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Summaries of Papers in This Issue

The Determination of Small Amounts of Mercury in Organic Matter

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

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1965, **90**, 515–530.

The Determination of Sulphaquinoxaline

Report prepared by the Prophylatics in Animal Feeds Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1965, **90**, 531–535.

Determination of β-Carotene in a Roller-dried Food

The standard saponification procedure failed to extract all the carotene from a dried protein - cereal infant-food preparation, fortified with β-carotene added as a water-dispersion. An alternative method has been used in which the foodstuff is treated with proteolytic and diastatic enzymes, and the carotene then extracted with solvent. Results by this method are higher than those obtained by saponification, and evidence is produced to show that low results are due to incomplete extraction.

A. E. BENDER and A. J. MACFARLANE
Research & Development Department, Farley's Infant Food Ltd., Colnbrook, Bucks.

Analyst, 1965, **90**, 536–540.

The Determination of Activation Products in Irradiated Steels by Anion-exchange Separation and γ-Ray Spectrometry

A method is described for determining chromium-51, manganese-54, cobalt-58 and -60, and iron-59 in irradiated mild and stainless steels. It involves the selective elution of these isotopes with different concentrations of hydrochloric acid from a strongly basic anion-exchange resin column. Each nuclide is determined by γ-ray spectrometry. The precision of the method is better than 2 per cent. for each determination.

D. A. HILTON and D. REED
Central Electricity Generating Board, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire.

Analyst, 1965, **90**, 541–544.

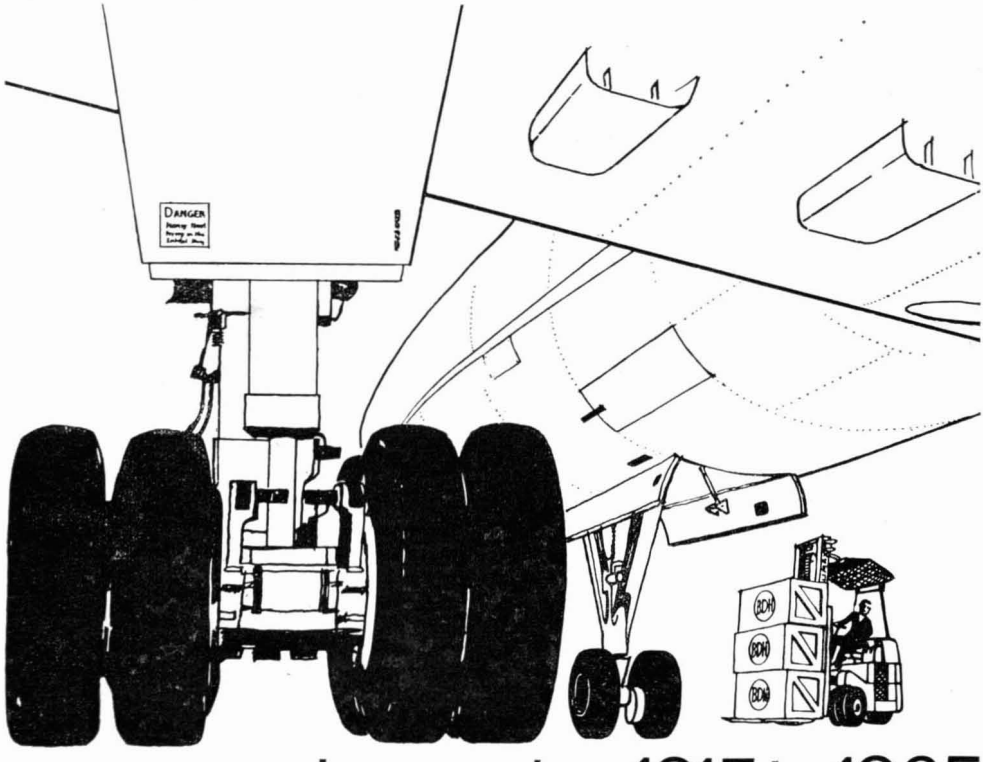
The Polarographic Determination of Trace Amounts of Lead in Stainless and Other Steels

A method is described for determining lead in stainless steels by successive extractions from a single phase, which is retained in the separating funnel throughout the procedure. An initial extraction as iodide and then one as diethyldithiocarbamate from a complexing solution gives effective separation from the rest of the sample.

When a differential cathode-ray polarograph is used, detection limits of 0·05 p.p.m. or better are possible with a 1-g sample, but reagent blank values generally limit the sensitivity to about 2 p.p.m.

R. C. ROONEY
Southern Analytical Limited, Frimley Road, Camberley, Surrey.

Analyst, 1965, **90**, 545–548.



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The Determination of Copper and Magnesium in Blood Serum by High-resolution Flame Spectrophotometry

The determination of copper and magnesium in human blood serum is used for demonstrating the potential of high-resolution flame spectrophotometry. A high-resolution monochromator provides the maximum differentiation between the spectrum line and the flame background and allows practical limits of detection to be obtained that approach the theoretical maximum as defined by spectrum line width. An automatic wavelength scanning and recording technique is used to obtain the value of the peak line intensity above the background in a single recording. Recordings of copper and magnesium lines illustrate the method and show the resolution required for separating the analytical line from adjacent lines or bands in the spectrum. Applications of the instrument to other analyses are also indicated.

R. L. WARREN

Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.1.

Analyst, 1965, **90**, 549–553.

Determination of Barium, Strontium and Calcium in Barium Peroxide

Improved methods have been developed for the determination of barium, strontium and calcium in barium peroxide. The barium is determined as barium chromate by using the technique of precipitation from homogeneous solution. The filtrate from the barium chromate is treated with ammonium oxalate and ethanol to precipitate the strontium and calcium oxalates, which are then converted to the nitrates. The calcium and strontium nitrates are separated by use of acetone, and the calcium and strontium are finally precipitated as the sulphates. The methods are applicable to the determination of barium, strontium and calcium in general.

GEORGE NORWITZ

Pitmann-Dunn Laboratories, Frankford Arsenal, Philadelphia 37, Pa., U.S.A.

Analyst, 1965, **90**, 554–563.

Analytical Results for the Paper-chromatographic Zone - Strip Technique

The use of paper chromatography for micro-scale quantitative analysis by the zone - strip technique gives accurate and reproducible results within certain concentration limits. Analytical results concerning these limits, obtained under specified experimental conditions, are given. Furthermore, some other conclusions concerning the mode of deviation outside these ranges are given.

IBRAHIM R. SHIMI and GAMAL M. IMAM

Biochemistry Department, Faculty of Science, Ain Shams University, Abbassiah, Cairo, Egypt.

Analyst, 1965, **90**, 564–567.

Determination of Cyclamates in Soft Drinks

Short Paper

D. I. REES

The Laboratories, J. Lyons & Co. Ltd., Kensington, London, W.14.

Analyst, 1965, **90**, 568–569.

Spectrophotometric Determination of Carbaryl in Insecticide Formulations

Short Paper

S. H. YUEN

Imperial Chemical Industries Limited, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berks.

Analyst, **1965**, **90**, 569–571.

A Rapid Method for Quantitative Separation of Vitamin D from Vitamin A

Short Paper

R. K. BARUA and M. V. K. RAO

Department of Chemistry, University of Gauhati, Assam, India.

Analyst, **1965**, **90**, 571–574.

Plastic Standard-taper Joints

Short Paper

G. M. LEET

Rukukia Soil Research Station, Ruakara Research Centre, Hamilton, New Zealand.

Analyst, **1965**, **90**, 574–575.

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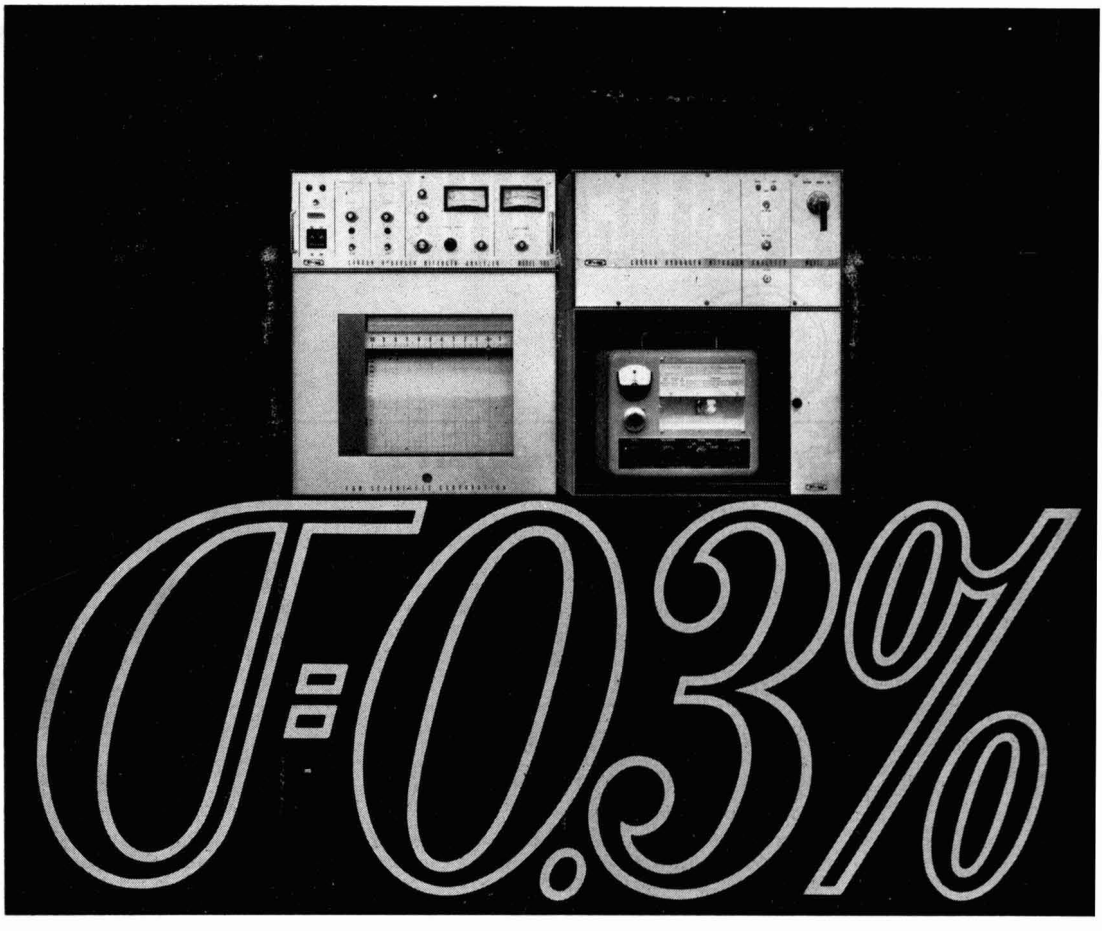

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THE ANALYST

Analytical Methods Committee

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

The Determination of Small Amounts of Mercury in Organic Matter

THE Analytical Methods Committee has received the following report from its Metallic Impurities in Organic Matter Sub-Committee. The report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The members of the Sub-Committee concerned with the preparation of this Report were Mr. W. C. Johnson (Chairman), Dr. J. C. Gage, Dr. T. T. Gorsuch, Mr. E. I. Johnson,* Miss E. M. Johnson, Dr. R. F. Milton, Dr. E. J. Newman, Mr. W. G. Sharples,* Mr. G. B. Thackray and Dr. J. F. C. Tyler, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

Mercury and its compounds are highly toxic. Organic mercurials are also widely used as pesticides and fungicides. The Sub-Committee decided, therefore, that a reliable method was required for determining amounts of mercury as small as 0·5 μg.

