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# Summaries of Papers in this Issue

## An Improved Procedure for the Determination of Thiamine

A modified thiochrome procedure for the assay of thiamine is described. It eliminates the use of ion-exchange columns and elutions with hot, saturated potassium chloride solution that are employed in most other methods. The times required for extraction and dephosphorylation have been examined critically and shortened.

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*Analyst*, 1975, **100**, 689–695.

## A Simple Method for the Determination of 5,7-Diiodoquinolin-8-ol and 5-Chloro-7-iodoquinolin-8-ol (Clioquinol)

A simple and accurate method is suggested for the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in a pure state as well as in various pharmaceutical preparations. The liberation of the iodine content of the compounds investigated is based on simple refluxing with 15 per cent. sodium hydroxide solution in the presence of zinc metal powder. Interference in the subsequent steps of the determination, due to the resulting organic products, is overcome by precipitation and filtration of these products prior to titration.

The suggested method is applicable to the determination of the compounds under investigation in a convenient concentration range that is suitable for the determination of different dosage forms without numerous dilutions. Any unnecessary dilutions may result in high experimental error.

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*Analyst*, 1975, **100**, 696–702.

## Differential Spectrophotometric Method for the Determination of Vitamin A (Retinol) by Using Trifluoroacetic Acid, and Its Application to Related Compounds

A differential spectrophotometric procedure for the determination of vitamin A (retinol) based on the formation of a pink colour in a trifluoroacetic acid - perchloric acid medium has been developed. Maximum absorbance of the pink colour with $\lambda_{max.}$ at 502 nm was attained within 7–12 min and remained stable for at least 30 s; the intensity of this colour was equivalent to 45 per cent. of the intensity of the blue colour produced by the well known antimony(III) chloride procedure. The pink colour could be destroyed within 2–4 min by the addition of pentane-2,4-dione followed by hydrogen peroxide. A rectilinear graph of absorbance *versus* amount of vitamin A was obtained for the 0–20-$\mu$g range, optimum results being achieved in the 5–10-$\mu$g range. The relative standard deviation of the method for 8·3 $\mu$g was $\pm$0·7 per cent. The reactions of vitamin A acetate, retinal, retinoic acid, $\beta$-carotene, ergocalciferol, cholecalciferol, cholesterol, ergosterol, phytonadione and $\alpha$-tocopherol were also investigated employing the same reagent additions.

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*Analyst*, 1975, **100**, 703–707.

## Spectrophotometric Determination of Trace Amounts of Vanadium by Formation of the Vanadium - 4-(2-Pyridylazo)resorcinol (PAR) - Crystal Violet Complex: Application to the Analysis of Plant Materials

The anionic complex of vanadium with 4-(2-pyridylazo)resorcinol (PAR), obtained at pH 4·6–5·1, forms an ion pair with Crystal Violet. This ion pair can be extracted with benzene - isobutyl methyl ketone (3 + 2). In the extracted ion pair the proportions of vanadium to PAR to Crystal Violet are 1 : 1 : 1. The coloured extract gives an absorption maximum at 585 nm, and Beer's law is obeyed in the concentration range 0·05–0·5 $\mu g\ ml^{-1}$ of vanadium. The molar absorptivity is equal to $1{\cdot}1 \times 10^5\ l\ mol^{-1}\ cm^{-1}$.

The method was applied to the determination of trace amounts of vanadium in dried plant materials after wet ashing of the sample and extraction of the vanadium as the *N*-benzoyl-*N*-phenylhydroxylamine complex. The results obtained for the determination of vanadium in serradella, rye straw and grain and trefoil showed a relative standard deviation of 8–15 per cent. at the 0·1–0·25 p.p.m. level of vanadium and of about 25 per cent. at the 0·05 p.p.m. level.

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*Analyst*, 1975, **100**, 708–715.

## Elimination of Interference from Aluminium in the Determination of Total Iron in Soils and Plant Materials Using 1,10-Phenanthroline Reagent

Aluminium, in solution, enhances the iron - 1,10-phenanthroline colour, leading to high results in the determination of iron. Both the iron and aluminium complexes of phenanthroline exhibit identical absorption characteristics. Attempts to mask the aluminium in solution with sodium fluoride have been unsuccessful as the fluoride ions suppress the colour formed with iron and the reagent. The determination of iron after the separation of aluminium and phosphates is simple and rapid. The method presented is reliable and recoveries are quantitative.

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*Analyst*, 1975, **100**, 716–720.

## Atomic-absorption Determination of Strontium in Silicate Rocks: A Study of Major Element Interferences in the Nitrous Oxide - Acetylene Flame

A study has been made of the major element interferences associated with the atomic-absorption determination of trace amounts of strontium in silicate rocks by using a nitrous oxide - acetylene flame. Aluminium causes suppression of the strontium signal, while calcium and magnesium act as partial releasing agents, thus reducing the effect of the aluminium. Exact matching of samples and standards can be avoided by the use of lanthanum, which has been shown to be an effective releasing agent in the nitrous oxide - acetylene flame.

Strontium levels in the United States Geological Survey rocks AGV1, BCR1 and GSP1, in the range 244–670 $\mu g\ g^{-1}$, have been determined with a precision of better than 2 per cent. A spiking and recovery experiment has also been carried out.

Strontium can be measured in silicate rocks with a limit of determination of 0·6 $\mu g\ g^{-1}$.

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The Analyst

# An Improved Procedure for the Determination of Thiamine

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A modified thiochrome procedure for the assay of thiamine is described. It eliminates the use of ion-exchange columns and elutions with hot, saturated potassium chloride solution that are employed in most other methods. The times required for extraction and dephosphorylation have been examined critically and shortened.

It was shown by Jansen[1] that the oxidation of thiamine to thiochrome using akaline potassium hexacyanoferrate(III) could be used for the determination of thiamine in natural materials and this reaction forms the basis of many assay procedures. In order to remove interfering substances the extracted thiamine was absorbed on an ion-exchange resin, the interfering substances removed by washing and the thiamine eluted with a hot, saturated solution of potassium chloride. In the procedure to be described, thiamine is converted into thiochrome while still on the resin, and the thiochrome then extracted into 2-methylpropan-1-ol. The method is simple, reproducible and has been found to be satisfactory in this laboratory.

## Experimental

### Reagents

All reagents should be of analytical-reagent grade.

*Hydrochloric acid*, 0·1 N.

*Sodium acetate trihydrate.*

*Clarase.* This is available from Miles Laboratories Inc., Elkhart, Indiana, USA, and P.O. Box 37, Stoke Poges, Slough, Berkshire.

*Decalso F (also called Zerolit S/F).* This resin is manufactured by Permutit Co. (Zerolit) and marketed by BDH Chemicals Ltd. and Hopkin and Williams Ltd. and is a sodium aluminosilicate cation-exchange resin of mesh size 60–85. It is prepared for use as follows. Spread 1 kg of the resin on a tray and run a strong magnet over it in order to remove any contaminating particles of iron or rust. Mix the resin well with a 25 per cent. solution of sodium chloride in dilute hydrochloric acid (2 ml of concentrated hydrochloric acid per litre). After settling, pour off the supernatant liquid together with much of the "fines." Repeat this treatment three times. Next, wash the resin repeatedly with acidified water (1 ml of glacial acetic acid per litre of de-ionised water) until the washings are free from chloride. At the end of this treatment only the coarser particles will be left and these should settle to the bottom rapidly. Finally, dry the treated resin in air.

NOTE—
We have observed batch variations in the activity of Decalso F; although most batches were satisfactory, some gave high blank fluorimeter readings and did not retain thiamine quantitatively (see under *Measurement of fluorescence*).

*Cyanogen bromide solution.* This solution must be freshly prepared before use. It is convenient to store the two reagents needed, *viz.*, saturated bromine water and 10 per cent. aqueous potassium cyanide solution, in a refrigerator at 0·4 °C. The required amount of bromine water is poured into a flask and the 10 per cent. potassium cyanide solution added dropwise until 1 drop causes the yellow colour of free bromine to disappear completely. ~~Any unused cyanogen bromide solution must be poured down the drain with plenty of running water.~~

Small amounts of unused cyanogen bromide, used tissue wipes and 689 glassware can be rendered harmless by treatment with sodium hypochlorite (10 per cent. available chlorine, diluted 2 or 3-fold) for 1–2 h before discharge, after dilution with a large volume of water.

*Mercury(II) chloride solution.* Dissolve 1 g of the solid in water and dilute to 100 ml.
*Sodium hydroxide solution.* Dissolve 30 g in water and dilute to 100 ml.
*Potassium hexacyanoferrate(III) solution.* Dissolve 1 g in water and dilute to 100 ml.
*2-Methylpropan-1-ol.* A fluorescence-free grade must be used.
*Repelcote.* This is a 2 per cent. solution of dimethyldichlorosilane in carbon tetrachloride.

## Preparation of Standards

As thiamine is deliquescent, it must be dried over phosphorus(V) oxide in a desiccator for several days. Dissolve an accurately weighed amount (say 100 mg) of the dried material in 100 ml of 0·1 N hydrochloric acid and make further dilutions with the 0·1 N acid in order to obtain standard solutions of 0·1–1 $\mu$g ml$^{-1}$. These solutions must be stored in dark bottles at 0–4 °C. As thiamine tends to become adsorbed on glass surfaces, it is advisable to treat these bottles with Repelcote. Standard solutions must be examined periodically for mould growth.

## Apparatus

*Fluorimeter.* A Locarte fluorimeter was used. An LF2 filter was used to select the 365-nm excitation wavelength and either an LF14 or LF3 plus LF5, both with a wedge monochromator in the fluorescent beam, to select a waveband in the region of 436 nm.

## General Procedure

### *Extraction*

*Solid samples.* Dice animal tissues, such as liver, brain or muscle, food concentrates or plant material and weigh about 2 g accurately into 1-oz Universal or Macartney bottles, adding 5–10 ml of 0·1 N hydrochloric acid. Place the loosely capped bottles in a boiling water bath and heat for 15 min. This procedure denatures the protein material and, at the same time, releases any protein-bound thiamine phosphates.

*Liquid samples.* Pipette liquid samples, such as blood (1 ml) or urine (5 ml), into 15 ml of 0·1 N hydrochloric acid in a Macartney bottle and heat as described for solid samples.

### *Dephosphorylation*

Adjust the pH of the extracts to 4·5 by adding small amounts of solid sodium acetate and using pH indicator paper. Next add about 100 mg of Clarase and mix it in thoroughly. Incubate the mixture for 3 h at 45 °C or 16 h at ambient temperature with occasional shaking and centrifuge at 3000 *g* for 10 min. Transfer the clear supernatant liquid to a calibrated flask (20- or 25-ml capacity) and re-extract the residue twice with 0·1 N hydrochloric acid. Then combine all of the extracts, add 1–2 drops of concentrated hydrochloric acid so that the pH is below 4, and dilute to the mark.

### *Purification*

Place about 1·5 g (about 2 ml) of activated Decalso in a stoppered test-tube (capacity about 17 ml) and pipette a portion (normally 1·0 ml) of the extract directly on to the Decalso, avoiding the sides of the tube. Mix the materials by using a mechanical vibrator. With all batches include a set of standards (at least three) and a reagent blank and treat them in the same way as the extracts. Fill the tubes nearly to the top with boiling, de-ionised water and shake them. After allowing the contents to settle pour off the wash water, taking care not to lose any Decalso particles.* Repeat the washing procedure three times.

### *Formation of thiochrome*

Pipette 3 ml of cyanogen bromide or 0·3 ml of mercury(II) chloride on to the wet Decalso and mix thoroughly. Then add 2 ml of 30 per cent. sodium hydroxide solution and shake the mixture vigorously for at least 30 s by use of a mechanical vibrator.

* It has been suggested that the procedure for decantation could be improved by having fine gauzes made to fit over the mouth of the test-tube and that even fine muslin held over the mouth could allow more efficient and safer washing procedures. Alternatively, the supernatant liquid could be removed by suction through a Pasteur pipette attached to a water-pump.

*Extraction into 2-methylpropan-1-ol*

To the mixtures in the tubes add 5·0 ml of 2-methylpropan-1-ol, stopper the tubes and shake them vigorously for 2 min. After centrifugation at 3000 *g* for 5 min, transfer the clear 2-methylpropan-1-ol extract into clean cuvettes.

*Measurement of fluorescence*

With all batches include a set of standards (at least three) and a reagent blank. Measure the fluorescence at 436 nm, and adjust the instrument so that the highest standard gives a convenient reading (say 100). The readings for the other standards should be linear and values for the samples can then be read off from a standard graph. The blank reading when the highest thiamine standard is 1·0 $\mu$g ml$^{-1}$ must be less than 8 divisions. If higher readings are obtained, it would be advisable to change the batch of Decalso.

## Critical Assessment of Method

### Time of Extraction

About 2 g of comminuted liver were analysed to determine the thiamine content as described above. The extraction time was varied, replicate determinations being made at six intervals during the first hour. The extraction was found to be complete within the first 15 min. There was a highly significant rise in mean thiamine content from 0 to 10 min ($P<0·01$) and a corresponding fall from 30 to 60 min (see Table I).

TABLE I

EFFECT OF EXTRACTION TIME ON THIAMINE CONTENT

| Time of extraction/min | 0 | 5 | 10 | 15 | 30 | 60 |
|---|---|---|---|---|---|---|
| Mean thiamine content in liver (wet mass)/$\mu$g g$^{-1}$ | 4·04 | 4·28 | 4·43 | 4·45 | 4·43 | 4·00 |
| Number of determinations | 4 | 3 | 4 | 4 | 4 | 4 |

Standard error of the mean of four determinations = 0·080.

### Optimum Amount of Clarase

The effect of varying the amount of Clarase was studied using bovine liver (about 2 g). It was found (Table II) that 50 mg of Clarase per gram of liver homogenate was the optimum amount.

TABLE II

EFFECT OF DIFFERENT AMOUNTS OF CLARASE

| Amount of Clarase per gram of homogenate/mg | Amount of thiamine in homogenate (wet mass)/$\mu$g g$^{-1}$ |
|---|---|
| 25 | 1·945 ± 0·015 |
| 50 | 2·225 ± 0·095 |
| 100 | 2·02 ± 0·02 |

### Optimum Amount of Decalso

The effect of using different amounts of Decalso in the assay procedure was examined by taking different amounts of the resin and adding 1·0 ml of a solution containing 0·5 $\mu$g of thiamine.

For each tube of Decalso plus thiamine a blank containing the same mass of Decalso, but no thiamine, was included. The remainder of the assay was as described under *Purification*, *Formation of thiochrome*, *Extraction into 2-methylpropan-1-ol* and *Measurement of fluorescence*. The results (Table III) show that the blank reading increased as the amount of Decalso was increased. However, this increase does not seriously interfere in the assay provided that the same amount of Decalso is used in all of the tubes.

## Limit of Thiamine Retention on Decalso

To ten tubes, each containing 1·5 g of Decalso, different amounts of thiamine (from 0·1 to 1000 μg) were added and thoroughly mixed. The Decalso was washed with boiling water as described in the assay procedure and the amount of thiamine remaining was assayed.

TABLE III

EFFECT ON THE ASSAY OF THIAMINE OF INCREASING THE AMOUNT OF DECALSO

| | Fluorimeter readings | | |
|---|---|---|---|
| Mass of Decalso/g | Blank | With thiamine | Corrected |
| 0·75 | 6 | 59 | 53 |
| 1·5 | 7·5 | 60 | 52·5 |
| 3·0 | 14·5 | 70 | 55·5 |

For the higher amounts of thiamine the extracts in 2-methylpropan-1-ol were suitably diluted with more 2-methylpropan-1-ol. The results (Table IV) show that with 1 mg of thiamine per 1·5 g of Decalso the limit of retention had not been reached.

TABLE IV

LIMIT OF THIAMINE RETENTION ON DECALSO

| Thiamine added/μg | Fluorimeter reading | Dilution factor | Thiamine found/μg |
|---|---|---|---|
| 0 (blank) | 6·0 | — | — |
| 0·1 | 14·5 | Nil | 0·1 |
| 0·25 | 28·5 | Nil | 0·25 |
| 0·5 | 51·5 | Nil | 0·5 |
| 0·75 | 72·0 | Nil | 0·73 |
| 1·0 | 100·0 | Nil | 1·03 |
| 250 | 56·0 | × 500 | 275 |
| 500 | 53·0 | × 1000 | 515 |
| 750 | 55·5 | × 1500 | 810 |
| 1000 | 52·0 | × 2000 | 1010 |

## Precision of the Method

The precision of the method was assessed by carrying out repeated assays on an acid extract of a sample of calf liver of low thiamine content. To another portion of the extract thiamine was added at the level of 10 μg $g^{-1}$ and the assay carried out in replicate. The mean values, together with the 95 per cent. confidence limits and standard deviations, are given in Table V.

TABLE V

PRECISION OF THE METHOD

| | Liver low in thiamine content | Extract plus added thiamine |
|---|---|---|
| Number of assays .. .. | 10 | 12 |
| Mean thiamine content/μg $g^{-1}$ .. | 0·305 | 10·22 |
| 95 per cent. confidence limits .. | 0·161–0·449 | 10·01–10·44 |
| Standard deviation .. .. | ± 0·201 | ± 0·344 |

The variation between replicates is greatly reduced with added thiamine.

## Over-all Recovery of Added Thiamine

Approximately 2-g portions of samples of comminuted liver were assayed (in duplicate) with and without added thiamine. From the results the percentage recoveries were calculated (Table VI). These recoveries lie within 10 per cent. of expected values.

## Comparative Use of Mercury(II) Chloride and Cyanogen Bromide for Thiochrome Formation

Standard graphs were prepared by using five levels of thiamine ranging from 0·1 to 4 μg. In the assay procedure either 0·3 ml of a 1 per cent. solution of mercury(II) chloride or 3 ml

TABLE VI

RECOVERY OF ADDED THIAMINE

| Thiamine added/μg | Amount of tissue/g | Thiamine/μg g$^{-1}$ Calculated | Found | Recovery, per cent. |
|---|---|---|---|---|
| 0 | 2·0021 | | 4·146 | |
| 0 | 2·0042 | | 4·341 | |
| | | | Mean: 4·250 | |
| 5 | 2·0031 | 6·74 | 6·59 | 98 |
| 5 | 2·0009 | 6·85 | 6·75 | 101 |
| 10 | 2·0004 | 9·25 | 9·9 | 107 |
| 10 | 1·9929 | 9·27 | 9·68 | 104 |
| 15 | 2·0039 | 11·74 | 12·48 | 106 |
| 15 | 1·9973 | 11·76 | 12·87 | 109 |

of cyanogen bromide solution were used for the oxidation stage. Fig. 1 shows the mean standard graphs evaluated for 14 assays using mercury(II) chloride and 13 using cyanogen bromide. The regression equations are:

Mercury(II) chloride: $y = 95{\cdot}13\ x + 0{\cdot}53$ (standard error of slope, $\pm$ 1·36)
Cyanogen bromide: $y = 94{\cdot}63\ x - 1{\cdot}31$ (standard error of slope, $\pm$ 1·28)

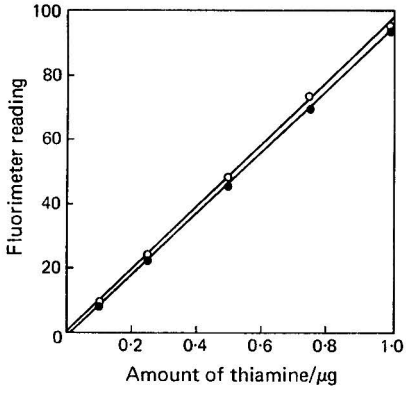


Fig. 1. Mean standard graph of thiochrome formation for 14 assays with mercury(II) chloride (○) and 13 assays with cyanogen bromide (●) as oxidant.

where $x$ = thiamine (μg) and $y$ = fluorimeter reading. The slopes did not differ significantly for the two methods.

The results are similar with either reagent. Mercury(II) chloride tends to give lower blanks.

## Comparative Use of Mercury(II) Chloride and Cyanogen Bromide for Determination of Thiamine

Thiamine was determined in liver, yeast and cattle concentrate nuts, using mercury(II) chloride or cyanogen bromide as the oxidant. Slightly higher values were obtained with mercury(II) chloride but Table VII shows that there is little difference between the results obtained by use of these two reagents.

## Assay of Thiamine in Urine: Comparison of Three Oxidising Agents

A sample of bovine urine was acidified by the addition of 2 drops of concentrated hydrochloric acid and held in a boiling water bath for a few minutes. Three sets of 1-, 2- and 3-ml aliquots were pipetted on to Decalso and washed as described before. The thiamine was then converted into thiochrome using cyanogen bromide or mercury(II) chloride, but in one

TABLE VII

ASSAY OF NATURAL MATERIAL USING MERCURY(II) CHLORIDE OR CYANOGEN BROMIDE

| Material | Thiamine/$\mu$g g$^{-1}$ | |
|---|---|---|
| | Cyanogen bromide | Mercury(II) chloride |
| Liver .. .. .. | 2·35 | 2·4 |
| Feed concentrate .. | 2·75 | 2·95 |
| Yeast .. .. .. | 17·2 | 18·9 |

set of tubes the oxidising agent was alkaline potassium hexacyanoferrate(III)[2] [3 ml of an oxidising solution made by mixing 1 per cent. potassium hexacyanoferrate(III) solution (1 ml) and 15 per cent. sodium hydroxide solution (24 ml)]. For each oxidising agent a standard graph was also prepared. The results (Table VIII) show that alkaline potassium hexacyanoferrate(III) is not a suitable reagent for the measurement of thiamine in urine as the fluorescence of its product does not appear to be linear.

TABLE VIII

ASSAY OF THIAMINE IN URINE

| Volume of urine/ml | Thiamine found ($\mu$g) after oxidation with— | | |
|---|---|---|---|
| | alkaline hexacyanoferrate(III) | cyanogen bromide | mercury(II) chloride |
| 1 | 0·55 | 0·42 | 0·48 |
| 2 | 1·25 | 0·90 | 1·04 |
| 3 | 2·17 | 1·33 | 1·55 |

## Discussion

A major step in the chemical assay of thiamine, its purification by adsorption on the synthetic cation-exchange material Decalso, was introduced by Hennessy and Cerecedo.[3] Following the removal of non-adsorbed impurities by washing with water, the thiamine was eluted with a hot, saturated solution of potassium chloride. However, this can be a tedious and time-consuming process, attended by many frustrations, such as the crystallisation of potassium chloride during elution and consequent clogging of the column. It has also been suggested that incomplete elution may result if the potassium chloride solution is allowed to cool, and on occasions high blanks have been known to result.[4] These drawbacks have been overcome in the present procedure by eliminating elution with potassium chloride. The thiamine adsorbed on the Decalso is oxidised to thiochrome and extracted directly into 2-methylpropan-1-ol, thus avoiding any losses.

The formation of thiochrome by the oxidation of thiamine with cyanogen bromide was first demonstrated by Fujiwara,[5] and later Fujiwara and Matsui[6] applied it to the determination of thiamine and found it to have distinct advantages over the use of alkaline potassium hexacyanoferrate(III). The conversion into thiochrome was shown to be quantitative over a wide range of concentrations of thiamine. Reducing impurities had little or no influence on thiochrome formation. With alkaline potassium hexacyanoferrate(III), however, such impurities can be a serious hindrance, even if the amount of hexacyanoferrate(III) is carefully adjusted to compensate for the interference. On the other hand, excess of the hexacyanoferrate(III) reagent can destroy thiochrome (Westenbrink and Goudsmit[7]) and some workers employ a further step to destroy the excess of hexacyanoferrate(III) with hydrogen peroxide.[8,9] Fujiwara and Matsui[6] found that cyanogen bromide can safely be used for the determination of thiamine in urine as it does not produce fluorescence with *N*-methylnicotinamide, whereas alkaline hexacyanoferrate(III) would do so. As this metabolite is likely to occur in urine it could be a source of error. Waring *et al.*,[10] who used the latter reagent, had to overcome this interference by separating thiamine and its phosphate esters in urine by thin-layer chromatography before assay.

Morita *et al.*[11] have used mercury(II) chloride instead of cyanogen bromide for the conversion of thiamine into thiochrome. This is a useful improvement as it has several advantages; the limit of sensitivity is said to be about 0·02 $\mu$g and although both reagents are highly

selective for thiamine, 1 per cent. mercury(II) chloride solution is easier to handle than cyanogen bromide, which must be used only in a fume cupboard and is stable for only 3 h at room temperature.[6] The procedure described in this paper is simple to use, reasonably accurate and considerably reduces the time taken to carry out the assay by conventional methods.[12,13]

The skilled technical assistance of Messrs. J. Carroll and K. James is acknowledged.

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# A Simple Method for the Determination of 5,7-Diiodoquinolin-8-ol and 5-Chloro-7-iodoquinolin-8-ol (Clioquinol)

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A simple and accurate method is suggested for the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in a pure state as well as in various pharmaceutical preparations. The liberation of the iodine content of the compounds investigated is based on simple refluxing with 15 per cent. sodium hydroxide solution in the presence of zinc metal powder. Interference in the subsequent steps of the determination, due to the resulting organic products, is overcome by precipitation and filtration of these products prior to titration.

The suggested method is applicable to the determination of the compounds under investigation in a convenient concentration range that is suitable for the determination of different dosage forms without numerous dilutions. Any unnecessary dilutions may result in high experimental error.

The compounds 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol (clioquinol) have been widely accepted as anti-amoebic compounds, both in the treatment of amoebic dysentery and intestinal amoebiasis, and as topical anti-infectives, because of their low toxicity. Official compendia provide different assay procedures for the determination of the two compounds. The methods of assay given in USP XVIII[1] and BP 1968[2] for the determination of 5,7-diiodoquinolin-8-ol, both as the pure compound and in tablets, depend on the determination of the halogen content by the oxygen-flask combustion technique. However, while USP XVIII employs an infrared spectrophotometric assay for the determination of 5-chloro-7-iodoquinolin-8-ol and its pharmaceutical preparations, BP 1968 again employs the oxygen-flask combustion technique. The method of BP 1968 was superseded in BP 1973 by a non-aqueous titration for total phenolic substances, coupled with gas - liquid chromatography for 5-chloro-7-iodoquinolin-8-ol. The NF XIII[3] describes different assay procedures for the determination of 5-chloro-7-iodoquinolin-8-ol in different pharmaceutical preparations.

Several methods are available in the literature for the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol as pure substances and in various formulations. Colorimetric methods involve the formation of coloured metal complexes in both instances. A colorimetric method,[4] based on a previously described reaction,[5] has been developed, which depends upon the formation of a coloured complex between 5-chloro-7-iodoquinolin-8-ol and the iron(III) ion. The method has been used in order to determine 5-chloro-7-iodoquinolin-8-ol in ointments.

The yellow complex formed between copper sulphate and 5,7-diiodoquinolin-8-ol in dimethylformamide has been the basis for a colorimetric determination of this compound in tablets and suspensions.[6] A method, which is based on the measurement of the orange - yellow colour formed when a solution of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol in glacial acetic acid is treated with sodium nitrite solution,[7] has been applied to the determination of these compounds in tablets, powders and ointments. Also, an analytical procedure that is based on the formation of a yellow-coloured copper chelate,[8] which is readily extractable into chloroform, has been used to determine either compound in tablets and creams. However, colorimetric methods that are based on chelate formation, in general, suffer from a marked sensitivity to variations in the moisture content of the system.[8]

The spectrophotometric determination of 5-chloro-7-iodoquinolin-8-ol has been carried out by measurement of the absorbance of a solution of the compound in ethanolic hydrochloric acid (0·1 N) at 257 or 325 nm.[9] It has also been determined spectrophotometrically at 390 nm.[10]

Infrared spectrophotometric methods include the semi-quantitative determination of 5-chloro-7-iodoquinolin-8-ol and its intermediates.[11] This method has been modified[12] to fulfil the conditions of specificity, accuracy and precision that are required for quantitative analysis. The compound has also been determined in various pharmaceutical preparations by use of infrared spectrophotometry.[13] Although infrared spectrophotometric methods are specific, sometimes the lengthy extractions required before the final measurement is made render the method impractical for routine analyses in the pharmaceutical industry.

Other methods described in the literature include the polarographic determination of 5,7-diiodoquinolin-8-ol,[14] the indirect X-ray spectrographic determination of 5-chloro-7-iodoquinolin-8-ol[15] and the determination of both compounds by gas - liquid chromatography of their trimethylsilylethers.[16]

Gravimetric and titrimetric methods that have been described include the precipitation of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol with cadmium iodide in acetone and then weighing the cadmium complex formed.[17] 5-Chloro-7-iodoquinolin-8-ol was also determined by precipitation from solution in ethyl acetate with a 6 per cent. solution of mercury(II) acetate in acetic acid, in the form of a mercury complex.[10] The total halogen content of 5-chloro-7-iodoquinolin-8-ol was determined by liberating the halogens by means of fusion with sodium carbonate, adding an excess of standard silver nitrate solution and determining the excess of the latter by titration against standard potassium thiocyanate solution. In addition, the iodide ion formed was determined by using standard iodate solution.[18] This compound has also been determined by precipitation in the form of a copper complex, the copper content of the residue being determined by iodimetric titration.[19] Other titrimetric methods that have been described include the fusion of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol with potassium carbonate and determination of the potassium iodide formed by titration against *N*-bromosuccinimide.[20] 5,7-Diiodoquinolin-8-ol has also been assayed by non-aqueous titration in dimethylformamide solvent against standard sodium methoxide solution, using thymol blue as the indicator.[21]

In this paper a simple and more direct analytical method that is applicable to the determination of relatively high concentrations of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol in pharmaceutical preparations without numerous dilutions of the sample is reported. The method has proved successful in determining accurately either compound alone or admixed with many other ingredients that are commonly encountered in dosage forms.

## Experimental

### Reagents

*5,7-Diiodoquinolin-8-ol.*
*5-Chloro-7-iodoquinolin-8-ol.*
*Zinc metal powder.*
*Chloroform, analytical-reagent grade.*
*Dilute hydrochloric acid,* 10 *per cent.*
*Concentrated hydrochloric acid, sp. gr.* 1·18.
*Sodium hydroxide,* 15 *per cent. solution.* Dissolve 15 g of sodium hydroxide in distilled water in order to make 100 ml of solution.
*Standard potassium iodate solution,* 0·025 M. Weigh accurately 5·35 g of potassium iodate and dissolve it in distilled water so as to give 1000 ml of solution.

### Procedure

*Determination of 5,7-diiodoquinolin-8-ol*

Weigh, accurately, about 0·3 g of 5,7-diiodoquinolin-8-ol, place it in a 250-ml Erlenmeyer flask, add about 0·5 g of zinc powder and then 30 ml of 15 per cent. sodium hydroxide solution. Boil the mixture for 30 min on a hot-plate under a reflux condenser. Disconnect the condenser, wash down any particles adhering to the walls of the flask and the condenser with about 5 ml of sodium hydroxide solution and a small amount of distilled water, and reflux again for a further 10 min. Cool, filter the contents and wash the flask and the filter with three 10-ml portions of distilled water. Next, neutralise the combined filtrate and washings to litmus by using 10 ml of concentrated hydrochloric acid and about 10–20 ml of the dilute

acid solution. Filter off the precipitate formed and wash it thoroughly by decantation, using four volumes, each of 10 ml, of distilled water and adding the washings to the filtrate. Add about 60 ml of concentrated hydrochloric acid for each 50 ml of flask contents and titrate the solution with 0·025 M potassium iodate solution until the dark brown solution becomes light brown. Then add 10 ml of chloroform and continue the titration, shaking well after each addition, until the chloroform layer becomes colourless.

Alternatively, separate the precipitate formed on neutralisation by centrifuging for 20 min at 5000 rev $min^{-1}$, transferring the clear supernatant liquid quantitatively to the titration flask, then wash the residue with distilled water until it is completely suspended, centrifuge it again for a further 10 min and combine this supernatant liquid with the solution in the titration flask.

*Determination of 5-chloro-7-iodoquinolin-8-ol*

Carry out the determination as described above. However, after separation of the precipitate formed on neutralisation by either filtration or centrifugation, transfer the filtrate and washings to a suitable separator and extract with two 20-ml portions of chloroform. Reject the chloroform layer, transfer the aqueous phase into the titration flask, run the concentrated hydrochloric acid through the separator into the flask and titrate as described above.

