although the lead chloronitrate precipitation procedure is not suitable as a referee method, it can give reasonably consistent results under

conditions that apply in a given laboratory and is to be preferred to the other procedures tried. The following disadvantages are noted—

(*i*) a negative bias can be expected;

(*ii*) the method is significantly less precise than the ion-exchange method; and

(*iii*) the procedure requires manipulative skill and is lengthy, requiring a period of standing overnight for precipitation.

Anionic interference is, however, less than that for the ion-exchange method, the analytical factor is good and the method is direct, *i.e.*, the fluorine being determined is precipitated.

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Book Reviews

STATISTICAL METHODS IN RESEARCH AND PRODUCTION WITH SPECIAL REFERENCE TO THE CHEMICAL INDUSTRY. Edited by OWEN L. DAVIES and PETER L. GOLDSMITH. Fourth Revised Edition. Pp. xiv + 478. Edinburgh: Oliver and Boyd for Imperial Chemical Industries Limited. 1972. Price £5.

The title of this book makes it clear that it is about the application of statistics to problems of research and production, and this must be borne in mind by the analyst who is wondering if he should buy it. For those for whom it is intended, it is an excellent exposition—logically set out, lucidly explained and with a wealth of practical examples that are fully worked out and based on I.C.I.'s actual experience.

The last (third) edition was published in 1957. The changes that have taken place since then have led to the production of a completely revised text, only two of the contributors to the new edition being contributors to the previous one. Attention is drawn in the introduction to the implications of the availability of computers, and the consequent reduction in use of desk calculating machines. This is illustrated later in the chapter on Multiple Regression, in which techniques of much practical use are described that could not practicably be used at all without the aid of a computer.

Although the chapter headings and general arrangement of the new edition remain almost the same as in the last edition, which makes it easy for anyone familiar with the latter to use the former, every chapter appears to have been completely re-written. The analyst engaged on quality control will be interested in the extension of Chapter 10, "Control Charts," to include the recently devised "Cusum" charts, by means of which undesirable trends can be detected sooner than with conventional Shewhart charts.

The chapter on decision-making has been completely re-written; it is very well done and should be most useful at management level, although perhaps not to the analyst who only provides the data on which the decisions will be based. But the analyst in question will find the first few chapters of this book a first-class description of standard statistical techniques, many of which may be of direct service to him in increasing the productivity of his department; and he would be well advised at least to skim through the later chapters also if he aspires to be, some day, the man at the aforesaid management level.

ERIC C. WOOD

PARTICLE MEASUREMENT, SIZE AND SURFACE AREA DETERMINATION. BIBLIOGRAPHY 1969–1972. By T. ALLEN. Pp. vi + 34. Published by the author at the University of Bradford. 1972. Price £2.

This book is a very worthwhile compilation. It comprises a literature survey for the period 1969 to 1972 and contains 1132 references. The survey review of recent publications on particle size and surface area determination is pertinent and sensibly arranged. The index to this survey provides a short cut to the original literature. The arrangement of the references cannot be faulted. The book is well produced and it is to be hoped that it can be made generally available. At present, this and other similar publications are available by direct application to the authors, at the Postgraduate School of Studies in Powder Technology, the University of Bradford. This book shows commendable initiative on the part of the staff at Bradford and they should be congratulated on their moves in this direction.

D. DOLLIMORE

ANALYTICAL APPLICATIONS OF EDTA AND RELATED COMPOUNDS. By R. PŘIBIL. *International Series of Monographs in Analytical Chemistry, Volume* 52. Pp. xxii + 368. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1972. Price £12·50.