For this purpose, a determination of mercury with dithizone seemed the obvious choice. Dithizone is the most widely used reagent for mercury; the extraction of mercury with dithizone from acid solution is fairly selective and the colour reaction is particularly sensitive.

The most important contribution that this Report makes to the determination of mercury is the procedure described for the preparation of the sample, and this replaces the previous method recommended by the Sub-Committee.[1] The destruction of organic matter presents a major problem because of the volatility of mercury and its covalent compounds. Dry ashing methods cannot, therefore, be used, and the likelihood of volatilisation losses must also be considered when wet digestion procedures are used. Two possibilities suggested themselves; either the volatility of mercury and its compounds could be used to advantage, and the mercury removed from the wet digestion mixture by a distillation method, or the digestion could be conducted in an apparatus in which volatilised mercury is trapped. The Sub-Committee decided to adopt the latter alternative and were able to devise a reliable wet digestion procedure. The recommended wet digestion method, involving use of the apparatus described, allows vigorous oxidising conditions to be used without risk of losses of mercury.

EXPERIMENTAL WORK

PREPARATION OF THE SAMPLE—

Losses of mercury during wet oxidations have been widely reported and attributed to the volatility of mercury and its compounds. The Sub-Committee decided, therefore, to investigate the possibility of loss of mercury by volatilisation with use of the modified Bethge apparatus described by Gorsuch[2] and shown in Fig. 1. In this apparatus, the distillate collects in the reservoir, B, and may be either returned to the digestion flask or tapped off through the two-way tap, A. The apparatus has previously been recommended by the Sub-Committee for the controlled oxidation of organic matter with mixed nitric, perchloric and sulphuric acids.[1] Gorsuch has shown that hydrochloric acid produced by the reduction of perchloric acid with organic matter causes volatilisation of mercury.[2] The Sub-Committee

* Since resigned

decided to study the use of nitric and sulphuric acids alone for the wet digestion of samples before mercury determinations.

A series of radiochemical experiments was carried out in one laboratory in which mercury labelled with mercury-203 was added to cocoa, butter and margarine. In the first of these, 5-g samples of cocoa, each containing 2 μg of labelled mercury, were wet digested with nitric and sulphuric acids. After the initial reaction had subsided, and the cocoa had dissolved, the mixture was heated to full reflux for 1 hour.

During the subsequent digestion, nitric acid distilled into the reservoir and when charring occurred a little of the nitric acid was run back into the flask to clear the solution. When no further charring occurred, the whole of the distillate was run back into the flask, heated under reflux for 10 minutes, and distilled to fumes of sulphuric acid. The distribution of the mercury between the distillate and digestate was determined by a radiochemical counting method. The results of four determinations are shown in Table I.

TABLE I

RECOVERY OF MERCURY FROM WET DECOMPOSITION OF COCOA

Test number	Mercury in digestate, per cent.	Mercury in distillate, per cent.
1	81	16
2	83	17
3	81	14
4	86	15

In two of these experiments the distillate was collected as three equal fractions and the mercury content of each fraction was measured. The results are shown in Table II.

In two further experiments the residue was removed from the flask and the distillate returned to it. A further 5 ml of sulphuric acid were added to the distillate and after being heated under reflux for 15 minutes the mixture was distilled just to fumes of sulphuric acid. The distribution of activity between the digestate and the distillate was then measured, and the results are shown in Table III.

TABLE II

DISTRIBUTION OF MERCURY IN THE DISTILLATE

Test number	Mercury in fraction, per cent.		
	Fraction 1	Fraction 2	Fraction 3
3	3	5	6
4	3	5	7

TABLE III

DISTRIBUTION OF MERCURY AFTER RE-TREATMENT OF DISTILLATE

Test number	Mercury in digestate, per cent.	Mercury in distillate, per cent.
5	83	17
6	76	24

NOTE—The percentages refer to the mercury content of the first distillate, and not to the original amount of added mercury.

The samples of butter and magarine were treated similarly, 5 μg of labelled mercury being added to each, except that the digestate could not be cleared in the final stages of charring by running back the nitric acid distillate. The nitric acid was therefore run off and reserved (distillate 1) and 10 ml of fresh nitric acid were added to complete the oxidation. When this was complete distillate 1 was returned to the flask and the whole mixture was heated under reflux for 10 minutes and then distilled to fumes. The mercury contents of the digestate and distillate were determined and the results are shown in Table IV.

TABLE IV

RECOVERY OF MERCURY FROM WET DECOMPOSITION OF BUTTER AND MARGARINE

Test number	Sample	Mercury in digestate, per cent.	Mercury in distillate, per cent.
7	Margarine	87	10
8	Margarine	84	14
9	Butter	88	9
10	Butter	89	6

The possible loss of mercury from large aqueous samples was also investigated radiochemically. Labelled mercury (5 μg) in the chloride form was added to 100 ml of distilled water and mixed with 25 ml of nitric acid and 10 ml of sulphuric acid. The solution was distilled and fractions of the distillate were collected and counted. The results are shown in Table V.

TABLE V

DISTILLATION OF MERCURY FROM AQUEOUS SOLUTION

Fraction number	Volume of fraction, ml	Mercury in fraction, per cent.
1	50	5
2	50	11
3	25	19
4	Residue	70

Losses of mercury by volatilisation were also determined by a series of collaborative experiments in which 5-μg amounts of mercury, as both mercuric chloride and phenylmercuric acetate, were added to 5-g samples of whole hens' egg. The samples were wet digested with nitric and sulphuric acids in the apparatus described. When digestion was complete, the solutions were distilled to fumes, and the mercury contents of the residues were determined by dithizone. (At this stage of the investigations, the choice of the separation and determination procedure was left to the discretion of each collaborator.) The results of these tests are shown in Table VI.

TABLE VI

RECOVERIES OF MERCURY FROM WET DIGESTATES OF EGGS

Laboratory	Mercury added as—	Mercury recovered, μg	Recovery, per cent.
A	Mercuric chloride	2·65	53
	Mercuric chloride	2·4	48
B	Mercuric chloride	3·8	76
	Mercuric chloride	3·7	74
	Mercuric chloride	2·7	54
	Phenylmercuric acetate	5·3	106
	Phenylmercuric acetate	5·3	106
	Phenylmercuric acetate	4·1	82
C	Mercuric chloride	3·0	60
	Mercuric chloride	3·7	74
	Mercuric chloride	3·2	64
	Phenylmercuric acetate	4·4	88
	Phenylmercuric acetate	2·5	50
	Phenylmercuric acetate	1·5	30
D	Mercuric chloride	4·5	90
	Mercuric chloride	4·7	94
	Phenylmercuric acetate	4·3	86
	Phenylmercuric acetate	4·7	94

In one of the collaborating laboratories (laboratory B) the mercury contents of the distillates were also determined. The results are shown in Table VII, together with the mercury contents of the digestates.

TABLE VII

DISTRIBUTION OF MERCURY FROM WET DIGESTIONS OF EGGS BETWEEN DIGESTATES AND DISTILLATES

Mercury added as—	Mercury in digestate, per cent.	Mercury in distillate, per cent.	Total recovery of mercury, per cent.
Mercuric chloride	76	12	88
Mercuric chloride	74	22	96
Mercuric chloride	54	28	82
Phenylmercuric acetate	84	12	96

Fat—During each radiochemical investigation of the wet oxidation of butter and margarine described above, a small amount of fat (about 200 mg) distilled into the reservoir. The undigested fats were separated from distillates 9 and 10 and their mercury contents were determined. The results indicated that less than 0·1 per cent. of the mercury was present in the fat.

The radiochemical check of the separation procedure carried out on cocoa (p. 524) also showed that a negligible proportion of mercury was associated with undigested fat (see Table XVI).

Chlorinated compounds—Gorsuch has shown that hydrochloric acid produced during wet oxidations with perchloric acid leads to enhanced volatility of mercury.[2] The hydrochloric acid was produced by the reduction of the perchloric acid by organic matter. In view of these findings, the Sub-Committee decided to carry out recovery experiments on chlorinated organic compounds, which would also be expected to produce hydrochloric acid under the conditions of wet oxidation.

In one of the collaborating laboratories, 5 g of aldrin to which 5 μg of mercury had been added was tested by the recommended procedure and 4·2 μg of mercury were recovered. In another laboratory, two 5-g amounts of *p*-chloroaniline were tested, one containing 1 μg and the other 2 μg of added mercury. The recoveries of mercury were 0·98 and 2·0 μg, respectively.

It was concluded that the recommended procedure is suitable for determining mercury in chlorinated compounds.

SUMMARY—

(*i*) Appreciable amounts of mercury are volatilised during wet decomposition. Volatilisation of mercury also occurs when an acidified aqueous solution containing inorganic mercury is evaporated. The rather large variations between the low recoveries reported in Table VI are probably due to variations of the heating time after the appearance of fumes of sulphuric acid, and hence to temperature variations.

The results indicate the need for collecting the distillate from the wet decomposition. The mercury content of the distillate could be determined separately or the distillate could be combined with the digestate on completion of the oxidation and the mercury determination carried out on the resulting solution.

(*ii*) Any small amount of fat remaining undigested after wet oxidation may be removed before proceeding with the mercury determination, because a negligible amount of the mercury is present in the fat.

(*iii*) The recommended wet-oxidation procedure is suitable for the determination of mercury in organic chloro-compounds.

CONCLUSIONS—

In order to overcome losses of mercury by volatilisation the Sub-Committee decided to use the apparatus shown in Fig. 1, and to combine the distillate and digestate after the completion of the wet decomposition.

The recommended method (see Appendix I) therefore supersedes the wet-decomposition procedure previously described by the Sub-Committee,[1] in which the decomposition could be controlled less readily. The proposed method (*a*) affords a more convenient means of adding nitric acid to maintain oxidising conditions during the decomposition, and (*b*) avoids losses of mercury through distillation. The earlier method was recommended for mercury contents in excess of 5 p.p.m. and it was reported that at much lower levels (0·1 to 0·01 p.p.m.)

the method failed to give good recoveries.[1] The method now recommended is satisfactory for the determination of mercury in urine; however, when this determination is to be carried out on a routine basis, a method involving oxidation with potassium permanganate would undoubtedly be more convenient.

Separation and determination of mercury

The Sub-Committee proposed to use dithizone for both the separation of mercury from the wet digest and its determination. The separation and determination procedure consists of the following stages—

(*i*) Extraction of mercury from the wet digest with excess of dithizone.
(*ii*) Back extraction of the mercury into 0·1 N hydrochloric acid, nitrous acid being used to destroy the mercuric dithizonate.
(*iii*) Re-extraction of the mercury with dithizone.
(*iv*) Determination.