Calculate the percentage recovery as follows:

$$\text{Recovery of 5,7-diiodoquinolin-8-ol, per cent.} = \frac{A \times 9{\cdot}925 \times 100}{W}$$

$$\text{Recovery of 5-chloro-7-iodoquinolin-8-ol, per cent.} = \frac{A \times 15{\cdot}275 \times 100}{W}$$

where A ml is the volume of 0·025 M potassium iodate solution and $W$ mg is the mass of the drug.

*Determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in tablets*

Weigh and powder 20 tablets. Transfer into a 250-ml Erlenmeyer flask an accurately weighed amount of the powder that is equivalent to about 300·0 mg of the active ingredient and proceed as described above under *Determination of 5,7-diiodoquinolin-8-ol* or *Determination of 5-chloro-7-iodoquinolin-8-ol.*

*Determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in suspensions*

Accurately measure a volume of the suspension equivalent to about 300·0 mg of active ingredient and centrifuge it for 30 min at 5000 rev $min^{-1}$. Discard the clear supernatant liquid and wash the residue with a 40-ml portion of distilled water until it is completely suspended, centrifuging for 30 min and rejecting the clear supernatant liquid. Repeat the washing and centrifugation with a further 40-ml portion of water. Transfer the residue quantitatively into a 250-ml Erlenmeyer flask by use of 30 ml of 15 per cent. sodium hydroxide solution and a small amount of distilled water and proceed as described above under *Determination of 5,7-diiodoquinolin-8-ol* or *Determination of 5-chloro-7-iodoquinolin-8-ol.*

## Results and Discussion

The methods that are in general use for liberating the iodine content of organic compounds are the double crucible method, which involves fusion with alkali-metal carbonates, or the oxygen-flask combustion technique. Fusion with alkali-metal carbonates has several dangers and disadvantages, among which are the long time needed to complete the reaction, the risk of loss of halogen by volatilisation and the possibility of significant contamination of the sample by impurities present in the flux required to fuse the sample. On the other hand, the oxygen-flask combustion technique can be performed only on a small amount of sample. Therefore, a method was sought for the assay of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol that would circumvent some of these difficulties and, at the same time, be applicable to various pharmaceutical preparations.

The analytical method reported in this paper is a modification of the BP 1968 method of assay of sodium acetrizoate injection.[2] It has been found that the direct application of this method, without any modification, to the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol is not possible in view of the fact that the organic products, resulting from the hydrolysis process, interfere in the subsequent steps of the analysis. Preliminary investigations on pure 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol have indicated that the presence of such organic compounds, which are believed to be quinolin-8-ol and/or decomposition products thereof, positively interfere with the titration against standard iodate solution, thus leading to higher recoveries. This interference can be explained as being due to partial iodination of these products under the conditions of the experiment, and/or consumption of iodate in an oxidation reaction. Such interference has been overcome by a suitable modification of the BP method in order to effect accurate determination of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol in the pure form as well as in various dosage forms. Further investigation has indicated that the interfering organic products can be precipitated quantitatively by neutralising the reaction mixture to litmus and filtering before the titration against the standard iodate solution is carried out. The solubility of the resulting precipitate increases in acidic or alkaline medium and therefore caution should be exercised not to overshoot the neutral point.

In the determination of 5,7-diiodoquinolin-8-ol or 5-chloro-7-iodoquinolin-8-ol in suspensions in which these drugs are not the only therapeutic agents, advantage is taken of the complete insolubility of the two drugs in water to separate them, by centrifugation, from soluble ingredients prior to determination. This step also eliminates any interference in the determination due to the presence of reducing sugars in suspensions.

In the determination of 5-chloro-7-iodoquinolin-8-ol as the pure substance, as well as in the dosage forms, it is necessary to shake the solution gently with chloroform just before the titration is carried out, discarding the organic solvent. This extraction will eliminate any colour, which, if present in solution, will interfere with the detection of the end-point.

Table I summarises the analytical results obtained in the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol by means of the developed method and the non-aqueous titration method. The non-aqueous titration[21] of 5,7-diiodoquinolin-8-ol was carried out with dimethylformamide as the solvent and 0·1 N sodium methoxide solution as the titrant. The end-point was detected visually, by using thymol blue as indicator. 5-Chloro-7-iodoquinolin-8-ol was determined in the same manner but using pyridine as the solvent. The mean percentage recoveries ($P' = 0·05$) obtained by applying the proposed method to the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol

TABLE I

DETERMINATION OF 5,7-DIIODOQUINOLIN-8-OL AND 5-CHLORO-7-IODOQUINOLIN-8-OL BY THE PROPOSED METHOD AND THE NON-AQUEOUS TITRATION PROCEDURE

| Proposed method | | | Non-aqueous titration procedure | | |
|---|---|---|---|---|---|
| Mass taken/mg | Mass found/mg | Recovery, per cent. | Mass taken/mg | Mass found/mg | Recovery, per cent. |
| *5,7-Diiodoquinolin-8-ol*— | | | | | |
| 302·8 | 303·7 | 100·3 | 399·5 | 404·5 | 101·3 |
| 300·1 | 301·7 | 100·5 | 402·1 | 404·5 | 100·6 |
| 302·0 | 294·3 | 97·4 | 400·4 | 404·5 | 101·0 |
| 301·8 | 300·7 | 99·6 | 388·4 | 398·9 | 102·7 |
| 301·0 | 296·7 | 98·6 | 401·5 | 416·8 | 103·8 |
| 319·6 | 320·5 | 100·3 | 422·1 | 441·4 | 104·6 |
| 298·0 | 290·3 | 97·4 | | | |
| Mean ($P' = 0·05$) = 99·2 ± 1·26 | | | Mean ($P' = 0·05$) = 102·3 ± 1·71 | | |
| *5-Chloro-7-iodoquinolin-8-ol*— | | | | | |
| 105·6 | 109·9 | 104·1 | 499·7 | 523·9 | 104·8 |
| 108·8 | 106·9 | 98·2 | 500·5 | 519·4 | 103·8 |
| 133·0 | 131·4 | 98·8 | 499·9 | 512·9 | 102·6 |
| 210·8 | 216·9 | 102·9 | | | |
| 304·4 | 294·8 | 96·9 | | | |
| Mean ($P' = 0·05$) = 100·2 ± 3·89 | | | Mean ($P' = 0·05$) = 103·7 ± 2·74 | | |

were 99·2 ± 1·26 and 100·2 ± 3·89, respectively, compared with 102·3 ± 1·71 and 103·7 ± 2·74, respectively, when the non-aqueous titration method was applied. The high results obtained by using the latter method may be attributable to the presence of such impurities as halogenated isomers in commercial samples of 5-chloro-7-iodoquinolin-8-ol and other halogenated isomers in 5,7-diiodoquinolin-8-ol.

The results obtained from the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in tablets are given in Table II. 5,7-Diiodoquinolin-8-ol was determined in tablets especially prepared so as to contain 300 mg of the drug in each. The mean recovery ($P' = 0·05$) was 100·3 ± 1·99 per cent. Tablet excipients, such as starch, gelatin, talc, soluble saccharin and fumed silica, did not interfere in the determination. The recovery of 5-chloro-7-iodoquinolin-8-ol from different batches of tablets that are marketed in Egypt and labelled to contain 250 mg of the drug was also investigated. The mean recovery ($P' = 0·05$) was 101·6 ± 4·55 per cent.

TABLE II

DETERMINATION OF 5,7-DIIODOQUINOLIN-8-OL AND 5-CHLORO-7-IODOQUINOLIN-8-OL IN SYNTHETIC AND COMMERCIAL TABLETS

| Brand | Average mass of tablet/mg | Mass of powdered tablets taken/mg | Content of drug/mg | Mass found/mg | Recovery, per cent. |
|---|---|---|---|---|---|
| *Synthetic 5,7-diiodoquinolin-8-ol tablets* (300 *mg*)— | | | | | |
| A | 500 | 334·0 | 200·4 | 202·9 | 101·3 |
| A | 500 | 498·4 | 299·0 | 298·5 | 99·8 |
| A | 500 | 500·3 | 300·2 | 304·4 | 101·4 |
| A | 500 | 501·9 | 301·1 | 297·5 | 98·8 |
| | | | | Mean ($P' = 0·05$) = | 100·3 ± 1·99 |
| *Commercial 5-chloro-7-iodoquinolin-8-ol tablets* (250 *mg*)— | | | | | |
| B | 399·2 | 479·7 | 300·2 | 307·0 | 102·3 |
| B | 399·2 | 328·1 | 205·5 | 209·3 | 101·8 |
| B | 399·2 | 326·7 | 204·6 | 200·1 | 97·8 |
| B | 399·2 | 163·0 | 102·1 | 106·9 | 104·7 |
| | | | | Mean ($P' = 0·05$) = | 101·6 ± 4·55 |
| C | 400·4 | 236·5 | 147·6 | 140·5 | 95·2 |
| D | 398·5 | 507·1 | 318·1 | 307·0 | 96·5 |
| E | 396·9 | 160·1 | 100·8 | 93·2 | 92·5 |
| E | 396·9 | 480·8 | 302·8 | 279·5 | 92·3 |

The proposed method has been applied to the determination of 5,7-diiodoquinolin-8-ol in suspensions. The results of these analyses can be seen in Table III. The mean recoveries ($P' = 0·05$) of the drug in specially prepared suspensions containing 2·5 per cent. *m/V*, and in commercial suspensions labelled to contain the same percentage, were 100·8 ± 3·26 and 101·5 ± 4·18 per cent., respectively. Substances that are commonly encountered in suspensions of 5,7-diiodoquinolin-8-ol, such as sulphaguanidine (8 per cent.), bismuth subcarbonate (5 per cent.), light kaolin (8 per cent.), sodium citrate (5 per cent.), sucrose (40 per cent.), carboxymethylcellulose (1 per cent.), Tween 80 (1 per cent.), methyl hydroxybenzoate (0·15 per cent.), propyl hydroxybenzoate (0·05 per cent.), liquid extract of belladonna (0·1 per cent.) and vanillin (0·1 per cent.), do not interfere in the determination. The calculated mean recovery ($P' = 0·05$) for the determination of 5-chloro-7-iodoquinolin-8-ol in commercial suspensions, as shown in Table III, was 97·9 ± 4·84 per cent. Up to 7 per cent. *m/V* of formosulphathiazole, the condensation product of sulphathiazole and formaldehyde, does not interfere in its determination in suspensions.

The recovery of 5,7-diiodoquinolin-8-ol, when added to commercial suspensions already containing it, was determined by adding a known amount of the pure drug to suspensions, the 5,7-diiodoquinolin-8-ol content of which had previously been determined. The results given in Table IV, obtained with some of these suspensions, are representative. These control experiments were conducted in order to differentiate between experimental errors and

TABLE III

DETERMINATION OF 5,7-DIIODOQUINOLIN-8-OL AND 5-CHLORO-7-IODOQUINOLIN-8-OL IN SYNTHETIC AND COMMERCIAL SUSPENSIONS

| Volume taken/ml | Content of drug/mg | Amount of drug found/mg | Recovery, per cent. |
|---|---|---|---|
| *Synthetic suspensions containing 2·5 per cent.* m/V *of 5,7-diiodoquinolin-8-ol*— | | | |
| 12·5 | 312·5 | 307·7 | 98·4 |
| 12·5 | 312·5 | 322·5 | 103·2 |
| 5·0 | 125·0 | 127·0 | 101·6 |
| 5·0 | 125·0 | 125·1 | 100·1 |
| | | Mean ($P' = 0·05$) = 100·8 ± 3·26 | |
| *Commercial suspensions labelled to contain 2·5 per cent.* m/V *of 5,7-diiodoquinolin-8-ol*— | | | |
| 10·0 | 250·0 | 252·1 | 100·8 |
| 10·0 | 250·0 | 252·1 | 100·8 |
| 10·0 | 250·0 | 242·2 | 96·9 |
| 10·0 | 250·0 | 258·0 | 103·2 |
| 10·0 | 250·0 | 264·9 | 106·0 |
| | | Mean ($P' = 0·05$) = 101·5 ± 4·18 | |
| *Commercial suspensions labelled to contain 3·0 per cent.* m/V *of 5-chloro-7-iodoquinolin-8-ol*— | | | |
| 10·0 | 300·0 | 297·9 | 99·3 |
| 10·0 | 300·0 | 296·3 | 98·8 |
| 10·0 | 300·0 | 287·2 | 95·7 |
| | | Mean ($P' = 0·05$) = 97·9 ± 4·84 | |

errors due to the interaction of other constituents encountered in the system or caused by bulk production.

The method developed in this investigation has the advantages of being simple and applicable, over a convenient range of concentrations, to the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol in the pure state or in different pharmaceutical formulations, without numerous dilutions of the sample. The high concentrations of the drugs

TABLE IV

RECOVERY OF 5,7-DIIODOQUINOLIN-8-OL FROM COMMERCIAL SUSPENSIONS LABELLED TO CONTAIN 2·5 PER CENT. WITH ADDED 5,7-DIIODOQUINOLIN-8-OL

| Number | Volume of suspension taken/ml | Labelled amount of drug/mg | Added amount of drug/mg | Total amount of drug/mg | Recovery/ mg | Recovery, per cent. |
|---|---|---|---|---|---|---|
| 1 | 5·0 | 125·0 | 0 | 125·0 | 125·1 | 100·1 |
| | 5·0 | 125·0 | 98·8 | 223·8 | 225·3 | 100·7 |
| | Recovery of added amount of drug = 100·2 mg (101·4 per cent.) | | | | | |
| 2 | 5·0 | 125·0 | 0 | 125·0 | 124·1 | 99·3 |
| | 5·0 | 125·0 | 204·0 | 329·0 | 326·5 | 99·2 |
| | Recovery of added amount of drug = 202·5 mg (99·3 per cent.) | | | | | |
| 3 | 5·0 | 125·0 | 98·6 | 223·6 | 215·9 | 96·6 |
| 4 | 10·0 | 250·0 | 100·6 | 350·6 | 362·2 | 103·3 |

usually encountered in their formulations cause no problems and render the great dilutions and low-level determinations that are inherent in instrumental methods unnecessary. Such dilutions can result in high experimental error. In addition, the method requires no special skills and does not necessitate the use of expensive instruments, which may not be readily available in developing and underdeveloped countries where preparations containing 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol are most needed.

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# Differential Spectrophotometric Method for the Determination of Vitamin A (Retinol) by Using Trifluoroacetic Acid, and Its Application to Related Compounds

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A differential spectrophotometric procedure for the determination of vitamin A (retinol) based on the formation of a pink colour in a trifluoroacetic acid - perchloric acid medium has been developed. Maximum absorbance of the pink colour with $\lambda_{max.}$ at 502 nm was attained within 7–12 min and remained stable for at least 30 s; the intensity of this colour was equivalent to 45 per cent. of the intensity of the blue colour produced by the well known antimony(III) chloride procedure. The pink colour could be destroyed within 2–4 min by the addition of pentane-2,4-dione followed by hydrogen peroxide. A rectilinear graph of absorbance *versus* amount of vitamin A was obtained for the 0–20-$\mu$g range, optimum results being achieved in the 5–10-$\mu$g range. The relative standard deviation of the method for 8·3 $\mu$g was $\pm$0·7 per cent. The reactions of vitamin A acetate, retinal, retinoic acid, $\beta$-carotene, ergocalciferol, cholecalciferol, cholesterol, ergosterol, phytonadione and $\alpha$-tocopherol were also investigated employing the same reagent additions.

Several methods are available for the determination of vitamin A (retinol)[1] in foods,[2] pharmaceutical preparations[3] and blood, but of these the colorimetric method with antimony(III) chloride[4] is the most widely used. The blue colour ($\lambda_{max.}$ 616–620 nm) that develops in the reaction with antimony(III) chloride, trichloroacetic acid[5] and other strong Lewis acids[6] is very sensitive but is subject to rapid fading within several seconds. Vitamin A gives the same colour reaction with trifluoroacetic acid (TFA)[6,7] with much less sensitivity to moisture[8] and this reagent is preferred, especially in micro-determinations.[9]

The blue colour produced with TFA decays to a more stable secondary pink colour ($\lambda_{max.}$ 520–550 nm) within 1·5–2 h, but the intensity of this pink colour is less than one third of that of the blue colour.[6] Thus, the difficulties with the colorimetric determination of vitamin A are that the blue colour, although very sensitive, is unstable while the pink colour, although more stable, is less sensitive and develops slowly.

During the investigation of chemical methods for the determination of vitamin A in vitamin mixtures, it was noted that temperature, solvents and oxidising agents all have an effect on the speed of development and the sensitivity of the pink colour. It was found that a mixture of trifluoroacetic acid and 0·1 N perchloric acid (5 + 1) added to a dichloromethane solution of vitamin A gave the best sensitivity within 7–12 min and the absorbance at 502 nm was stable for at least 30 s. This absorbance could be destroyed rapidly within 2–4 min by the addition of pentane-2,4-dione followed by hydrogen peroxide. In this paper, the optimum conditions for a differential spectrophotometric procedure for determining vitamin A are described. In addition, the reactions of related compounds that may be present with vitamin A in pharmaceutical preparations or in blood are reported.

## Experimental

### Reagents

*Trifluoroacetic acid.* "OR" grade. (CAUTION—Trifluoroacetic acid is an extremely corrosive acid and should be handled with care.)

*Perchloric acid, 0·1 N solution in glacial acetic acid.*

*Dichloromethane.* Spectroscopic quality.

*Pentane-2,4-dione (acetylacetone).*

*Hydrogen peroxide solution, 30 per cent.* Analytical-reagent grade.
*All*-trans-*vitamin A.*
*Vitamin A acetate.* USP reference standard in oil.
*All*-trans-*retinal.*
*All*-trans-*retinoic acid.*
*β-Carotene, ergosterol, cholesterol and cholecalciferol, 100 per cent. pure, crystalline.*
*Ergocalciferol.* USP reference standard.
*Phytonadione.* USP reference standard.
DL-α-*Tocopherol.*

## Apparatus

*Perkin-Elmer, Coleman Model 124, spectrophotometer.*
*Rotary evaporator.*
*Fast-delivery pipette.* Capacity, 2·0 ml.

## Procedure

Transfer an aliquot of a solution of the sample, preferably dry, in a volatile organic solvent, containing 5–10 μg of vitamin A, into a 10- or 25-ml flask and evaporate it to dryness under vacuum at 35–40 °C. Add 1 ml of dichloromethane to the flask and again evaporate to dryness. To the residue in the flask, add 0·20 ml of dichloromethane, mix well to effect dissolution, and quickly add 2·0 ml of the freshly mixed solvent trifluoroacetic acid - 0·1 N perchloric acid (5 + 1). Gently swirl the flask in order to mix the contents and immediately transfer the mixture into a 1-cm spectrophotometer cuvette. Monitor the absorbance at 502 ± 2 nm and record the maximum value attained (usually within 7–12 min of mixing), zeroing the instrument with the mixed solvent as blank. After the maximum absorbance has been recorded, add 1 drop of pentane-2,4-dione, with mixing, followed by 1 drop of hydrogen peroxide solution, with mixing, and measure the absorbance at the same wavelength 3 min ± 30 s after the addition of the hydrogen peroxide. Subtract the second absorbance reading from the maximum absorbance reading and calculate the amount of vitamin A by comparison with a similarly treated standard solution.

## Results and Discussion

Efforts to utilise the pink colour developed in the reaction of vitamin A with trifluoroacetic acid, led to an investigation of this reaction and the parameters that affect it. Simply allowing vitamin A to react with TFA at room temperature gives an initial blue colour that decays to a pink colour. It was found that a fairly stable isosbestic point was reached within 5–10 min at 532 ± 2 nm, which was unsatisfactory as the basis of an analytical procedure, as the absorbance value obtained was relatively weak and easily affected by solvent and pH variations. Heating vitamin A in various mixtures of chloroform and TFA at 50 °C for time intervals of 1–4 min demonstrated that the pink colour could be developed more rapidly and completely, but again it was easily affected by slight variations in experimental conditions.

Encouraging results were obtained initially when it was found that the addition of hydrogen peroxide as an oxidant, in conjunction with TFA, gave increased sensitivity to the pink colour as the maximum absorbance at 502 nm developed within 10 min and remained stable for about 1 min. The effects of nitric acid, perchloric acid and mixtures of these acids, in lieu of hydrogen peroxide, were then investigated. The most satisfactory combination was found to be a 5 + 1 mixture of TFA with 0·1 N perchloric acid in glacial acetic acid. The presence of dichloromethane before the addition of TFA was found to be advantageous, but the sensitivity decreased somewhat with the use of 1,2-dichloroethane, chloroform, carbon tetrachloride and benzene, in that order, with absolute ethanol and acetone being definitely inferior.

During the initial work with hydrogen peroxide as an aid to TFA for the rapid development of the pink colour, it was found that the addition of a small amount of acetone could destroy the colour within 15 min. Then, when developing the colour with a trifluoroacetic acid - 0·1 N perchloric acid (5 + 1) mixture, it was found that the colour was only slightly affected by the addition of a small amount of either hydrogen peroxide or acetone alone; however, if

hydrogen peroxide and acetone were added successively or in a 1 + 1 mixture, the colour was removed within 15–20 min. On the assumption that the keto group of the acetone had a role in this reaction, pentane-2,4-dione was substituted for acetone with the result that the absorbance of the colour was reduced to less than 0·02 within 2–4 min. Based on these observations, the conditions described in the procedure were deemed to be the optimum for the determination of vitamin A.

The visible spectrum obtained in the reaction of trifluoroacetic and perchloric acid with vitamin A at maximum intensity (Fig. 1A) illustrates the absorbance band at 502 nm with only a slight shoulder at 528 nm. The absorbance at 502 nm increases rapidly in the first 5 min, attains maximum intensity within 12 min and remains stable for at least 30 s, before decreasing slowly. The primary blue colour, which appears at 595 nm and which decays rapidly within a few seconds of the addition of the reagent, has about twice the intensity of the secondary pink colour. On the addition of pentane-2,4-dione, with mixing, the absorbance falls only slightly, but on subsequent addition of hydrogen peroxide, with mixing, the absorbance falls rapidly in the first 1 min and then attains a stable intensity (less than 0·02 absorbance unit) within 2–4 min (Fig. 1B). Addition of the last two reagents in the reverse order gives essentially the same result. The spectrum shown in Fig. 1B is also given by a blank reaction without vitamin A. It is important that the sample and standard solution are treated in a similar way as the concentration of TFA may vary from batch to batch and degree of use. The perchloric acid should be mixed with the TFA shortly before use (preferably less than 1 h) as the mixture tends to lose potency with time.

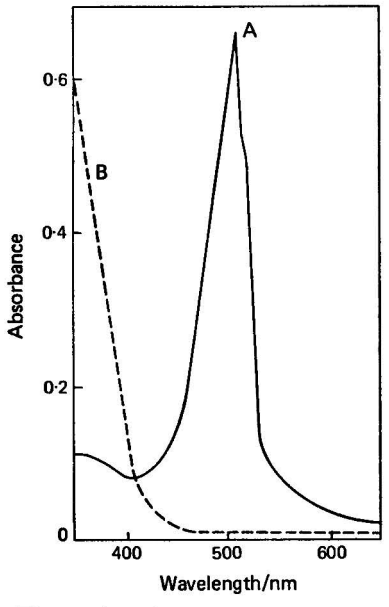


Fig. 1. A typical spectrum of colour developed in the reaction of vitamin A with trifluoroacetic acid - perchloric acid mixture before (A) and after (B) the addition of pentane-2,4-dione, then hydrogen peroxide.

Various dilutions of vitamin A [Fig. 2(*a*)] and of the USP reference standard vitamin A acetate [Fig. 2(*b*)] in chloroform were used to establish the linearity of response. The graphs of maximum absorbance at 502 nm *versus* the amount of vitamin A and that of the acetate was rectilinear in the range 0–20 μg, with optimum results being obtained in the 5–10-μg range. The relative standard deviations of the absorbance for six aliquots of vitamin A containing 8·3 μg and of the acetate containing 8·4 μg were found to be 0·66 and 1·07 per cent., respectively.

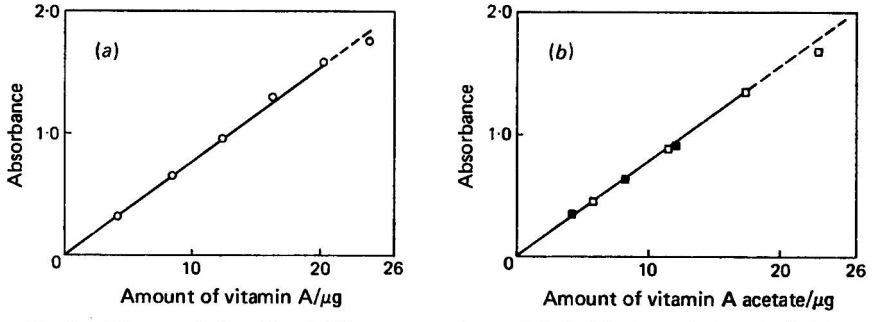


Fig. 2. Linear relationship of different samples and their dilutions of vitamin A (*a*) and reference standard vitamin A acetate (two samples) (*b*) to the colour development in the trifluoroacetic acid - perchloric acid procedure.

The $E^{1\%}_{1cm}$ values calculated from the average of several determinations of vitamin A and its acetate (Table I) indicate that the value of the acetate is 2 per cent. higher than that of the alcohol, which is in contrast to a value expected to be 15 per cent. lower based on the difference in relative molecular masses and according to the comparable literature values for the blue colour with TFA.[6,9] Hydrolysis of vitamin A acetate by the USP XVIII[10] procedure and determination of the resulting alcohol by both the ultraviolet[10] and the colorimetric procedures gave 97·9 ± 1·8 and 96·9 ± 2·4 per cent. recoveries, respectively. Vitamin A acetate was adsorbed on deactivated neutral silica, chromatographed on neutral alumina, eluted with acetone and the acetate determined in the eluate by both the ultraviolet and the colorimetric procedures, which gave 99·9 ± 0·7 and 97·5 ± 1·5 per cent. recoveries, respectively. Apparently, the acetate does indeed produce a higher colour yield than does the alcohol on an equimolar basis.

The colorimetric procedure was applied to some related compounds that may be present with vitamin A in foods, pharmaceutical preparations and in blood, and the results are shown in Table I. The spectra of all of the compounds in the table showed single peaks except for β-carotene, which showed a secondary peak at 475 nm, and α-tocopherol, which showed an additional shoulder at 430 nm. All the peaks disappeared on the addition of pentane-2,4-dione and hydrogen peroxide. However, it is evident that differential spectrophotometry could not be used for compounds that have their maxima at wavelengths below 460 nm as the blank solvents begin to make a significant contribution to the absorbance (Fig. 1B).

TABLE 1

COMPARATIVE RESULTS OF THE APPLICATION OF THE DIFFERENTIAL SPECTROPHOTOMETRIC PROCEDURE FOR THE DETERMINATION OF VITAMIN A TO RELATED COMPOUNDS

| Compound | Wavelength ($\lambda_{max.}$ ± 2)/ nm | $E^{1\%}_{1cm}$ at $\lambda_{max.}$ | Time taken to reach maximum absorbance/ min | Minimum time of stability of absorbance/s | $E^{1\%}_{1cm}$ at 502 nm |
|---|---|---|---|---|---|
| Vitamin A | 502 | 1670 | 10 ± 2 | 30 | 1670 |
| Vitamin A acetate | 502 | 1704 | 10 ± 2 | 30 | 1704 |
| Retinal | 408 | 2138 | 8 ± 2 | 180 | 142 |
| Retinoic acid | 450 | 1870 | 13 ± 3 | 60 | 122 |
| β-Carotene | 735 | 332 | 7 ± 1 | 30 | 92 |
| | 475 | 108 | 17 ± 2 | 60 | 102 |
| Ergocalciferol | 485 | 1151 | 3 ± 1 | 60 | 641 |
| Cholecalciferol | 485 | 1096 | 3 ± 1 | 30 | 517 |
| Cholesterol | 412 | 163 | 2 ± ½ | 10 | 2 |
| Ergosterol | 383 | 213 | 2·5 ± 1 | 20 | 34 |
| Phytonadione | 400 | 125 | 60 ± 5 | 120 | 0·8 |
| DL-α-Tocopherol | 458 | 28* | >90 | — | 2·5* |

* At 90 min.

Several advantages over other colorimetric methods that are used for the determination of vitamin A could be attributed to the method described in this paper. The developed

colour, in addition to giving an absorbance graph that is rectilinear with concentration, is relatively stable, sensitive and rapidly formed. The procedure is simple and provides a distinct advantage in the use of differential spectrophotometry in the presence of spectral interferences of a general background nature. There were no significant day-to-day variations in results noted under similar conditions.

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# Spectrophotometric Determination of Trace Amounts of Vanadium by Formation of the Vanadium - 4-(2-Pyridylazo)resorcinol (PAR) - Crystal Violet Complex: Application to the Analysis of Plant Materials

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The anionic complex of vanadium with 4-(2-pyridylazo)resorcinol (PAR), obtained at pH 4·6–5·1, forms an ion pair with Crystal Violet. This ion pair can be extracted with benzene - isobutyl methyl ketone (3 + 2). In the extracted ion pair the proportions of vanadium to PAR to Crystal Violet are 1 : 1 : 1. The coloured extract gives an absorption maximum at 585 nm, and Beer's law is obeyed in the concentration range 0·05–0·5 $\mu$g ml$^{-1}$ of vanadium. The molar absorptivity is equal to 1·1 $\times$ 10$^5$ l mol$^{-1}$ cm$^{-1}$.

The method was applied to the determination of trace amounts of vanadium in dried plant materials after wet ashing of the sample and extraction of the vanadium as the *N*-benzoyl-*N*-phenylhydroxylamine complex. The results obtained for the determination of vanadium in serradella, rye straw and grain and trefoil showed a relative standard deviation of 8–15 per cent. at the 0·1–0·25 p.p.m. level of vanadium and of about 25 per cent. at the 0·05 p.p.m. level.

Ion pairs composed of an anionic complex of the metal ion to be determined and a cationic dye molecule are known to have high molar absorptivities.[1] It was assumed that a sensitive spectrophotometric method for the determination of vanadium in plant materials would make it possible to determine vanadium in a sample of not more than 5–10 g of dry material. The concentration of vanadium in plant material varies in the range 1–0·01 p.p.m. and therefore the 5-g (10-g) sample would contain 5–0·05 $\mu$g (10–0·1 $\mu$g) of vanadium. Assuming that this amount of vanadium is finally contained in 10 ml of solution, then the range of concentrations for the solution of the coloured vanadium complex corresponds to 1–0·01 $\mu$g ml$^{-1}$ of vanadium. To obtain reasonable values for the absorbance, the molar absorptivity of the coloured compound must be of the order of about 10$^5$ l mol$^{-1}$ cm$^{-1}$. The ion pairs mentioned above have this property but sufficiently sensitive methods for the determination of vanadium are not known. The purpose of this work was to devise such a method, using the anionic complex of vanadium with 4-(2-pyridylazo)resorcinol (PAR)[2–5] as the anionic component of the ion pair. Crystal Violet has been chosen as the cationic component. This paper presents the characterisation of this complex and its use in the determination of vanadium in plant materials.

## Experimental

### Reagents

*Vanadium stock solutions, 100 $\mu$g ml$^{-1}$ and 5 $\times$ 10$^{-3}$ M.* Vanadium(V) oxide (0·1785 g and 0·4547 g, respectively), freshly heated to 500 °C, was dissolved in 100 ml of 1 M sodium hydroxide solution. Lower concentrations were obtained by diluting the stock solutions with water.

*Crystal Violet solution, 10$^{-3}$ M.* Crystal Violet (0·5701 g) was dissolved in water and the solution made up to 100 ml with water.

*PAR solution, 5 $\times$ 10$^{-4}$ M.* PAR (0·0064 g) was dissolved in two drops of 1 M sodium hydroxide solution and the solution made up to 100 ml with water.

*Cerium(IV) sulphate, 1 per cent. m/V solution in 1 N sulphuric acid.*

*Buffer solution, pH 4·8.* This solution was obtained by mixing the appropriate volumes

of 1 M potassium dihydrogen orthophosphate solution and 1 M disodium hydrogen orthophosphate solution.

*Extraction solvent, benzene - isobutyl methyl ketone* (3 + 2). Benzene was previously washed three times with water. Isobutyl methyl ketone was washed with 1 M sodium hydroxide solution and then with water until it gave a neutral reaction.

## Apparatus

*Wet-ashing apparatus.* The apparatus described by Bethge[6] was used.
*Spectrophotometers.* Perkin-Elmer 450 and Spekol, Carl Zeiss, Jena.
*pH meter.* Elpho N-512.