The formation of complexes in aqueous solution is of great importance in analytical chemistry and EDTA and its derivatives provide some of the most efficient chelating agents available for these reactions. Dr. Přibil is a well known authority on the reactions of these compounds and his extensive published work and stimulating lectures clearly demonstrate his mastery of their mode of action and ingenuity in their application. When confronted with a new monograph by this author, therefore, the reader is assured that the work will contain a wealth of material that will be of immediate practical value. The monograph is divided into two sections. Part I takes the form of two chapters that make up a theoretical introduction. The first of these, written by

Prof. R. Belcher and Dr. A. Townshend, describes the development of EDTA as an analytical reagent. The second chapter, written by Prof. J. Koryta, contains an excellent brief account of the nature of the equilibria of complexes and methods of study. Part II commences with Chapter 3 and is devoted to the reactions of classical gravimetric analysis, titrimetric analysis, colorimetry and other instrumental techniques. The monograph is thus concerned only with the "passive" rôle of EDTA and other complexans, *i.e.*, their screening or masking properties. The author states clearly that he considers the "active" rôle of EDTA to be in its use as a reagent in complexometric titrations, and that the latter is not included in the book as it forms an independent sector of analytical chemistry. The text contains a vast amount of information on the "passive" applications of the complexans; the use of some of the metallochromic indicators, such as those derived from the triphenylmethane dyestuffs, is also considered in relation to colorimetry in Chapter 5.

This volume is a translation of an entirely new Czech manuscript, except for Chapter 2, which is essentially a translation of Prof. Koryta's contribution to the 1957 Czech edition, and is stated to have little in common with the Czech editions published in 1953 and 1957 as regards extent or concept of the whole subject. It is authoritative, well balanced and clearly written and illustrated; despite its relatively high price, it should be extremely valuable to all workers concerned with wet-chemical procedures for the separation and determination of inorganic species in solution.

G. F. KIRKBRIGHT

COURS DE CHIMIE ANALYTIQUE GÉNÉRALE. Tome II. MÉTHODES ÉLECTROCHIMIQUES ET ABSORPTIOMÉTRIQUES, CHROMATOGRAPHIE. By GASTON CHARLOT. Pp. iv + 201. Paris: Masson et Cie. 1971. Price FF41.

This book is the second of the volumes written by the author especially for students in the "Licence et Maîtrise des Sciences" course in analytical chemistry in Universities and Colleges of Technology in France. As these courses correspond approximately to the B.Sc. and M.Sc. degree courses in some U.K. universities, it is perhaps timely to consider what is thought to be appropriate for these levels by a well known advocate of analytical chemistry. There may be some desire to have closer academic relations between the universities and colleges in the countries of the E.E.C. and it is probable that we may have some transfer of professional analysts within multinational industries. A knowledge of what is recommended for these courses may be useful. The first volume dealt with reactions in aqueous and non-aqueous solutions. This small volume is concerned with the basic theory of electrochemical reactions and their applications in analytical chemistry, as well as separation methods, including chromatography. The application of optical methods, such as absorptiometry, to photometric titrimetry is also considered. There is also a chapter dealing with accuracy and precision. The aim is reasonable but the range of subjects covered in 200 pages is so wide that much of the treatment is of necessity very superficial. The level of the work would not be accepted at the Finals level for a first degree in the U.K. It is not detailed enough in its practical aspects for L.R.I.C. courses in Colleges of Technology. As a revision text-book it is quite acceptable, and as a book for students of chemistry who do not specialise in analytical chemistry it gives a good coverage of the subject; a translation would certainly prove of value to many undergraduates in the U.K.

L. S. BARK

Errata

JULY (1972) ISSUE, p. 533, line 47. *For* "0·05-μCi" *read* "0·5-μCi."

IBID., p. 533, line 48. *For* "2×10^{-2} mrad h^{-1}" *read* "0·2 mrad h^{-1}."

IBID., p. 534, line 26. *For* "10 μCi" *read* "100 μCi."

IBID., p. 534, line 27. *For* "10 μCi g^{-1}" *read* "10 Ci g^{-1}."