The investigation of each of these stages is described below, but before proceeding with these experiments it was necessary to select and study a suitable method for the final determination (stage (*iv*)).

Choice of method for determination of mercury

Three methods were considered for the determination of mercury. The method of mixed-colour titration[3] in which a chloroform solution of dithizone is used can, with practice, be used to determine small amounts of mercury with a sensitivity at least equal to that obtainable by spectrophotometry. In this method, the mercury is extracted with dithizone solution until a "mixed colour" is produced, showing that the mercury has been extracted and the organic phase contains a slight excess of dithizone. The same amount of dithizone solution is added to the reagent blank solution, which is then titrated with a standard mercury solution until the colour of the organic layer matches that of the test. The volume of mercury solution required is a measure of the mercury content of the sample.

The Sub-Committee found this method reliable for determining amounts of mercury within the range 0·5 to 5·0 μg, but decided to adopt instead a spectrophotometric method that would be more convenient in practice, particularly for handling large numbers of samples.

In the spectrophotometric method of Milton and Hoskins,[4] mercury is extracted from 0·1 N hydrochloric acid solution with a solution of dithizone in chloroform. Extraction is carried out with successive small volumes of dithizone solution until the last extract produces a mixed colour instead of the characteristic orange colour of mercuric dithizonate, showing that all the mercury has been removed from the aqueous phase. In the presence of copper, the last extract produces a purplish mixed colour instead of a greenish one, since after the mercury has been extracted the copper begins to form its dithizonate. The method thus provides a means of separating mercury from limited amounts of copper. The extracts are combined and the excess of dithizone is removed by washing with ammonia. After dilution to a known volume with chloroform, the optical density of the organic phase is measured at a wavelength of 490 mμ.

The method of Rolfe, Russell and Wilkinson[5] is similar, except that the mercury is extracted in a toluene solution of dithizone.

Preliminary tests carried out by the Sub-Committee confirmed the accuracy and reliability of both these methods, and the principle of the method of Milton and Hoskins was preferred because of the practical advantage of using an organic solvent denser than water. This method was therefore studied further and modified to adapt it to the purposes of the Sub-Committee. The further study of the method and its modifications are described below.

It was also decided to add the extracts to dilute acetic acid solution to stabilize the mercuric dithizonate against light, as recommended by Reith and Gerritsma.[6]

Choice of experimental conditions for colorimetric method

Effect of washing the extracts with ammonia—

Small amounts of mercury were extracted from 0·1 N hydrochloric acid solutions with small portions of a 0·005 per cent. solution of dithizone in chloroform. Each extraction was continued until a mixed colour was produced, the excess of dithizone was then removed from the combined

extracts by washing with dilute ammonia solution (1 + 19) and the organic phase was diluted to 25 ml with chloroform. The optical densities of the chloroform solutions of mercuric dithizonate prepared in this way were measured against a similarly prepared blank solution, in 1-cm cells, at a wavelength of 492 mμ.

A parallel series of experiments was carried out in which the excess of dithizone was not removed, but the combined extracts from each mercury extraction were diluted to 25 ml with chloroform and the optical densities were measured as described above. The results of these measurements are shown in Table VIII.

It can be seen that the omission of the ammonia wash results in a small increase in the optical density, but in both series Beer's law is obeyed, and there appears to be no advantage in washing the extracts with ammonia solution.

TABLE VIII

EFFECT OF WASHING THE EXTRACTS WITH AMMONIA SOLUTION

Concentration of mercury in the chloroform extract, μg per ml	Optical densities	
	Washed with ammonia solution	Not washed with ammonia solution
0·4	0·123	0·133
0·8	0·253	0·266
1·2	0·366	0·385
1·6	0·500	0·530
2·0	0·627	0·665

EFFECT OF SOLVENT—

Two series of experiments were carried out to examine the effect of the solvent on the sensitivity of the method. In one series, amounts of mercury in the range 1 to 10 μg were each extracted from 0·1 N hydrochloric acid solution with a 0·001 per cent. solution of dithizone in chloroform until a mixed colour was produced. Each extract was then diluted to 4·0 ml with chloroform and its optical density was measured against a similarly prepared blank solution in 1-cm cells, at a wavelength of 492 mμ, the wavelength of maximum light absorption. The other series of experiments was performed similarly, except that a carbon tetrachloride solution of dithizone was used, and the optical density measurements were made at 485 mμ, which is the wavelength of maximum light absorption of mercuric dithizonate in carbon tetrachloride.

The results of these two series of measurements are shown in Table IX.

TABLE IX

COMPARISON OF OPTICAL DENSITIES OF MERCURIC DITHIZONATE IN CHLOROFORM AND CARBON TETRACHLORIDE

Concentration of mercury in the organic extract, μg per ml	Optical densities	
	In carbon tetrachloride at 485 mμ	In chloroform at 492 mμ
0·25	0·083	0·080
0·5	0·163	0·161
1·0	0·323	0·321
1·5	0·481	0·480
2·0	0·642	0·639
2·5	0·804	0·800

The results in Table IX show that the molar extinction coefficients of mercuric dithizonate in chloroform and carbon tetrachloride are similar and that Beer's law is obeyed whichever solvent is used.

EFFECT OF COPPER—

The only metals other than mercury that will react appreciably with dithizone in 0·1 N hydrochloric acid solution are copper and the noble metals. Series of tests were carried out in which 1-μg amounts of mercury were extracted with dithizone in the presence of different amounts of copper. Both carbon tetrachloride and chloroform were used as solvents for the dithizone and in each case a 0·001 per cent. solution of the reagent was used.

With a carbon tetrachloride solution of dithizone, as little as 5 μg of copper gave a red colour with dithizone after the mercury had been extracted, instead of the green colour that is normally produced, but it was possible to separate mercury from the copper. In extractions with the chloroform solution of dithizone, 15 μg of copper had no effect on the extraction of 1 μg of mercury and 25 μg of copper caused a red extract to be produced *after* the mercury had been extracted. These observations confirm the findings of Milton and Hoskins,[4] and Irving, Andrew and Risdon[7] that the separation of mercury and copper is better with chloroform than with carbon tetrachloride.

Similar series of tests were carried out in which 1 ml of a 2·5 per cent. solution of EDTA was added to each test before the mercury was extracted as recommended by Fabre, Truhaut and Boudène.[3] When a carbon tetrachloride solution of dithzone was used, 60 μg of copper had no effect on the mercury extraction, and 100 μg caused red cupric dithizonate to form after the mercury had been extracted. In extractions for which a chloroform solution of dithizone was used, at least 600 μg of copper had no effect on the extraction of mercury.

It was decided, therefore, to carry out the final extraction of mercury in the presence of EDTA as this enhances the separation of mercury and copper. Also, it was decided to recommend the use of chloroform as the solvent for the final colorimetric determination for samples containing relatively large amounts of copper.

Study of separation and determination procedure

Final determination: stage (*iv*)—

Having extracted mercury from the diluted wet digestate with excess of dithizone, it was proposed to destroy the mercuric dithizonate with acidified sodium nitrite solution to reverse the mercury to the aqueous phase, discard the organic layer, add hydroxylammonium chloride and urea to the aqueous solution to destroy the excess of nitrite and any remaining oxides of nitrogen, and complete the mercury determination by the proposed colorimetric method. This final stage of the procedure was investigated as follows.

Calibration—

Calibration figures were obtained for the colorimetric determination of 1-, 2- and 5-μg amounts of mercury. A series of volumes of standard mercury solution (1 μg of mercury per ml, prepared from mercuric chloride in 0·1 N hydrochloric acid) were diluted to 10 ml with 0·1 N hydrochloric acid. To each was added 1 ml of 20 per cent. hydroxylammonium chloride solution, 1 ml of 10 per cent. urea solution and 1 ml of 2·5 per cent. EDTA solution. Each was extracted with 0·001 per cent. dithizone solution in carbon tetrachloride and the extract was diluted to 4·0 ml, as described under "Effect of Solvent," p. 520. The optical densities were measured in 1-cm cells against a reagent blank. The results are shown in Table X.

Table X

Calibration figures obtained by the proposed method

Mercury taken, μg	1	1	1	2	2	2	5	5	5
Optical density at 485 mμ	0·080	0·082	0·083	0·170	0·170	0·169	0·417	0·419	0·420

These results showed that Beer's law was obeyed and that the method had satisfactory precision.

Use of urea—

The use of urea as well as hydroxylammonium chloride for the destruction of nitrogen oxides was an additional safeguard that was introduced in view of the observations of Fabre, Truhaut and Boudène that hydroxylammonium chloride and oxidising nitrogen compounds can co-exist in solution.[3] During the later investigations of the Sub-Committee, there was some uncertainty as to whether the use of urea formed an essential part of the method and it was therefore investigated by one of the collaborating laboratories.

Known amounts of mercury were each extracted from decinormal hydrochloric acid solutions with 10 + 1 + 1 ml portions of a 0·001 per cent. solution of dithizone in carbon tetrachloride. Each combined extract was treated with 10 ml of 0·1 N hydrochloric acid and 1 ml of 5 per cent. sodium nitrite solution and the organic layer was discarded. The

mercury content of the aqueous solution was determined by the recommended colorimetric procedure, both hydroxylammonium chloride and urea being added to the solution.

A parallel series of experiments was carried out similarly except that the urea was omitted in the final stage of the determinations.

The results of these experiments are given in Table XI.

TABLE XI

RECOVERIES OF MERCURY IN THE PRESENCE AND ABSENCE OF UREA

		Urea present			Urea absent		
Mercury added, μg	Calibration, optical density	Optical density	Recovery of mercury, per cent.	Mean recovery of mercury, per cent.	Optical density	Recovery of mercury, per cent.	Mean recovery of mercury, per cent.
2	0·163	0·161	100		0·174	109	
2		0·153	96	99·3	0·166	104	104·0
2		0·164	102		0·160	99	
5	0·404	0·407	102		0·430	108	
5		0·386	96	98·3	0·439	110	108·0
5		0·389	97		0·426	106	

These results show that apparently high results are obtained in the absence of urea, and it was therefore the view of the Sub-Committee that the use of urea should be retained in the recommended procedure.

RE-EXTRACTION OF MERCURY DITHIZONATE: STAGE (*iii*)—

Calibration results for 1- and 2-μg amounts of mercury were repeated in the same way except that 1 ml of 5 per cent. sodium nitrite solution was added before the hydroxylammonium chloride solution, and the solution was set aside for 15 minutes before proceeding further. The optical densities obtained for two 1-μg amounts of mercury were 0·083 and 0·083, and for two 2-μg amounts 0·172 and 0·173, showing that the addition of sodium nitrite and its subsequent destruction had no effect on the recovery of mercury. The results also confirmed that the conditions selected were suitable for the quantitative destruction of nitrite ions.

BACK EXTRACTION INTO 0·1 N HYDROCHLORIC ACID: STAGE (*ii*)—

It was proposed to dilute the combined digestate and distillate from the wet decomposition to about N in mineral acid and then remove the mercury by extraction with an excess of dithizone solution in carbon tetrachloride or chloroform, and to revert the mercury to the aqueous phase by destruction of its dithizonate with sodium nitrite solution in 0·1 N hydrochloric acid.