## Choice of Dye and Extraction Solvent

The type of dye used has an important influence on the sensitivity of the method and on the absorbance of the blank. The use of Crystal Violet, Methyl Violet, Diamond Green CB, Malachite Green, Rhodamine B and Rhodamine 6G has been investigated. The extraction solvent used should extract completely the vanadium - PAR - dye complex and should not extract the dye itself or its complex with PAR. Use of benzene and its mixtures with acetone, isobutyl methyl ketone and diisopropyl ether, and also of n-pentyl acetate, has been studied and the results are shown in Fig. 1. It can be seen that the best results were obtained when using the solvent benzene - isobutyl methyl ketone (3 + 2).

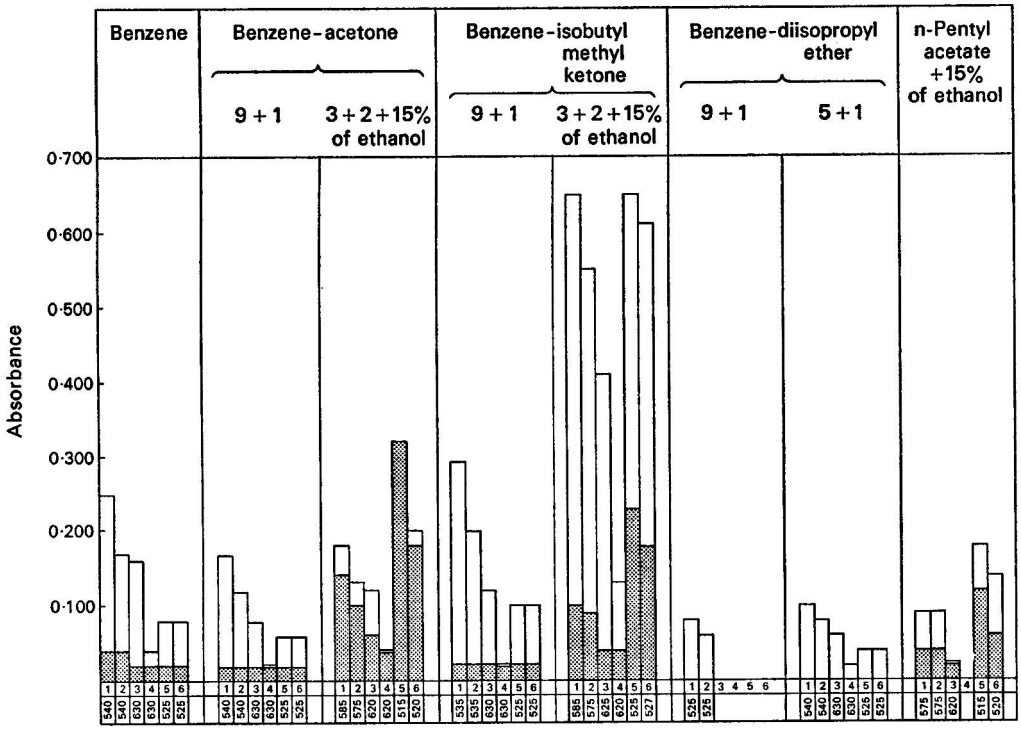



Fig. 1. Absorbance and blank values for the vanadium - PAR - dye complex in various solvents: unshaded regions, absorbances of complex; shaded regions, blank values. [Vanadium] = 5 × $10^{-6}$ M; [PAR] = 3 × $10^{-5}$ M; [dye] = 1 × $10^{-4}$ M. Path length, 1 cm. Wavelengths (nm) are given at the bottom of each column. Dyes: 1, Crystal Violet; 2, Methyl Violet; 3, Diamond Green CB; 4, Malachite Green; 5, Rhodamine B; and 6, Rhodamine 6G.

After extraction the organic phase was often turbid owing to the presence of water. The addition of 15 per cent. *V/V* of ethanol made this phase fully transparent. The addition of ethanol also caused a shift in the absorption maximum and an increase in the absorbance (Table I).

The highest values for the absorbance were obtained when using Crystal Violet and Rhodamine B, the blank absorbance with the latter, however, being twice as high as that with Crystal Violet. In the system finally chosen, Crystal Violet was used as the dye in the vanadium - PAR - dye complex and benzene - isobutyl methyl ketone (3 + 2) as extraction solvent. After extraction 15 per cent. *V*/*V* of ethanol was added.

TABLE I

EFFECT OF THE ADDITION OF ETHANOL TO THE EXTRACTION SOLVENT ON THE ABSORBANCE OF THE VANADIUM - PAR - CRYSTAL VIOLET COMPLEX

| Solvent | $\lambda_{max.}$/nm | Absorbance |
|---|---|---|
| Benzene - isobutyl methyl ketone (2 + 1) .. | 545 | 0·440 |
| + 15 per cent. *V*/*V* of ethanol .. .. | 585 | 0·510 |
| Benzene - isobutyl methyl ketone (3 + 2) .. | 547 | 0·430 |
| + 15 per cent. *V*/*V* of ethanol .. .. | 585 | 0·550 |

## Characteristics of Absorption Spectra

In Fig. 2 the absorption spectra of Crystal Violet (1), the vanadium - PAR complex in water (3), the vanadium - PAR - Crystal Violet complex (2) and the reagent blank (4), all except (3) in solution in benzene - isobutyl methyl ketone (3 + 2) plus 15 per cent. *V*/*V* of ethanol, are given. The vanadium complex gives an absorption maximum at a wavelength of 580–585 nm. Curve 2, for the vanadium complex, is very similar to that for the Crystal Violet alone (1) and shows a small deformation at 540–570 nm, caused by absorption due to the vanadium - PAR complex.

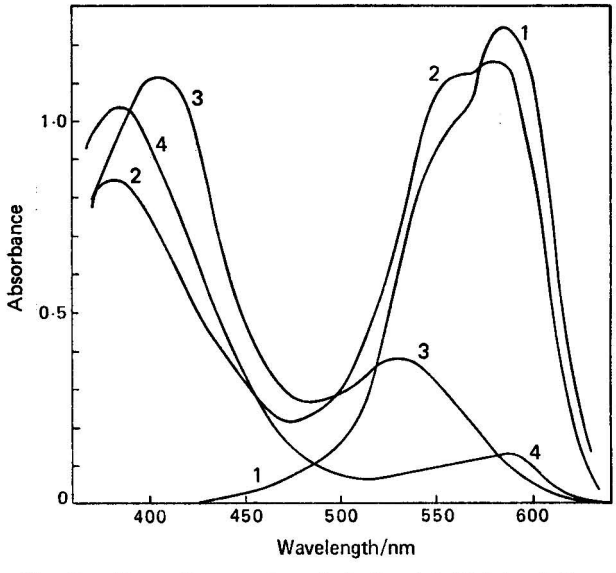



Fig. 2. Absorption spectra of: 1, Crystal Violet solution in the solvent benzene - isobutyl methyl ketone (3 + 2) + 15 per cent. of ethanol ([Crystal Violet] = $1 \times 10^{-5}$ M); 2, vanadium - PAR - Crystal Violet complex in the same solvent ([vanadium] = $0{\cdot}96 \times 10^{-5}$ M, [PAR] = $5 \times 10^{-5}$ M, [Crystal Violet] = $1 \times 10^{-5}$ M); 3, vanadium - PAR complex in water ([vanadium] = $0{\cdot}96 \times 10^{-5}$ M; [PAR] = $5 \times 10^{-5}$ M); and 4, reagent blank. Path length, 1 cm.

## Formation and Extraction of the Vanadium - PAR - Crystal Violet Complex

The effect of the pH of the aqueous phase and of the initial concentrations of PAR and Crystal Violet on the absorbance of the extract has been investigated. The effect of pH in

the range 3–9 has been examined and it can be seen from Fig. 3 that maximum absorbance is obtained in the pH range 4·6–5·1. At pH values above 5·1 the blank absorbance increases considerably owing to the fact that at pH values greater than 6 the PAR is completely in the form of an anion and the possibility of PAR - Crystal Violet complex formation is much higher.

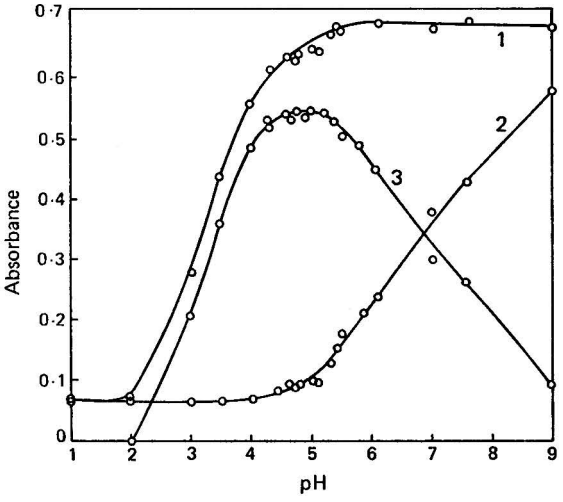



Fig. 3. Variation of absorbance with pH for the vanadium - PAR - Crystal Violet complex in aqueous solution (curve 1). Curve 2, reagent blank. Curve 3, the difference between curves 1 and 2. [Vanadium] = $5 \times 10^{-6}$ M; [PAR] = $3 \times 10^{-5}$ M; [Crystal Violet] = $1 \times 10^{-4}$ M. Wavelength, 585 nm. Path length, 1 cm.

To maintain the pH in the range 4·6–5·1, phosphate buffer solution was used. It was shown that 2 ml of buffer solution were sufficient to keep the pH constant and did not influence the formation of the vanadium - PAR - Crystal Violet complex. The dependences of the absorbance of the extract and of the blank value on the initial concentration of PAR and on the initial concentration of Crystal Violet are shown in Figs. 4 and 5, respectively. For a concentration of vanadium of $5 \times 10^{-6}$ M the most efficient extraction of the complex occurs with an initial concentration of PAR above $2{\cdot}5 \times 10^{-5}$ M and with an initial concentration of Crystal Violet above $1 \times 10^{-4}$ M. Therefore the proportions of vanadium to PAR to Crystal Violet are 1:5:20.

After extraction, the complex is stable for 5 min, after which the absorbance continuously decreases. The extraction proceeds rapidly and it is sufficient to shake the mixture for 2 min in order to achieve 100 per cent. extraction of the complex.

Beer's law is obeyed by the coloured solution of the complex for concentrations of vanadium in the range 0·05–0·5 $\mu$g ml$^{-1}$ and the molar absorptivity is equal to $1{\cdot}1 \times 10^5$ l mol cm$^{-1}$.

## Composition of the Complex

Job's method and that of Asmus[7] have been used for investigation of the ratio of vanadium to PAR and of vanadium - PAR to Crystal Violet. The results obtained show that the complex formed in a weakly acidic medium consists of vanadium, PAR and Crystal Violet in equimolar proportions. These results confirm the literature data,[2,4] which show that vanadium forms with PAR a 1:1 anionic complex that has a single negative charge. This complex associates with one molecule of Crystal Violet. In solution at a pH of approximately 4·5 vanadium is mainly in the form of $VO_3^-$.[8,9] Thus, the following mechanism for the formation of the final complex can be formulated:

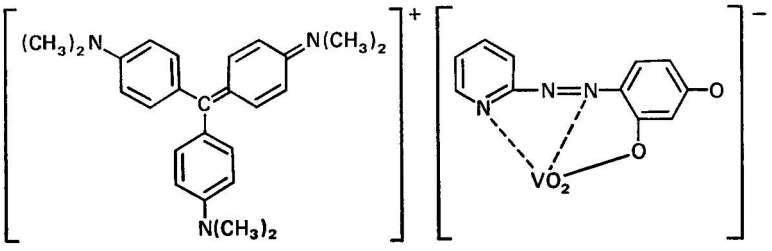
$$HVO_3 + HR^- \rightleftharpoons VO_2R^- + H_2O$$

where $HR^-$ represents PAR, and

$$VO_2R^- + CV^+ \rightleftharpoons VO_2R^-.CV^+$$

where CV represents Crystal Violet.

The following structural formula for the ion-association complex can be assumed:




## Interference by Anions

The proposed method for the determination of vanadium by formation of a complex with PAR and Crystal Violet is not selective. The PAR anion forms coloured, extractable complexes with some cations and the Crystal Violet cation can form extractable ion-association complexes with simple anions. Thus, if the concentration of interfering ions considerably exceeds the concentration of vanadium, erroneous results for the absorbance of the vanadium - PAR - Crystal Violet complex will be obtained.

Table II shows the absorbances of the vanadium - PAR - Crystal Violet complex and of the blank solution in the presence of some common anions. It can be concluded that only sulphate and acetate ions do not interfere in the determination. Chlorides, fluorides, oxalates, citrates and tartrates do not interfere at concentrations lower than 100 times the vanadium concentration. The anions that interfere most are perchlorate, thiocyanate, nitrate and iodide ions. The EDTA anion does not influence the blank determination, but interferes with the formation of the coloured complex, acting as a masking agent on the vanadium ion.

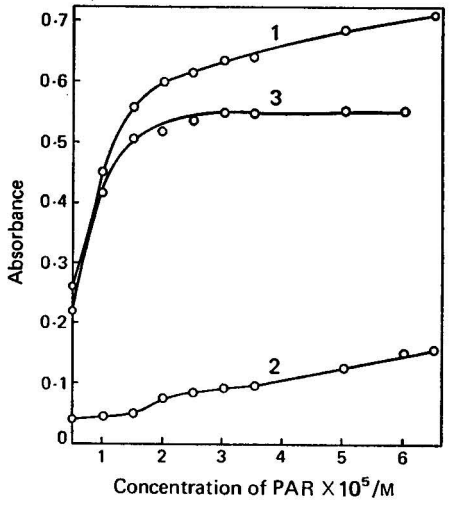



Fig. 4. Variation of absorbance with the initial concentration of PAR for the vanadium - PAR - Crystal Violet complex (curve 1). Curve 2, reagent blank. Curve 3, the difference between curves 1 and 2. [Vanadium] = $5 \times 10^{-6}$ M; [Crystal Violet] = $1 \times 10^{-4}$ M. pH, 4·8. Wavelength, 585 nm. Path length, 1 cm.

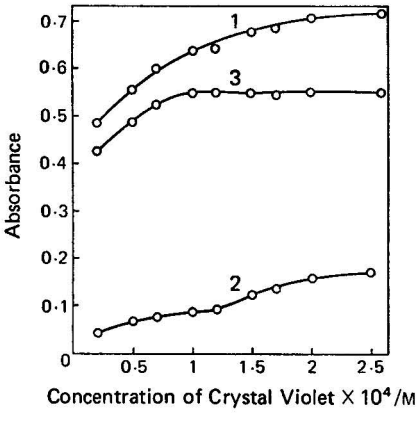



Fig. 5. Variation of absorbance with the initial concentration of Crystal Violet for the vanadium - PAR - Crystal Violet complex (curve 1). Curve 2, reagent blank. Curve 3, the difference between curves 1 and 2. [Vanadium] = $5 \times 10^{-6}$ M; [PAR] = $3 \times 10^{-5}$ M. pH, 4·8. Wavelength, 585 nm. Path length, 1 cm.

TABLE II

EFFECT OF SOME COMMON ANIONS ON THE ABSORBANCE OF THE VANADIUM - PAR - CRYSTAL VIOLET COMPLEX

| Anion added | Molar ratio of anion to vanadium | Absorbance at 585 nm: Blank | Absorbance at 585 nm: Vanadium - PAR - Crystal Violet | Anion added | Molar ratio of anion to vanadium | Absorbance at 585 nm: Blank | Absorbance at 585 nm: Vanadium - PAR - Crystal Violet |
|---|---|---|---|---|---|---|---|
| — | — | 0·100 | 0·550 | $ClO_4^-$ | 2 | 1·400 | — |
| $F^-$ | 5 | 0·100 | 0·550 | $SCN^-$ | 5 | 1·900 | — |
| | 50 | 0·125 | 0·555 | $CH_3CO$ | 5 | 0·100 | 0·540 |
| | 100 | 0·145 | 0·545 | | 100 | 0·110 | 0·560 |
| $Cl^-$ | 5 | 0·105 | 0·545 | | 2000 | 0·170 | 0·500 |
| | 100 | 0·170 | 0·510 | $C_2O_4^{2-}$ | 5 | 0·100 | 0·560 |
| | 2000 | 0·225 | 0·485 | | 50 | 0·145 | 0·495 |
| $Br^-$ | 5 | 0·115 | 0·565 | | 100 | 0·150 | 0·490 |
| | 100 | 0·170 | 0·630 | $C_6H_5O_7^{3-}$ | 5 | 0·125 | 0·535 |
| $I^-$ | 5 | 0·770 | 0·430 | | 50 | 0·145 | 0·525 |
| $BrO_3^-$ | 5 | 0·120 | 0·530 | | 100 | 0·170 | 0·510 |
| $NO_3^-$ | 5 | 0·270 | 0·450 | $C_4H_4O_6^{2-}$ | 5 | 0·095 | 0·565 |
| | 50 | 0·860 | 0·150 | | 50 | 0·110 | 0·560 |
| $SO_4^{2-}$ | 5 | 0·100 | 0·540 | | 100 | 0·140 | 0·545 |
| | 100 | 0·120 | 0·540 | EDTA | 5 | 0·100 | 0·420 |
| | 2000 | 0·120 | 0·530 | | 100 | 0·100 | 0·060 |
| | | | | DCTA* | 5 | 0·105 | 0·515 |
| | | | | | 100 | 0·160 | 0·520 |

* 1,2-Diaminocyclohexane-*NNN'N'*-tetraacetic acid.

## Determination of Vanadium in Plant Material

### Extraction of Vanadium from the Plant Material

After wet ashing of the plant material it is necessary to separate the trace amounts of vanadium from micro-amounts of other elements that are present in the solution. The extraction of vanadium, in the form of its complex with *N*-benzoyl-*N*-phenylhydroxylamine (BPHA), from a solution that was 4 N in hydrochloric acid[10] was used for this purpose. This method, devised for vanadium in the concentration range 20–500 $\mu$g ml$^{-1}$, was not suitable for amounts of vanadium of a few micrograms. In the presence of iron, which is always present in plant material, it was impossible to extract such small amounts of vanadium quantitatively. It was found necessary to carry out the extraction from a highly oxidising solution [containing cerium(IV) sulphate], immediately after adjustment of the concentration of hydrochloric acid to 4 N (using 6 N hydrochloric acid).

From a solution that is 4 N in hydrochloric acid, BPHA can completely extract niobium, hafnium, tungsten and molybdenum and can partially extract zirconium, tantalum and titanium. Zirconium, molybdenum and titanium may be present in plant materials in concentrations comparable with that of vanadium. As can be seen from the literature, and from our own experiments, these elements do not influence the determination of vanadium using the method devised.

In order to return the vanadium into aqueous solution three procedures were examined: a re-extraction with nitric acid, sodium hydroxide and hydrogen peroxide solutions; wet ashing with nitric acid - perchloric acid; and dry ashing after evaporation of the solvent. The last method gave the best results and proceeded rapidly, without additional reagents. The vanadium(V) oxide obtained could be dissolved easily in a small volume of sodium hydroxide solution.

### Procedure

Powder the sample of dried plant material and weigh an amount containing 1–5 $\mu$g of vanadium. Apply the wet-ashing procedure of Minczewski *et al.*,[11] using Bethge's apparatus, with 4 ml of concentrated nitric acid and 2 ml of 60 per cent. perchloric acid per 1 g of sample. When the wet ashing is complete, boil off the acids until the residue in the flask is nearly dry. Dissolve the residue in a small amount of water and filter off any undissolved material.

Add 0·2 ml of 0·1 per cent. cerium(IV) sulphate solution to the sample solution and leave

for 5 min. Then add sufficient 6 N hydrochloric acid to adjust the concentration of hydrochloric acid in the solution to 4 N. Immediately add to the solution two 5-ml portions of a 0·1 per cent. solution of *N*-benzoyl-*N*-phenylhydroxylamine in chloroform, each time shaking for 1 min. Combine the extracts and wash them with 5 ml of 6 N hydrochloric acid. Transfer the solution into a quartz crucible, evaporate the solvent on a water-bath and then gently heat the crucible with a burner in order to destroy the complex, finally heating vigorously to ash the residue. After cooling the crucible, add 0·5 ml of 0·1 N sodium hydroxide solution, then 5 ml of water, and heat the solution gently. After cooling again, add 1 ml of PAR solution, adjust to pH 5 with 0·1 N sulphuric acid and transfer the solution quantitatively to the separator. Add 2 ml of phosphate buffer solution, 1 ml of the Crystal Violet solution and 8 ml of benzene - isobutyl methyl ketone (3 + 2). Shake the mixture for 2 min, separate the organic phase quantitatively and transfer it to a 10-ml calibrated flask containing 1·5 ml of ethanol. Make up to the mark with the extraction solvent, mix well and immediately measure the absorbance at wavelength 585 nm, using the extraction solvent as a blank. Carry out the blank experiment in parallel.

Prepare a calibration graph for the concentration range 0·1–0·5 $\mu$g ml$^{-1}$ of vanadium, including vanadium taken through the procedure. The solutions used for calibration should contain a concentration of iron similar to that in the sample of plant material.

## Results and Conclusion

By using the procedure described above vanadium was determined in four types of dry plant material, *viz.*, in serradella (*Ornithopus*), rye (*Secale*) straw, rye grain and trefoil (*Trifolium*). Five-gram samples were used. For serradella and rye straw, the full procedure was checked by addition of vanadium standard solution containing 0·5 $\mu$g of vanadium. The results are given in Tables III and IV. As can be seen from Table III, the recovery of vanadium is good (94–96 per cent.) in spite of the rather complicated procedure.

TABLE III

RECOVERY OF VANADIUM FROM PLANT MATERIAL

The results given are the means of six determinations.

| Plant material | Vanadium added/$\mu$g | Vanadium found/$\mu$g | Recovery, per cent. |
|---|---|---|---|
| Serradella .. .. .. | 0·50 | 0·48 | 96·0 |
| Rye straw .. .. .. | 0·50 | 0·47 | 94·0 |

For vanadium concentrations in the plant material in the range 0·13–0·25 p.p.m. the relative standard deviation is equal to 8–15 per cent. For concentrations below 0·1 p.p.m. the relative standard deviation is higher, *i.e.*, 24 per cent. (Table IV). Taking into account the rather complicated procedure the results can be considered to be acceptable.

TABLE IV

STATISTICAL EVALUATION OF THE RESULTS

| Plant material | Number of determinations | Concentration of vanadium, p.p.m. | Standard deviation, p.p.m. | Relative standard deviation, per cent. |
|---|---|---|---|---|
| Serradella .. .. | 6 | 0·15 | 0·015 | 10·0 |
| Rye straw .. .. | 6 | 0·13 | 0·019 | 14·6 |
| Rye grain .. .. | 6 | 0·05 | 0·012 | 24·0 |
| Trefoil .. .. .. | 5 | 0·25 | 0·022 | 8·8 |

The method described has been used to determine vanadium in the concentration range 0·05–0·5 p.p.m. Sensitive methods such as this are not usually specific and the procedure for the determination is rather complicated. It is necessary to observe the specified conditions, the reagent concentrations and the composition of the organic solvent. It is especially important in this method because of the blank absorbance, which is constant only under specified conditions. In spite of the rather high value of the blank absorbance, which is about 0·1, the level of absorbance of the vanadium complex is considerably higher than that

of the blank (molar absorptivity of the complex is equal to $1{\cdot}1 \times 10^5$ l mol$^{-1}$ cm$^{-1}$). From Table IV it can be seen that the error of the method is typical for spectrophotometric methods for the determination of trace elements.

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# Elimination of Interference from Aluminium in the Determination of Total Iron in Soils and Plant Materials Using 1,10-Phenanthroline Reagent

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Aluminium, in solution, enhances the iron - 1,10-phenanthroline colour, leading to high results in the determination of iron. Both the iron and aluminium complexes of phenanthroline exhibit identical absorption characteristics. Attempts to mask the aluminium in solution with sodium fluoride have been unsuccessful as the fluoride ions suppress the colour formed with iron and the reagent. The determination of iron after the separation of aluminium and phosphates is simple and rapid. The method presented is reliable and recoveries are quantitative.

There are several methods for the determination of iron in soils and plant materials. The method commonly adopted is that involving the use of 1,10-phenanthroline. However, there appears to be considerable uncertainty when this method is used to determine iron in biological materials. Sandell[1] lists a number of ions that interfere in this method and indicates that pyrophosphates could cause serious errors. However, he also indicates that one of the main advantages of the method is that it can be used in a weakly acidic medium, so that the hydroxides and phosphates of many metals are not precipitated. He also discusses the degree to which interfering ions can be tolerated and suggests means of overcoming such interferences.

Many workers have drawn attention to low recoveries of iron as a result of the interference caused by aluminium and phosphates. Cowling and Benne[2] have shown that if a relatively large amount of aluminium is present together with phosphates, low results are obtained for iron because iron is co-precipitated with aluminium phosphate, which precipitates in a weakly acidic medium. They overcame this interference by adding citrate before the adjustment of pH with sodium acetate. However, there has been no reference in the literature to obtaining high results for iron as a result of a positive interference from the presence of aluminium in the solution.

That this last effect occurs was established during the course of an investigation in which tea soils, originally known to be high in aluminium, had to be analysed to determine total iron and phosphates. Yamamura and Sikes[3] have presented a method with which they claim that *in situ* masking using a double masking reagent is effective in determining iron in the presence of a 1000-fold increase in the concentration of aluminium. The authors decided to examine the method of Yamamura and Sikes[3] and it was found, for reasons that will be discussed below, that their masking agent was ineffective in preventing the interference caused by aluminium in solution. It was therefore decided to examine the original method and effect the necessary modifications. The results of the investigation are reported and a modified method is presented.

## Experimental

### Reagents

*Sodium acetate buffer solution.* Dissolve 20·75 g of analytical-reagent grade sodium acetate in about 50 ml of glass-distilled water in a beaker, add 30 ml of glacial acetic acid, mix well and transfer the mixture quantitatively into a 250-ml calibrated flask, washing the beaker with several small volumes of distilled water. Then stopper the flask and mix the contents well. The pH of this solution should be 4·0.

*Hydroquinone (quinol) solution, 1 per cent.* m/V. Dissolve 1 g of hydroquinone in a little water in a beaker, heating gently to dissolve the solid, transfer the solution quantitatively into a 100-ml calibrated flask and dilute to the mark with distilled water. Stopper the flask and mix the contents well. The reagent should be prepared freshly and discarded if there is any slight discoloration.

1,10-*Phenanthroline reagent*, 0·50 *per cent.* m/V. Dissolve 0·50 g of the reagent in about 40 ml of distilled water in a beaker, warming gently to effect dissolution, and transfer the solution quantitatively into a 100-ml calibrated flask. Dilute to the mark with distilled water, stopper the flask and mix well. If the solid tends to recrystallise on standing, warm the flask in order to redissolve the reagent before use.

*Ammonia solution, sp. gr.* 0·92. Analytical-reagent grade.

*Ammonia solution*, 4 N. Dilute the analytical-reagent grade ammonia solution.

*Bromocresol purple indicator solution.*

*Standard iron solution.* Dissolve 0·1000 g of pure iron wire in 15 ml of 0·6 N hydrochloric acid plus 2 ml of concentrated nitric acid and when it has completely dissolved transfer the solution quantitatively into a 1-l calibrated flask, adjusting the volume to the mark.

1 ml of solution ≡ 100 $\mu$g of iron.

## Methods

### Calibration of Standard Graph

Transfer, by pipette, into 25-ml calibrated flasks aliquots of solutions containing from 0 to 90 $\mu$g of iron; adjust the volumes to approximately 15 ml with distilled water. Add, in order, 5 ml of the sodium acetate buffer solution, 2 ml of the hydroquinone solution and, finally, 1 ml of the phenanthroline reagent. Mix the solutions and dilute to the mark with distilled water, stopper the flasks and mix again. A reference blank is prepared in the same manner except that the iron is omitted. By use of a colorimeter read the values of the colours produced against the reference blank in 2-cm cells at a wavelength of 490 nm. The resulting graph of instrument reading *versus* concentration of iron should be linear, passing through the origin. Any suitable colorimeter can be used.

### Procedure for Separation of Iron from Interfering Ions

To centrifuge tubes marked with a 50-ml calibration, transfer, by pipette, suitable portions (say 10–20 ml) of solutions from plant materials or soils. Add 10 drops of bromocresol purple indicator to each and place the tubes in a boiling water bath for 3 min. Next add ammonia solution, sp. gr. 0·92, dropwise until precipitation just begins. Thereafter, add 4 N ammonia solution dropwise until the indicator turns purple; then add a further 2 ml of the 4 N ammonia solution. Stir the solution well with an air jet and place the tubes in a boiling water bath for 5 min in order to complete the precipitation. When the solution has cooled, adjust the volume to 50 ml, stir it well with the air jet and centrifuge at 200 rev $min^{-1}$ for 5 min. Discard the supernatant liquid. To the residue in the tube add 3 ml of hot, 6 N hydrochloric acid and place the tubes in a boiling water bath for 2 min. Thereafter add 10 ml of hot, 25 per cent. $m/V$ sodium hydroxide solution and again place the tubes in a boiling water bath for 5 min. After cooling, adjust the volume of solution to 50 ml with distilled water, stir well with the air jet and centrifuge at 2000 rev $min^{-1}$ for 5 min. Then discard the supernatant solution.

To the residues in the centrifuge tubes add 3 ml of hot, 6 N hydrochloric acid and warm them in a boiling water bath for 2 min in order to effect complete dissolution. Transfer the contents quantitatively into 50-ml calibrated flasks, washing the tubes with several small portions of water. Next, make the contents of the flasks up to the mark with distilled water, stopper and mix well. The solutions in the 50-ml calibrated flasks contain iron free from interfering ions. Transfer, by pipette, portions containing 0–90 $\mu$g of iron from the separated solutions into 25-ml calibrated flasks, develop the iron colour and proceed as described under Calibration of Standard Graph.

### Sample Preparation

*Ashing of plant samples*

Weigh 0·20 g of finely ground sample into a number of glass ignition tubes held in place with a stainless-steel tray and leave them overnight in a muffle furnace at 450 °C. Cool, add a few drops of distilled water to the tubes in order to moisten the ash, then add by pipette 2 ml of the digestion mixture, nitric acid - hydrochloric acid - water (25 + 25 + 50 $V/V$), and evaporate the mixture to dryness on a hot-plate. Add exactly 10 ml of 0·05 N hydrochloric acid, warm the tubes so as to dissolve the contents, stopper and shake them to mix the

solution well. Leave the tubes aside for 1 h so as to allow any silica to settle, then use suitable portions for the determination of iron.

*Acid digestion of soil samples*

To 2–5 g of a soil sample placed in the digestion tubes, 10 ml of 10:1 digestion mixture (100 ml of 60 per cent. perchloric acid mixed with 10 ml of concentrated sulphuric acid) are added. Several such tubes are slowly heated in an orthophosphoric acid bath until the perchloric acid has boiled off and only about 1–2 ml of solution remain. If the samples are not digested to a clear white residue, a few more millilitres of the digestion mixture are added and the digestion is continued. When the tubes have cooled they are removed from the bath and the digested samples are diluted to about 15 ml with distilled water. The solutions are then filtered into 250-ml calibrated flasks using filter-paper No. 542. The residues in the digestion tubes are washed repeatedly with hot distilled water and the washings passed through the filter-papers into the flasks. Finally, the soil extracts in the flasks are diluted to the mark with distilled water, mixed well and suitable aliquots used for the determination of iron. The method is that described by Jackson.[4]

## Results and Discussion

### Interference from Aluminium

The calibrated standards obeyed Beer's law over the range 0–90 μg of iron per 25 ml. When aluminium was added, however, there was a definite enhancement in colour that resulted in positive error. The results are shown in Table I.

TABLE I

INTERFERENCE FROM ALUMINIUM WHEN ADDED TO SAMPLES BEING ANALYSED TO DETERMINE IRON

| Iron present/ μg per 25 ml | Aluminium added/ μg per 25 ml | Iron found/ μg per 25 ml |
|---|---|---|
| 50·0 | Nil | 50·0 |
| 50·0 | 500 | 52·0 |
| 50·0 | 1000 | 57·0 |
| 50·0 | 2000 | 63·0 |
| 50·0 | 4000 | 76·0 |

In order to find out whether aluminium in solution also formed a complex with phenanthroline reagent, aluminium standards containing from 200 to 8000 μg per 25 ml were developed alone in the same manner as the iron standards and the colours were measured against a blank solution at a wavelength of 490 nm in 2-cm cells. The results obtained are shown in Table II.