LECTURES AND COURSES

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APPOINTMENTS VACANT

The Identification and Semi-quantitative Assay of Some Fat-soluble Vitamins and Antioxidants in Pharmaceutical Products and Animal Feeds by Thin-layer Chromatography

A thin-layer chromatographic method for the identification and semi-quantitative assay of vitamin A (alcohol), its acetate and palmitate, vitamin D, α-tocopherol, α-tocopheryl acetate, BHA (butylated hydroxyanisole; 2-t-butyl-4-methoxyphenol), BHT (butylated hydroxytoluene; 2,6-di-t-butyl-4-methylphenol) and ethoxyquin in vitamin preparations is described. The sample solutions are applied to thin layers of silica gel and the vitamins and antioxidants are separated by using n-hexane - ethyl methyl ketone - di-n-butyl ether (34 + 7 + 6) as the developing solvent. Decomposition of vitamins A and D when applied to the adsorbent layer is inhibited by the presence of triethylamine in the spotting solvent. The compounds are identified by means of their R_F values, their appearance in ultraviolet radiation of wavelengths 254 and 360 nm and their response to iron(III) chloride - bipyridyl and iron(III) chloride - potassium hexacyanoferrate(III) spray reagents; they are assayed by visual comparison with standards. The method has been applied to gelatin-protected vitamin beads, animal feed additives, multivitamin tablets, oily vitamin concentrates and halibut-liver oil samples.

A simple colour test for distinguishing vitamin D_2 from vitamin D_3 after removal of vitamin A and its esters is also described.

G. W. JOHNSON and C. VICKERS

Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham.

Analyst, **1973**, **98**, 257–267.

Gas - Liquid Chromatographic Determination of Vitamin D in Cod-liver Oil

A gas - liquid chromatographic method for the determination of vitamin D (cholecalciferol) in cod-liver oil is described. It involves saponification of the oil, extraction of the unsaponifiable matter, removal of interferences such as cholesterol and retinol (vitamin A) by precipitation, and column chromatography on Sephadex LH-20 and Florisil; the final determination of cholecalciferol is carried out by gas - liquid chromatography. A determination can be completed in less than 2 days.

J. G. BELL and A. A. CHRISTIE

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, **1973**, **98**, 268–273.

The Determination of Trace Amounts of Cobalt and Other Metals in High-purity Water by Using Ion-exchange Membranes

Microgram amounts of cobalt, chromium, copper, iron, nickel and zinc are concentrated on ion-exchange resin impregnated membranes from large volumes of reactor cooling waters. Atomic-absorption measurement on the acid-extracted membranes has permitted the determination of cobalt down to 0·01 $\mu g\ l^{-1}$ in water samples, with an analytical precision of ±12 per cent. at the 96 per cent. confidence limit, based on twelve replicate observations. Gamma-spectrometric measurement of the nuclide cobalt-60 present in the reactor cooling waters has enabled membrane efficiency to be determined; at cobalt levels of 0·01 and 0·1 $\mu g\ l^{-1}$, the efficiencies are shown to be 85 per cent. and greater than 99 per cent., respectively.

H. JAMES

Atomic Energy Establishment, Winfrith, Dorchester, Dorset.

Analyst, **1973**, **98**, 274–288.

Indirect Complexometric Titration of Barium and Strontium after Stepwise Precipitation as Sulphate from Homogeneous Solution

An indirect method has been developed for the stepwise titration of barium and strontium with EDTA, based on precipitation of the elements as sulphate from a homogeneous solution. Barium, strontium and other multivalent cations are complexed by titration with EDTA at pH 10·0 by using a mixed indicator comprising Eriochrome black T, Titan yellow and Naphthol green B. Barium is then selectively replaced from its EDTA complex and precipitated as the sulphate with an excess of magnesium sulphate solution. The excess of magnesium is determined by titration with EDTA and the equivalent concentration of barium is calculated. Strontium is determined in the same solution in the same way, with an excess of zinc sulphate solution.