Known amounts of mercury were extracted from a series of solutions containing 10 ml of 0·1 N hydrochloric acid by extracting each solution first with 10 ml and then with 1 ml of 0·001 per cent. dithizone solution in carbon tetrachloride. The combined extracts from each solution were treated with 10 ml of 0·1 N hydrochloric acid and 1 ml of 5 per cent. sodium nitrite solution, the mixture was shaken for 1 minute, the layers were allowed to separate, and the lower layer was discarded. Then 1 ml of 20 per cent. hydroxylammonium chloride solution was added, the solution was set aside for 15 minutes, urea and EDTA were added, and the determination of mercury was completed as before. The results are shown in Table XII.

TABLE XII

RECOVERIES OF MERCURY AFTER BACK-EXTRACTION STAGE

Mercury taken, μg	.. ..	1	1	2	2	5	5
Optical density at 485 mμ	..	0·083	0·083	0·168	0·172	0·410	0·424

The results were quite satisfactory and showed that the destruction of the first dithizone extract with nitrous acid and the back-extraction of the mercury into the aqueous phase were quantitative.

EXTRACTION OF MERCURY FROM THE WET DIGEST: STAGE (*i*)—

A series of solutions containing known amounts of mercury was prepared, each containing 5 ml of sulphuric acid, sp.gr. 1·84, and 18 ml of nitric acid, sp.gr. 1·42, diluted to 400 ml with water. The solutions were prepared to simulate wet digestates that had been diluted to be approximately N in total acidity. Each solution was heated to boiling, treated with 40 ml of 20 per cent. hydroxylammonium chloride solution, set aside for 15 minutes, cooled to room temperature and transferred to a 500-ml separating funnel.

Mercury was separated from each solution by extraction with 0·001 per cent. dithizone solution in carbon tetrachloride, the dithizonate in the combined extracts was decomposed with nitrite and the mercury was determined colorimetrically as previously described. The results of these experiments are shown in Table XIII, together with calculated percentage recoveries based on the calibration figures of Table X.

TABLE XIII

RECOVERIES OF MERCURY FROM SIMULATED WET DIGESTATES

Mercury taken, μg	Optical density	Average optical density	Average recovery of mercury, per cent.
1	0·078		
1	0·085	0·078	95·4
1	0·072		
2	0·168		
2	0·163	0·166	97·6
2	0·168		
5	0·390		
5	0·419	0·410	98·0
5	0·421		

These results are slightly low and it was the opinion of the Sub-Committee that this was associated with the large volumes of aqueous solution from which mercury had been separated.

Accordingly, a further series of simulated wet digestates containing mercury was prepared, each containing the same amounts of nitric and sulphuric acids as before, but diluted to 100 ml with water. Each solution was neutralised with about 40 ml of ammonia solution, sp.gr. 0·910, and then made N with respect to sulphuric acid by adding 4 ml of sulphuric acid, sp.gr. 1·84. The solutions were boiled, treated with 5 ml of 20 per cent. hydroxylammonium chloride solution, and the mercury separated and determined as before. The results of these experiments are shown in Table XIV.

TABLE XIV

RECOVERIES OF MERCURY FROM PARTIALLY NEUTRALISED SIMULATED WET DIGESTATES

Mercury added, μg	Optical density	Average optical density	Average recovery of mercury, per cent.
1	0·081		
1	0·082	0·082	100·2
1	0·083		
2	0·170		
2	0·173	0·170	100·0
2	0·168		
5	0·439		
5	0·427	0·431	102·6
5	0·428		

For work of the highest accuracy it may be advisable, therefore, to limit the volume of the solution prepared from the wet digestate by neutralising and re-acidifying to N with sulphuric acid. Nevertheless, it was the view of the Sub-Committee that this need not form a part of the recommended procedure, since the results obtained by simply diluting the digestate, shown in Table XII, are sufficiently accurate for most purposes.

USE OF CHLOROFORM AS SOLVENT FOR EXTRACTION IN STAGE (*i*)—

Since a chloroform solution of dithizone can be used for the final colorimetric determination of mercury, it was decided to investigate the possibility of using the same solution for the initial extractions of mercury from wet digestion mixtures.

A series of solutions was prepared to simulate wet digestates after dilution. Each solution contained 5 ml of sulphuric acid, sp.gr. 1·84, and 18 ml of nitric acid, sp.gr. 1·42, and sufficient water to produce a volume of 400 ml. A known amount of mercury was then added to each solution, which was then heated to boiling, treated with 40 ml of 20 per cent. hydroxylammonium chloride solution and allowed to cool.

The mercury was extracted from each solution with 10 + 1 + 1 ml of a 0·001 per cent. solution of dithizone in chloroform. The determinations were completed by the recommended procedure with carbon tetrachloride as the solvent in the final stage of the colorimetric method.

The recoveries of mercury obtained in this way are shown in Table XV and are compared with those obtained similarly but with carbon tetrachloride as the solvent for the initial extraction (taken from Table XIII).

TABLE XV

RECOVERIES OF MERCURY FROM SIMULATED WET DIGESTATES WITH CHLOROFORM AS SOLVENT FOR THE INITIAL EXTRACTIONS

Mercury added, μg	Calibration, optical density	Chloroform solvent: Optical density	Chloroform solvent: Recovery of mercury, per cent.	Chloroform solvent: Mean recovery of mercury, per cent.	Carbon tetrachloride solvent: mean recovery of mercury, per cent.
1	0·082	0·075	92		
1		0·078	95	92·5	95·4
1		0·073	90		
2	0·163	0·153	94		
2		0·159	98	96·6	97·6
2		0·159	98		
5	0·407	0·417	102		
5		0·403	99	100·0	98·0
5		0·402	99		

The results obtained with the chloroform solution of dithizone compared favourably with those obtained with carbon tetrachloride as solvent, except at the 1-μg level.

A radiochemical check of the 1-μg results was carried out similarly. The chloroform solution of dithizone extracted 93·6 per cent. and 94·4 per cent. of the mercury from the digestion mixture. These results confirmed the lower efficiency of this solvent for the extraction of smaller amounts of mercury.

RADIOCHEMICAL CHECK OF THE DIGESTION AND SEPARATION PROCEDURES—

The efficiency of the recommended procedure was checked in one of the collaborating laboratories by a radiochemical counting method in which mercury labelled with mercury-203 was used. The results obtained are shown in Table XVI and compare well with those reported above.

TABLE XVI

RADIOCHEMICAL CHECK OF THE PROCEDURE

Ten grams of cocoa containing 0·4 p.p.m. of labelled mercury and wet digested with nitric and sulphuric acids was used

Stage investigated	Mercury found, per cent.
Mercury present in combined digestate and distillate after oxidation	96
Mercury extracted with 5 ml of carbon tetrachloride (used to remove fat)	Negligible
Mercury remaining in aqueous phase after extraction with dithizone: stage (*i*)	1·3
Mercury remaining in organic phase after back extraction: stage (*ii*)	2
Mercury present in aqueous phase after back extraction	93

SUMMARY

These experiments show that good recoveries of small amounts of mercury, down to 1 μg, are obtained by the recommended separation and determination procedure. The

method developed from considerations of these results and those described under "Preparation of the Sample" is outlined below.

It was generally accepted that dithizone in chloroform was less satisfactory for the initial extraction of mercury than was a solution of dithizone in carbon tetrachloride. It was agreed to recommend the use of a solution of dithizone in carbon tetrachloride for both extractions, except in the presence of relatively large amounts of copper, for which purpose a solution of dithizone in chloroform is preferable for the second extraction.

OUTLINE OF THE METHOD—

The proposed method, based on the above experiments, consists of the following stages—

(*a*) The sample is oxidised with nitric and sulphuric acids and the distillate from this oxidation is collected and subsequently combined with the residue.

(*b*) The combined digestate is diluted until it is approximately N in acid. It is then treated with hydroxylammonium chloride to remove oxides of nitrogen.

(*c*) The mercury is extracted with excess of dithizone in carbon tetrachloride. Any copper present in the sample will be extracted at this stage with the mercury.

(*d*) The dithizone in the extract is destroyed with sodium nitrite in 0·1 N hydrochloric acid. The mercury reverts to the aqueous phase and the organic phase is discarded.

(*e*) After treatment with hydroxylammonium chloride, urea and EDTA, the mercury is extracted with successive small portions of a solution of dithizone in carbon tetrachloride. Mercury is separated from copper at this stage. The extracts are combined and diluted to 4·0 ml with carbon tetrachloride.

If more than 60 μg of copper is present, it is recommended that the extraction be carried out with a solution of dithizone in chloroform and the combined extracts diluted to 4·0 ml with chloroform.

(*f*) The mercury is determined by spectrophotometric measurement of the carbon tetrachloride solution at a wavelength of 485 mμ, or of the chloroform solution at a wavelength of 492 mμ.

TESTS OF PROPOSED METHOD—

(*a*) *Series* 1—Collaborative tests for the determination of mercury were made on samples of milk powder to which known amounts of mercury were added as (*i*) mercuric chloride and (*ii*) phenylmercuric acetate. The results are shown in Table XVII.

TABLE XVII

DETERMINATION OF MERCURY ADDED TO MILK POWDER

	Mercury (Hg) added as mercuric chloride			Mercury (Hg) added as phenylmercuric acetate		
Laboratory	Amount added, μg	Amount recovered, μg	Recovery, per cent.	Amount added, μg	Amount recovered, μg	Recovery, per cent.
A	1	0·90	90	0·96	0·95	99
	1	0·85	85	0·96	0·98	102
	1	0·82	82			
	1	0·88	88			
	2	1·80	90	1·92	1·88	98
	2	1·95	98	1·92	2·00	104
	2	2·09	105			
	2	1·82	91			
	3	2·82	94	2·88	2·57	89
	5	4·75	95	2·88	2·69	93
	5	4·46	89			
	5	4·57	91			
B	5	4·3	86	2·0	1·9	95
	5	4·4	88			
C	1	0·85	85	1·03	1·09	106
	5	4·44	89	5·16	4·69	91

Quite good results were obtained except that at the 1-μg level the recoveries when mercuric chloride was used were significantly lower then those of the higher levels, and also lower than the corresponding results for phenylmercuric acetate. It was considered that,

since the 1-μg results for mercuric chloride were the first that had been obtained by the proposed method, these low recoveries could simply have been due to lack of experience with the technique. Consequently, this work was repeated by one of the laboratories. It was indeed found that quantitative recoveries were obtainable at the 1-μg level of mercury added as mercuric chloride. The results are shown in Table XVIII.

TABLE XVIII

FURTHER DETERMINATION OF MERCURY ADDED TO MILK POWDER

Carried out by collaborating laboratory A: 1·0 μg of mercury added as mercuric chloride to each test

Test number	1	2	3	4	5	6
Mercury recovered, μg	0·96	1·02	1·03	1·00	0·98	0·99

(*b*) *Series* 2—One of the collaborating laboratories carried out recovery experiments on amounts of mercury added to 10-g samples of cocoa. The mercury was added as mercuric chloride. Initially, when the acid mixture was added to the sample, much frothing occurred, and the mixture was set aside for 10 minutes for this to subside before the mixture was heated. After digestion, some fat remained in the mixture and this was removed by extraction with carbon tetrachloride before proceeding with the mercury determination. In the separation stage, some copper was extracted with the mercury, imparting a mauve hue to the extract. There was no interference from copper in the subsequent mercury determination. The results of these tests are shown in Table XIX.