TABLE II

COLOUR DEVELOPMENT OF ALUMINIUM STANDARDS WITH PHENANTHROLINE

| Aluminium present/ μg per 25 ml | Apparent iron concentration/ μg per 25 ml | Aluminium present/ μg per 25 ml | Apparent iron concentration/ μg per 25 ml |
|---|---|---|---|
| 200 | 3·0 | 2000 | 13·0 |
| 400 | 4·0 | 4000 | 24·0 |
| 800 | 5·0 | 8000 | 53·0 |
| 1000 | 7·0 | | |

Colours developed with phenanthroline by iron and aluminium standards were read against a blank at various wavelengths in order to study their absorption characteristics. The absorption spectra are shown in Fig. 1.

It can be seen from Fig. 1 that both the iron and aluminium complexes of phenanthroline exhibited identical absorption characteristics under the conditions of colour development, the only difference being that the iron - phenanthroline complex was more sensitive than the aluminium - phenanthroline complex. That being so, it is evident that the presence of aluminium in the system will cause a positive error in the determination of iron. Efforts to suppress the interference from aluminium using sodium fluoride were unsuccessful because the fluoride ions markedly suppressed the colour formed by iron with phenanthroline. The results are shown in Table III.

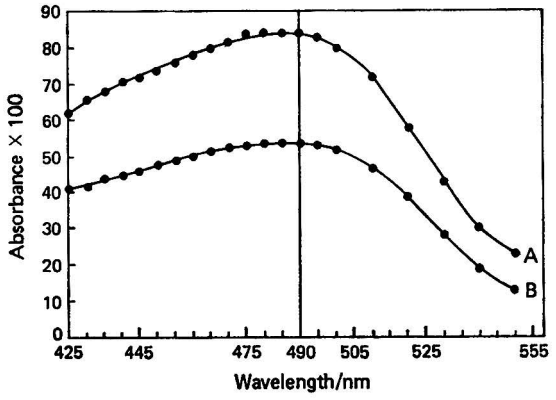


Fig. 1. Absorption spectra of iron and aluminium complexes of phenanthroline at pH = 4. A, iron(II) - phenanthroline complex; B, aluminium - phenanthroline complex.

TABLE III

EFFECT OF DIFFERENT VOLUMES OF SODIUM FLUORIDE SOLUTION (5 per cent. *m/V*) ON IRON - PHENANTHROLINE COLOURS

| Iron present/ μg per 25 ml | Volume of sodium fluoride solution/ml | Colorimeter reading | Apparent iron concentration/ μg per 25 ml |
|---|---|---|---|
| 50·0 | Nil | 48·0 | 45·0 |
| 50·0 | 0·50 | 44·0 | 41·5 |
| 50·0 | 1·00 | 33·0 | 31·0 |
| 50·0 | 2·00 | 22·0 | 21·0 |
| 50·0 | 4·00 | 7·0 | 6·5 |

## Suppression of Aluminium Interference by the Method of Yamamura and Sikes[3]

The colours from pure aluminium standards were developed by the method described by Yamamura and Sikes[3] and the absorbances of the solutions were measured in 2-cm cells at 507 nm. The results obtained are given in Table IV.

TABLE IV

INEFFECTIVENESS OF THE DOUBLE MASKING REAGENT IN PREVENTING INTERFERENCE FROM ALUMINIUM IN SOLUTION

| Al present/μg per 25 ml | Colorimeter reading in 2-cm cells at 507 nm | Apparent iron concentration/ μg per 25 ml |
|---|---|---|
| 1000 | 15·0 | 14·0 |
| 2000 | 21·0 | 20·0 |
| 3000 | 26·0 | 25·0 |
| 4000 | 32·0 | 31·0 |
| 5000 | 39·0 | 38·0 |

It can be seen from the results in Table IV that despite the presence of the double masking reagent, the aluminium formed a complex with phenanthroline and the absorbance readings of the colour given by the aluminium - phenanthroline complex increased linearly with the increase in aluminium concentration. This proportionality indicated that the aluminium - phenanthroline complex was much stronger and more stable than either the aluminium - citrate or aluminium - EDTA complexes under the conditions of colour development. Because the masking agents that were tried failed to achieve the desired results, it was decided to separate iron from all other interfering ions by use of the proposed method. The results obtained for iron in soil and tea plant foliage by using the proposed procedure are presented in Tables V and VI.

TABLE V

TOTAL IRON IN SOIL WITH AND WITHOUT SEPARATION OF ALUMINIUM

| Original soil (without separation), p.p.m. | | | Soil after separation of Al and P, p.p.m. | |
|---|---|---|---|---|
| Fe | Al | P | Fe | Fe + added Al equivalent to Al in original soil |
| 100 000 | 50 000 | 10 000 | 81 250 | 100 000 |

TABLE VI

IRON IN TEA LEAVES BY THE EXISTING AND PROPOSED METHODS

| Aluminium present, p.p.m. | Iron by existing method, p.p.m. | Iron by proposed method, p.p.m. | Iron by atomic-absorption method, p.p.m. |
|---|---|---|---|
| 600 | 200·0 | 160·0 | 155·0 |
| 1700 | 320·0 | 200·0 | 205·0 |
| 2400 | 350·0 | 290·0 | 290·0 |
| 4200 | 485·0 | 365·0 | 362·0 |

The results given in Tables V and VI clearly illustrate the enhancing effect of aluminium, the amount of which was determined by the method of Jayman and Sivasubramaniam,[5] on the iron values. Once the aluminium in solution was eliminated, the iron values obtained agreed well with the results obtained by atomic-absorption spectrophotometry. In the soil samples (Table V), when the calculated amount of the aluminium contained in the original soil was added to an aliquot of the extract from which aluminium had been separated, the value obtained for iron agreed with the apparent iron content of the unseparated soil extract, illustrating clearly the interference caused by aluminium.

The modified method is simple and, with a little practice, the separations can be carried out rapidly and quantitatively, enabling large numbers of samples to be analysed in one working day.

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# Atomic-absorption Determination of Strontium in Silicate Rocks: A Study of Major Element Interferences in the Nitrous Oxide - Acetylene Flame

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A study has been made of the major element interferences associated with the atomic-absorption determination of trace amounts of strontium in silicate rocks by using a nitrous oxide - acetylene flame. Aluminium causes suppression of the strontium signal, while calcium and magnesium act as partial releasing agents, thus reducing the effect of the aluminium. Exact matching of samples and standards can be avoided by the use of lanthanum, which has been shown to be an effective releasing agent in the nitrous oxide - acetylene flame.

Strontium levels in the United States Geological Survey rocks AGV1, BCR1 and GSP1, in the range 244–670 $\mu g\ g^{-1}$, have been determined with a precision of better than 2 per cent. A spiking and recovery experiment has also been carried out.

Strontium can be measured in silicate rocks with a limit of determination of 0·6 $\mu g\ g^{-1}$.

The determination of strontium by means of atomic-absorption spectrophotometry can be accomplished by using an air - acetylene flame or, with greater sensitivity, by using a nitrous oxide - acetylene flame. The determination with the air - acetylene flame is subject to a variety of serious interferences, which can be controlled by using lanthanum as a releasing agent.[1–3] The higher temperature and greater reducing capacity of the nitrous oxide - acetylene flame generally serves to reduce a number of inter-element interferences. Magill and Svehla[3] investigated the effect of determining strontium in the presence of a number of interferents (using a strontium - interferent ratio of 1:6) and found that use of the nitrous oxide - acetylene flame successfully eliminated them. However, Bowman and Willis[4] found, when determining strontium in rock solutions, that appreciable interference did occur, even in the nitrous oxide - acetylene flame, and recommended that the method of standard additions be used in order to compensate for variations in the levels of major elements found in different rocks.

During recent work[5] on the determination of trace elements in silicate rocks we also found that the nitrous oxide - acetylene flame did not completely eliminate the interferences with strontium, even when the standards used contained a background matrix that was similar to, but not identical with, that of the rock sample. As the standard additions method suggested by Bowman and Willis is time consuming it was decided to study the effects that the varying amounts of major components present in silicate rock (aluminium, calcium, iron and magnesium) had on the strontium signal, and to determine whether lanthanum would prove to be effective in overcoming the interferences.

## Experimental

### Apparatus

A Perkin-Elmer 403 atomic-absorption spectrophotometer, fitted with a single-slot nitrous oxide - acetylene burner head, was used with a Telsec chart recorder of 10-mV full-scale deflection. A low-pressure PTFE bomb from Perkin-Elmer Ltd. was also used.

### Reagents

The following Specpure materials (Johnson Matthey Chemicals Ltd.) were used: aluminium rod, iron sponge, magnesium crystals, calcium carbonate, strontium nitrate, potassium chloride

and sodium chloride. The other reagents were of analytical-reagent grade: boric acid, hydrochloric acid (sp. gr. 1·16), hydrofluoric acid (40 per cent.), nitric acid (sp. gr. 1·42), perchloric acid (60 per cent.) and lanthanum chloride solution (10 per cent. *m/V* lanthanum content) for atomic-absorption purposes.

## Dissolution of Rock

The dissolution procedure used in this study has already been reported[5] in connection with an atomic-absorption method for the determination of trace amounts of copper, vanadium, chromium, nickel, cobalt and barium in silicate rocks. Briefly, it consists in digesting the rock with a hydrofluoric - nitric - perchloric acid mixture at 100 °C in a low-pressure PTFE bomb, followed by evaporation until perchloric acid fumes appear and final dissolution in a mixture of hydrochloric and boric acids. A suitable amount of potassium chloride (ionisation buffer) is then added to the mixture, which is diluted with distilled water to give a solution containing 1 per cent. of rock (original mass).

## Study of Interferences

Potential interferences were monitored by observing whether the signal produced from a standard strontium solution was influenced by the presence of the major element under investigation. The concentration of strontium used throughout this study was 8 $\mu$g ml$^{-1}$, which is equivalent to 800 $\mu$g g$^{-1}$ in the original rock. The concentration ranges of the major elements studied were chosen to be similar to those normally found in silicate rocks. All of the interference studies were carried out on solutions that were also 0·1 per cent. in potassium, 0·8 per cent. in boric acid, 2 per cent. in perchloric acid and 5 per cent. in hydrochloric acid, so that the results obtained in this study would be directly applicable to our previously reported[5] atomic-absorption procedure for rock analysis.

### *Effect of aluminium, calcium, magnesium and iron on the strontium signal*

The effects that aluminium, calcium, magnesium and iron have separately on strontium were determined as follows.

*Aluminium on strontium.* Six solutions that contained 8 $\mu$g ml$^{-1}$ of strontium were prepared so as to contain 0, 159, 397, 794, 1191 and 1588 $\mu$g ml$^{-1}$ of aluminium. These levels correspond to 0, 3·0, 7·5, 15·0, 22·5 and 30·0 per cent. of aluminium oxide in the original rock.

*Calcium on strontium.* Six solutions that contained 8 $\mu$g ml$^{-1}$ of strontium were prepared so as to contain 0, 179, 357, 536, 714 and 1071 $\mu$g ml$^{-1}$ of calcium. These levels correspond to 0, 2·5, 5·0, 7·5, 10·0 and 15·0 per cent. of calcium oxide in the original rock.

*Magnesium on strontium.* Five solutions that contained 8 $\mu$g ml$^{-1}$ of strontium were prepared so as to contain 0, 121, 241, 482 and 723 $\mu$g ml$^{-1}$ of magnesium. These levels correspond to 0, 2·0, 4·0, 8·0 and 12·0 per cent. of magnesium oxide in the original rock.

*Iron on strontium.* Five solutions that contained 8 $\mu$g ml$^{-1}$ of strontium were prepared so as to contain 0, 280, 560, 840 and 1400 $\mu$g ml$^{-1}$ of iron. These levels correspond to 0, 4·0, 8·0, 12·0 and 20·0 per cent. of iron oxide (total iron expressed as $Fe_2O_3$) in the original rock.

### *Effects of calcium, magnesium and iron on strontium in the presence of aluminium*

The effects that calcium, magnesium and iron have separately on strontium in the presence of aluminium were determined on solutions containing 8 $\mu$g ml$^{-1}$ of strontium and 794 $\mu$g ml$^{-1}$ of aluminium. These levels correspond to 800 $\mu$g g$^{-1}$ of strontium and 15 per cent. of aluminium oxide in the original rock. The concentrations of calcium, magnesium and iron used in this experiment were the same as in the investigations of the effects of aluminium, calcium, magnesium and iron alone.

### *Effect of lanthanum*

The above two interference experiments were repeated with duplicate solutions containing 1 per cent. *m/V* of lanthanum, so that the effectiveness of lanthanum as a releasing agent in the nitrous oxide - acetylene flame could be observed.

## Results and Discussion

*Effects of aluminium, calcium, magnesium and iron on the strontium signal*

*Aluminium on strontium.* The influence that the aluminium concentration has on the strontium signal is shown in Fig. 1. It can be seen from line A that while a solution of 8·0 $\mu$g ml$^{-1}$ of strontium (800 $\mu$g g$^{-1}$ in the rock) gave an absorbance of 0·420, the addition of aluminium decreased the signal to give an absorbance of 0·180 at a level of 1588 $\mu$g ml$^{-1}$ of aluminium (30 per cent. of aluminium oxide in rock). The effect of repeating the atomic-absorption measurements on duplicate solutions containing 1 per cent. of lanthanum (as described under *Effect of lanthanum*) is shown in Fig. 1, line B, and illustrates the effectiveness of lanthanum as a releasing agent. A slight decrease in absorbance (from 0·420 to 0·410) was observed in the range corresponding to 22·5–30·0 per cent. of aluminium oxide in the original rock. However, this decrease was completely eliminated when solutions containing 2 per cent. of lanthanum were used.

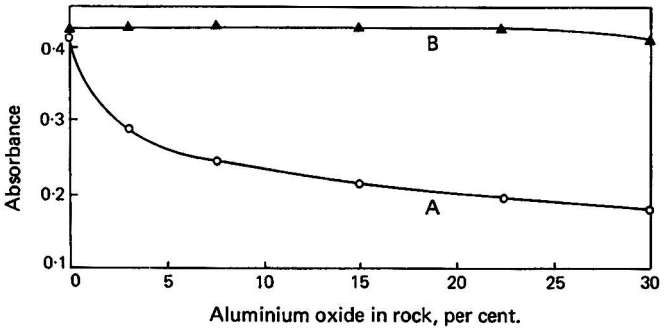


Fig. 1. The effect of aluminium on the absorbance of strontium (800 $\mu$g g$^{-1}$ in rock): A, aluminium; and B, aluminium with 1 per cent. of lanthanum.

*Effects of calcium, magnesium and iron on strontium.* The presence of these elements had no effect on the strontium signal over the ranges investigated in this work. The addition of 1 per cent. of lanthanum to the solutions also had no effect, and the measured absorbance of 0·420 indicated that no interference occurred.

*Effects of calcium, magnesium and iron on strontium in the presence of aluminium*

Because the experiment to determine the effects of aluminium, calcium, magnesium and iron separately indicated that of the elements investigated only aluminium interfered directly with strontium, it was decided to study separately the effect that calcium, magnesium and iron had on the aluminium plus strontium system so as to determine if they had any indirect effect on the interference mechanism. The influence of 1 per cent. of lanthanum on this system was also studied, as described under *Effect of lanthanum.*

The results of the experiment to determine the effect of calcium, magnesium and iron in the presence of aluminium are shown graphically in Fig. 2, and from line A it can be seen that iron does not influence the interference of aluminium in the determination of strontium. Calcium and magnesium do, however, cause a reduction in the interference effect by acting as releasing agents, and lines B and C indicate that the releasing action is greater for calcium than magnesium. The addition of 1 per cent. of lanthanum to the iron, calcium and magnesium systems completely eliminates the interference, as is shown by line D, which represents an absorbance of 0·420.

*Possible interference mechanism*

It is possible that in the flame, aluminium salts are converted into aluminate, which combines with the strontium[2] in a manner similar to that of other oxyanions, such as phosphate and silicate, perhaps giving rise to mixed strontium - aluminium oxides, which would inhibit the formation of ground-state strontium atoms.

Magnesium, calcium and lanthanum would seem to combine with aluminium in a manner similar to that of strontium, thus acting as releasing agents for strontium. The order of effectiveness as releasing agents was found to be lanthanum > calcium > magnesium. This observed effectiveness is probably due to the relative affinity of the elements for aluminium. Increasing the concentration of the releasing elements also increases their effectiveness, as would be expected from the Law of Mass Action. However, it appears, from Fig. 2, that neither calcium nor magnesium at acceptable levels would effect a complete release of strontium.

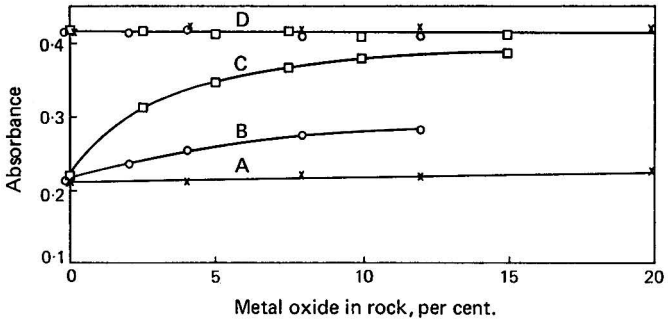


Fig. 2. The effect of iron, magnesium and calcium on the absorbance of strontium (800 $\mu g\ g^{-1}$ in rock) in the presence of aluminium (15 per cent. of aluminium oxide in rock): A, iron; B, magnesium; C, calcium; and D, iron, magnesium and calcium with 1 per cent. of lanthanum.

*Determination of strontium in silicate rocks*

The previous experiments indicate that the effect on strontium of the major elements normally present in silicate rocks can be overcome by the addition of 1 per cent. of lanthanum to the analytical spray solution. A high aluminium content rock containing aluminium oxide at a level of 22·5–30 per cent. should be analysed in the presence of 2 per cent. of lanthanum. Therefore, it was decided to determine the strontium content of some silicate rocks by using a previously reported[5] atomic-absorption method with the modification of adding lanthanum chloride to the rock solutions and standards to give a final concentration of 1 per cent. $m/V$ of lanthanum. It was found that these solutions had a shelf-life of approximately 3 d before precipitation of lanthanum fluoride occurred. Because of this the lanthanum was added just prior to the atomic-absorption determination.

An attempt was made to assess the accuracy and precision of this modified atomic-absorption method by analysing the United States Geological Survey rocks AGVI, BCRI and GSPI. From the results shown in Table I it can be seen that the precision is approximately 1–2 per cent. and that there is reasonable agreement between the atomic-absorption values and the reported literature values.[6,7] However, it can also be seen that the individual literature values cover a wide range, and this must influence the credibility of the literature averages. Because of this drawback it was decided to obtain a further indication of the accuracy by carrying out some recovery experiments on a rock that had been spiked with 100 $\mu g\ g^{-1}$ of strontium. The rock used was a sample of olivine-dolerite that contained 17·3 per cent.

TABLE I

ATOMIC-ABSORPTION DETERMINATION OF STRONTIUM IN UNITED STATES GEOLOGICAL SURVEY ROCKS

| Rock | Literature average/$\mu g\ g^{-1}$ | Literature range/$\mu g\ g^{-1}$ | Mean of atomic-absorption results/$\mu g\ g^{-1}$ | Coefficient of variation of atomic-absorption results, per cent. | Number of determinations |
|---|---|---|---|---|---|
| AGV-1 | 657 | 348–1050 | 670 | 0·75 | 9 |
| BCR-1 | 330 | 244–525 | 344 | 1·6 | 10 |
| GSP-1 | 233 | 148–400 | 244 | 1·6 | 15 |

of aluminium oxide, 10·8 per cent. of iron oxide (total iron as $Fe_2O_3$), 9·0 per cent. of magnesium oxide and 10·7 per cent. of calcium oxide. The spiking was carried out in a PTFE bomb by adding 20 ml of 10 $\mu$g ml$^{-1}$ strontium solution to 2 g of rock. The contents of the bomb were evaporated to dryness before the dissolution procedure was commenced. Five replicate portions of spiked and unspiked rock were analysed and from the results shown in Table II it can be seen that the recoveries determined in this way, and at these levels, varied from 98·2 to 101·6 per cent.

TABLE II

SPIKING EXPERIMENT WITH 100 $\mu$g g$^{-1}$ OF STRONTIUM

| Strontium concentration in unspiked rock/ $\mu$g g$^{-1}$ | Strontium concentration in spiked rock/ $\mu$g g$^{-1}$ | Recovery, per cent. |
|---|---|---|
| 191·6 | 291·0 | 99·4 |
| 190·5 | 291·0 | 100·5 |
| 192·8 | 291·0 | 98·2 |
| 190·5 | 292·1 | 101·6 |
| 189·3 | 289·5 | 100·2 |

The limit of determination of strontium in silicate rocks by use of this atomic-absorption method is 0·6 $\mu$g g$^{-1}$.

The authors thank the Government Chemist for permission to publish this paper.

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# A Potentiostatic Method and Apparatus for Multiple Determination of Copper in Free-cutting Aluminium Alloys

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A simple and direct method for the deposition of copper at a controlled cathode potential from a mixed tartrate - sulphosalicylate electrolyte has been developed. This method is used in the routine determination of the copper content of free-cutting aluminium alloys in conjunction with a multi-potentiostatic electro-deposition apparatus.

A method for the determination of copper that is suitable for use in the analysis of free-cutting aluminium alloys and that confers the speed and ease that is customary with the determination of copper in Duralumins has long been required. Potentiostatic methods were obviously needed for solution of the problem, with the proviso that multiple determinations would require a multi-potentiostatic apparatus. This condition in turn required the development of suitable equipment based on miniaturised circuitry. The analyte can be used with other potentiostatic deposition systems.

## Experimental

Many transistorised potentiostat designs have been published in recent years, among which are those of Wadsworth,[1] Juniper,[2] Tackett and Knowles[3] and Lindstrom and Davis.[4] For our purposes all seemed to be unnecessarily elaborate and costly. The literature[5,6] and *a priori* reasoning suggested the use of a control circuit based on an operational amplifier with a high-input impedance, drawing only nanoamperes from a reference cell of sufficient output to bias a transistor base and thus giving a sufficiently low output impedance. A fast response and high transition frequency would be advantageous, although most inorganic reactions are slow. Our choice was a ZEL-1 operational amplifier (obtainable from Advance Industrial Electronics Ltd., Bishop's Stortford, Herts.). This amplifier, coupled with a 2N 3055 transistor in the anode lead of the electrolysis cell, provided the miniaturisation needed for a multi-station electro-deposition apparatus (Fig. 1).

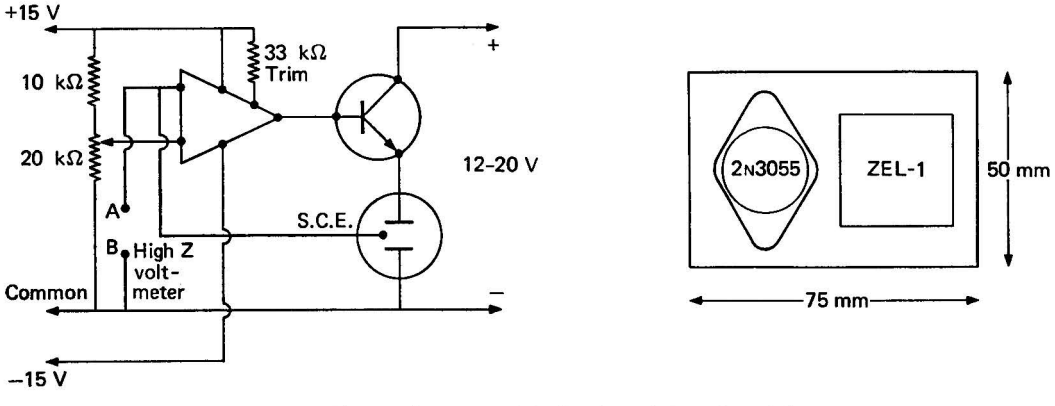


Fig. 1. Circuit diagram and indication of size of module.

The ZEL-1 amplifier needs a positive and negative voltage supply of 15–18 V to common earth; it has an open loop gain of $5 \times 10^5$ and a unity gain (a.c.) at 1·5 MHz, its slew rate is 6 $V\mu s^{-1}$ and the bias current at each input is 50 nA. Also, the output impedance is 1 kΩ and the input 1 MΩ (d.c.) and the common mode rejection ratio is 20 000. The transition frequency

of the transistor is 1 MHz, and is reasonably well matched to the voltage swing of the ZEL-1 instrument.

Because of the low bias current drawn by the operational amplifier we designed a high-impedance voltmeter to monitor the voltage at its negative input terminal. This was based on a Radiospares Ltd. FET-MOPA with an impedance of $10^{14}\ \Omega$ (Fig. 2). Connections to each module in sequence were made by a rotary wavechange switch, using every other switch point of the make-before-break action and connecting switch points 2 and 3, 5 and 6, and 7 and 8 through resistors to avoid excessive meter swings. The circuit has provision for calibration and zero adjustment.

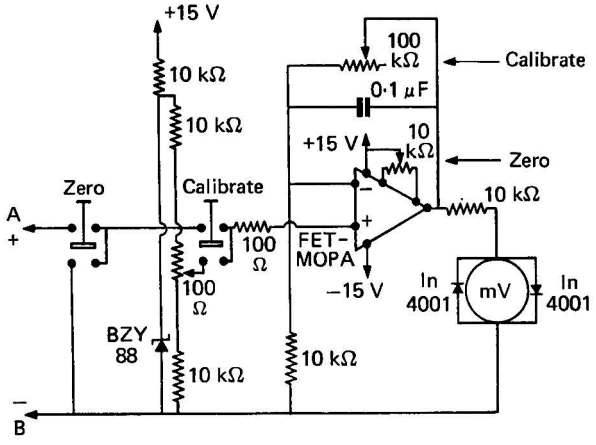


Fig. 2. Circuit for high-impedance voltmeter (correct polarity must be observed).

The d.c. supplies for both the operational amplifier and the cells were found to need a high level of regulation. The cell supply is provided from mains a.c., which is fed into the 230-V tapping of a transformer with a 250-V Varistor connected across the 250-V tappings in order to eliminate any transients. The 42-V output is rectified by a full-wave diode rectifier and fed into a Radiospares HPV regulator circuit, which provides adequate smoothing and decoupling.

The adjustable output is dissipated across a precision resistor of 110 Ω and a 25-Ω rheostat in series. The rheostat facilitates fine adjustment, while the useful voltage level is pre-set by a potentiometer in the HPV regulator circuit at 12–15 V. An under-damped meter movement reads the voltage output and detects variation (Fig. 3).

The cell supply unit and the operational amplifier supply are mounted inside a steel box, 12 × 8 × 7½ in, that is fitted with louvres. The operational amplifier supply is assembled from a Radiospares universal transformer that delivers 2 × 18 V into a full-wave diode rectifier, using the centre tap as common earth and smoothing the positive and negative outputs with respect to it by use of two 3300-mF condensers. Surge across the condensers is dissipated in a 1-kΩ resistor. The outputs are regulated by a Raytheon 1495M dual-tracking regulator and are then decoupled (Fig. 3).

Both supplies are constant to within 0·1 per cent. of the load voltage. This value represents 12–15 mV at the transistor collector, but feedback in the operational amplifier reduces it to less than 1 mV at the cathode.

The potentiostats themselves are easily made by mounting the 2N 3055 transistor and the ZEL-1 operational amplifier on a 3 × 2 in piece of peg-board. The only other components needed are the 10 000-Ω limiting resistor for the reference voltage, supplied to the non-inverting terminal, and the offset bias resistor of 33 000 Ω. The reference voltage is selected by use of a 20 000-Ω precision potentiometer mounted on the control panel of the instrument. It is best to fit a digital control dial for control of shaft position, and this is conveniently arranged in front of each mounting post that carries the control panel above the stirrer bank. Leads to the reference electrode and working electrodes can be screened

if necessary. In the present work it was unnecessary, but all of the cathodes were connected to a common earth.

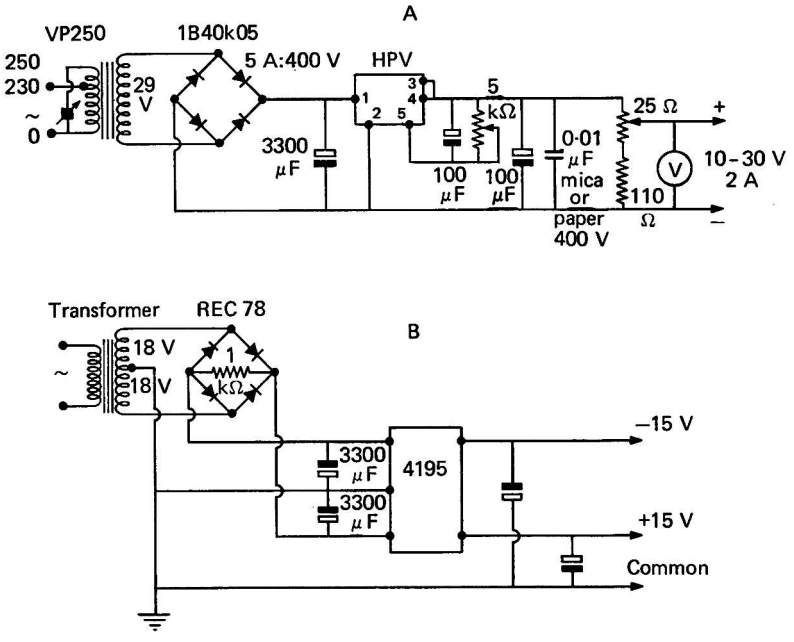


Fig. 3. Power supplies to the cell (A) and the operational amplifier (B).

Each potentiostatic module is mounted at the rear of its reference voltage potentiometer inside a box section, 22 × $2\frac{3}{4}$ × $3\frac{1}{2}$ in, that is supported over a four-bank magnetic stirrer made by Voss Ltd. The Lasky MR65 milliammeter (500 mA) and the reference voltmeter are mounted symmetrically between the digital dials and the reference voltage "read" switch mounted in the middle of the box panel. On the top of the box at each station is mounted a "read"/"short" switch for reading the current in each cell circuit. Connections are carried through tight rubber grommets in the underside of the box, while a slot at the top right-hand surface allows access to the "zero" and "calibrate" voltmeter controls.

Each stirrer position has a rod at the rear supporting the box control panel and acting as the support for the electrode carrier. This last structure is made of 1 × 5 × 1 in Tufnol soaked in high-viscosity silicone oil; it carries the reference electrode, a Pye - Ingold saturated calomel electrode. This electrode is wound with 25 cm of 1 mm diameter platinum wire, which acts as the anode, and it carries a Perspex probe cemented to its base that is drilled to carry the slow feed of potassium chloride from the electrode. The edges of the tip are chamfered to avoid "screening." The cathode is fitted from below, through the Tufnol and into a brass contact that is protected against corrosion. The carrier is cemented to a circular Perspex disc on its underside, which acts as a cover to the 250-ml, squat Pyrex beaker containing the analyte. The carrier slides on the rod and is clamped by nickel - tin plated bolts and wing-nuts.

The total cost of building a three-position instrument was just under £200 (December, 1974). A fourth position will be used for a modified module, but each of the three present modules cost just under £20. This represents a very considerable saving on previous designs.

Considering again the circuit shown in Fig. 1, the non-inverting input of the operational amplier is fed with a positive potential derived from the operational amplifier supply unit. A connection is made through residual resistance (much less than 1 MΩ) to common earth. A second potential, derived from the reference cell, is supplied to the non-inverting terminal of the operational amplifier and is balanced by the first potential. Any change in this second potential referred to common earth causes an opposing change in the output of the

operational amplifier. The gain factor ($Av$) is

$$Av = \frac{R_1 + R_2}{R_1}$$

where $R_1$ is the reference cell resistance and $R_2$ is the total resistance of the cell and emitter - base transistor junction.

As the cell resistance increases during deposition, the cathode potential tends to become more negative, so that the bias supplied to the transistor and the current flow across the transistor are reduced, thus reducing the anode - cathode potential. The gain factor, $Av$, tends to increase during electrolysis and thus the precision of control increases. Most of the absolute gain of the operational amplifier is used as negative feedback stabilisation. In our circuit there appears to be no need for isolation of the reference electrode from the ZEL-1 instrument by a voltage follower amplifier.