The optimum conditions for precipitation and the interferences due to various anions and cations have also been studied. Copper, nickel, cobalt and iron interfere and a second method for the determination of barium alone has been derived wherein the interfering elements are complexed with triethanolamine, potassium cyanide and ascorbic acid. Phosphate and chromate interfere and require to be separated.

B. C. SINHA and S. K. ROY

Central Glass and Ceramic Research Institute, Calcutta-32, India.

Analyst, 1973, **98**, 289–292.

Molecular Interaction Errors in Phase-solubility Analysis

Results are presented for the assay by phase-solubility analysis of ibufenac (4-isobutylphenylacetic acid) and of saccharin in the presence of various impurities. The results for impurities obtained for ibufenac mixtures were almost twice those expected, while those for saccharin mixtures were normal. From a consideration of these results, the effect of compound formation by molecular interaction is discussed.

D. THORBURN BURNS, J. B. GALLAGHER, R. J. STRETTON

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

and J. S. WRAGG

Analytical Research, Quality Control, The Boots Company Ltd., Pennyfoot Street, Nottingham.

Analyst, 1973, **98**, 293–296.

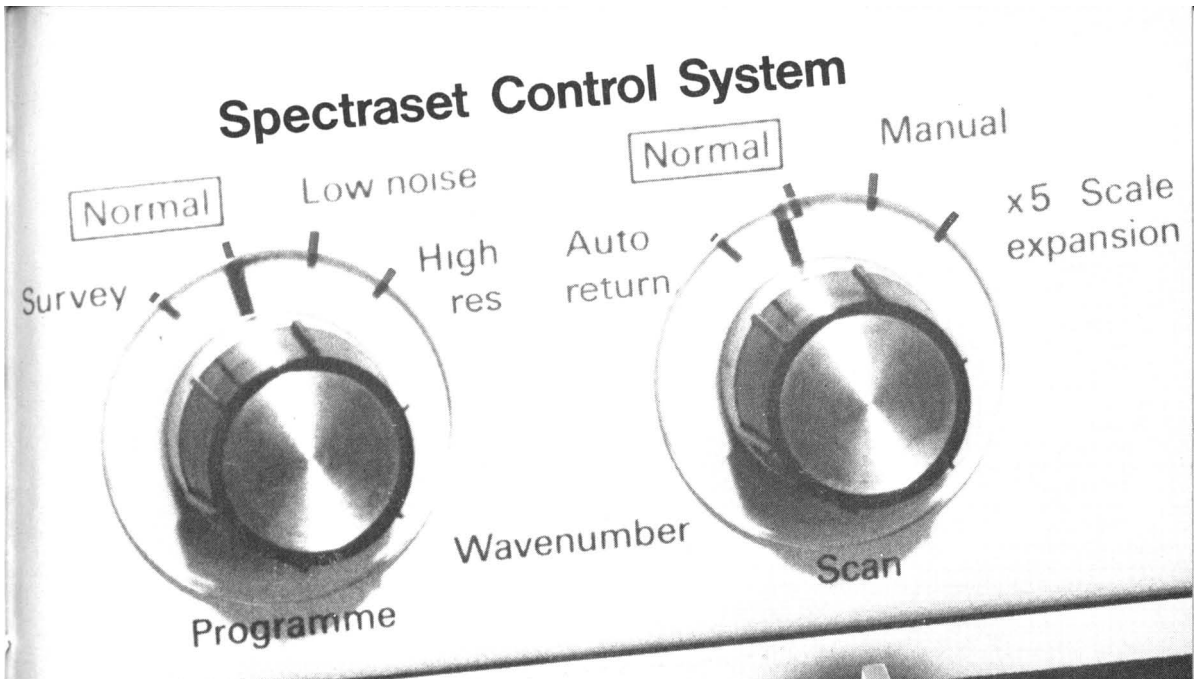
A Critical Examination of Procedures for the Assay of Sodium Fluoride

Report prepared by the Fluorine Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W1X 1AF.

Analyst, 1973, **98**, 297–302.



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