TABLE XIX

DETERMINATION OF MERCURY ADDED TO COCOA

Mercury added, μg	Mercury recovered, μg	Recovery, per cent.
1	0·89	89
1	0·83	83
1	0·89	89
2	2·04	102
2	1·84	92
2	1·78	89
5	4·25	85

Another laboratory carried out a radiochemical tracer determination of cocoa, adding 4 μg of mercury labelled with mercury-203. A recovery of 93 per cent. was obtained.

(*c*) *Series* 3—One laboratory carried out recovery tests by adding mercury, as mercuric chloride solution, to 5-g samples of pure lard. Digestion was carried out as before, and the oxidation took 7 hours to complete. Frothing occurred during the early stages of the oxidation, but it was controllable. Only a few drops of fat remained at the end of the oxidation and it was removed by extraction with carbon tetrachloride. The results are shown in Table XX.

TABLE XX

DETERMINATION OF MERCURY ADDED TO LARD

Mercury added, μg	Mercury recovered, μg	Recovery, per cent.
1	1·04	104
1	1·04	104
1	0·97	97
2	2·05	103
2	1·90	95
2	1·86	93

CONCLUSION

1. The recommended procedure described in the Appendix has been shown to give reliable results for the mercury content of organic matter down to 0·1 p.p.m., calculated with reference to the dried sample.

2. The sensitivity of the method is such that it should be possible to reduce the lower level of mercury determined to 0·05 p.p.m., calculated with reference to the dried sample.

Appendix

Recommended method for the determination of small amounts of mercury

Principle of method—

After destruction of the organic matter by wet oxidation with nitric and sulphuric acids, in the apparatus shown in Fig. 1, dilution of the resulting solution to give an acid concentration of approximately N, and reduction with hydroxylammonium chloride to destroy oxides of nitrogen, the mercury is separated by extraction with an excess of a solution of dithizone in carbon tetrachloride.

The mercury is removed from this extract and returned to the aqueous phase by oxidation with sodium nitrite in 0·1 N hydrochloric acid solution. Excess of nitrite is destroyed with hydroxylammonium chloride, and any remaining oxides of nitrogen are removed by treating the solution with urea. After the addition of EDTA, which hinders the reaction of copper with dithizone, mercury is extracted titratively with a solution of dithizone in carbon tetrachloride. The combined extracts are diluted to a standard volume of 4·0 ml by adding carbon tetrachloride, and the content of mercury in the sample is determined by measuring the optical density of this solution against a reagent blank, in 1-cm cells, at a wavelength of 485 mμ, and reference to a calibration graph.

In the presence of more than 60 μg of copper, it is recommended that the final colorimetric determination should be made with a solution of dithizone in chloroform instead of in carbon tetrachloride (see p. 521). In this instance, the combined extract is diluted to 4·0 ml with chloroform and measurement is made at a wavelength of 492 mμ. In other respects the procedure is identical with that described below.

Range—

For mercury contents down to 0·5 μg (as Hg) in the sample taken.

Applicability—

The method is suitable for the analysis of most types of organic materials. The method is specific for mercury in all ordinary circumstances. At least 60 μg of copper can be present when carbon tetrachloride is used, and at least 600 μg of copper can be present when chloroform is used in the final colorimetric determination, without interfering. The possibility of interference from noble metals such as gold, palladium and platinum, has not been investigated.

Apparatus—

Digestion apparatus—See "Destruction of Organic Matter," page 528.

Separating funnels, 150-, 500- and 1000-ml capacity—Pear-shaped separating funnels with well fitting glass stopcocks and stoppers.

Note—

All glassware should be of borosilicate glass and must be thoroughly cleaned with nitric and sulphuric acids, and then washed with distilled water, immediately before use.

Reagents—

All water must be distilled or de-mineralised and free from mercury or other impurities that react with dithizone.

The acids supplied as "low in lead" or "for foodstuffs analysis" are suitable for the determination of mercury without further treatment.

Other reagents used should be of analytical-reagent quality. Certain of the reagent solutions used may be purified in order to reduce blank values and so increase the accuracy of the method at low levels of mercury. The purification of hydroxylammonium chloride solution is described.

Sulphuric acid, sp.gr. 1·84.

Nitric acid, sp.gr. 1·42.

Hydroxylammonium chloride solution—Prepare a 20 per cent. w/v solution in water and purify it as follows. Transfer the solution to a separating funnel. Add a few millilitres of dithizone stock solution, shake for 2 minutes, and allow the layers to separate. Reject the organic layer. Repeat the extraction with dithizone until the organic layer has the colour of pure dithizone solution. Finally, extract the solution with successive small amounts of chloroform until the extracts are colourless, and discard the extracts.

Dithizone stock solution—Prepare a 0·05 per cent. w/v solution in chloroform. This solution should be stored in a dark glass bottle in a refrigerator.

NOTE—

Commercially available analytical-reagent grade dithizone can usually be used without purification. If, however, purification of the reagent is considered to be desirable, the reagent solution may be prepared as follows.

Dissolve 0·1 g of dithizone in 150 ml of chloroform in a separating funnel and shake for 10 minutes. Filter the solution through an ashless filter-paper into a second separating funnel, add about 100 ml of approximately 0·1 N ammonia solution and shake vigorously for 1 minute. Allow the layers to separate and run the organic layer back into the first separating funnel and shake it for 1 minute with a further 100-ml portion of approximately 0·1 N ammonia solution. Discard the organic phase and combine the ammoniacal solutions in a large separating funnel. Wash the solution with three successive 5-ml portions of chloroform and discard the washings. Add 200 ml of chloroform, neutralise with approximately N sulphuric acid and add 10 ml of the acid in excess. Extract the dithizone into the chloroform by shaking vigorously for 2 minutes, allow to separate, and run the organic layer through a dry, ashless filter-paper into a dark-glass bottle. Store the solution in a refrigerator.

Dilute dithizone solution in carbon tetrachloride—Dilute 2 ml of the stock solution to 100 ml with carbon tetrachloride.

Dilute dithizone solution in chloroform—Dilute 2 ml of the stock solution to 100 ml with chloroform.

NOTE—

Dilute dithizone solutions should be freshly prepared.

Hydrochloric acid solution, 0·1 N.*

Sodium nitrite solution, 5 *per cent. w/v, aqueous.**

Urea solution, 10 *per cent. w/v, aqueous.**

*EDTA solution**—Dissolve 2·5 g of EDTA (disodium salt dihydrate) in 100 ml of water.

Acetic acid solution, approximately 4 N.*

Carbon tetrachloride.

Chloroform.

Standard mercury stock solution—Dissolve 0·1354 g of mercuric chloride in 1 litre of 0·1 N hydrochloric acid.

1 ml of solution ≡ 100 μg of mercury (Hg).

Dilute standard mercury solution—Dilute 10 ml of the stock solution to 1 litre with 0·1 N hydrochloric acid.

1 ml of solution ≡ 1 μg of mercury (Hg).

This solution should be prepared freshly as required.

PROCEDURE

Reagent blank value—Carry out a blank test by the entire procedure; use the precise amounts of the reagents used in the test and omit only the sample.

DESTRUCTION OF ORGANIC MATTER—

The apparatus shown in Fig. 1 should be used for the wet decomposition of the sample. The method described below is suitable for the oxidation of most materials. Sample weights of up to about 10 g of dry solid can be oxidised by this procedure with 50 ml of nitric acid.

Care should be taken in applying the method of wet oxidation to samples containing fats since, although this method has been applied to several such substances, some workers who have possibly used other conditions have experienced explosive reactions.

Members of the Sub-Committee have also carried out wet digestions on 100-g samples of potatoes, equivalent to about 18 g of dry matter, and in this instance no water was added to the digestion mixture and 10 to 15 ml more nitric acid was needed.

Other procedures may be found more suitable for the wet oxidation of particular types of organic matter. For example, the destruction of sugars and other carbohydrates is facilitated by heating the sample under reflux with nitric acid and water for some time before cooling, adding sulphuric acid, and completing the digestion. Nevertheless, the apparatus described in this Report should be used throughout.

* These solutions can be purified as described for hydroxylammonium chloride solution.

Description of apparatus—The flask has a capacity of 250 ml and the reservoir, B, has a capacity of 150 to 200 ml. The condenser is a standard double-surface or spiral-surface reflux type. The thermometer is calibrated for temperatures up to 200° C and all the connections are made through standard ground-glass joints.

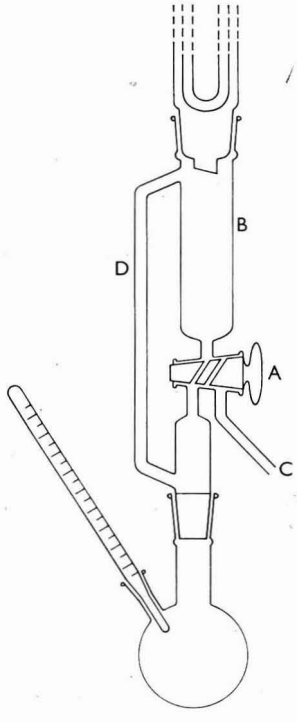

Fig. 1. Apparatus for the wet decomposition of organic matter

Procedure—Transfer a weighed amount of sample to the oxidation flask and add a cooled mixture of 20 ml of water, 5 ml of sulphuric acid and 50 ml of nitric acid. (If the sample is wet, reduce the volume of water added, and if the sample weight exceeds 10 g of dry solid add up to a further 5 ml of nitric acid for each gram of dry solid in excess.) Add a few anti-bumping granules or glass beads and assemble the apparatus as illustrated. Allow any initial reaction to subside and then heat, cautiously at first, collecting the distillate in the reservoir, B, with tap A closed. When the temperature indicated by the thermometer reaches 116° C (see Note 1), run off the contents of the reservoir through the drain-tube, C, and collect in a measuring cylinder.

Continue collecting the distillate in the reservoir and when the oxidation mixture darkens run a little of the distillate from the reservoir to the flask. Continue this procedure, maintaining a slight excess of nitric acid in the oxidation flask, until the solution ceases to darken and fumes of sulphuric acid are evolved. Allow the mixture to cool, run the contents of the reservoir into the flask and add to the first distillate in the measuring cylinder (see Note 2).

Titrate 1 ml of this solution with standard sodium hydroxide solution to determine the normality of acid present. Dilute with water to produce a solution with a total acidity of about N (see Note 3), heat to boiling, remove from the source of heat, and add rapidly, with mixing, a volume of hydroxylammonium chloride solution equal to one-tenth of the total bulk; then set aside for 15 minutes, and cool to room temperature.

NOTES—

1. This temperature is close to the boiling-point of nitric acid.
2. The volume of the residue *plus* distillate is usually about 80 to 90 ml.
3. The volume after dilution to N is about 400 ml.