Each potentiostatic module will allow the passage of a current of 1 A for short periods of time, but this is a maximum. For larger currents a higher rating transistor should be used. With the 2N 3055 transistor operating in a circuit supplied with 20–30 V across the cell, the temperature of the transistor will be raised to a case temperature of 50 °C by the continuous passage of a 300-mA current. A current of 150 mA will give a case temperature of 40 °C. At 13·5 V, with a mean current of 500 mA, the maximum case temperature is 32 °C in still air, allow- in a current variation of 500–520 mA in 30 min. Temperatures were measured in a rubber push-on cap that was fitted over the transistor and contained 1 ml of silicone oil.

In practice, the demand on the circuit is much less than the instances quoted because the current decays exponentially. The 2N 3055 is rated to dissipate 15 W at 25 °C and with a supply of 13·5 V and a current of 400 mA the dissipation is only 5 W at a cell voltage drop of 2 V. This is the normal operating condition of the module for the deposition of 0·06 g of copper in 200 ml of electrolyte.

The electrolysis time is, of course, related to the concentration of reducible ions by the equation:

$$C_t = C_0 e^{-Kt}$$

where $C_t$ is the concentration of metal after $t$ s, $C_0$ the initial concentration and $K$ a constant inversely dependent on the volume and character of the electrolyte, and directly dependent on the area of the cathode and the depolariser. It is therefore dependent only on analytical technique and normally an analysis using a standard 45 × 45 mm platinum-gauze cathode or 25 cm of 1 mm diameter wire is complete within 50 min.

The electrolyte used is a slightly acidic tartrate solution at pH 5–5·5 as used by Lingane and Jones,[7] but it was modified to permit the deposition of copper in the presence of alum- inium. The original base solution contained a concentration of 0·25–0·5 M of tartrate ion and had a pH variable between 5·8 and 6·0; hydrazinium chloride was added to a maximum concentration of 1 g per 100 ml, and succinic acid - succinate buffers were found necessary to absorb the hydrogen ion generated by electrolysis. In the presence of 5000 mg $l^{-1}$ of alum- inium, a normal level in our application, we found that the deposition of copper was partially suppressed. However, additions of sulphosalicylic acid, instead of succinic acid, to the electrolyte before adjustment of the pH to the optimum range for plating nullified the in- hibitory effect. Sulphosalicylic acid is a chelating agent for aluminium and is used as a screening agent in complexometry. Its action is likely to be that of suppression of adion exclusion of copper at the electrode surface by aluminium tartrate.

Naturally, the conditions derived by Lingane and Jones were found to be changed by the presence of the sulphosalicylic acid, although the qualitative effects were generally similar. The concentration of the acid itself was linearly related to the relaxation of inhibition up to the level of 2 g per 200 ml, but thereafter its effect tailed off, so that at least 8 g per 200 ml were required to permit 100 per cent. copper deposition (Fig. 4).

In the potentiostatic deposition of copper from solutions of aluminium alloys, the element most likely to interfere by co-deposition is bismuth. A family of curves of current (measured in μA) against cathode potential (in mV), using a 1-$mm^2$ micro-electrode in a base solution con- taining aluminium (1 g per 200 ml) and bismuth (50 mg per 200 ml), is shown in Fig. 5. The electrode shows hysteresis because of the large activation energy involved[7] and the points on the curve are not very reproducible[8]; however, the important values are those at initiation of deposition.

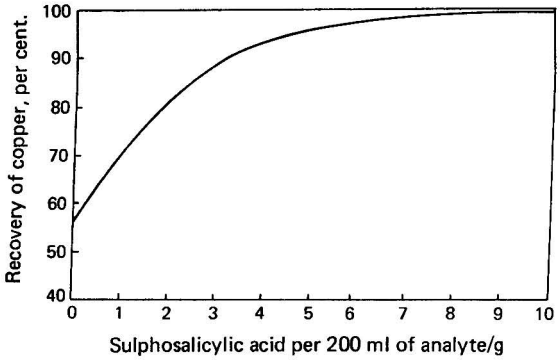


Fig. 4. Effect on the electrolysis of the addition of sulphosalicylic acid to the electrolyte.

A further graph shows the minimum reference voltages for the deposition of bismuth and copper over a range of pH values (Fig. 6). The maximum separation occurs between pH 4·5 and 5·5, while the maximum rate of deposition of copper occurs at pH 3·5, with a reference potential of 250 mV *versus* S.C.E.

Because the likely change of pH, during the course of a routine analysis, is to a lower value, we decided to adopt pH 5·5 as our initial optimum and checked the sequential deposition of binary combinations of ions using the 1-mm$^2$ micro-electrode, again in the presence of 5 mg ml$^{-1}$ of aluminium. The family of curves is shown in Fig. 7. Good separation is shown in each instance with a maximum safe reference potential of 300 mV for deposition of copper. We later found that the change in pH is not significant when using sulphosalicylic acid.

The concentration of the depolariser, hydrazinium chloride, is very important. For normal amounts of copper in solution the optimum amount seems to be 0·06 M, larger amounts leading to a lower final pH.

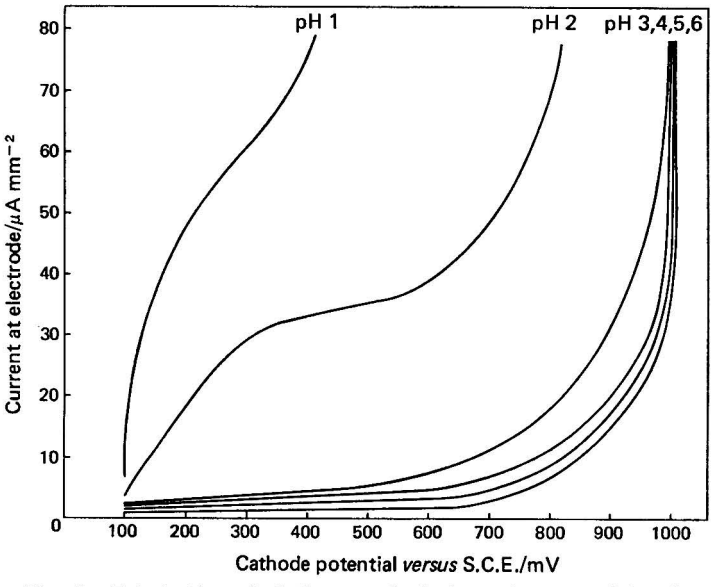


Fig. 5. Polarisation of platinum cathode in analyte containing 1 g of aluminium and 50 mg of bismuth per 200 ml. Temperature, 18 °C; nitrogen purge.

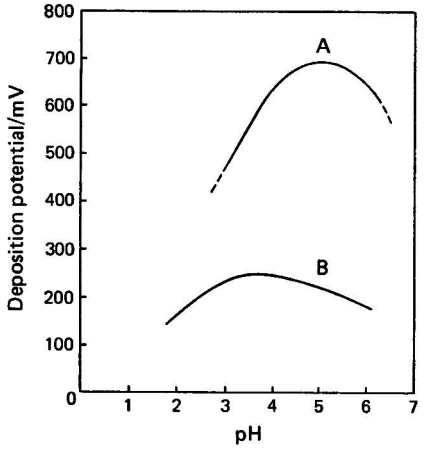


Fig. 6. Deposition curves for bismuth (A) and copper (B) at a concentration of 50 mg per 200 ml in the analyte containing 1 g of aluminium per 200 ml at 18 °C.

The concentration of tartrate ion can be lowered, in the presence of sulphosalicylic acid, to increase the rate of deposition (Fig. 8). However, it cannot be lowered safely to below 0·25 M per gram of aluminium. If hydrolysis occurs during pH adjustment then the tartrate concentration should be increased to 0·35 M per gram of aluminium. The deposition of copper is accompanied by electrophoretic deposition of trace amounts of insoluble bismuth and lead salts. The formation of dispersions of these salts is most likely to occur at the neutralisation stage. The temperature at which this operation is carried out is critical and should be about

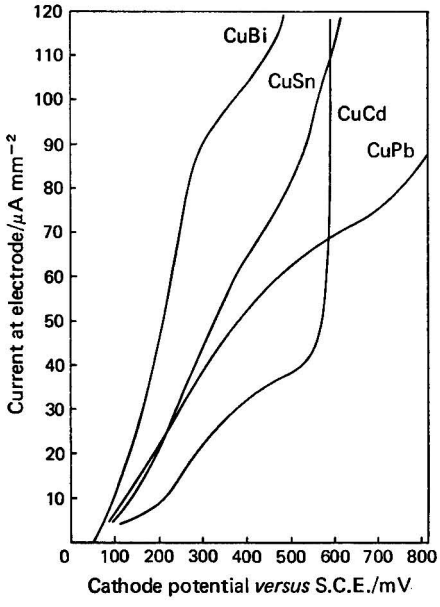


Fig. 7. Polarisation curves of binary mixtures of ions in the analyte (50 mg of each mixture and 1 g of aluminium per 200 ml).

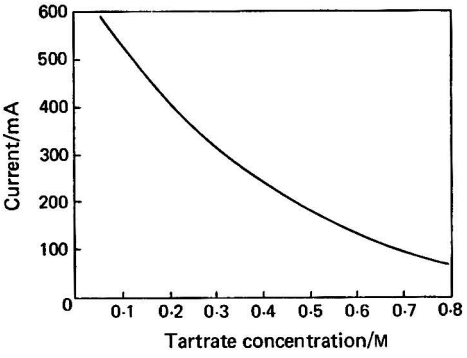


Fig. 8. Influence of tartrate on deposition of 50 mg of copper in the analyte containing 10 g of sulphosalicylic acid.

70 °C at up to pH 4·5. In order to use the pH meter, it is permissible to decrease the temperature to 30 °C for the final additions of dilute alkali solution. If the lead and bismuth contents are very high, *e.g.*, 1·5 per cent., then the precipitate which might otherwise form should be re-dissolved by addition of 0·1 M tartaric acid and heating. If the resultant analyte solution is not clear, it must be filtered, preferably through a pulp pad.

## Method

A 1-g amount of the alloy is weighed into a 200-ml tall-form beaker and dissolved in the minimum amount of sodium hydroxide solution (20 g per 100 ml). When the main reaction is complete the beaker walls are washed down with water and dissolution completed by boiling. The beaker and contents are cooled to 70 °C and 10 ml of dilute sulphuric acid (1 + 1) are added, then the insoluble residue is dissolved by dropwise addition of dilute nitric acid (1 + 1). After boiling the solution, washing down the beaker walls with water and re-boiling, the solution is cooled to about 50 °C. Then, 50 ml of 1 M sodium D(+)-tartrate solution, 10 g of sulphosalicylic acid and 1·25 g of hydrazinium chloride are added and dissolved by use of a magnetic stirrer. The solution is then neutralised by the addition first of pellets of sodium hydroxide and then with dilute sodium hydroxide solution (2 + 98). In the correct pH range (5·0–5·5) the colour of the solution is orange. At this stage the solution is cooled to 30 °C and the dilute alkali added, the volume being controlled by reference to a pH meter. The solution is next diluted to 200 ml and electrolysed with an initial current of 10–20 mA for 30–60 s in order to deposit coherent copper, then the reference potential can be raised to 300 mV and the electrolysis completed.

The electrodes used are standard 45 × 45 mm platinum-gauze cathodes and the spiral anode is as detailed above. Most of the copper is plated out within 25 min, but we normally continue for another 20 min to ensure completeness. The electrode carrier is raised without switching off the current, the electrodes are then rinsed with water, removed from the carrier, washed in alcohol and peroxide-free diethyl ether and dried in warm air. They are finally cooled and weighed.

## Results

The method described has been used to determine copper in a variety of alloys and synthetic mixtures. Our initial synthetic solutions, made by dissolving 0·06 g of copper in nitric acid and the addition of 0·0900 g of basic lead carbonate and 0·0800 g of bismuth(III) oxide to solutions of aluminium sulphate made by dissolving 1 g of aluminium (99·9 per cent. pure) as described in the recommended method, yielded copper recoveries of 100–101·3 per cent. Results of analyses carried out by using the preferred method are given in Table I. In routine use for the analysis of FC1 (AA.2011) alloy (copper 5·0–6·0, magnesium 0·1 maximum, silicon 0·1–0·3, iron 0·3–0·5, manganese 0·1 maximum, lead 0·35–0·65, bismuth 0·35–0·65 per cent.) we have obtained a standard deviation of 0·03 per cent. at the 5·5 per cent. of copper level.

TABLE I

DETERMINATION OF COPPER IN ALUMINIUM ALLOYS AND SYNTHETIC MIXTURES

| Sample | No. of analyses | Composition, per cent. | Copper content by other methods, per cent. | Copper content by potentiostatic method, per cent. |
|---|---|---|---|---|
| AU4 Pb Bi (1) (Calibration standard) | 12 | Cu 3·5, Mg 0·80, Mn 0·30, Fe 0·26, Zn 0·29, Pb 0·80, Bi 0·40 (nominal but unconfirmed) | 3·50 | 3·51 (S* = 0·011) |
| AU4 Pb Bi (2) (Spectrographic sample) | 12 | Cu 6·5, Mg 1·50, Mn 1·00, Fe 0·86, Zn 0·60, Pb 1·50, Bi 0·60 (nominal but unconfirmed) | 6·83 (duplicate sample of that used for determination by potentiostatic method) | 6·82 (S = 0·11) |
| Cast No. L 9065 | 6 | Cu 5·1, Mg 0·17, Mn 0·05, Fe 0·36, Zn 0·01, Pb 0·65, Bi 0·60 | 5·10 | 5·15 |
| DTD 5074 rod | 6 | Cu 1·7, Zn 5·98, Mg 2·44 | 1·74 | 1·77 |
| Cast No. MW 46 (Int. alloys) | 6 | Cu 3·3, Mg 0·64, Fe 0·56, Pb 0·02, Sn 0·18, Sb 0·53 | 3·30 | 3·28 |
| Alloy "A" BCS 181 | 6 | Cu 4·64, Mg 1·28, Fe 0·51, Zn 2·37, Pb 1·51 | 4·60–4·70 | 4·66–4·68 |
| Synthetic aluminium alloy solutions | 10 | Cu 0·01, Pb 8·0, Bi 8·5, Fe 0·75 (nominal) | — | Gravimetric, 0·02; visual inspection showed no discoloration of cathode |
| Weighed mixture of L 9065 and MW 46 (1 + 1) | 2 | Cu 4·22, Pb 0·33, Bi 0·30, Sn 0·08, Sb 0·27 (expected) | — | 4·25 |

* S = standard deviation.

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## APPENDIX

### List of Components

| Quantity | Component | | Supplier |
|---|---|---|---|
| *Module* | | | |
| 1 | ZEL-1 | Operational amplifier | Advance Industrial Electronics |
| 1 | 2N 3055 | Transistor, p–n–p | R.S. Components |
| 1 | 10 kΩ | ½-W high-stability resistor | R.S. Components |
| 1 | 20 kΩ | 10-turn potentiometer | Bourne or Varley |
| 1 | 33 kΩ | ½-W high-stability resistor | R.S. Components |
| 1 | | Digital-reading dial | R.S. Components |

| Quantity | Component | | Supplier |
|---|---|---|---|
| *High-impedance voltmeter* | | | |
| 1 | FET-MOPA | Operational amplifier | R.S. Components |
| 1 | 500 mA f.s.d. MR 65 | Meter movement | Lasky's |
| 1 | BZY 88 | Zener diode | R.S. Components |
| 2 | 1N 4001 | Diodes | R.S. Components |
| 1 | 100 Ω | ¼-W high-stability resistor | R.S. Components |
| 5 | 10 kΩ | ¼-W high-stability resistors | R.S. Components |
| 1 | 10 kΩ | 16-mm potentiometer | R.S. Components |
| 1 | 100 kΩ | 16-mm potentiometer | R.S. Components |
| 1 | 100 Ω | Mini pre-set potentiometer | R.S. Components |
| 1 | 0·1 μF | 250-V P.D.C. condenser | R.S. Components |
| 2 | Press 2P | Push-button switches | R.S. Components |
| 1 | | Rotary wave-change switch, midget, 12-way | R.S. Components |
| *Cell supply unit* | | | |
| 1 | 2 A, 30 V | Output mains transformer with 200–250-V tapped input | As available |
| 1 | VP250 | GEC varistor | Jermyn Industries |
| 1 | 1B40k05 | Rectifier bridge | Texas Instruments |
| 1 | 3300 μF | Electrolytic smoothing condenser, computer grade, high ripple | R.S. Components |
| 2 | 100 μF | Electrolytic smoothing condensers, tubular | R.S. Components |
| 1 | 0·01 μF | 400-V d.c. polyester condenser | R.S. Components |
| 1 | 5 kΩ | Midget potentiometer | R.S. Components |
| 1 | 25 Ω, 2 A | Potentiometer, wire-wound | Berco |
| 1 | 110 Ω, 2 A | Precision resistor, wire-wound | As available |
| 1 | 0–35 V | Voltmeter | As available |
| 1 | HPV | Regulator | R.S. Components |
| *Operational amplifier supply unit* | | | |
| 1 | 2 × 18 V | Output 500 mA mains transformer | As available |
| 1 | REC78 | Bridge rectifier | R.S. Components |
| 1 | kΩ | 1-W resistor, wire-wound | R.S. Components |
| 2 | 3300 μF | Electrolytic smoothing condensers, computer grade, high ripple | R.S. Components |
| 1 | Raytheon 1495 | Dual-tracking voltage regulator | Jermyn Industries |
| 2 | 4·7 μF | Electrolytic condensers, 63 V, tubular | R.S. Components |
| *Other components* | | | |
| 1 | | "Read"/"short" D.P.D.T. switch per module | R.S. Components |
| 1 | 500 mA f.s.d. MR65 | Ammeter | Lasky's |
| 1 | | Steel box housing voltage supply units | Olson Electronics |
| 1 | | Multi-position magnetic stirrer to order | Voss Ltd. |
| 1 | | Pye - Ingold saturated calomel reference electrode per module | Pye - Unicam |

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# The Voltammetric Determination of Sodium Hydroxymethanesulphinate

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The determination of sodium hydroxymethanesulphinate by voltammetry using a rotating platinum micro-electrode is described with operating details. The method is sufficiently specific for it to have definite advantages over methods based on redox titrations.

Sodium hydroxymethanesulphinate (SHMS) is a reducing agent that is widely used in the textile industry. Methods for its determination based on redox titrations[1–6] are subject to errors because of impurities, such as sulphite, metabisulphite and the formaldehyde - hydrogen sulphite complex, which occur in commercial samples,[7] and they are therefore unsuitable in many instances.

The use of voltammetry for the determination of SHMS was first proposed in 1948,[8] when a stationary platinum micro-electrode was used to give a wave for a 0·01 M concentration of SHMS in 0·1 N nitric acid ($E_{\frac{1}{4}} \approx +600$ mV; all potentials are relative to a standard calomel electrode). In a more detailed study with a similar micro-electrode a well defined wave from 0·1 M citrate buffer ($E_{\frac{1}{4}} \approx +250$ mV) was reported, but it was concluded that the method was unsuitable for quantitative analysis, mainly because of the inherent characteristics of stationary micro-electrodes.[9]

The work reported here has established the criterion on which a quantitative voltammetric determination of SHMS could be based. While such a method may not be applicable to routine dyehouse testing, it is hoped that it will foster renewed interest in the chemical reactions of this compound.

## Experimental

SHMS, obtained from BDH Chemicals Ltd. under the name sodium formaldehydesulphoxylate (laboratory-reagent grade), was recrystallised at 60 °C from 40 per cent. aqueous ethanol and dried *in vacuo* over silica gel at room temperature (more powerful drying agents remove the water of crystallisation).[1]

### Base Electrolyte

The following buffer solutions were investigated as base electrolytes: glycine - hydrochloric acid (pH 3·0), 0·1 M citrate (pH 4·4), 0·2 M acetate (pH 4·6) and a series of McIlvaine buffers (citric acid - disodium hydrogen orthophosphate) ranging from pH 2·0 to 8·0 but of constant ionic strength (1·0 M).[10] The McIlvaine buffer of pH 4·0 was found to be the most satisfactory because the linearity of the graph of wave height *versus* concentration was considerably better than that obtained in other buffers, although the shape of the wave was not as well defined as in glycine - hydrochloric acid buffer. Maximum suppression was not necessary.

### Polarograph

The polarograph used was a Polariter PO4 (Radiometer Ltd.), with a standard calomel electrode (Radiometer K501) as reference. The connections of the micro-electrode and the calomel electrode to the polarograph were interchanged so as to allow the micro-electrode potential to be varied between +3195 and −605 mV.

### Micro-electrode

The micro-electrode was a 0·5 mm diameter platinum wire that extended 6 mm from the

side of a 6 mm diameter soda-glass tube, electrical contact between the platinum wire and the polarograph being made through a pool of mercury.

A rotating micro-electrode assembly similar to that available from Radiometer Ltd. (E70) was constructed by using a Quickfit stuffing-box gland (ST 4/2F). In order to reduce turbulence set up in the solution by precession of the electrode, the felt washer in the stuffing-box gland was consolidated by squeezing it in a vice and re-drilling to 6 mm diameter. The assembly was belt-driven at a constant speed of approximately 600 rev $min^{-1}$. Provision was made for passing humidified pure nitrogen through the cell solution but this requirement was found to be unnecessary owing to the relatively slow rate of autoxidation of SHMS.

*Regeneration of the micro-electrode*

The anodic films formed on platinum electrodes have been discussed by Gilman,[11] and are considered to be of a complex nature. However, under the conditions of this determination it is probable that it is a platinum oxide film, which gives rise to an anodic wave at a half-wave potential of approximately +600 mV, thus causing the residual current to become unacceptably irregular after about 20 determinations.

Regeneration of the electrode surface was effectively carried out by allowing the electrode to stand overnight in 5 N sulphuric acid that was 0·1 M in iron(II) and iron(III) ions, then rinsing in distilled water and cycling the electrode at 200 mV $min^{-1}$ between 0 and +1000 mV in pH 4·0 McIlvaine buffer until a stable residual current was obtained (usually between five and ten cycles were required). Care had to be taken not to allow the electrode to remain at potentials greater than about +700 mV for longer than was necessary in order to establish concentration polarisation, as anodic films formed rapidly on the platinum surface at these potentials. While not in use the micro-electrode was stored in de-ionised water and the calomel electrode in saturated potassium chloride solution.

## Procedure

The anodic wave of SHMS was recorded at 20 °C between 0 and +1200 mV at 200 mV $min^{-1}$, with minimum damping and no compensation for condenser or diffusion currents. Current sensitivities of 100 or 200 μA, full scale, were required.

## Results

Fig. 1 shows the anodic wave of SHMS in pH 4·0 McIlvaine buffer, together with the corresponding base electrolyte curve.

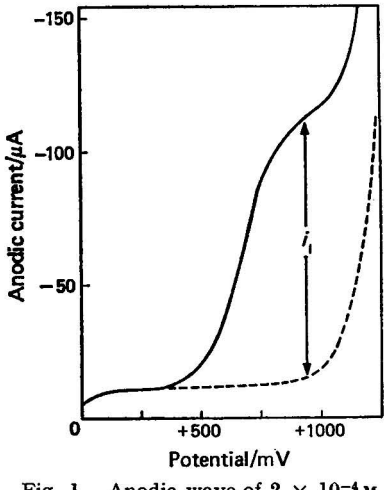


Fig. 1. Anodic wave of 2 × $10^{-4}$ M SHMS in pH 4·0 McIlvaine buffer (continuous line) and the residual current curve (broken line) showing the measurement of $i_l$.

The point of inflection on the plateau was taken as the minimum in a graph of $\Delta i/\Delta E$ against $E$, and was found to be at +935 mV. The wave height ($i_1$) was then taken as the ordinate at +935 mV limited by the intercepts of the wave and the residual current curves (see Fig. 1).

The relationship between the wave height and the SHMS concentration is shown in Fig. 2. At each concentration, five waves were recorded and the mean wave height was calculated. The spread of the wave-height measurements at each concentration are shown in Fig. 2.

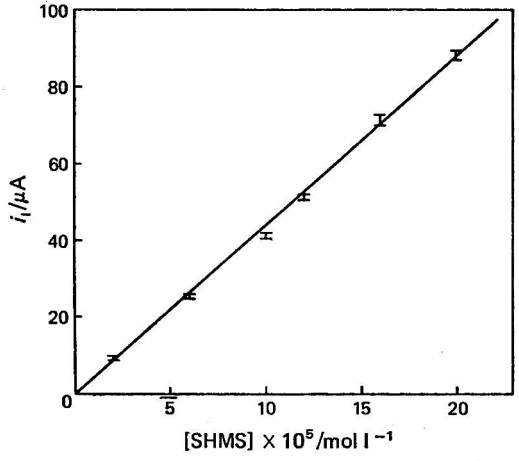


Fig. 2. Graph of wave height ($i_1$) *versus* concentration of SHMS. Base electrolyte - pH 4·0 McIlvaine buffer.

The line of best fit was found by the method of least squares to be

$$i_1 = 4{\cdot}38 \times 10^5[\text{SHMS}] - 0{\cdot}26$$

with a correlation coefficient $r = 0{\cdot}892$, which was significant at the 1 per cent. level. The standard errors in the estimates of the gradient and intercept were 0·04 and 0·46, respectively, and the standard error in the measurement of any one wave height was 1·58.

## Interference by Impurities

The most serious interference in this analysis by impurities commonly present in SHMS, which arise either during manufacture or from subsequent degradation, is that caused by hydrogen sulphite. Under the conditions used to measure the SHMS wave, a $2 \times 10^{-4}$ M hydrogen sulphite solution gave a wave between +400 and +700 mV with $i_1 \approx 20\ \mu$A. Thus the error introduced by the latter wave into the measurement of $i_1$ for SHMS is negligible when the hydrogen sulphite is present as an impurity in a concentration of less than about 10 per cent.

If the hydrogen sulphite ion is present in higher concentrations, say as a reaction product, it can conveniently be removed by the addition of a small excess of formaldehyde, which forms electro-inactive sodium formaldehydesulphonate.[9] Formaldehyde itself has no effect on the SHMS wave.

A $2 \times 10^{-4}$ M solution of dithionite ions produces a wave similar to that of hydrogen sulphite, with $i_1 \approx 25\ \mu$A. The wave is not markedly affected by the presence of formaldehyde at 20 °C for 0·5 h. At low concentrations interference by dithionite ions is negligible, but at concentrations higher than about 10 per cent. of the SHMS concentration the dithionite should be determined independently by the method of Lem and Wayman.[12]

Sulphide, thiosulphate and sulphate ions, and colloidal sulphur, are not oxidised under the conditions used and should not interfere in the analysis. However, sulphide ions may well be a factor in the poisoning of the micro-electrode and they should therefore be removed

from the solution if their concentration is suspected to be higher than about $10^{-5}$ M in the cell solution.

## Conclusion

The relationship between the concentration of SHMS and the anodic wave height has been established and found to be linear over the range $2 \times 10^{-5}$–$2 \times 10^{-4}$ M. The determination of SHMS should preferably be carried out at a concentration of about $1 \times 10^{-4}$ M.

The procedure outlined is satisfactory for the determination of the purity of samples of SHMS, but in kinetic studies, for example, precautions against interference from reactants and/or products must be taken.

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# Mutual Interference Effects During the Successive Determination of Iodide, Bromide, Chloride and Fluoride in a Single Sample Using Halide-selective Electrodes

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The determination, using halide-selective electrodes, of iodide, bromide, chloride and fluoride in a single 100-mg sample of an orthophosphate mineral is described. Mutual halide interference does not occur if the level of iodide is less than one twentieth of that of bromide (although with care this can be extended to one tenth) and if the chloride concentration is between 50- and 200-fold in excess of that of bromide. The extent of other interferences in the determination is discussed briefly.

The method is applicable to the determination of iodide over the concentration range $5 \times 10^{-5}$ to $10^{-1}$ M, bromide from $5 \times 10^{-4}$ to $10^{-1}$ M, chloride from $3 \times 10^{-4}$ to $10^{-1}$ M and fluoride from $3 \times 10^{-6}$ to $10^{-1}$ M, provided that the described precautions are taken to limit mutual interference effects.

In our previous papers[1–4] we have discussed the determination of fluoride and chloride ions individually, using both a citrate buffered and a triethanolamine buffered medium. The latter medium has also been successfully used in the determination of both chloride and fluoride in a single sample.[5] These two ions do not mutually interfere when detected by the appropriate halide-selective electrode as the composition of the membrane in the fluoride electrode (doped lanthanum fluoride) differs substantially from that in the chloride electrode (silver chloride - silver sulphide or silver chloride). Chloride-, bromide- and iodide-selective electrodes, having membranes that are similar in construction, suffer to a greater or lesser extent from mutual interference; iodide may interfere in both bromide and chloride determinations, and bromide may interfere in chloride determinations. Electrode manufacturers provide data on selectivity coefficients, usually for aqueous solutions, but data on the mutual interference of these ions in buffer sytems are lacking. These data are required before a practical method can be developed for the determination of iodide, bromide, chloride and fluoride in the same sample.

Numerous potentiometric methods are available for the determination of a single halide ion and the iodide electrode has been used for the sequential titrimetric determination of iodide, bromide and chloride[6] with silver nitrate. This method suffers from the imprecise end-point of the bromide titration and, more seriously, from the adsorption of ions on to the silver halide detector crystal.[7] Addition of large amounts of potassium nitrate[8] or the application of Fajan's adsorption rule can be used to minimise adsorption effects. Alternatively, a mixed silver nitrate - thallium(IV) nitrate solution, pH 7·2, has been employed[9] and a sodium-selective glass electrode has been used[10] in place of the more usual silver billet electrode in argentimetric determinations. The use of individual halide-selective electrodes in total halide determinations has received little attention.

In this paper the determination, using halide-selective electrodes, of iodide, bromide, chloride and fluoride in a single 100-mg sample of an orthophosphate mineral is described.

## Experimental

Potentiometric measurements for solutions of inorganic or biological orthophosphates containing trace amounts of halide ions were obtained in a citrate buffered aqueous medium using the system

| Halide electrode | Buffered solution | Reference electrode |
|---|---|---|

The detector meter was a Radiometer, PHM 52, digital pH meter reading to ± 0·1mV. The electrode systems used are shown in Table I.

TABLE I

ELECTRODE SYSTEMS USED

| Halide determined | Detector electrode | Reference electrode |
|---|---|---|
| Fluoride | Orion 94–09A (solid state) | Orion 90–01 liquid junction calomel |
| Chloride | Orion 94–17A (solid state) | (*i*) Cambridge Instruments, Type 42528, ceramic plug, filled with saturated $KNO_3$ solution |
| Bromide | Radiometer F1022 Selectrode (solid state) | (*ii*) Radiometer K601 ceramic plug Hg - $HgSO_4$ |
| Iodide | Orion 94–53A (solid state) | |

## Reagents

The reagents were of analytical-reagent grade when available and de-ionised and distilled water was used in the preparation of the reagent and standard solutions.

*Stock standard solutions.* Standard solutions that were $10^{-1}$ M in the relevant halide ion were prepared initially (as the sodium or, occasionally, the potassium salt). The stock solutions were kept in polyethylene vessels and were diluted as required. Iodide-containing solutions decompose rapidly and should be freshly prepared.

*Dissolution acid.* A solution that was 1 M in perchloric acid and 2 M in citric acid was prepared by adding 83 ml of 70–72 per cent. perchloric acid to 750 ml of a solution containing 420 g of citric acid, and diluting to 1 l.

*pH adjusting solutions.* (*a*), 1 M and 2 M solutions of trisodium citrate were prepared by dissolving 294·2 g and 588·4 g, respectively, of trisodium citrate in 1 l of water. The 2 M solutions were prepared freshly each day in order to ensure that no precipitation had occurred. (*b*), A 10 M solution of sodium hydroxide was prepared by dissolving 400 g of sodium hydroxide in 1 l of water.

## Method

Approximately 100 mg of the orthophosphate mineral were weighed accurately into a dark polyethylene 50-ml container. Five millilitres of the dissolution acid were added and the solid was dissolved by means of gentle agitation. The pH was adjusted to about 2·5 by the addition of 5 ml of de-ionised, distilled water and 5 ml of 1 M trisodium citrate solution.

The iodide activity was determined and the concentration derived from a calibration graph, in the construction of which the 5 ml of water were replaced by 5 ml of a standard iodide solution. Bromide and chloride activities were then determined in a similar manner.

In order to obtain the optimum pH for the determination of fluoride, 10 ml of 2 M trisodium citrate solution were then added, followed by 2 ml of 10 M sodium hydroxide solution. These additions gave a pH of about 5·3, and the fluoride activity was then determined.

The ranges of halide concentrations for which this method is applicable are indicated in Table II.