SEPARATION OF MERCURY—

Transfer the solution to a separating funnel of suitable capacity and extract with carbon tetrachloride, if necessary, to remove any fat. Add 10 ml of dilute dithizone solution in carbon tetrachloride, shake for 1 minute, allow the layers to separate, and run the lower layer into a 150-ml separating funnel. Continue the extraction with successive 1-ml portions of dithizone solution until two successive extracts remain green, and combine the extracts in the second separating funnel.

Add 10 ml of 0·1 N hydrochloric acid and 1 ml of sodium nitrite solution, shake vigorously for 1 minute, allow the layers to separate and carefully discard the lower layer. Add 1 ml of hydroxylammonium chloride solution and set aside for 15 minutes, shaking occasionally. Add 1 ml of urea solution and 1 ml of EDTA solution.

DETERMINATION OF MERCURY—

Add 0·5 ml of dilute dithizone solution in carbon tetrachloride* from a 10-ml burette. Shake the funnel vigorously for 10 seconds and allow the layers to separate. Run the lower layer into another separating funnel containing 5 ml of 4 N acetic acid (see Note 4) and repeat the operation until the separated layer is greenish-orange; the shaking time should then be extended to 30 seconds and the increments of dithizone solution reduced to 0·2 ml. Continue the titration and separation, combining the extracts, until the organic layer has a greyish mixed colour, showing that the mercury has been extracted completely and that the extract contains a slight excess of dithizone: note the volume of dithizone solution required. From another 10-ml burette add sufficient carbon tetrachloride (or chloroform) to adjust the volume of the extract to 4·0 ml. Mix, dry the stem of the funnel, and run the lower layer through a small glass-wool plug, supported in a small glass funnel, into a 1-cm glass spectrophotometer cell. Measure the optical density at a wavelength of 485 mμ with the blank solution as reference.

Read the number of micrograms of mercury equivalent to the measured optical density from the calibration graph established as described below, and calculate the mercury content of the sample.

NOTE—

4. Solutions of mercuric dithizonate in organic solvents are sensitive to light. Exposure to daylight causes the solutions to fade, but the original colour is slowly restored if the faded solutions are kept in the dark, and is more rapidly restored on shaking with dilute acids. It has been shown by Reith and Gerritsma[6] that the light sensitivity is eliminated in the presence of acetic acid.

PREPARATION OF CALIBRATION GRAPH—

Transfer aliquots of dilute standard mercury solution to cover the range 0·5 to 10·0 μg of mercury to a series of separating funnels and dilute each to 10·0 ml, if necessary, by adding 0·1 N hydrochloric acid. Transfer 10·0 ml of 0·1 N hydrochloric acid to another separating funnel to be used as a blank solution. Treat each solution as described below.

Add 1 ml of sodium nitrite solution and 1 ml of hydroxylammonium chloride solution, mix, and set aside for 15 minutes. Add 1 ml of urea solution and 1 ml of EDTA solution and complete the extraction and measurement of each extract as described under "Determination of Mercury"; use the same solution of dithizone as was used in the tests.

Construct a graph relating optical density to the number of micrograms of mercury. The plot should be linear and should, on extrapolation, pass through the origin.

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* If copper is present a solution of dithizone in chloroform should be used, and final measurements should be made at 492 mμ.

Analytical Methods Committee

REPORT PREPARED BY THE PROPHYLACTICS IN ANIMAL FEEDS SUB-COMMITTEE

The Determination of Sulphaquinoxaline

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

REPORT

The Prophylactics Panel originally set up under the Chairmanship of Dr. R. F. Phipers by the Additives in Animal Feeding Stuffs Sub-Committee of the Analytical Methods Committee to study methods of analysis suitable for the determination of prophylactics in feeds medicated at low dose levels was reconstituted in 1963 as a Sub-Committee of the Analytical Methods Committee. The composition of the Panel and the Sub-Committee was Dr. R. F. Phipers (Chairman until 1964), Mr. N. C. Brown, Mr. P. J. Cooper, Dr. H. G. Dickenson, Mr. G. Drewery, Mr. A. W. Hartley, Mr. R. S. Hatfull, Mr. A. Holbrook, Mr. D. H. Mitchell, Mr. H. E. Monk, Mr. S. G. E. Stevens (Chairman since 1964), Mr. J. A. Stubbles and Mr. D. C. Thomas, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

A method for determining sulphaquinoxaline in medicated feeds has been described by Merwin.[1] This was based on extraction of the drug and determination after diazotisation and subsequent coupling with naphthylethylenediamine as recommended in the Bratton - Marshall technique.

When this study was initiated, the usual drug level was about 0·015 per cent., but the use of other active compounds in conjunction with sulphaquinoxaline has tended to reduce the level of the sulphonamide to below 0·01 per cent.

Drug-extraction procedures involving the use of hot aqueous alkali were unsatisfactory, since materials other than sulphaquinoxaline were also extracted, and these interfered with the results obtained by the Bratton - Marshall method. Difficulties also arose when the medicated feeds contained drugs other than sulphaquinoxaline that were capable of being diazotised and coupled. Acinitrazole and procaine from procaine penicillin are examples of such additives.

The efforts of the Sub-Committee have been directed towards establishing a satisfactory drug-extraction procedure and providing a concentration of sulphaquinoxaline suitable for the diazotisation and coupling reactions.

A preliminary study in which chloroform was used as the solvent for extracting the drug was made on two feeds medicated with sulphaquinoxaline at a level of approximately 0·0125 per cent. When this method was applied to a mixture having a wheatfeed basis, a mean recovery of 83 per cent. (range 74 to 93 per cent.) was obtained from ten analyses. For a medicated product containing grass meal, a mean recovery of 91 per cent. (range 46 to 109 per cent.) was found from twenty-one analyses. It might be considered that the results for the grass-meal samples were satisfactory, but tests revealed that extraneous material, equivalent to about one-third of the sulphaquinoxaline present, accounted for the apparent higher drug recoveries.

A study was then made of the effects of different initial extraction media. The results are shown in Tables I, II and III.

Although the mean recoveries by the three methods were similar, it was decided to reject the ether technique in view of the wider spread of the results and the additional hazard introduced when ether is used as an extraction solvent.

Table I

Recovery of sulphaquinoxaline from laboratory-medicated feed by extraction with cold dimethylformamide and chloroform

Laboratory	Sulphaquinoxaline added, mg	Sulphaquinoxaline found, mg	Recovery, per cent.
B	1·32	1·26	93
	1·28	1·23	96
	1·44	1·34	95
	1·28	1·30	101
	1·26	1·15	91
	1·25	1·03	82
C	1·15	1·046	91
	1·18	1·18	100
	1·25	1·18	94
	1·24	1·17	94
D	1·260	1·125	89
	1·141	1·100	96
	1·152	1·213	105
	1·388	1·338	95
F	1·21	1·09	90
	1·63	1·52	93
G	1·180	1·026	87
	1·510	1·337	89
	1·218	1·068	88
	1·202	1·105	92
	1·230	1·118	97
	1·490	1·245	84
	1·255	1·076	86
	1·205	1·053	88
H	1·25	1·49	119
		Mean ..	93

Table II

Recovery of sulphaquinoxaline from laboratory-medicated feed by extraction with hot dimethylformamide and cold chloroform*

Laboratory	Sulphaquinoxaline added, mg	Sulphaquinoxaline found, mg	Recovery, per cent.
B	1·22	1·16	95
	1·24	1·18	95
C	1·25	1·07	86
	1·25	1·08	86
D	1·172	1·125	96
	1·106	1·050	95
	1·094	1·113	102
	1·179	1·063	89
F	1·46	1·30	89
	1·16	1·02	88
G	1·34	1·245	93
	1·31	1·076	82
H	1·25	1·28	102
		Mean ..	93

* This method of extraction led to the formation of emulsions in the latter stages of the analysis, but in all instances they broke down fairly easily.

TABLE III

RECOVERY OF SULPHAQUINOXALINE FROM LABORATORY-MEDICATED FEED BY EXTRACTION WITH COLD DIMETHYLFORMAMIDE AND ETHER

Laboratory	Sulphaquinoxaline added, mg	Sulphaquinoxaline found, mg	Recovery, per cent.
B	1·22	1·24	102
	1·28	1·28	100
C	1·25	0·98	78
	1·25	1·01	80
D	1·372	1·513	110
	0·953	1·175	123
	1·251	1·513	121
	1·378	1·650	120
F	1·58	1·49	94
	1·26	1·18	94
G	1·59	1·17	74
	1·17	1·00	86
H	1·25	0·65	55
		Mean ..	95

It has been reported that medicated feeds in the form of hard pellets gave more consistent results when the warm dimethylformamide extraction method was used, and, as this was equally suitable for application to medicated feeds, it was agreed to make use of this solvent in all further work.

Tests were then made on various commercial feeds containing sulphaquinoxaline added at a level of approximately 0·012 per cent. The results are shown in Table IV.

TABLE IV

RECOVERY OF SULPHAQUINOXALINE FROM COMMERCIAL FEEDS

Laboratory	Sulphaquinoxaline, per cent., recovered from— wheatfeed	broiler feed	high-fat feed
B	79	96	94
C	92	79	82
	89	86	74
			81
			87
D	93	93	95
	93	93	94
	93		
F	93	96	80
	90	96	83
G	72	70	78
	72	74	76
H	68	85	86
	60	92	94
	90	81	96
Mean ..	83	87	86

Difficulties often arose from somewhat intractable emulsions that were present after the vigorous shaking of the separator at the drug-purification stage. The attention of the members of the Sub-Committee was drawn to a Report on the determination of rotenone in lonchocarpus,[2] in which similar problems arose that were resolved by the adoption of a gentle liquid-rolling technique. A slow multiple-inversion technique as applied to the contents of the separator was also found to be satisfactory. When this modification was included in the analytical process, the results obtained were improved, and the technique has been incorporated in the recommended method.

During the progress of the investigation, attention was directed to a paper by Kunze and Espinoza,[3] which queried the concentration of acid required to give a satisfactory Bratton - Marshall reaction.

A collaborative study was made on a factory feed medicated with sulphaquinoxaline at the 0·010 per cent. level, in which the volumes of concentrated hydrochloric acid were set at 2 and 5 ml. The mean drug recovery at the 2-ml level of added acid was 93 per cent., whereas at the 5-ml level it was 98 per cent. Four of the six laboratories involved in this study reported no difference in the apparent sulphaquinoxaline recovery, but two laboratories found that better recoveries were possible when the larger volume of acid was used.

In subsequent work, a volume of 5 ml of concentrated hydrochloric acid was retained, and this has been incorporated in the recommended method.

The method, including the various modifications, was applied to samples of bulk medicated feed containing sulphaquinoxaline at the 0·005 and 0·010 per cent. levels. The results are shown in Table V.

TABLE V

RECOVERY OF SULPHAQUINOXALINE FROM BULK MEDICATED FEEDS

Laboratory	Sulphaquinoxaline found at 0·005 per cent. level, per cent.	Recovery, per cent.	Sulphaquinoxaline found at 0·010 per cent. level, per cent.	Recovery, per cent.
B	0·0063	126	0·0095	95
	0·0072	144	0·0091	91
D	0·0059	118	0·0104	104
	0·0060	120	0·0099	99
	0·0056	112		
F	0·0051	102	0·0095	95
	0·0051	102	0·0091	91
G	0·0063	126	0·0118	118
	0·0053	106	0·0090	90
K	0·0043	86	0·0093	93
	0·0043	86	0·0093	93
L	0·0056	112	0·0095	95
	0·0050	100	0·0094	94
	0·0049	98	0·0090	90
	0·0048	96	0·0094	94
	0·0048	96	0·0094	94
	0·0046	92	0·0099	99
	Mean ..	107	*Mean* ..	96

MEDICATED PELLETS—

The current practice of providing pellets as a convenient form of therapy prompted some members of the Sub-Committee to apply the proposed method to crushed pellets.

From the limited evidence available, it appears that the sulphaquinoxaline recoveries approximate to 90 per cent. of those for the corresponding feeds.

CONCLUSION—

The recommended method, given in the Appendix, is suitable for determining sulphaquinoxaline in medicated feeds at drug levels of 0·01 per cent. or more. At lower drug levels, the method is less reliable.

Appendix

RECOMMENDED METHOD FOR DETERMINING SULPHAQUINOXALINE IN MEDICATED FEEDS

REAGENTS—

Dimethylformamide—Laboratory grade.

Chloroform—B.P. grade.

Alkaline brine—An aqueous solution containing 2 per cent. of sodium hydroxide and 5 per cent. of sodium chloride.

Hydrochloric acid, sp.gr. 1·17—Laboratory grade.

Sodium nitrite solution, 0·1 *per cent. w/v*—Prepare freshly before use.

Ammonium sulphamate solution, 0·5 *per cent. w/v.*

N-1-*Naphthylethylenediamine dihydrochloride solution (coupling agent)*—A 0·1 per cent. w/v solution of the reagent in 0·1 per cent. v/v hydrochloric acid. Store the solution in a brown-glass bottle, but discard if not colourless.

Sulphaquinoxaline—B.Vet.C. grade.

PROCEDURE—

Transfer 10 ± 0·1 g of a representative sample of the feed to a 250-ml conical flask, and add 20 ml of dimethylformamide. Heat the flask on a bath of boiling water for 20 minutes. Cool the flask and contents, add 60 ml of chloroform, stopper the flask, and shake it for 30 minutes. Filter the liquid through a 3-inch sintered-glass funnel (porosity 3) under mild suction, wash the flask with four 5-ml portions of chloroform, and pass the washings through the funnel. Transfer the filtrate to a separating funnel, rinse the filter flask with about 15 ml of chloroform, and transfer the rinsings to the funnel. Add 50 ml of alkaline brine and 5 ml of ethanol. Thoroughly mix the layers, either by slow inversion of the funnel about twenty times or by rotating it about the horizontal axis of the stem of the stopper. Allow the layers to separate (separation is usually complete in about 15 minutes). Transfer the upper aqueous layer to a 250-ml calibrated flask. Repeat the extraction of the chloroform layer with three further 50-ml portions of alkaline brine, and add each aqueous extract to the contents of the calibrated flask.

Dilute the solution in the flask to the mark with water. Transfer 25 ml of the solution to a 50-ml calibrated flask, add 5 ml of hydrochloric acid, and dilute to volume with water. Normally, a clear solution is obtained, but if insoluble material is present filtration is necessary, the first 15 ml of filtrate being discarded.

Transfer 10 ml of the acidified solution to a 6-inch × 1-inch boiling-tube, add 2·0 ml of sodium nitrite solution, shake well to mix, and set the tube aside for 3 minutes; then add 2·0 ml of ammonium sulphamate solution, mix, and set the tube aside for 2 minutes. Add 1·0 ml of the coupling agent. By means of a water-pump, apply a vacuum to the tube through rubber connections in order to remove dissolved nitrogen. Transfer the coloured solution to a 20-mm cell, and measure the optical density of the solution at 545 mμ about 10 minutes after the coupling agent has been added.

SULPHAQUINOXALINE STANDARDS—

Weigh accurately 0·250 g of sulphaquinoxaline and dissolve it in a mixture of 25 ml of 0·1 N sodium hydroxide and 25 ml of water. Transfer the solution to a 500-ml calibrated flask, and dilute to the mark with water. Mix, transfer 5·0 ml of the solution to a 100-ml calibrated flask, and dilute to the mark with water. Into separate 100-ml calibrated flasks transfer 2·0, 4·0, 6·0, 8·0 and 10·0 ml of the dilute sulphaquinoxaline solution; to the contents of each flask add 8 ml of hydrochloric acid, and dilute to volume with water.

By pipette place 10·0 ml of each of the standard solutions (equivalent to 5, 10, 15, 20 and 25 μg of sulphaquinoxaline) in 6-inch × 1-inch boiling-tubes, and continue as described above under "Procedure," beginning at the words "add 2·0 ml of sodium nitrite solution. . . ."

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Determination of β-Carotene in a Roller-dried Food

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The standard saponification procedure failed to extract all the carotene from a dried protein - cereal infant-food preparation, fortified with β-carotene added as a water-dispersion. An alternative method has been used in which the foodstuff is treated with proteolytic and diastatic enzymes, and the carotene then extracted with solvent. Results by this method are higher than those obtained by saponification, and evidence is produced to show that low results are due to incomplete extraction.

THE food under examination was a protein and cereal mixture prepared from a roller-dried slurry. β-Carotene was added to the wet material as an aqueous suspension, prepared by heating water-dispersible gelatin-coated beadlets in water. The food thus differed in two respects from common carotene-rich foods; it was low in fat content and the carotene was not in the fat phase.

The accepted method of determining carotene in foodstuffs is by saponification with subsequent solvent extraction and measurement of the depth of colour of the extract.[1] When this method was applied to the protein - cereal food, low results were obtained. Direct solvent extraction produced higher results, but required unusually long periods of boiling under reflux. An alternative method was developed, based on a publication of Hoffman-La Roche and Co. Ltd.,[2] for determining vitamin A in foodstuffs enriched with dry beadlets of water-dispersible vitamin A. In principle, the food was digested enzymically and then the carotene extracted from the aqueous mixture by solvent. The recovery of carotene was higher than that obtained by saponification, and further experiments indicated that the lower recoveries were due to incomplete extraction rather than to destruction.

EXPERIMENTAL

MATERIALS USED—

The principal material examined was a powdered infant food (marketed as Farlene) manufactured by roller-drying a batter prepared from a mixture of cereal and protein-rich foodstuffs. It contained 25 per cent. of protein (N × 6·25), 5·5 per cent. of fat (by acid hydrolysis), 3·5 per cent. of water and the remainder was carbohydrate and ash. The food was fortified with an aqueous suspension of carotene added to the batter before drying. The carotene suspension was prepared by adding water-dispersible gelatin-coated beadlets of β-carotene (Roche Products Ltd.) to water at 80° C, whereupon they formed a stable suspension. After the material had been dried, it was powdered and sieved, and to ensure uniformity of samples for assay, a large amount was mixed for 30 minutes in a planetary mixer.

METHODS

APPARATUS—

Spectrophotometric measurements of the carotene solutions were made at 452 mμ in 1-cm cells in a Unicam SP600 instrument.

REAGENTS—

Buffer solution—Prepare a 0·4 M solution of disodium hydrogen orthophosphate and adjust the pH of the solution to 7·0 with 0·2 M citric acid.

Extraction solvent—Prepare a 10 per cent. v/v solution of 99 per cent., 74° over proof industrial methylated spirits in peroxide-free analytical-reagent grade diethyl ether.

Light petroleum—Analytical-reagent grade, boiling-range 40° to 60° C.

Carotene—β-Carotene crystals finely dispersed in a protective matrix of gelatin and starch, and termed "15% water-soluble beadlets" (obtainable from Roche Products Ltd.).

Industrial methylated spirits—Use 99 per cent., 74° over proof material.

Ethanol—Absolute.

* Present address: Nutrition Department, Queen Elizabeth College, London, W.8.

Bacterase—An enzyme preparation of bacterial origin, mainly diastatic (α-amylase) in activity, with some proteolytic action (obtainable from Associated British Maltsters Ltd., Stockport, Cheshire).

Enzyme 2261—A proteolytic enzyme with no diastatic activity (obtainable from Associated British Maltsters Ltd.).

Pancreatin—Supplied as dry powder by Koch-Light Laboratories Ltd., Colnbrook, Bucks.

Takadiastase—Supplied by Parke Davis & Co., Hounslow, Middlesex.

Lipase—Supplied by Koch-Light Laboratories Ltd.

ENZYME METHOD—

Weigh out 10·00 g of powdered food and 0·3 g of Bacterase and mix in a 150-ml beaker. Add 40 ml of pH 7·0 buffer solution at 50° C, mix to a smooth paste and incubate the mixture for 5 minutes at 50° C. Add sufficient ammonia solution to raise the pH value to 8·5 and incubate the mixture at 50° C for a further 5 minutes.

Transfer the mixture to a 250-ml separating funnel with the minimum amount of water, and extract it with successive 50-ml portions of extraction solvent until all the colour has been removed. Combine the extracts, spin them in a centrifuge if necessary, and read the optical density at 452 mμ. Convert this figure to international units of β-carotene from a calibration curve, and express the results as i.u. of β-carotene per oz of foodstuff.

As the food we used had been fortified with pure β-carotene and it was known that the natural carotenoid pigments of the raw materials contributed only a small proportion to the total, purification by chromatography was not essential. It is realised that this would not necessarily be true for other foodstuffs.

SAPONIFICATION METHOD—

Follow the procedure laid down by the Analytical Methods Committee, Additives in Animal Feeding Stuffs Sub-Committee, Part 5,[1] except for small modifications of the volumes of alcohol and potassium hydroxide necessitated by the high water absorbancy of the material, *viz.*, use a 10-g sample with 75 ml of absolute ethanol, 0·2 g of quinol and 10 ml of potassium hydroxide solution (160 g in 100 ml of water). Read the optical density as under "Enzyme Method."

DIRECT SOLVENT EXTRACTION—

Shake 10 g of the food with 50 ml of light petroleum, filter the mixture, and wash the residue with light petroleum. Re-suspend the residue in fresh solvent, filter and wash. Repeat this procedure until a colourless filtrate is obtained. Heat the residue under reflux with 75 ml of industrial methylated spirits for 90 minutes, filter the mixture, and wash the residue with fresh industrial methylated spirits. Repeat the refluxing, filtering and washing until a colourless filtrate is obtained. Combine all filtrates and read the optical density as under "Enzyme Method."

RESULTS

Table I shows the carotene content of the foodstuff assayed by each of the three methods. Saponification, the Society's recommended method,[1] gave results considerably lower than did the other two methods. Solvent extraction required at least 5 hours because of the prolonged boiling with ethanol needed to liberate the carotene, whereas the enzyme method could be completed in 1½ hours.

TABLE I

CAROTENE CONTENT BY THREE METHODS OF ASSAY

Method	Number of assays	Carotene content, i.u. per oz	Standard deviation, i.u. per oz
Enzyme treatment	7	672	±15
Saponification	6	476	±28
Solvent extraction	7	659	±40

EXPERIMENTAL CONDITIONS—

Enzymes—It was found that digestion with a proteolytic enzyme alone (2261) or a diastatic enzyme alone (takadiastase) did not break down the material sufficiently to permit

complete extraction of the carotene. Both starch and protein digestion were necessary, and this could be achieved by using enzyme 2261 and takadiastase in succession, or with an enzyme preparation, such as pancreatin or Bacterase, which possess both diastatic and proteolytic activity. Lipase was found to be unnecessary.