TABLE II

CONCENTRATION RANGE IN BUFFERED SOLUTION OVER WHICH THERE IS A LINEAR RESPONSE OF ELECTRODE POTENTIAL FOR EACH HALIDE-SELECTIVE ELECTRODE

| Halide | Concentration/mol $l^{-1}$ Minimum | Maximum |
|---|---|---|
| Iodide | $5 \times 10^{-5}$ | $10^{-1}$ |
| Bromide | $5 \times 10^{-4}$ | $10^{-1}$ |
| Chloride | $3 \times 10^{-4}$ | $10^{-1}$ |
| Fluoride | $3 \times 10^{-6}$ | $10^{-1}$ |

## Results and Discussion

It is perhaps pertinent to discuss in some detail the levels of concentration of halide ions

at which mutual interference occurs (Table III). A comparison is given of the interferences that occur in aqueous solution with those that occur in the buffered system described above.

TABLE III

LEVELS OF HALIDE IONS AT WHICH MUTUAL INTERFERENCE OCCURS

| Halide | Maximum concentration |
|---|---|
| Iodide .. .. .. | Other halides may be present to 1000-fold excess |
| Bromide .. .. .. | $I^-$ must be $\leqslant \frac{1}{500} \times Br^-$ |
| | $Cl^-$ must be $\leqslant 200 \times Br^-$ |
| | $F^-$ has no effect at 1000-fold excess |
| Chloride .. .. .. | $I^-$ must be $\leqslant \frac{1}{500} \times Cl^-$ |
| | $Br^-$ must be $\leqslant \frac{1}{50} \times Cl^-$ |
| | $F^-$ has no effect at 1000-fold excess |
| Fluoride .. .. .. | Not affected by the presence of other halides |

## Iodide Interference in the Determination of Bromide

Fig. 1 shows a graph of the electrode potential of the bromide Selectrode against the $I^-$ to $Br^-$ ratio for solutions that are $10^{-2}$ and $10^{-3}$ M in bromide ions. In aqueous solutions, iodide interference in bromide determinations occurs at an $I^-$ to $Br^-$ ratio of greater than 1:3000 while in the buffered system, interference occurs when the ratio is greater than 1:500. This

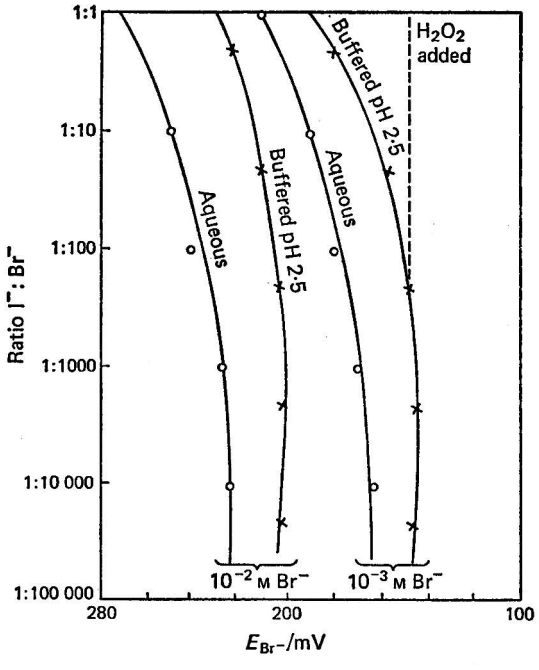


Fig. 1. Iodide interference in bromide determination.

latter ratio becomes 1:10 on addition of 0·5 ml of 20-volume hydrogen peroxide, followed by standing at room temperature for 20 min. The oxidation of iodide to iodine at room temperature proceeds without effect on the bromide-ion concentration; if, however, the temperature is allowed to rise, oxidation of bromide to bromine may become a serious problem, as would the peroxide-induced decomposition of perchloric acid.

### Chloride - Bromide Interferences

These interactions are probably the most difficult of the halide mutual interferences to overcome. However, in the majority of mineralogical and biological samples the chloride level is in considerable excess of that of the bromide. If the bromide concentration is not required, a direct reading of the chloride concentration can be made after removal, by means of oxidation, of the interference due to bromide. Care should be exercised in this procedure in order to prevent loss of chloride.

Chloride will interfere in bromide determinations when the chloride is present in greater than 200-fold excess. This interference can be largely overcome by the addition of one or more known amounts of bromide to the sample solution after the chloride activity has first been determined, in order to raise the $Br^-$ to $Cl^-$ ratio to greater than 1:200. This method is reasonably satisfactory provided that the bromide concentration is not too low, *i.e.*, if the required additions are relatively small.

Alternatively, techniques to remove the interference can be attempted. Several of these techniques have already been described, and they include the oxidation of bromide to bromine in concentrated nitric acid,[11] with the removal of the bromine by flushing with air. The bromine is absorbed in ammonia solution; chloride is stated to be unaffected by the oxidation procedure. If fluoride were present it would also be removed to a certain extent, but any difficulty could be overcome by determination of the residual fluoride and of the fluoride carried over to the ammonia solution. However, this technique could not be applied to determinations in the buffered system we have developed as perchloric acid would be oxidised to chloride, giving high and uncontrolled results for chloride. Substitution of nitric acid for perchloric acid in the buffer system may prove a satisfactory alternative.

Other methods that have been described include gas - liquid chromatography,[12] spectrophotometric determinations with the halides complexed with palladium(II) sulphate,[13] and the removal of bromide with iodide by oxidation at 90 °C with 6 per cent. hydrogen peroxide in the presence of quinolin-8-ol.[14] Using this last procedure, iodide, total halide and chloride can be determined and the concentration of bromide deduced. The method would require the substitution of nitric acid for perchloric acid and is perhaps worth investigating further. We cannot, however, see any advantage, in most circumstances, of that method over the procedure we have described.

### Interferences by Other Ions

In addition to the mutual interferences of halides, which are minimised by the use of the perchloric acid - citric acid - trisodium citrate buffer solution, certain other ions interfere. Any ion, *e.g.*, sulphide or thiosulphate, which complexes with the silver in the detector crystal of halide electrodes other than the fluoride electrode will interfere in the determinations. Interferences of bi- and tervalent cations are largely avoided in the buffer system which we have described, chiefly as a result of the masking of these ions by citrate.

### Interferences in the Determination of Iodide

The electrode potential of the system is virtually independent of pH over the pH range 1–7 provided that sufficient time for stabilisation is allowed. However, the interference of other ions is dependent on pH. Some of these interferences are summarised in Table IV. The most serious interferences are those attributable to oxidising agents, and to copper. With copper, the interference is possibly due to the formation of a precipitate of copper(I) iodide on the electrode surface, which not only reduces the iodide activity in solution but also affects the electrode response.

The optimum electrode response was at pH 2·5–2·7; in this pH range the maximum response time was usually between 5 and 10 min, 8 min being chosen as the optimum for normal working. The choice of reference electrode is critical to the sequential determination described here and for the determination of iodide is ideally the Orion 90–01 liquid junction calomel electrode, or the Radiometer K401 ceramic plug calomel electrode, with which linear response occurs at concentrations of iodide down to approximately $10^{-6}$ M in the buffered solution. If a Cambridge Instruments, Type 42528, ceramic plug reference electrode, filled with saturated potassium nitrate solution was used, the response was linear only down to $2 \times 10^{-4}$ M, although the results were reproducible for concentrations down to $10^{-6}$ M. The

TABLE IV

INTERFERENCE (PER CENT.) OF VARIOUS IONS IN THE DETERMINATION OF IODIDE IN A PERCHLORIC ACID - CITRIC ACID - TRISODIUM CITRATE BUFFER SYSTEM AT VARIOUS pH VALUES

| Ion added (X) | Ratio X:I⁻ | pH of solution 0·8 | 2·6 | 3·7 | 4·4 | 5·0 | 8·0 |
|---|---|---|---|---|---|---|---|
| $Cl^-$ .. .. .. | 2000 | −10 | 0 | 0 | 0 | −10 | −10 |
| $F^-$ .. .. .. | 1000 | −30 | −4 | 0 | 0 | 0 | +10 |
| $Br^-$.. .. .. | 1000 | 0 | 0 | 0 | 0 | −10 | 0 |
| $Cu^{2+}$ .. .. | 1000 | −60 | −60 | −60 | −60 | −60 | −60 |
| $Co^{2+}$ .. .. | 100 | −20 | 0 | 0 | 0 | 0 | 0 |
| $Sr^{2+}$ .. .. | 100 | 0 | 0 | 0 | −10 | 0 | −10 |
| $Cr_2O_7^{2-}$ .. .. | 100 | −100 | −80 | −70 | 0 | 0 | 0 |
| $IO_3^-$ .. .. | 100 | −100 | −90 | −80 | −10 | 0 | 0 |
| $Fe^{2+}$ .. .. | 100 | −20 | 0 | 0 | +15 | 0 | +70 |
| $Fe(CN)_6^{4-}$ .. .. | 100 | −20 | 0 | 0 | +10 | 0 | +50 |
| $Cr^{3+}$ .. .. | 100 | —* | −10 | 0 | 0 | −25 | 0 |
| $Al^{3+}$ .. .. | 100 | —* | 0 | 0 | −10 | −15 | −40 |
| $Mg^{2+}$ .. .. | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Ca^{2+}$ .. .. | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| $MnO_4^-$ .. .. | 100 | −100 | −100 | −100 | | Precipitation | |

* Not determined.

Radiometer K601 electrode showed similar responses. These last two electrodes, unlike the Orion 90–01 and the Radiometer K401, do not leak chloride ions. The Cambridge reference electrode was generally preferred for this work, although we have also used the Orion 90–01 electrode.

## Copper Interference in the Determination of Chloride and Bromide

The removal of iodide from solution by the addition of copper ions was investigated as a possible means of removing iodide interference in chloride and bromide determinations. The masking worked reasonably well for the determination of bromide. For chloride, however,

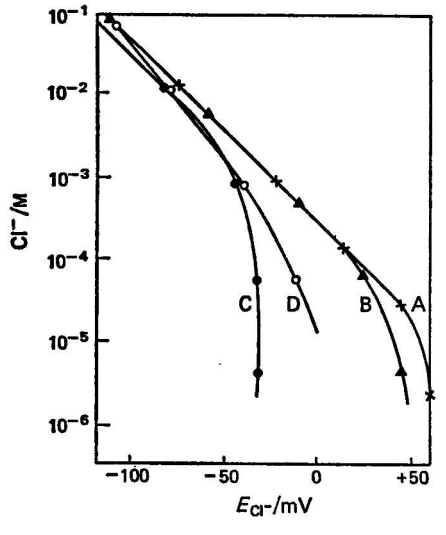


Fig. 2. Calibration graphs in standard potassium chloride solutions, showing effect of copper(II) and iodide ions on chloride-selective electrodes: A, electrode in pH 2·5 buffer solution; B, electrode in aqueous solution; C, 1 h after immersion in solution containing $Cu^{2+}$ and $I^-$ ions; and D, 2 weeks after immersion in $Cu^{2+}$ and $I^-$ solution.

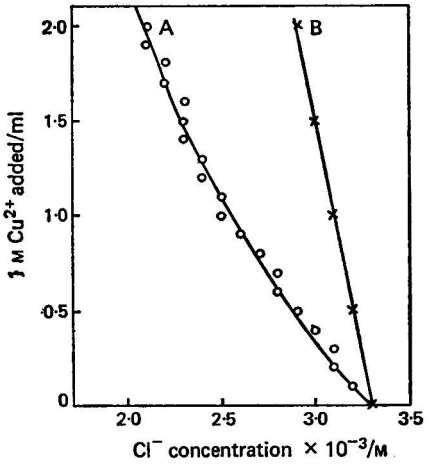


Fig. 3. Interference of $Cu^{2+}$ in pH 2·5 buffer on 94–17A chloride electrode. A, chloride found; B, chloride calculated.

considerable electrode damage was caused. It was necessary to soak the electrode for several weeks in $10^{-1}$ M potassium chloride solution and to polish the electrode crystal frequently before the electrode response returned reasonably closely to its previous level (Figs. 2 and 3), although even after two months, the response time was impaired both in aqueous and in buffer solutions. It is possible that some interaction between the silver chloride - silver sulphide crystal membrane of the Orion chloride electrode and copper ions occurred, yielding a deposit of copper(I) sulphide. This interaction could be the reason why the Radiometer bromide electrode, which has a pure silver bromide crystal membrane, was relatively unaffected by copper ions, although the electrode exhibited a sluggish response. Subsequently we have found that the damage caused by copper ions can be repaired by treatment with silver nitrate, which confirms the possibility described above.[15]

## Bromide

The Orion 90–01 and the Radiometer K401 reference electrodes were unsatisfactory for use in conjunction with the bromide Selectrode, chiefly because of leakage of chloride ions. Both the Radiometer K601 and an electrode filled with saturated potassium nitrate solution proved satisfactory as reference electrodes. Table III indicates the chief interference with the bromide Selectrode. The presence of both copper and iodide ions in the buffer solution should be avoided as a yellow precipitate is formed on the Selectrode surface, which may cause crystal damage.

## Chloride

We have previously reported[2] a method for the determination of chloride in a perchloric acid - citric acid - triethanolamine buffer system and we have extended[5] this method to include the determination of other ions. This buffer system appears to be excellent for the determination of fluoride[3] (giving a faster response time) but may possibly cause crystal damage to the chloride electrode over a long period. It cannot, however, be used with either the iodide or the bromide electrode without causing immediate crystal damage by the formation of a silver - triethanolamine complex. The buffer system described in this paper gives very similar electrode response and precision without damage to the detector membrane.

Interference by bromide (Fig. 4) was shown to occur below a 50-fold excess of chloride over bromide.

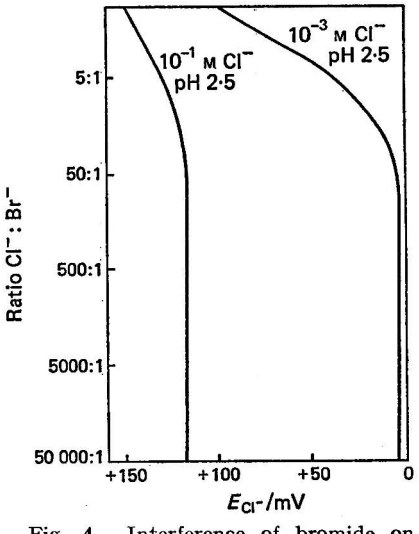


Fig. 4. Interference of bromide on 94–17A chloride electrode.

Aluminium ions caused little interference unless present in a concentration in excess of that of chloride.

## Fluoride

The technique for the determination of fluoride is too well known to require comment. It is sufficient to say that the buffer system reported here is slightly better than the systems we have previously reported,[1,3] and minor interferences by bi- and tervalent cations and some anions were reduced. The only significant interferences were due to aluminium ions and to permanganate.

## Conclusions

The buffer system described here has a wide application in the determination of halide ions within the limits we have discussed, the chief interferences (other than mutual halide interference) being by oxidising agents and copper ions. Tables V–VIII give results of the determination of halides in rock samples.

TABLE V

HALIDE CONTENT (PER CENT.) OF PHOSPHATE ROCK SAMPLES*

Results are given as the mean of ten determinations.

| Sample† | Fluoride | Chloride | Bromide |
|---|---|---|---|
| 1 | — | 0·035 ± 0·005 | 0·0035 ± 2 × $10^{-4}$ |
| 2 | 4·23 | 0·057 ± 0·0058 | 0·015 ± 5 × $10^{-4}$ |
| 3 | 2·80 | 0·052 ± 0·0067 | 0·016 ± 3·6 × $10^{-3}$ |
| 4 | 4·30 | 0·053 ± 0·0057 | 0·015 ± 1·5 × $10^{-3}$ |
| 5 | 4·14 | 0·048 ± 0·0061 | 0·016 ± 2·2 × $10^{-3}$ |
| 6 | 2·39 | 0·042 ± 0·0010 | 0·011 ± 3·5 × $10^{-3}$ |
| 7 | 2·14 | 0·014 ± 0·0045 | 0·004 ± 1·0 × $10^{-4}$ |

* Samples supplied by Albright and Wilson, Marchon Division.
† Amount of iodide present was below the electrode detection limit.

TABLE VI

RECOVERY OF CHLORIDE FROM PHOSPHATE ROCK SAMPLES

| | Chloride added/mg | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 0·0355 | | 0·0710 | | 0·1056 | |
| | Chloride found | | | | | | |
| Sample | Amount/mg | Amount/mg | Recovery, per cent. | Amount/mg | Recovery, per cent. | Amount/mg | Recovery, per cent. |
| A | 0·0852 | 0·124 | 102·7 | 0·160 | 102·4 | 0·195 | 102·2 |
| B | 0·0746 | 0·114 | 103·5 | 0·150 | 102·2 | 0·185 | 102·7 |
| C | 0·0746 | 0·110 | 99·9 | 0·145 | 99·5 | 0·180 | 99·9 |
| D | 0·0603 | 0·099 | 103·3 | 0·137 | 104·3 | 0·175 | 105·6 |
| E | 0·0514 | 0·085 | 97·8 | 0·120 | 98·0 | 0·155 | 98·7 |
| Mean recovery, per cent. .. .. | | | 101·4 | | 101·3 | | 101·8 |

Mean recovery for all samples = 101·5 ± 2·38 per cent.

TABLE VII

RECOVERY OF BROMIDE FROM PHOSPHATE ROCK SAMPLES

| | Bromide added/mg | | |
|---|---|---|---|
| | 0 | 0·008 | |
| | Bromide found | | |
| Sample | Amount/mg | Amount/mg | Recovery, per cent. |
| A | 0·0140 | 0·0208 | 94·5 |
| B | 0·0220 | 0·0300 | 100 |
| C | 0·0182 | 0·0270 | 103 |
| D | 0·0150 | 0·0230 | 100 |
| E | 0·0110 | 0·0184 | 97 |
| Mean recovery, per cent. .. .. .. | | | 98·9 ± 3·24 |

## Table VIII

### Recovery of iodide from phosphate rock samples

| | Iodide added/mg | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 0·126 | | 0·252 | | 0·378 | |
| | Iodide found | | | | | | | |
| | Amount/mg | Recovery, per cent. | Amount/mg | Recovery, per cent. | Amount/mg | Recovery, per cent. | Amount/mg | Recovery, per cent. |
| *Standard*— | | | | | | | | |
| 0·63 mg $I^-$ as KI | 0·63 | 100·0 | 0·75 | 99·2 | 0·87 | 98·6 | 0·98 | 97·2 |
| *Rock samples*— | | | | | | | | |
| A | 0 | — | 0·124 | 98·4 | 0·245 | 97·2 | 0·380 | 100·5 |
| B | 0 | — | 0·132 | 104·8 | 0·273 | 108·3 | — | — |
| C | 0 | — | 0·126 | 100 | 0·252 | 100 | 0·370 | 98·2 |
| D | 0 | — | 0·128 | 101·6 | 0·246 | 97·6 | 0·359 | 95·0 |

Mean recovery for all samples = 99·74 ± 3·74 per cent.

Table V indicates that the rocks have a fluoride content close to that expected for fluoroapatite [fluoride content of $Ca_5F(PO_4)_3$, 3·77 per cent.]. The chloride and bromide contents are typical for apatitic materials. Table VI indicates that the mean recovery of chloride added to rock samples was 101·51 per cent. The mean recovery of added bromide (Table VII) was 98·90 per cent. Table VIII indicates a 99·74 per cent. recovery of iodide from rocks that contain no naturally occurring iodide.

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# Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

## Part VII.* The Determination of *O*-Alkyl Dithiocarbonates and Metal Iodides

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Lead, nickel, potassium, sodium and zinc *O*-alkyl dithiocarbonates have been determined in amounts down to 0·002 mequiv by catalytic thermometric iodimetric titration of their solutions in dimethylformamide, acrylonitrile or chloroform. The end-points of the titrations correspond to the formation of the metal polyiodides, and the method is more sensitive than conventional aqueous iodimetry for the determination of potassium and sodium alkyl dithiocarbonates, in which a monoiodide is the final product. The results show precisions ranging from 0·20 to 1·15 per cent. when a 0·05 M titrant is used.

The mechanisms of the titration reactions have been confirmed by titrating solutions of the metal iodides in dimethylformamide by the iodimetric method, and this procedure can be used for the determination of nickel, potassium, sodium and zinc iodides in non-aqueous solution.

Impurities such as sulphides, sulphites, thiosulphates, sulphates, carbonates and trithiocarbonates can be separated from technical-grade dithiocarbonates before analysis by using the appropriate solvent for the sample and centrifuging the solution.

Sodium and potassium alkyl dithiocarbonates in aqueous solution have been determined as the corresponding nickel salts by a modification of an established solvent-extraction method.

The metal *O*-alkyl dithiocarbonates (xanthates) {$M[SC(:S)OR]_n$; M = metal and R = alkyl (or aryl)} have a wide range of industrial applications. Sodium alkyl dithiocarbonates find use as flotation agents for the recovery of sulphide ores and also as weed killers, fungicides and oil additives. The main application of the alkyl dithiocarbonates of zinc is as rubber accelerators, and some of those of sodium are also used for this purpose. Nickel alkyl dithiocarbonates function as anti-ozonants when incorporated in rubber.

Technical-grade dithiocarbonates contain various impurities and decomposition products, such as sulphides, sulphites, thiosulphates, sulphates, carbonates and trithiocarbonates. Assay procedures must, therefore, be selective for the determination of the dithiocarbonate in the presence of interferents, or must involve a separation step.

Numerous titrimetric, gravimetric and spectrophotometric methods for the determination of dithiocarbonates have been reported, many of which are based on the formation of derivatives with copper or mercury.[1–3] Both acid - base[4,5] and iodimetric[1,6] titrimetry are used for the assay of technical-grade dithiocarbonates, and when the titrations are carried out on acetone extracts of the sodium and potassium salts interference effects are substantially eliminated.[5,6] Iodimetry is probably more selective than acid - base titrimetry; it is recommended not only for the direct determination of dithiocarbonates but also for the indirect determination of metal dithiocarbamates. A standard procedure for the assay of the latter compounds[7] involves their decomposition to yield carbon disulphide, which is absorbed in sodium methoxide solution. The sodium methyl dithiocarbonate thus formed is determined by iodimetric titration.

Interference by impurities in the determination of technical-grade dithiocarbonates can also be eliminated, or substantially reduced, by converting the latter into derivatives as a first step. Thus, Veibel and Wroński[8] used a method in which the dithiocarbonates were decomposed by the addition of dimethylamine, and the sulphide formed was titrated with

* For Part VI of this series, see *Analyst*, 1974, 99, 360.

a solution of *o*-hydroxymercuribenzoate. Bičovský and Bičovská[9] determined water-soluble dithiocarbonates by converting them into the nickel(II) derivatives, extracting them into chloroform and titrating the extracts with mercury(II) acetate reagent.

In an earlier short communication[10] it was noted that potassium n-butyl dithiocarbonate could be determined by thermometric iodimetry in non-aqueous solution. The present, more detailed, study is concerned with the evaluation of this technique for the determination of dithiocarbonates in general and the technical-grade salts in particular. The effect of impurities and decomposition products on the analytical results has been investigated and methods for the elimination of these interferents have been developed.

## Experimental

### Reagents

Laboratory-reagent grade acrylonitrile and dimethylformamide were dried over molecular sieve 4A before use. AnalaR-grade chloroform was washed with distilled water in order to remove ethanol and dried over molecular sieve 4A shortly before use.

Sodium trithiocarbonate was prepared by the method of Lamotte, Porthault and Merlin,[11] and zinc trithiocarbonate was prepared from the sodium salt and zinc sulphate. Anhydrous nickel iodide and sodium sulphide were prepared from the hydrated salts by azeotropic distillation with benzene.

Iodine (AnalaR grade) and ethyl vinyl ether (laboratory-reagent grade) were used without purification.

*Iodine solution, 0·05 M in dimethylformamide.* Standardise by the method described in Part V.[12]

### Dithiocarbonates

Technical-grade sodium and zinc ethyl dithiocarbonates, sodium and zinc isopropyl dithiocarbonates, and bis(ethoxythiocarbonyl) and bis(isopropoxythiocarbonyl) disulphides were gifts from Robinson Brothers Limited. Potassium ethyl and potassium n-butyl dithiocarbonates were laboratory-reagent grade materials. Nickel ethyl dithiocarbonate was prepared by adding nickel nitrate solution to a solution of the potassium ethyl salt and crystallising the precipitate formed from chloroform solution.[13] Zinc n-butyl and lead ethyl dithiocarbonates were prepared in a similar manner from the appropriate potassium alkyl salts and zinc sulphate and lead nitrate, respectively.

Purified specimens of sodium ethyl, sodium isopropyl, potassium ethyl, potassium n-butyl, zinc ethyl, zinc isopropyl, zinc n-butyl and lead ethyl dithiocarbonates were prepared by recrystallising the crude or technical-grade materials from ethanol - diethyl ether, propan-2-ol, acetone, butan-1-ol, benzene, benzene, toluene and toluene, respectively.

### Apparatus

Use the automatic apparatus described in Part III[14] with an 8-ml titration flask.

### Procedure

*Thermometric titration*

Prepare a solution containing 1·0 mequiv of the sample in 10 ml of dimethylformamide, chloroform or acrylonitrile as required (the concentration will depend on the stoicheiometry of the reaction with iodine), and centrifuge the solution for 30 min in order to remove insoluble impurities. Transfer 1 ml of the supernatant solution into the reaction flask and add 3 ml of ethyl vinyl ether. To the stirred solution add titrant at the rate of 0·2 ml $min^{-1}$ from the motor-driven syringe. The titrant volume at the end-point is taken to be the volume corresponding to the point of inflection in the titration graph. When this inflection is indistinct, the end-point is taken to be the point where the tangent to the main heat rise leaves the graph at its lower-temperature end.

Carry out a blank titration by using an equal volume of the same batch of solvent with the same water content as that used for the sample solution.

The chart recorder of the automatic titration apparatus is conveniently operated at a chart speed of 600 mm $h^{-1}$ and in the range 0–100 mV.

*Solvent extraction of aqueous solutions of dithiocarbonates*

To 10 ml of a 0·1 N aqueous solution of the dithiocarbonate add 20 ml of a 1 per cent. solution of nickel nitrate [$Ni(NO_3)_2.6H_2O$] in a 5 + 95 *V/V* mixture of acetic acid and water, and shake the mixture for 1 min. Extract the nickel alkyl dithiocarbonate suspension with two accurately measured 5-ml portions of chloroform and combine the extracts. Ensure that no extract remains in the separating funnel, by allowing some of the aqueous layer to run into the separated chloroform layer. Transfer about 6 ml of the chloroform solution, free from water droplets, into a dry container. Determine the nickel alkyl dithiocarbonate content of the chloroform extract by the above thermometric procedure.

*Aqueous iodimetric titration*[6]

Weigh accurately into a dry 100-ml B19 flask about 0·4 g of the dithiocarbonate, add 30 ml of acetone, stopper the flask, and shake it for 15 min. Filter the solution through a porosity 4 sintered-glass funnel, washing the insoluble residue with small amounts of acetone, dilute the filtrate to 400 ml with water, add starch indicator and titrate the solution with 0·1 N aqueous iodine solution to the first permanent blue colour.

## Results and Discussion

In Figs. 1 and 2 are shown the titration curves for solutions of recrystallised and technical-grade dithiocarbonates, respectively, in dimethylformamide. Typical titration curves for solutions of dithiocarbonates in acrylonitrile and chloroform are shown in Fig. 3.

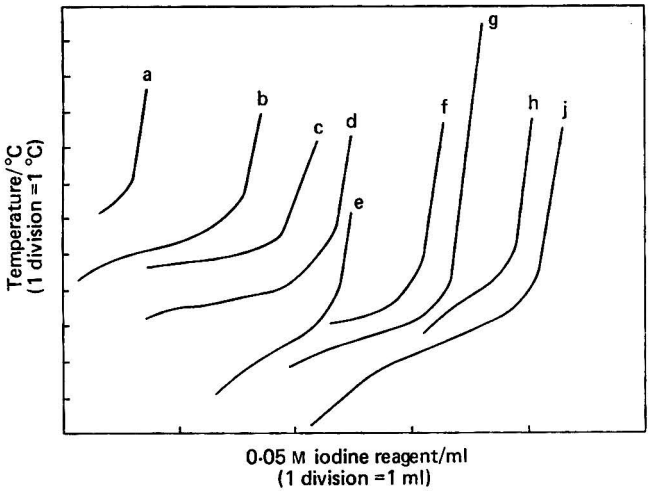


Fig. 1. Catalytic thermometric titration of recrystallised metal dithiocarbonates in solution in dimethylformamide. Solvent: a, dimethylformamide. Dithiocarbonate/mg (with reaction stoicheiometry in parentheses): b, potassium n-butyl 6·1 (3·0); c, potassium ethyl 5·7 (3·0); d, zinc n-butyl 11·59 (3·9); e, zinc ethyl 7·0 (3·9); f, zinc isopropyl 3·1 (4·1); g, sodium isopropyl 5·6 (3·0); h, lead ethyl 7·6 (2·9); and j, nickel ethyl 7·3 (7·0).

The titration curves for the above solvents differ in shape: that for dimethylformamide has a distinct inflection, that for acrylonitrile indicates a sharp temperature rise but the inflection is difficult to locate, while that for chloroform, although steep, has two rounded inflections and it is necessary to draw a tangent to the main temperature rise to locate a reproducible "blank" titration value. The titration values of dithiocarbonates in solution in each of the three solvents yield linear calibration graphs over the range 0–2 ml of 0·05 M titrant, and the precise location of the blank titration value is not essential when the method is used for assay purposes.

In the titrations of the sodium and potassium alkyl dithiocarbonates the temperature rise at the end-point occurs when three atoms of iodine are consumed by one molecule of the

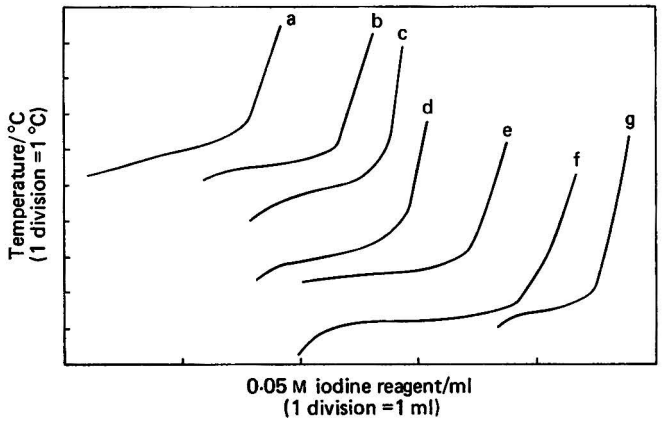


Fig. 2. Catalytic thermometric titration of technical-grade metal dithiocarbonates, sodium sulphide and sodium trithiocarbonate in solution in dimethylformamide. Dithiocarbonate/mg (with reaction stoicheiometry in parentheses): a, zinc ethyl 13·0 (2·7); b, zinc isopropyl 9·6 (3·2); c, sodium isopropyl 5·2 (3·2); d, sodium ethyl 4·8 (2·7); and e, potassium ethyl 4·2 (3·1). Sodium trithiocarbonate/mg: f, 4·5 (5·8); and sodium sulphide/mg: g, ~0·35 (~10).

dithiocarbonate. This reaction stoicheiometry can be accounted for by the formation of a triiodide:

$$3I_2 + 2MSC(:S)OR = 2MI_3 + [ROC(:S)S]_2$$

(M = Na or K). The titration in non-aqueous solution is, therefore, three times as sensitive

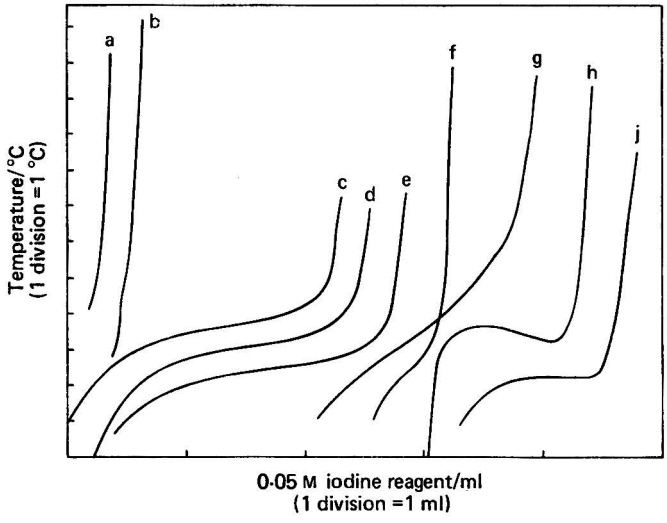


Fig. 3. Catalytic thermometric titration of metal dithiocarbonates in solution in acrylonitrile and chloroform. Solvent: a, acrylonitrile; and b, chloroform. Dithiocarbonate/mg (with reaction stoicheiometry in parentheses): c, sodium isopropyl* 8·3 (2·6); d, sodium ethyl* 8·1 (2·5); e, sodium isopropyl† 6·8 (3·0); f, lead ethyl† 6·4 (3·1); g, potassium ethyl† 8·0 (3·0); h, nickel ethyl‡ 6·4 (2·4); and j, nickel ethyl† 4·7 (7·0). c–g, acrylonitrile solvent; h and j, chloroform solvent. *Technical grade; †recrystallised; ‡chloroform extract of the nickel derivative of 6·4 mg of technical-grade sodium ethyl dithiocarbonate. The reaction stoicheiometry is calculated for the sodium ethyl compound assuming a 7·0 reaction stoicheiometry for the nickel derivative.

as conventional iodimetric titration in aqueous solution, in which the starch indicator acquires its blue colour when monoiodide is formed.

Polyiodides are also formed in the titrations of lead, nickel and zinc alkyl dithiocarbonates, but the reaction stoicheiometries are more difficult to explain. With the zinc compounds, the end-point corresponds approximately to the formation of a tetraiodide, which could have the structure $ZnI^+I_3^-$, or it could be a mixture of mono- and triiodides. With lead and nickel ethyl dithiocarbonates, the iodimetric reactions lead, apparently, to the formation of $PbI_3$ and $NiI_7$, respectively. A possibility is that higher iodides are formed in addition to $PbI_2$ and $NiI_6$, and the final stoicheiometry is dependent on the reaction conditions.

The stoicheiometries of the iodimetric reactions in the various solvents are given in the legends to the figures. Those in the legend to Fig. 1 are used in calculating the dithiocarbonate contents of the corresponding technical-grade compounds.

It should be noted that the lead, nickel and zinc alkyl derivatives are insoluble in water and, consequently, are difficult to determine directly by aqueous iodimetry.

The mechanisms of the iodimetric reactions in non-aqueous solution were investigated by titrating solutions of sodium, potassium, zinc, lead and nickel iodides in dimethylformamide, using the thermometric procedure. The titration curves are shown in Fig. 4. The end-points in the titrations of dilute solutions of sodium, potassium and nickel iodides correspond to the formation of $NaI_3$, $KI_3$, $Ni(I_3)_2$ and some higher halides, but with the zinc and lead iodides the reaction stoicheiometries are less well defined and correspond, approximately, to the formation of $ZnI_{3.4}$ and $PbI_{2.3}$. The formation of $NaI_3$ and $KI_3$ confirms the reaction with dithiocarbonates shown in the above equation. With the nickel ethyl salt the formation of triiodide ions would appear to be confirmed but, in addition, some higher iodide ions must be formed as noted above.

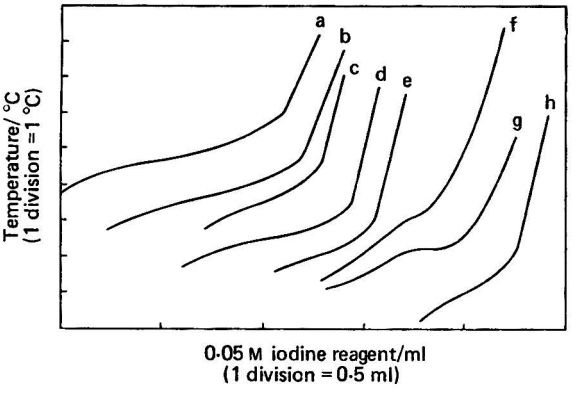


Fig. 4. Catalytic thermometric titration of metal iodides and bromides in solution in dimethylformamide. Iodide/mg (with reaction stoicheiometry in parentheses): a, sodium 5·2 (1·8); b, potassium 4·7 (1·9); c, zinc 3·1 (1·4); d, nickel 4·4 (4·1); and e, lead 6·0 (0·28). Bromide/mg: f, sodium 2·1 (~1·8); g, potassium 2·1 (~2·0); and h, zinc 3·4 (0·14). Solvent system: 2 ml of dimethylformamide + 2 ml of ethyl vinyl ether.

It is probable that the stoicheiometry of the reaction of iodine with dithiocarbonates in non-aqueous solution is related to the structure of the metal iodides, as they normally exist in the solid state. Thus, if these iodides are fully dissociated in the solvent, triiodide ions can be formed in the course of the iodimetric titration, but if there is little or no dissociation of the metal iodide, then the thermometric indicator reaction will be initiated soon after the addition of iodine in amount corresponding to that required for the formation of this particular iodide. The reaction stoicheiometries of the titrations of dithiocarbonates suggest that, in dimethylformamide solution at least, sodium, potassium and nickel iodides are substantially dissociated, but zinc iodide is only partially, and lead iodide only slightly, dissociated.

In these titrations, formation of the simple iodides does not necessarily occur before the

further reaction with iodine to yield triiodide ions; it is possible that the production of these ions and undissociated metal iodide are competing reactions during the oxidation of the dithiocarbonate.

The catalytic thermometric method can be used for the determination of sodium, potassium, nickel and zinc iodides in non-aqueous solution. The calibration graphs for these compounds (Fig. 5) are not linear because the stoicheiometry of the iodimetric reaction is dependent on the extent of dissociation of the iodide in solution, and this, in turn, depends on the concentration of the sample.

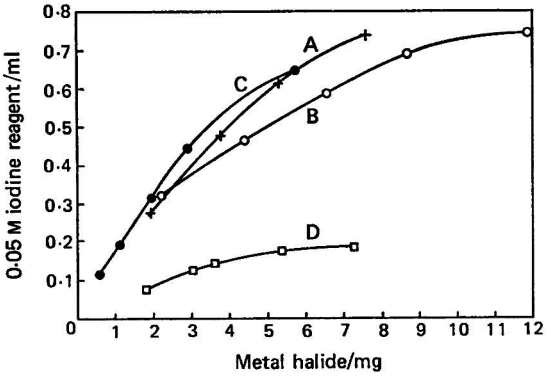


Fig. 5. Calibration graphs for the determination of sodium, potassium, nickel and zinc iodides. Graph: A, sodium iodide; B, potassium iodide; C, nickel iodide; and D, zinc iodide. Solvent system: 2 ml of dimethylformamide + 2 ml of ethyl vinyl ether.

Of the impurities in technical-grade dithiocarbonates (as quoted above), the zinc salts, including the trithiocarbonate, are insoluble in dimethylformamide, while the dithiocarbonate is soluble, hence this solvent is suitable for the assay of technical-grade zinc alkyl dithiocarbonates. The sodium alkyl compounds cannot be determined in dimethylformamide solution because two impurities, sodium sulphide and sodium trithiocarbonate, are soluble and both compounds are titrated iodimetrically. However, all the sodium salts, other than the dithiocarbonates, are insoluble in acrylonitrile and satisfactory results for the dithiocarbonate content of technical-grade sodium ethyl and sodium isopropyl salts are obtained by using this solvent. It is, of course, necessary to remove the insoluble impurities before carrying out the titration, and this can be achieved by centrifuging the sample solutions.

The organic products of the reaction of iodine with metal ethyl and isopropyl dithiocarbonates in aqueous solution, namely bis(ethoxythiocarbonyl) and bis(isopropoxythiocarbonyl) disulphides, respectively, were found to undergo no further reaction when their solutions in dimethylformamide were titrated with iodine titrant. The possibility that the high stoicheiometries found in the non-aqueous determinations could be attributed in part to the formation of iodine derivatives of these disulphides is therefore eliminated.

Dithiocarbonates in aqueous solution have been determined by using an indirect procedure based on the solvent-extraction method of Bičovský and Bičovská.[9] Nickel nitrate is added to the sample solution and the precipitated nickel salt is extracted with chloroform. The chloroform solution is then titrated by the catalytic thermometric method instead of using mercury(II) acetate titrant and a visual indicator, as proposed in the original paper. Bičovský and Bičovská have shown that the impurities normally present in technical-grade sodium alkyl dithiocarbonates are insoluble in chloroform and do not form chloroform-soluble nickel derivatives.

The results obtained in the determination of dithiocarbonates by the catalytic thermometric method are similar in precision to those obtained earlier[12] in the determination of organic bases and hydrazine derivatives by the same iodimetric method. Typical values for the coefficients of variation are shown in Table I, and range from 0·20 to 1·15 per cent. Precision results obtained in the determination of the metal iodides are also included in this table; the coefficients of variation range from 0·76 to 1·43 per cent.

TABLE I

RESULTS FOR PRECISION FROM THE THERMOMETRIC TITRATION OF METAL ALKYL DITHIOCARBONATES AND IODIDES WITH 0·05 M IODINE SOLUTION

| Compound | Amount taken/mg | Solvent* | Mean titre/ml | n† | Standard deviation | Coefficient of variation, per cent. |
|---|---|---|---|---|---|---|
| *Dithiocarbonate—* | | | | | | |
| Sodium ethyl‡ .. | 8·24 | A | 1·43 | 3 | 0·013 | 0·88 |
| Sodium isopropyl§ | 6·82 | A | 2·37 | 3 | 0·015 | 0·65 |
| Sodium isopropyl‡ | 10·2 | A | 2·01 | 3 | 0·012 | 0·58 |
| Potassium ethyl§ .. | 7·55 | A | 1·52 | 4 | 0·013 | 0·85 |
| Zinc ethyl‡ .. .. | 9·04 | D | 1·63 | 3 | 0·003 | 0·20 |
| Zinc isopropyl§ .. | 3·14 | D | 0·84 | 3 | 0·009 | 1·01 |
| Zinc isopropyl‡ .. | 4·28 | D | 0·72 | 4 | 0·008 | 1·11 |
| Nickel ethyl§ .. | 7·27 | D | 1·96 | 3 | 0·010 | 0·52 |
| Nickel ethyl¶ .. | (7·38) | C | 1·25 | 3 | 0·010 | 0·80 |
| Lead ethyl§ .. | 7·61 | D | 0·79 | 3 | 0·009 | 1·15 |
| *Iodide—* | | | | | | |
| Potassium .. .. | 7·28 | D | 1·97 | 3 | 0·023 | 1·19 |
| Sodium .. .. | 5·20 | D | 0·77 | 3 | 0·006 | 0·76 |
| Zinc .. .. | 11·64 | D | 0·69 | 3 | 0·007 | 1·02 |
| Nickel .. .. | 7·46 | D | 1·43 | 3 | 0·015 | 1·05 |
| Lead .. .. | 20·9 | D | 0·49 | 3 | 0·007 | 1·43 |

* A = acrylonitrile; D = dimethylformamide; C = chloroform.
† Number of determinations.
‡ Technical grade; § recrystallised; ¶ prepared as a derivative from an aqueous solution of potassium ethyl dithiocarbonate (7·38 mg).
(Mean titres are not corrected for the blank titre, and the titrant molarity is a nominal value.)

In Table II are compared the values, determined by using different analytical methods, for the dithiocarbonate contents of recrystallised samples of the potassium ethyl and sodium isopropyl compounds, and of technical-grade samples of the sodium ethyl and sodium isopropyl compounds. It can be seen that catalytic thermometric titration of acrylonitrile solutions and aqueous iodimetric titration of acetone extracts lead to results that differ by about 1 per cent. This difference is of the same order as the experimental error observed with the former analytical method. The dithiocarbonate contents determined by using the solvent-extraction method are 2–3 per cent. lower than those obtained by the direct methods; this difference may be a measure of the efficiency of the solvent-extraction step.

TABLE II

DETERMINATION OF SODIUM AND POTASSIUM ALKYL DITHIOCARBONATES BY DIFFERENT ANALYTICAL METHODS

| Dithiocarbonate | Dithiocarbonate content, per cent., by different methods* A | B | C |
|---|---|---|---|
| Potassium ethyl† .. .. | 99·1 | 98·8 | 96·2 |
| Sodium isopropyl† .. .. | 97·3 | 97·9 | 96·0 |
| Sodium ethyl‡ .. .. .. | 82·5 | 83·4 | 80·3 |
| Sodium isopropyl‡ .. .. | 87·0 | 85·9 | 84·1 |

* Method A: catalytic thermometric titration of the acrylonitrile solution; method B: aqueous iodimetry of the acetone extract; method C: determination as the nickel derivative (solvent-extraction procedure).
† Recrystallised; ‡ technical grade.

Sodium trithiocarbonate, one of the dimethylformamide-soluble compounds known to occur as an impurity in technical-grade sodium alkyl dithiocarbonates, has been determined by the catalytic thermometric method; its titration curve is shown in Fig. 2. The end-point corresponds to the combination of six atoms of iodine with one molecule of the trithiocarbonate, which suggests the following iodimetric reaction:

$$Na_2CS_3 + 3I_2 = 2NaI_3 + \frac{1}{n}(CS_3)_n$$

Although sodium sulphide is only slightly soluble in dimethylformamide, its titration curve (Fig. 2) indicates a high reaction stoicheiometry, possibly exceeding 10 atoms of iodine to 1 molecule of sulphide, and it is important that this particular impurity be separated from the dithiocarbonates before they are titrated.

In addition to the metal iodides, sodium, potassium and zinc bromides have been titrated; the titration curves are included in Fig. 4. The end-points correspond, presumably, to the formation of iodobromide ions but they are not sharply defined with sodium and potassium bromides and the titration values cannot be determined precisely. A very low titre is obtained with the zinc bromide, suggesting that this compound is only slightly dissociated in solution in dimethylformamide.

Catalytic thermometric iodimetry is a particularly useful method for the direct determination of dithiocarbonates that are insoluble in water but are soluble in solvents such as dimethylformamide, acrylonitrile and chloroform. Alternative titrimetric methods for determining these compounds require their prior decomposition followed by the titration of the decomposition product, sometimes as a derivative. The thermometric method is as convenient as aqueous iodimetry for the determination of sodium and potassium alkyl dithiocarbonates, and it offers the additional advantage of higher sensitivity.

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NOTE—References 12 and 14 are to Parts V and III of this series, respectively.

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# The Indirect Colorimetric Determination of Sulphate with 2-Aminoperimidine

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An indirect colorimetric procedure is described for the determination of sulphate. After precipitating the sulphate as 2-aminoperimidinium sulphate, the remaining 2-aminoperimidine reagent is determined colorimetrically following treatment with nitrous acid and alkali in order to give a red colour with maximum absorbance at 525 nm and a molar absorptivity of 13 600 l $mol^{-1}$ $cm^{-1}$. The mean absorbance difference for a sulphate-ion concentration of 10 mg $l^{-1}$ is 0·283 with a relative standard deviation of 4 per cent.

The sulphate of 2-aminoperimidine is relatively insoluble in water[1,2] and the base was introduced as a reagent for the determination of trace amounts of sulphate by nephelometry[2] and spectrophotometry.[3] A modified spectrophotometric[4] procedure has been proposed for the determination of sulphate in rain water. In the spectrophotometric method, the decrease in absorbance resulting from the precipitation of the amine sulphate was measured by comparison with a blank reagent solution and was found to be proportional to the original sulphate concentration. In an attempt to improve the sensitivity of this method, by coupling the base with diazotised aromatic amines, it was found that treatment with nitrous acid and alkali converted 2-aminoperimidine into a stable, water-soluble red product. As the resulting molar absorptivity was almost double that of the original amine, the colorimetric procedure was examined in detail and the method developed was applied to the determination of sulphate in water samples.

## Method

Absorbance measurements were made with a Varian Techtron 635 spectrophotometer.

### Reagents

*2-Aminoperimidinium bromide.* This reagent was prepared from 1,8-diaminonaphthalene and cyanogen bromide as described by McClure[5] and its aqueous solution gave a molar absorptivity (at 305 nm) of 7250 l $mol^{-1}$ $cm^{-1}$; the value reported[3] for the chloride is 7230 l $mol^{-1}$ $cm^{-1}$.

*2-Aminoperimidinium bromide solution, 0·3 per cent.* m/V. Dissolve, by warming, 0·3 g of 2-aminoperimidinium bromide in about 50 ml of water, cool the solution and dilute it to 100 ml with water.

*Hydrochloric acid, approximately* 0·1 N. Dilute 10 ml of concentrated hydrochloric acid to 1 l with water.

*Sodium nitrite solution, 1 per cent.* m/V.

*Sodium hydroxide solution, 4 per cent.* m/V.

*Standard sulphate solutions.* Dissolve 0·9071 g of dried potassium sulphate in water and dilute the solution to 500 ml with water. Dilute 10 ml of this solution to 100 ml with water, then dilute 5, 10, 15 and 20 ml of the latter solution to 100 ml with water so as to give solutions containing 5, 10, 15 and 20 $\mu g$ $ml^{-1}$ of sulphate ion, respectively.

### Procedure

Pipette 2 ml of distilled water, to act as a blank, 2 ml of each of the standard sulphate solutions and 2 ml of sample solution (containing not more than 20 $\mu g$ $ml^{-1}$ of sulphate ion) into a series of centrifuge tubes. Add to each tube 0·2 ml of 2-aminoperimidinium bromide solution, mix the solutions and allow them to stand for 30 min at room temperature.

Centrifuge the tubes at 2000 rev $min^{-1}$ for 2 min and pipette 1 ml of the clear supernatant liquid from each tube into a series of test-tubes. Add to each tube 1 ml of 0·1 N hydrochloric

acid and 1 ml of sodium nitrite solution, mix, add 1 ml of sodium hydroxide solution and dilute to 10 ml with water.

Measure the absorbance difference at 525 nm of each standard and sample against the blank in 10-mm cells (Note). Plot absorbance differences for the standard solutions against sulphate concentration and hence determine the sulphate concentration in the sample.

NOTE—
For double-beam spectrophotometers, place the blank solution in the sample position and the standard and sample solutions in the blank position. For single-beam spectrophotometers, zero the instrument with the 20 $\mu$g ml$^{-1}$ standard sulphate solution and record the apparent absorbance of the remaining solutions including the blank; this calibration graph will have a negative slope.

## Discussion

The procedure for the precipitation of the 2-aminoperimidinium sulphate was that used by Jones and Stephen[3] and was not examined further. When treated with nitrous acid 2-aminoperimidine gave an immediate yellow colour ($\lambda_{max.}$ 430 nm), which when treated with alkali gave an immediate red colour ($\lambda_{max.}$ 525 nm). The absorbance at 525 nm was double that at 430 nm and gave a molar absorptivity of 13 600 l mol$^{-1}$ cm$^{-1}$. Preliminary experiments led to the concentrations of reagents described under Method. An increase in acid concentration to 0·5 N produced a slight decrease in absorbance but the addition of an excess of sodium hydroxide solution, up to 5 ml of 1 M solution, or an excess of sodium nitrite solution, up to 3 ml of 1 per cent. solution, or varying the time interval between the addition of the sodium nitrite solution and the sodium hydroxide solution from 30 s to 30 min, produced no significant change in absorbance. The final red colour showed no change in absorbance when allowed to stand in indirect sunlight for 1·5 h. The absorbance at 525 nm was proportional to the amount of 2-aminoperimidinium bromide present up to a concentration of 300 $\mu$g ml$^{-1}$. The precision of the colorimetric procedure is shown in Table I.

TABLE I

PRECISION OF THE COLORIMETRIC PROCEDURE

| 2-Aminoperimidinium bromide/$\mu$g | Number of determinations | Mean absorbance at 525 nm | Relative standard deviation, per cent. |
|---|---|---|---|
| 100 | 10 | 0·491 | 1·2 |
| 200 | 10 | 1·022 | 1·75 |
| 300 | 10 | 1·501 | 1·4 |

TABLE II

SULPHATE CONTENT OF WATER SAMPLES DETERMINED BY NEPHELOMETRIC AND COLORIMETRIC METHODS

Treated samples Nos. 4, 7 and 8 were chlorinated samples containing about 0·1 p.p.m. of free chlorine. In addition samples 4 and 7 contained an added 1 p.p.m. of fluoride ion.

| Sample No. | Origin and type of water sample | Sulphate, mg l$^{-1}$,* by— nephelometry | colorimetry |
|---|---|---|---|
| 1 | Broken Hill, reservoir | 140 | 135 |
| 2 | Broken Hill, treated | 153 | 155 |
| 3 | Windemere, well | 282 | 295 |
| 4 | Sydney, tap water | 9·0 | 9·0 |
| 5 | Murwillumbah, river | 11·0 | 10·5 |
| 6 | Temora, bore | 7·7 | 9·2 |
| 7 | Cowra, treated | 44 | 45 |
| 8 | Bowral, treated | 9·2 | 10·5 |
| 9 | Mungindi, bore | 30 | 27 |
| 10 | Tuncurry, bore | 9·2 | 9·0† |
| 11 | Sample 10 + 10 mg l$^{-1}$ of $SO_4^{2-}$ | 19·4 | 19·4‡ |

* Mean of two determinations.
† Mean of ten determinations; relative standard deviation 3·9 per cent.
‡ Mean of ten determinations; relative standard deviation 2·1 per cent.

Factorial experiments were carried out, on 100, 200 and 300 $\mu$g of 2-aminoperimidinium bromide, in which three factors were examined at two levels, *i.e.*, hydrochloric acid, 0·5 and 1·5 ml, sodium nitrite solution, 0·75 and 1·25 ml, and sodium hydroxide solution, 0·5 and 1·5 ml. No significant effect was found at any of the three concentrations of 2-aminoperimidinium bromide examined. The mean absorbance difference for a sulphate-ion concentration of 10 mg $l^{-1}$ was 0·283 (ten determinations) with a relative standard deviation of 4·0 per cent, which compares favourably with the value of 0·167 (relative standard deviation 5·0 per cent.) reported[3] for the spectrophotometric procedure. The colorimetric method was used to determine the sulphate content of natural and treated water samples, which were also analysed by a standard nephelometric method.[6] The results are recorded in Table II and show no significant difference between the results obtained by each method.

## Conclusion

A simple colorimetric modification to the previously reported[3] indirect spectrophotometric procedure for the determination of low levels of sulphate is described. The colorimetric procedure gives almost twice the sensitivity with similar precision and, when applied to natural and treated water samples, the results obtained were not significantly different from those found by using a standard nephelometric method.[6]

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# An Enzymic Method for the Determination of [1-$^{14}$C]Lactose

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A simple, rapid and specific method for the determination of [1-$^{14}$C]lactose in biological fluids is described. It is based on the enzymic removal of the 1-$^{14}$C atom of lactose as [$^{14}$C]carbon dioxide, using commercially available enzymes. The assay involves only one critical addition and the entire reaction can be carried out in a scintillation vial.

Lactose occurs uniquely in milk, and there is considerable interest in both its biosynthesis and subsequent degradation after ingestion. Studies of the biosynthesis of lactose are commonly based on the method of Babad and Hassid[1] in which the [1-$^{14}$C]lactose is separated from the reaction mixture by use of ion-exchange chromatography. Studies of its subsequent metabolism after ingestion, on the other hand, frequently involve only the determination of the [$^{14}$C]carbon dioxide produced in the expired air,[2] despite the fact that in lactose intolerance the plasma lactose concentration may rise to approximately 0·02 per cent. *m/V*.[3]

In this paper a simple reproducible method is described for the assay of [1-$^{14}$C]lactose. β-Galactosidase is used to hydrolyse [1-$^{14}$C]lactose to galactose and [1-$^{14}$C]glucose; the latter is then decarboxylated to ribulose-5-phosphate and [$^{14}$C]carbon dioxide by a modification of the method of Costello *et al.*[4] The nature of the assay makes it ideal for application in biological systems in which [1-$^{14}$C]glucose is likely to occur together with [1-$^{14}$C]lactose; and in such a situation the two compounds can be determined concurrently.

## Materials

*Enzymes and coenzymes.* β-Galactosidase (β-D-galactoside galactohydrolase E.C. 3.2.1.23) from *Escherichia coli*, 150 u ml$^{-1}$; hexokinase (ATP: D-hexose-6-phosphotransferase E.C. 2.7.1.1) and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase E.C. 1.1.1.49) from yeast, both in the same solution at 140 u ml$^{-1}$ (HK/G-6-PDH); 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating) E.C. 1.1.1.44] from yeast, 120 u ml$^{-1}$ (6-PGDH); and glutamate dehydrogenase (L-glutamate: NADP(P) oxidoreductase (deaminating) E.C. 1.4.1.3) from beef liver, 450 u ml$^{-1}$ in 50 per cent. glycerol (GLDH).

All of the above were obtained from Boehringer, West Germany.

*ATP, disodium salt, crystalline.*

*NADP, disodium salt.*

*NAD, lyophilised free acid.*

*α-Ketoglutaric acid* (free acid).

D-[1-$^{14}$C]glucose, 58·8 mCi mmol$^{-1}$, and [D-*glucose*-1-$^{14}$C]lactose (referred to as [1-$^{14}$C]-lactose), 20 mCi mmol$^{-1}$, were obtained from the Radiochemical Centre, Amersham; 2,5-diphenyloxazole (PPO) from Intertechnique; and triethanolamine from Sigma Chemical Company.

All other chemicals were of AnalaR grade.

## Method

The relative contributions of [1-$^{14}$C]lactose and [1-$^{14}$C]glucose in a solution of a mixture of the two can be determined simply and with good reproducibility by assaying aliquots of up to 1 ml of the mixture in three separate counting vials: (*i*), to which no enzymes are added; (*ii*), in which [1-$^{14}$C]glucose is converted into ribulose-5-phosphate and [$^{14}$C]carbon dioxide; and (*iii*), in which [1-$^{14}$C]lactose is hydrolysed to [1-$^{14}$C]glucose and the total [1-$^{14}$C]glucose is converted into ribulose-5-phosphate and [$^{14}$C]carbon dioxide. The amounts of [1-$^{14}$C]lactose and [1-$^{14}$C]glucose can then be obtained by difference after the [$^{14}$C]carbon dioxide has diffused off. Details of the reaction mixtures contained in the vials are summarised in Tables I and II. Glutamate dehydrogenase and α-ketoglutarate are added to vials (*ii*) and

TABLE I

COMPOSITION OF REACTION MIXTURES IN THE VIALS

| Contents | Vial (*i*) | Vial (*ii*) | Vial (*iii*) |
|---|---|---|---|
| Sample volume up to .. .. | 1·0 ml | 1·0 ml | 1·0 ml |
| Analytical cocktail .. .. .. | — | 0·8 ml | 0·8 ml |
| Blank cocktail .. .. .. | 0·8 ml | — | — |
| HK/G-6-PDH .. .. .. | — | 10 µl | 10 µl |
| GLDH .. .. .. .. | — | 20 µl | 20 µl |
| β-Galactosidase .. .. .. | — | — | 10 µl |
| 6-PGDH* .. .. .. .. | — | 5 µl | 5 µl |

* Added after incubating the mixture for 1 h at 37 °C.

(*iii*) in order to recycle NADP, thereby increasing the reaction rate with a substantial reduction in cost.

These reactions are carried out at 37 °C for 1 h before the addition of the 6-PGDH, which catalyses the final decarboxylation reaction (Table I). This interval allows the non-enzymatic hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate, the limiting step in the overall reaction,[4,5] to reach completion. After a further 1 h at 37 °C the reactions are stopped by the addition of 0·2 ml of 2 N hydrochloric acid. The samples are placed in a fume cupboard and the diffusion of carbon dioxide is complete within 30 min. To each vial 18 ml of toluene - Triton X-100 scintillation fluid[6] are added and the remaining radioactivity is then determined with a Packard liquid scintillation counter, Model No. 3375. The d.p.m. values are calculated by using the channels ratio method. The d.p.m. value in vial (*i*) minus that in vial (*ii*) is then equivalent to the d.p.m. value for [1-$^{14}$C]glucose. Similarly, the d.p.m. value in vial (*ii*) minus that in vial (*iii*) is equivalent to the d.p.m. value for [1-$^{14}$C]lactose.

TABLE II

COMPOSITION OF THE COCKTAILS

| Contents | Concentration/M | Amount, ml, in— analytical cocktail | Amount, ml, in— blank cocktail |
|---|---|---|---|
| Triethanolamine buffer, pH 7·4 .. | 0·9 | 0·4 | 0·4 |
| ATP .. .. .. .. .. | 0·16 | 0·1 | — |
| NADP .. .. .. .. | 0·012 | 0·1 | — |
| α-Ketoglutaric acid, pH 7·0 .. | 0·2 | 0·1 | — |
| KCl .. .. .. .. .. | 0·6 | 0·05 | — |
| $MgCl_2$ .. .. .. .. | 0·2 | 0·05 | — |
| Water .. .. .. .. | | — | 0·4 |
| Final volume .. .. | | 0·8 | 0·8 |

## Results

Volumes from 0·1 to 0·5 ml of a 0·04 per cent. *m*/*V* standard solution of lactose (containing [1-$^{14}$C]lactose) added to 0·5 ml of untreated dog plasma (containing 0·110 per cent. *m*/*V* of glucose) were assayed both in the presence (Table III) and absence (Table IV) of [1-$^{14}$C]-glucose. The recovery of added [1-$^{14}$C]lactose was consistently between 89 and 91·5 per cent. The results show that the determination of [1-$^{14}$C]lactose is unaffected by the presence of [1-$^{14}$C]glucose in the reaction mixture. The determination of the latter is also unaffected by the presence of [1-$^{14}$C]lactose and the recovery is consistently between 97 and 98·5 per cent. Similar results were obtained with human plasma and with urine and milk. The assay is applicable under conditions such as those of lactose intolerance and lactation when lactose concentrations of up to 0·050 per cent. *m*/*V* occur.[7] Recovery of [1-$^{14}$C]lactose is increased to 95 per cent. by allowing the 1-h reaction at 37 °C before the addition of 6-PGDH to proceed for 12 h. Under these conditions a slight contamination with β-galactosidase activity was observed, which would reduce the accuracy of the method in the presence of [1-$^{14}$C]glucose. No such activity was detectable up to 3 h and the shorter reaction time was therefore preferred.

## Discussion

The method of assay for [1-$^{14}$C]lactose described above can be applied equally well to milk, plasma and urine. Recovery of added [1-$^{14}$C]lactose is consistently between 89 and

## TABLE III

DETERMINATION OF INCREASING AMOUNTS OF [1-$^{14}$C]LACTOSE, IN THE PRESENCE OF A CONSTANT AMOUNT OF [1-$^{14}$C]GLUCOSE (17340 d.p.m.), BY ENZYMIC RELEASE OF [$^{14}$C]CARBON DIOXIDE

Vial (*i*): total d.p.m. before reaction; vial (*ii*): d.p.m. remaining after removal of [$^{14}$C]carbon dioxide from the 1-position of [1-$^{14}$C]glucose; (*i*) − (*ii*): d.p.m. due to [1-$^{14}$C]glucose; vial (*iii*): d.p.m. remaining after removal of [$^{14}$C]carbon dioxide from the 1-position of both [1-$^{14}$C]glucose and [1-$^{14}$C]lactose; (*ii*) − (*iii*): d.p.m. due to [1-$^{14}$C]lactose.

| Volume of standard lactose solution/ ml | Vial (*i*), d.p.m. | Vial (*ii*), d.p.m. | (*i*) − (*ii*), ≡ [1-$^{14}$C]glucose d.p.m. | Mean d.p.m. | Vial (*iii*), d.p.m. | (*ii*) − (*iii*), ≡[1-$^{14}$C]-lactose, d.p.m. | 0·1 ml of standard lactose solution d.p.m. | Mean d.p.m. | Mean recovery of [1-$^{14}$C]-lactose, per cent. |
|---|---|---|---|---|---|---|---|---|---|
| 0·1 | 29 432 | 12 434 | 16 998 | 16 955 ± 213 | 1822 | 10 612 | 10 612 | 10 734 ± 134 | 89·2 |
| 0·2 | 41 751 | 24 475 | 17 276 | | 3233 | 21 242 | 10 621 | | |
| 0·3 | 53 494 | 36 693 | 16 801 | | 3882 | 32 811 | 10 937 | | |
| 0·4 | 65 348 | 48 623 | 16 725 | | 5479 | 43 144 | 10 786 | | |
| 0·5 | 77 576 | 60 599 | 16 977 | | 7021 | 53 578 | 10 715 | | |

## TABLE IV

DETERMINATION OF [1-$^{14}$C]LACTOSE AND [1-$^{14}$C]GLUCOSE, SEPARATELY, IN PLASMA BY ENZYMIC RELEASE OF [$^{14}$C]CARBON DIOXIDE

Vial (*i*): total d.p.m. before reaction; vial (*ii*): d.p.m. remaining after removal of [$^{14}$C]carbon dioxide from the 1-position of [1-$^{14}$C]glucose; (*i*) − (*ii*): d.p.m. due to [1-$^{14}$C]glucose; vial (*iii*): d.p.m. remaining after removal of [$^{14}$C]carbon dioxide from the 1-position of [1-$^{14}$C]lactose; (*i*) − (*iii*): d.p.m. due to [1-$^{14}$C]lactose.

| Sub-stance | Volume of standard solution/ ml | Vial (*i*), d.p.m. | Vial (*ii*), d.p.m. | (*i*) − (*ii*), ≡ [1-$^{14}$C]-glucose, d.p.m. | Vial (*iii*), d.p.m. | (*i*) − (*iii*), ≡ [1-$^{14}$C]-lactose, d.p.m. | 0·1 ml of standard solution d.p.m. | Mean d.p.m. | Mean recovery, per cent. |
|---|---|---|---|---|---|---|---|---|---|
| Lactose | 0·1 | 12 343 | — | — | 1318 | 11 025 | 11 025 | 10 883 ± 91 | ·2 |
| | 0·2 | 24 330 | — | — | 2778 | 21 552 | 10 776 | | |
| | 0·3 | 36 852 | — | — | 4129 | 32 723 | 10 907 | | |
| | 0·4 | 48 696 | — | — | 5265 | 43 431 | 10 857 | | |
| | 0·5 | 60 150 | — | — | 5879 | 54 271 | 10 854 | | |
| Glucose | 0·1 | 17 095 | 474 | 16 621 | — | — | — | | 97·22 |

91·5 per cent. from all of these biological fluids. Neither [1-$^{14}$C]glucose nor cold glucose up to a concentration of 0·5 per cent. *m/V* interferes in the assay using the above recycling method for NADP. Any [1-$^{14}$C]glucose present can itself be determined concurrently with 97–98·5 per cent. recovery.

We thank the Irish Stone Foundation and The Medical Research Council of Ireland for generous financial support.

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# Analytical Methods Committee

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

# The Determination of Small Amounts of Cadmium in Organic Matter

## Part II.* Determination of Amounts Down to the Sub-microgram Level

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

### Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Dr. L. E. Coles (Chairman), Mr. J. R. Bishop (resigned May, 1973), Mr. W. Cassidy, Mr. S. Greenfield (appointed July, 1973), Mr. W. H. Hill (appointed May, 1973), Dr. R. A. Hoodless, Mr. E. E. J. King, Mr. D. A. Lambie, Dr. R. F. Milton, Mr. W. L. Sheppard and Mr. C. A. Watson, with Mr. P. W. Shallis as Secretary and Mr. J. J. Wilson as Assistant Secretary.

### Introduction

Spectrophotometric, polarographic and atomic-absorption spectrometric methods for determining small amounts of cadmium in organic matter have previously been recommended by the Sub-Committee.[1,2] These methods are, however, not particularly suitable for determining the very low levels of cadmium in which there is considerable environmental interest at the present time. With EEC regulations to limit the levels of cadmium in food, drink and packaging materials impending, the Sub-Committee was of the opinion that it would be desirable to recommend a method, preferably based on atomic-absorption spectrometry, for determining sub-microgram amounts of cadmium.

An unpublished report[3] was made available to the Sub-Committee in which a method was proposed that was claimed to improve the effective sensitivity of the atomic-absorption procedure after only a simple preliminary treatment. The method requires few reagents, thereby minimising the risk of contamination. A sample concentration step is included in order to improve the sensitivity; it involves a solvent extraction with a liquid ion-exchange resin, Amberlite LA-2.[4,5] By using the proposed method cadmium can be determined in the presence of several metals that interfere in other procedures.[2]

### Experimental

A collaborative exercise was arranged in which two samples of a proprietary tomato soup, one as supplied and the other with 0·2 p.p.m. of added cadmium, were sent to the participating laboratories. In each laboratory 20-g portions of both samples were wet oxidised with sulphuric acid and hydrogen peroxide.[6] Following dilution of the digests with water the proposed method was applied, and the concentration of cadmium in the samples was calculated by reference to a calibration graph constructed from the results obtained by taking known amounts of cadmium through the entire procedure.

The results obtained in the collaborative exercise are given in Table I.

### Discussion

The method was first investigated by a member of the Sub-Committee who had the particular problem of determining small amounts of cadmium in zinc compounds.[3,5] It was found that the recovery of cadmium at the 10-$\mu$g level was unaffected by the presence of large amounts of zinc and, either separately or collectively, of 100 $\mu$g of iron, tin, lead and

* For details of Part I of this series, see reference list, p. 763.

TABLE I

DETERMINATION OF CADMIUM IN TOMATO SOUP BY THE PROPOSED METHOD

| | Cadmium found, p.p.m. | | | |
|---|---|---|---|---|
| Laboratory | Soup as supplied | Mean | Soup with added cadmium | Mean |
| A | 0·05, 0·05, 0·01 | 0·04 | 0·23, 0·22, 0·22 | 0·22 |
| B | 0·02, 0·01, 0·02 | 0·02 | 0·22, 0·22, 0·21 | 0·22 |
| C | 0·05, 0·03, 0·06 | 0·05 | 0·23, 0·24, 0·26 | 0·24 |
| D | 0·02, 0·03, 0·02 | 0·02 | 0·21, 0·22, 0·21, 0·22, 0·21, 0·21 | 0·21 |
| E | −0·02, −0·02, 0·06, 0·02, 0·00, 0·01 | 0·01 | 0·20, 0·19, 0·20, 0·20, 0·22, 0·21 | 0·20 |
| F | 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01, 0·01 | 0·01 | 0·20, 0·20, 0·21, 0·20, 0·20, 0·20, 0·21, 0·21, 0·20, 0·21, 0·20, 0·21, 0·21, 0·20, 0·21, 0·21, 0·22, 0·22, 0·21, 0·20, 0·20, 0·19, 0·19, 0·20 | 0·21 |
| G | <0·01 | | 0·20, 0·20 | 0·20 |

antimony. The recovery of the same level of cadmium was unaffected by the presence of large amounts of alkaline earth and alkali metals in the sulphuric acid solution. Extracts were shown to be stable for at least 24 h and the use of a ketone solution gave a four-fold increase in sensitivity over that obtained for an equivalent concentration of cadmium in aqueous solution. The great advantage of the method for routine use after wet oxidation lies in the fact that the formation of the iodocadmate anion, and its subsequent extraction into the ketone phase, are not dependent on the concentration of the sulphuric acid as they are for other complex iodo anions, such as those of zinc, tin, lead and antimony. The method was found to be simple to operate and with so few steps involved was considered to be well suited for the Sub-Committee's purpose.

In view of the results of the collaborative work carried out in seven laboratories the Sub-Committee recommends the use of the method given in the Appendix for the determination of amounts of cadmium in organic matter down to the sub-microgram level.

## APPENDIX

### Recommended Method for the Determination of Amounts of Cadmium in Organic Matter Down to the Sub-microgram Level

#### Principle of Method

Following wet oxidation of the organic matter, the cadmium in sulphuric acid solution is converted to the iodocadmate ion with potassium iodide and is extracted with a solution of a liquid ion-exchange resin in 4-methylpentan-2-one. The ketone phase is aspirated directly into an atomic-absorption spectrometer.

#### Applicability

The method can be applied to the sulphuric acid extract resulting from any wet oxidation. By suitable adjustment of the amount of sample a wide range of cadmium contents down to sub-microgram amounts can be determined.

#### Reagents (Note 1)

*Sulphuric acid, density* 1·84 *g ml*$^{-1}$. Analytical-reagent grade.

4-*Methylpentan-2-one*. For atomic-absorption spectrometry.

*Potassium iodide solution*, 0·1 M.

*Amberlite LA-2 solution*. A 1 per cent. *V*/*V* solution of Amberlite LA-2 [*N*-lauryl(trialkylmethyl)amine] in 4-methylpentan-2-one.

*Cadmium standard solution*. Dissolve 2·282 g of cadmium sulphate ($3CdSO_4.8H_2O$) in 50 ml of water, add 10 ml of sulphuric acid and dilute to 1 l with water.

1 ml of solution ≡ 1000 μg of cadmium.

Dilute 10 ml of this solution to 1 l with water.

1 ml of solution ≡ 10 μg of cadmium.

Dilute 10 ml of the latter solution to 100 ml with water.

1 ml of solution ≡ 1 μg of cadmium.

## Method

### Preparation of Sample

Destroy the organic matter present in an appropriate amount of sample (see Note 2) by wet oxidation with 10 ml of sulphuric acid and any of the other reagents recommended for this purpose,[7] but preferably with 50 per cent. hydrogen peroxide.[6] Observe all of the precautions that are necessary to avoid mechanical loss of solution. When oxidation is complete, cool the digest, add, with care, 20 ml of water and again cool the digest.

### Extraction of Cadmium

Transfer the cooled and diluted digest to a 100-ml separating funnel with the minimum amount of water necessary to effect the transfer. Add 5 ml of 0·1 M potassium iodide solution, dilute to about 50 ml with water, and mix well. Then add, by use of a pipette, 10 ml of Amberlite LA-2 solution, and shake the funnel vigorously for 20 s. Allow the phases to separate, run off and reject the aqueous phase, and filter the ketone phase through a dry Whatman No. 541 or equivalent filter-paper in order to remove suspended droplets of aqueous phase. Collect the filtrate in a small, glass-stoppered vessel.

### Preparation of Calibration Standards

Transfer volumes of cadmium standard solution equivalent to 0, 2, 4, 6, 8 and 10 μg of cadmium to a series of separating funnels, each containing a cooled mixture of 10 ml of sulphuric acid, about 20 ml of water and 5 ml of 0·1 M potassium iodide solution. Dilute all of the solutions to about 50 ml with water and extract each with Amberlite LA-2 solution as described under Extraction of Cadmium. Collect the filtrate after each extraction in a separate, small, glass-stoppered vessel (see Note 3).

### Measurement

Set the atomic-absorption spectrometer to the optimum conditions for cadmium at a wavelength of 228·8 nm. Aspirate the ketone alone (not the Amberlite LA-2 solution) and set the instrument to zero, adjusting the air flow to give a flame that is just short of luminous (see Note 4). Then aspirate successively the standard, reagent blank and sample solutions and after each reading aspirate the ketone alone, thus carrying out frequent checks on the zero. Prepare a calibration graph by plotting the reading obtained for each standard solution against its known cadmium content. Calculate the cadmium content of the sample by reference to the calibration graph.

NOTES—

1. If any of the reagents (or samples) has been in contact with coloured polythene stoppers some cadmium could have been picked up from the pigment. A check should be made on the Amberlite LA-2 solution in ketone in order to ensure that it is free from cadmium.
2. The effective sensitivity required can be controlled simply by adjusting the amount of sample taken.
3. This range of standards will, with most instruments, give a calibration graph with little deviation from linearity. For a 20-g sample the absorbance of the 10-μg standard will be equivalent to 0·5 p.p.m.
4. Care must be taken with some burner assemblies, *e.g.*, the three-slot Boling burner, when changing solutions as the removal of the ketone renders the flame weak and there is a tendency to flash back.

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

## Communication

*Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication precludes the use of diagrams, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.*

*Manuscripts are* not *subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.*

# The Separated Flame as a Resonance Detector*

The phenomenon of resonance radiation provides a means of "isolating" atomic resonance lines, and the use of resonance detectors exploiting this fact has been described previously.[1–4] The requisite atomic vapour has, in the past, been produced by electrical heating or by cathodic sputtering in a low-pressure electrical discharge. Such resonance detectors have been used as "monochromators" in atomic-absorption[2,3] and atomic-emission[4] spectroscopy. The possibility of using a flame, and in particular a separated flame, as the resonance detector does not appear to have been considered.

A flame can be made a detector for any given element simply by spraying into it a pure solution of that element. When this flame is illuminated, the fluorescence signal can be due only to radiation of wavelength(s) corresponding to the absorption line(s) of that element. A non-dispersive flame-fluorescence spectrophotometer[5,6] can therefore be converted into an atomic-absorption spectrophotometer by interposing a second flame, into which is sprayed the solution for analysis, between the light source and the separated flame. My colleague P. L. Larkins has confirmed this by experiments that we shall report in detail in a future paper. In the meantime, workers using flame-fluorescence techniques will be able to carry out similar experiments with equipment already available to them.

The separated flame has an outstanding advantage over previous types of resonance detectors in the ease with which it can be changed from the detection of one element to another. It is necessary only to spray the appropriate solution into the flame. Sequential analysis can be achieved by using a multi-element light source and an automatic sample changer in order to introduce sequentially into the flame solutions of the appropriate elements.

There are other intriguing possibilities.[7] If, in the above arrangement, the absorbing flame is replaced by a de-mountable sputtering cell in which the cathode is the sample for analysis, it is possible to carry out the analysis of solids by absorption, fluorescence or emission measurements. It therefore appears possible to develop a versatile, non-dispersive atomic spectrophotometer for the analysis of solutions or solids using obvious permutations of the arrangements suggested above. Multi-element systems could be achieved by using either type of detector in the various series - parallel arrangements described previously.[3]

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* This communication is based on comments made during the Chairman's address to the Fifth International Conference on Atomic Spectroscopy, Melbourne, August 25th, 1975.

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# Book Reviews

HANDBOOK OF REACTIVE CHEMICAL HAZARDS. By L. BRETHERIC. Pp. xx + 976. London: Butterworths. 1975. Price £20.

Every chemist knows that all chemicals are potentially hazardous and is aware of a number of dangerous reactions, a number that may be small for the student and researcher alike. Even the most experienced chemist is usually less knowledgeable than he would like to be and, when faced with a combination of chemicals that is new to him, is often driven to utilise dimly remembered "facts" by the difficulty of finding, in the widely scattered literature, references to the hazards of the reaction. How useful , then, to have available a well documented compilation of the reactivity hazards of over 4000 single elements, compounds and their combinations, as well as general information on types or groups of elements or compounds that possess similar hazardous characteristics.

The author does not claim complete coverage, of course, but the handbook is the result of an obviously extensive survey of published information and includes a good deal of unpublished information that has been culled from many sources. Information on toxic hazards has been specifically excluded on the reasonable grounds that much of it is available collectively elsewhere, and no attempt has been made to include details of all flammable materials capable of burning explosively when mixed with air and ignited. However, about 550 gases and liquids with flash points below 25 °C and/or autoignition temperatures below 225 °C have been included, with figures for flash point, autoignition temperature and explosive limits in air when known. Elements and compounds that ignite on exposure to air are also included.

In my opinion, the book should be available in every establishment where "novel" chemical reactions are undertaken, and I think that most libraries would agree that £20 is a small enough price to pay for what is, in effect, the result of 10 years' dedicated work. Making it available to the experimentalist is one thing, but in cynical vein one must only hope that it is consulted before an accident or incident rather than after it. In either event, some relevant information is likely to be found in this book and the author is to be congratulated on producing it, as are his employers, British Petroleum, for being public spirited enough to encourage and support him.

G. E. PENKETH

ANALYTICAL METHODS APPLIED TO AIR POLLUTION MEASUREMENTS. Edited by R. K. STEVENS and W. F. HERGET. Pp. viii + 303. Ann Arbor, Michigan: Ann Arbor Science Publishers Inc. Distributed by John Wiley & Sons Ltd.: New York, Sydney, Tokyo, Mexico City and London. 1974. Price £9·80.

From the title, one would expect this book to be a collection of methods for the measurement of air pollutants. In fact, it is a review by various authors, all experts in their fields, of techniques and applications and emanated from a symposium held during the 165th National Meeting of the American Chemical Society in Dallas, Texas, 1973. The introduction, by Dr. A. P. Altshuller, outlines the US Environmental Protection Agency's requirements for air pollution monitoring, which in the 1970s stimulated the development of new instruments and the application of existing methods to these special and often stringent requirements.

The book is divided into three main sections, describing techniques for measuring "gaseous pollutants at ambient concentrations," "chemical and physical properties of particles in the atmosphere" and "pollutants from stationary and mobile sources." The reports within each section are well illustrated with photographs and diagrams and there is a good selection of up-to-date (1974) references.

The book is well produced and I detected no serious factual errors, although there are a few minor editorial errors. For example, "per cubic centimeter" appears as "/cc" "/cm$^3$" and "cm$^{-3}$" in various parts of the book, although the nomenclature is consistent within a particular report.

On the whole, the book succeeds in its purpose of bringing together the latest techniques available for the measurement of air pollutants and could be a useful addition to the library of workers in the field. However, with the rapid advances being made, much of the content of the book may well be only of historical value within a few years.

A. F. SMITH

OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Twelfth Edition. Edited by WILLIAM HORWITZ. Pp. xxii + 1094. Washington: Association of Official Analytical Chemists. 1975. Price $41.

When the Tenth Edition was published in 1965, the inside cover carried a notice that it should not be destroyed upon the appearance of the Eleventh Edition because it contained surplus methods that would not be repeated in the Eleventh Edition. A similar note appeared in the Eleventh Edition, and in this Edition, and there are numerous references in the text to the Tenth and Eleventh Editions. There can be no analyst who has ever deliberately *destroyed* a copy of this publication, but it would now seem that, as with *The Analyst*, our shelves must make room for sets of "AOAC" editions and that old editions will acquire a scarcity value.

Methods that it is proposed to declare as surplus, and therefore to be excluded from the next edition, are marked with asterisks and analysts are requested to notify the Association if they wish any such methods to be retained.

The Preface comments that the Twelfth Edition is an accumulation of only 4 instead of the customary 5 years of approved work, but that in these 4 years, more new methods (about 280) were approved than in the previous 5 years (about 250).

Although there is no obvious change in style or format, the Twelfth Edition has been produced by a computer-controlled photocomposition method, which it is hoped will slow down the rate of increase of publishing costs.

An earlier review,[1] of the Tenth Edition, commented upon the absence of statistical evaluation of the methods, an absence that is continued in the Twelfth Edition, apart from a simple test of acceptance applied to microbiological methods. The Association is, however, well aware of the necessity for such evaluation as shown by the publication of the "Youden Manual"[2] and the Preface to the Twelfth Edition observes that such procedures are now applied to the collaborative studies from which the approved methods emerge, but with the warning that individual laboratories should not take such an evaluation as a measure of what their individual analysts produce. An individual report is a statistically confounded combination of the random error of the method, intermingled with components generated by the individual environment and the analysts' experience, skill and bias, to say nothing of the contribution of a heterogeneous sample!

An apparently statistically poor performance of a method does not always mean that the method is at fault unless it is certain that there is no need for improved laboratory quality control, as shown by recovery studies and the ability of the individual analyst to perform the method properly. Analysts, who take pride in this skill, can take heart from this pronouncement that statistics are no substitute!

The publication of the AOAC Style Manual[3] was an important contribution to the presentation of methods and it is pointed out that the success of a method in hands other than those of the developers depends upon the completeness and clarity of the text.

The number of chapters has been increased from 47 to 52, but there is actually only one new Chapter, "Forensic Sciences," the other additional chapters resulting from the sub-division of the previous chapter on "Drugs."

The new chapter on "Forensic Sciences" contains only two methods, one on the examination of glass fragments by an optical dispersion technique and the other on the development of latent fingerprints. Clearly, some rapid expansion of this chapter is to be expected and it is wondered whether the decision to include these methods is a "holding action" pending the appearance of a separate volume on such a wide ranging subject. Other additions include a number of biological methods, including an eye irritation test for cosmetics and a haemoglobin repletion test for biologically available iron. Automatic methods have been introduced for fertilisers, milks, waters and a number of drugs.

The progress in instrumental methods of analysis is evident, as only about one in every eight new methods now makes use of classical titrimetric and gravimetric procedures.

With the exception of the table of refractive indices of sugar solutions, which has been enlarged as a result of computer calculations, the very valuable reference tables appear largely unchanged from the Eleventh Edition. A change that may not be welcomed is the replacement of page numbers by section numbers in the Index, a change made necessary by economic considerations so that the index can be prepared in advance. On first usage, it did take longer to find the matter sought, but improvement should come with familiarity.

There has been criticism of this book for the omission of explanations or *raison d'être* from the methods, but the lists of references to the original work should satisfy this need. The unusually

forthright Preface makes several references to the extent of the collaborative work behind the methods.

There is no question but that this book must be on every analyst's bookshelf, if not more often on the bench, and considering the use that it will have it is regrettable that the binding is not more robust.

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R. S. HATFULL

MODERN PHYSICAL TECHNIQUES IN MATERIALS TECHNOLOGY. Edited by T. MULVEY and R. K. WEBSTER. *Harwell Series*. Pp. xiv + 321. London: Oxford University Press. **1974**. Price £9·50.

This book gives a clear and up-to-date account of the instrumental methods available for the characterisation of materials. The chapters, which are grouped broadly into diffraction, electron-beam, microscopic and spectroscopic techniques, are in essence expanded versions of the lectures that have been presented at interdisciplinary courses at the Education and Training Centre, A.E.R.E., Harwell.

The chapters treat Crystallography and Diffraction by Crystals; X-ray Diffraction; Neutron Diffraction; Electron Diffraction; Field-ion Microscopy; Quantitative Optical Microscopy and Interferometry; Electron Microscopy, (a) Principles, (b) Preparation Techniques, (c) High-voltage Techniques and (d) Scanning Techniques; Electron-probe Microanalysis; Auger Spectroscopy; X-ray Spectrometry; Atomic-absorption Spectroscopy; Optical Emission Spectroscopy; Mass Spectrometry; Activation Analysis; Mössbauer Spectroscopy; and Magnetic Resonance Spectroscopy. There is a combined Author and Subject Index.

The chapters vary in length from 8 to 24 pages; although the four separate chapters on electron microscopy account for 30 per cent. of the book, the editors have succeeded in achieving a remarkable uniformity of style, presentation and standard of treatment, which gives an outline of basic theory and a review of typical instruments and applications. Clearly, little other than a general and somewhat superficial account of the techniques discussed in this book could be given by any author; in the space available to them, the authors involved have given clear, concise and authoritative accounts that must be regarded as models of their kind.

For students, this book will serve as an excellent introduction to much of modern instrumental analysis at first- or second-year level; it would also make an excellent book to stimulate senior school pupils in the transition from school to university. There is no doubt, however, that this book is best suited for the categories of person for whom it was intended—"for those who carry responsibility for research programmes and who must be able to judge the potential contribution from alternative techniques, and for specialists in any one technique who require an overview in adjacent fields of work." None of us can cover more than a few selected areas nowadays: this is a good book to have available on the shelf.

D. M. W. ANDERSON

X-RAY DIFFRACTION PROCEDURES FOR POLYCRYSTALLINE AND AMORPHOUS MATERIALS. By H. P. KLUG and L. E. ALEXANDER. Second Edition. Pp. xxiv + 966. New York, London, Sydney and Toronto: Wiley-Interscience. **1974**. Price £18·55.

X-ray diffraction is a widespread technique of great usefulness in analysis, and it is therefore a pleasure to welcome the second edition of a basic standard text. The new edition has been enlarged and for the greater part re-written so that it is best regarded as a new book in its own right. To provide a work of standard reference that would cover all aspects of the subject remained the basic aim of the authors and is one which they have achieved. It is not easy to be all things to all people and the price that must be paid for completeness is length; with over 900 pages, nothing has been omitted. Thus this book covers elementary ideas about X-rays and crystals, as well as a sophisticated treatment of curve stripping, gives detailed descriptions of the various types of diffractometers and also an extensive discussion of experimental results.

In layout, the book is logical and straightforward. Elementary ideas of crystallography and X-rays are dealt with in detail in the first two chapters. A virtue of this book is the care that the authors have taken with the clarity of mathematical derivations, even the most elementary. This treatment may be regarded as superfluous by the sophisticated expert but greatly enhances the value of the book for the novice, as it enables him to understand the basic principles of the subject (and even experts sometimes like to be able to refer back to some elementary derivation!). In the third introductory chapter, the fundamentals of diffraction are discussed, followed by two detailed and important chapters concerned with experimental techniques. The Debye - Scherrer method is of course described and discussed in depth but very useful sections on experimental techniques for obtaining diffraction data at high and low temperatures and at high pressures are also included. The powder diffractometer is described exhaustively, special attention being paid to the all-important setting-up procedures of calibration and alignment. A most interesting section of this chapter is concerned with the factors that determine line profiles, their shape and structure. In a later chapter, modern computer techniques for "unfolding" broadened lines are discussed, which enables the reader to understand the factors that give rise to the observed shapes of diffraction peaks and how some of these effects can be removed. Considerable attention is also paid to a detailed description of the electronic equipment associated with modern diffractometers. This is most useful information for understanding what goes on inside the various "black boxes" and is invaluable as a basis for reasoned and intelligent fault detection.

The remainder of this book, that is to say rather more than half, is devoted to a discussion of the applications of X-ray diffraction. The first chapter describes the general methods for the interpretation of diffraction data and particular attention is paid to the use of computers in indexing powder patterns. A most important chapter describes the role of X-ray diffraction in the qualitative and also quantitative analysis of crystalline powders. A detailed introduction to the Powder Diffraction File is given, together with numerous examples of its application. Also discussed in detail are the problems associated with sample self-absorption upon diffraction peak intensities—a problem that can be particularly severe with unknown mixtures. The chapter on the precise determination of lattice constants includes a valuable discussion on the sources of error in X-ray diffraction. From consideration of the degree of line broadening, it is possible to deduce important information about crystal size and also lattice strains. Before this analysis can be made, however, other more mundane sources of broadening must be systematically removed. Both the Fourier transform method and the simpler approach of iterative folding are described in detail. Subsequent chapters show how topics such as stress in metals and preferred crystallite orientation (*e.g.*, in fibres) can be tackled and what results can be expected from the application of diffraction techniques to non-crystalline materials (carbon black, liquids, biological compounds, etc.).

In all of the topics covered, the great virtue of this book is the same—a complete and comprehensive yet very readable and understandable coverage from elementary basic principles through to modern research applications. This is clearly a basic reference book that no X-ray diffraction laboratory can afford to be without. The range of readership should, however, be much wider, embracing all who would call themselves materials scientists and all who would call themselves analysts. Physicists and chemists will also find the book of interest and of value for its up-to-date description of the breadth and power of the X-ray diffraction technique. The book is well produced and, relative to today's prices (when the merest monograph can cost £5), is good value for money.

D. S. URCH

INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS. Third Edition. PART I. GENERAL CONSIDERATIONS AND REACTIONS AT CONVENTIONAL RATES. Edited by E. S. LEWIS. *Techniques of Chemistry*. Volume VI. Pp. xiv + 838. New York, London, Sydney and Toronto: Wiley-Interscience. 1974. Price £21.

This series represents a comprehensive treatment of the techniques used by practical chemists and most analysts will no doubt find particular volumes of use in their own work. The present volume would be of special application to those analytical chemists who are interested in the rates and mechanisms of analytical reactions. The quantitative analytical application of rate measurements is not included but there are excellent chapters, by experts in each field, on the measurement of rates of reactions and on the mechanistic theories of all types of reactions. The book represents the most comprehensive up-to-date compilation of information in this field. J. M. OTTAWAY

## A Potentiostatic Method and Apparatus for Multiple Determination of Copper in Free-cutting Aluminium Alloys

A simple and direct method for the deposition of copper at a controlled cathode potential from a mixed tartrate - sulphosalicylate electrolyte has been developed. This method is used in the routine determination of the copper content of free-cutting aluminium alloys in conjunction with a multi-potentiostatic electro-deposition apparatus.

**T. S. RANDHAWA and R. L. SOUTHWELL**

E. & E. Kaye Ltd., Ponders End, Enfield, Middlesex, EN3 4SS.

*Analyst*, 1975, **100**, 726–734.

## The Voltammetric Determination of Sodium Hydroxymethanesulphinate

The determination of sodium hydroxymethanesulphinate by voltammetry using a rotating platinum micro-electrode is described with operating details. The method is sufficiently specific for it to have definite advantages over methods based on redox titrations.

**J. S. EDGAR**

Wool Research Organisation of New Zealand Inc., Christchurch, New Zealand.

*Analyst*, 1975, **100**, 735–738.

## Mutual Interference Effects During the Successive Determination of Iodide, Bromide, Chloride and Fluoride in a Single Sample Using Halide-selective Electrodes

The determination, using halide-selective electrodes, of iodide, bromide, chloride and fluoride in a single 100-mg sample of an orthophosphate mineral is described. Mutual halide interference does not occur if the level of iodide is less than one twentieth of that of bromide (although with care this can be extended to one tenth) and if the chloride concentration is between 50- and 200-fold in excess of that of bromide. The extent of other interferences in the determination is discussed briefly.

The method is applicable to the determination of iodide over the concentration range $5 \times 10^{-5}$ to $10^{-1}$ M, bromide from $5 \times 10^{-4}$ to $10^{-1}$ M, chloride from $3 \times 10^{-4}$ to $10^{-1}$ M and fluoride from $3 \times 10^{-6}$ to $10^{-1}$ M, provided that the described precautions are taken to limit mutual interference effects.

**E. J. DUFF and J. L. STUART**

Department of Preventive Dentistry, Turner Dental School, University of Manchester, Manchester, M15 6FH.

*Analyst*, 1975, **100**, 739–746.

## Ionic Polymerisation as a Means of End-point Indication in Non-aqueous Thermometric Titrimetry

### Part VII. The Determination of *O*-Alkyl Dithiocarbonates and Metal Iodides

Lead, nickel, potassium, sodium and zinc *O*-alkyl dithiocarbonates have been determined in amounts down to 0·002 mequiv by catalytic thermometric iodimetric titration of their solutions in dimethylformamide, acrylonitrile or chloroform. The end-points of the titrations correspond to the formation of the metal polyiodides, and the method is more sensitive than conventional aqueous iodimetry for the determination of potassium and sodium alkyl dithiocarbonates, in which a monoiodide is the final product. The results show precisions ranging from 0·20 to 1·15 per cent. when a 0·05 M titrant is used.

The mechanisms of the titration reactions have been confirmed by titrating solutions of the metal iodides in dimethylformamide by the iodimetric method, and this procedure can be used for the determination of nickel, potassium, sodium and zinc iodides in non-aqueous solution.

Impurities such as sulphides, sulphites, thiosulphates, sulphates, carbonates and trithiocarbonates can be separated from technical-grade dithiocarbonates before analysis by using the appropriate solvent for the sample and centrifuging the solution.

Sodium and potassium alkyl dithiocarbonates in aqueous solution have been determined as the corresponding nickel salts by a modification of an established solvent-extraction method.

**E. J. GREENHOW and L. E. SPENCER**

Department of Chemistry, Chelsea College, University of London, Manresa Road, London, SW3 6LX

*Analyst*, 1975, **100**, 747–754.

## The Indirect Colorimetric Determination of Sulphate with 2-Aminoperimidine

An indirect colorimetric procedure is described for the determination of sulphate. After precipitating the sulphate as 2-aminoperimidinium sulphate, the remaining 2-aminoperimidine reagent is determined colorimetrically following treatment with nitrous acid and alkali in order to give a red colour with maximum absorbance at 525 nm and a molar absorptivity of 13 600 l $mol^{-1}$ $cm^{-1}$. The mean absorbance difference for a sulphate-ion concentration of 10 mg $l^{-1}$ is 0·283 with a relative standard deviation of 4 per cent.

**A. W. ARCHER**

Research Section, Division of Analytical Laboratories, Health Commission, P.O. Box 162, Lidcombe, N.S.W., Australia, 2141.

*Analyst*, 1975, **100**, 755–757.

## An Enzymic Method for the Determination of [1-$^{14}$C]Lactose

A simple, rapid and specific method for the determination of [1-$^{14}$C]lactose in biological fluids is described. It is based on the enzymic removal of the 1-$^{14}$C atom of lactose as [$^{14}$C]carbon dioxide, using commercially available enzymes. The assay involves only one critical addition and the entire reaction can be carried out in a scintillation vial.

**E. DAVIES, E. BOURKE and J. COSTELLO**

Department of Clinical Medicine, Trinity College, University of Dublin, Meath Hospital, Dublin 8.

*Analyst*, 1975, **100**, 758–760.

## The Determination of Small Amounts of Cadmium in Organic Matter

### Part II. Determination of Amounts Down to the Sub-microgram Level

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

**ANALYTICAL METHODS COMMITTEE**

The Chemical Society, Burlington House, London, W1V 0BN.

*Analyst*, 1975, **100**, 761–763.

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