Amount of enzyme and time of treatment—Table II shows that 0·3 g of enzyme is adequate and that a 5 minute digestion time at each pH value (7·0 and 8·5) is sufficient.

TABLE II

AMOUNT OF ENZYME AND TIME OF TREATMENT

Bacterase and pancreatin were used for these experiments

	Carotene extracted, i.u. per oz	
Digestion time, minutes	0·3 g of each enzyme	0·5 g of each enzyme
10 (5 + 5)	571	542
20 (10 + 10)	573	555
30 (15 + 15)	—	536

Solvents—By using completeness of extraction and speed of separation of the emulsion as criteria, an ethanol - diethyl ether (1 + 9) solvent mixture was found to be the most effective. Higher concentrations of ethanol gave emulsion trouble at later stages and coagulated the material in suspension. Further, as the ethanol concentration in the aqueous phase increased in successive extractions, a yellow pigment other than β-carotene was extracted by the ether. It could be removed by washing with water, but this lengthened the procedure. On the other hand, alcohol was found to be essential for proper extraction and the quoted amounts of solid, aqueous liquid and solvent gave the least trouble with emulsions for this particular foodstuff.

Replacement of ethanol with methanol caused troublesome emulsions; hexane and cyclohexane extracted less of the carotene; isopropanol gave results similar to those with ethanol. The diethyl ether - ethanol solvent was a more effective extracting agent than a light petroleum - ethanol mixture.

pH of buffer solution—Low yields of carotene were obtained unless the digestion was carried out in two stages, namely at pH 7·0 and at pH 8·5, the optimum pH values for amylolysis and proteolysis, respectively. Values of pH greater or less than 8·5 for the second stage resulted in poor extraction or the formation of intractable emulsions. Adjustment of the pH first to 8·5 and subsequently to 7·0 gave very poor yields, presumably owing to the inactivation of the diastase by proteolysis. Buffer solutions at molarities greater and less than those recommended (0·4 M disodium hydrogen orthophosphate and 0·2 M citric acid) interfered with the separation of the solvent - aqueous phases. Buffer solutions of potassium chloride - borate - sodium hydroxide and disodium hydrogen orthophosphate - citric acid with second-stage pH adjustment with sodium hydroxide instead of ammonia solution, also interfered with the separation of the phases.

EVIDENCE OF FAILURE TO EXTRACT THE CAROTENE—

The lower recoveries of carotene after saponification as compared with enzymic digestion, could have been due either to destruction of carotene or failure to extract. Cold saponification

TABLE III

INFLUENCE OF TIME AND TEMPERATURE OF SAPONIFICATION ON RECOVERY OF CAROTENE

	Carotene, i.u. per oz	
Time, minutes	Sample A (replicates)	Sample B (replicates)
Hot saponification—		
7	542, 506, 568	—
15	610, 667, 590, 568, 633	585, 651
30	565, 656, 624, 654	646, 631
60	—	631, 610, 636
Cold saponification—		
7	527	—
15	511, 588	—
30	533	—
60	488	—

was effected by treating the food with alcoholic potassium hydroxide for 1 hour at room temperature. The results were not higher than by hot saponification (see Table III), suggesting that the normal hot procedure did not destroy any carotene. Further evidence for this conclusion was provided by the finding that hot saponification for longer periods did not yield lower results. However, when the residue from saponification was assayed by the enzyme method, an additional yield was obtained amounting to approximately 25 per cent. of the total carotene extracted (see Table IV). To investigate whether the method of carotene addition was a cause of the problem, a batch of material was prepared in which the carotene was added as a solution in arachis oil instead of as an aqueous supension. The fat content of the finished product was thereby increased from 5·5 to 6·5 per cent. Enzyme treatment again gave a higher result than saponification (see Table V).

TABLE IV

RECOVERY OF CAROTENE BY SAPONIFICATION AND SUBSEQUENT ENZYME TREATMENT

Sample	Recovery of carotene, i.u. per oz, after— saponification	Recovery of carotene, i.u. per oz, after— enzyme treatment of residue	Total carotene recovery, i.u. per oz	Total carotene extracted by saponification, per cent.
C	641	190	831	77
D	563	228	791	71
E	525	218	743	71
F	537	157	694	77

TABLE V

COMPARISON OF CAROTENE RECOVERIES AFTER ADDITION AS A SOLUTION IN ARACHIS OIL OR AS A DISPERSION IN WATER

Sample	Form of carotene	Carotene recovered, i.u. per oz, after— enzyme treatment	Carotene recovered, i.u. per oz, after— saponification
G	Aqueous dispersion	905, 931, 900, 915	644, 701, 711, 672
H	Oily solution	723, 778, 727, 757	525, 563, 641, 558

These findings demonstrate that the standard saponification procedure is not reliable when applied to the material under examination.

DISCUSSION

Lease and Mitchell[4] reported that carotene was difficult to extract by saponification from cooked sweet potatoes, carrots and squash, because of the formation of a gummy resin that bound the carotene mechanically. The problem was overcome by boiling the saponified material with water and re-extracting with solvent. When this treatment is applied to our material, the yield is not increased, so it is assumed that there is no resin formation. Other workers have also reported incomplete extraction or destruction of carotene by saponification.[5,6,7]

The material used in the present experiment differs from most other foods and feeding stuffs in two respects, the fat content is low (5·5 per cent.) and the carotene is added not in oil solution, but as an aqueous dispersion. The carotene is associated with a film of starch and protein and may possibly be linked with the protein.[3] Alternatively, it is possible that the carotene is mechanically trapped in the gelatinised starch mass. The fact that both proteolytic and diastatic enzymes are necessary to release the carotene does not indicate which of these two alternatives predominates. A protein - carotene complex could be protected from the proteolytic enzyme by a starch sheath, or alternatively, starch-enclosed carotene could be protected from the diastatic enzyme by a protein barrier. The failure of saponification to liberate the carotene may be explained by the finding that the residue after saponification still retains the undissolved granular structure of the original powder.

Direct solvent extraction yielded as much carotene as the enzyme method, but needed a long period of treatment. The fact that about one-third of the carotene could be easily extracted at room temperature with light petroleum, but the remaining two-thirds required prolonged boiling with ethanol, suggests that part of the carotene is present in a bound form. Finer grinding had no effect on the efficiency of extraction with light petroleum.

The stability of carotene in the product throws some light on this problem. About 10 per cent. disappears fairly rapidly on storage (in 2 to 4 weeks at room temperature) and the remainder is stable over a period of several months. It is possible that the unstable carotene is that on the outside of the powder particles, while the remainder is retained in the interior. Carotene dispersed and dried in wheat starch is extremely unstable and 75 per cent. disappears in 3 weeks at room temperature. Thus the protecting influence of the protein is again implied.

We thank the Directors of Farley's Infant Food Ltd. for permission to publish this paper and also Miss C. M. Holloway who bore the brunt of the analytical work.

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The Determination of Activation Products in Irradiated Steels by Anion-exchange Separation and γ-ray Spectrometry

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A method is described for determining chromium-51, manganese-54, cobalt-58 and -60, and iron-59 in irradiated mild and stainless steels. It involves the selective elution of these isotopes with different concentrations of hydrochloric acid from a strongly basic anion-exchange resin column. Each nuclide is determined by γ-ray spectrometry. The precision of the method is better than 2 per cent. for each determination.

Determination of the principal γ-active nuclides in irradiated stainless and mild steels provides useful information for shielding calculations and permits the integrated fast and thermal neutron fluxes in the reactor to be calculated. Although many neutron reactions occur when mild steel or chromium - nickel stainless steel is irradiated, the main γ-ray-emitting activation products present after a decay of several days are iron-59, chromium-51 and cobalt-60 from (n,γ) reactions and manganese-54 and cobalt-58 from (n,p) reactions. Details of the formation and nuclear properties of these isotopes are shown in Table I. In some steels small amounts of elements with high activation cross-sections, *e.g.*, tantalum, tungsten, antimony and zinc, will form γ-ray-emitting isotopes by (n,γ) reactions that may also contribute significantly to the total γ-ray specific activity. These were found to be only of minor importance in the steels examined.

Table I

Principal γ-ray-emitters present in neutron irradiated steels after several days' decay

Steel constituent	Neutron reaction	Abundance of parent isotope in natural element, per cent.	Cross section, for reaction, barns	Half life of activation product, days	Main energies, MeV, and radiations emitted, per cent.
Iron	Fe^{54} (n,p) Mn^{54}	5·84	0·065	300	0·84 (100)
	Fe^{58} (n,γ) Fe^{59}	0·31	1·0	45·1	1·10 (56) 1·29 (44)
Chromium	Cr^{50} (n,γ) Cr^{51}	4·31	13·5	27·8	0·323 (8)
Nickel	Ni^{58} (n,p) Co^{58}	67·8	0·090	71	0·81 (100)
Cobalt	Co^{59} (n,γ) Co^{60}	100	36	5·24 years	1·17 (100) 1·33 (100)

Direct determination of the principal activation products cannot be achieved precisely by γ-ray spectrometry with conventional equipment, since several of the nuclides have similar energies. However, since nuclides can be determined in the presence of each other provided their γ-ray energies are sufficiently different, it is only necessary to separate them into groups of activities containing no similar γ-ray energies.

Kraus and Nelson[1] have described the adsorption of various elements as complex chlorides from hydrochloric acid solutions by using strongly basic anion-exchange resins. This information provided the basis for the selective elution of chromium *plus* manganese, cobalt and iron at different hydrochloric acid concentrations from a strongly basic anion-exchange resin, and was examined in detail.

Experimental

Behaviour of chromium-51, manganese-54, cobalt-60 and iron-59—

To establish the conditions for the separation of manganese-54 and chromium-51 from cobalt-58 and -60 and iron-59, a solution was prepared in 12 M hydrochloric acid that contained 40 mg per ml of $iron^{III}$, and 5 mg per ml of manganese, cobalt and chromic chromium. To this solution were added tracer amounts of iron-59, manganese-54, cobalt-60 and chromium-51. A 1-ml aliquot of the solution was then placed on an anion-exchange column of Deacidite FF

(52 to 100 mesh, 7-cm long by 0·5-cm diameter column) that had been previously conditioned by washing with 12 M hydrochloric acid. The elution of chromium-51 and manganese-54 by 10 M hydrochloric acid was followed by counting the eluate at approximately 0·5-ml intervals with a γ-ray scintillation spectrometer. On removal of these activities from the column, the elution of cobalt-60 with 6 M hydrochloric acid was followed in a similar manner. Finally, the iron-59 fraction was eluted with 0·1 M hydrochloric acid. A flow rate of 1·5 ml per minute per sq. cm was used throughout the elution. Fig. 1 illustrates the selective elution of these isotopes and clearly shows that a satisfactory separation of cobalt and iron from the manganese *plus* chromium fraction had been achieved.

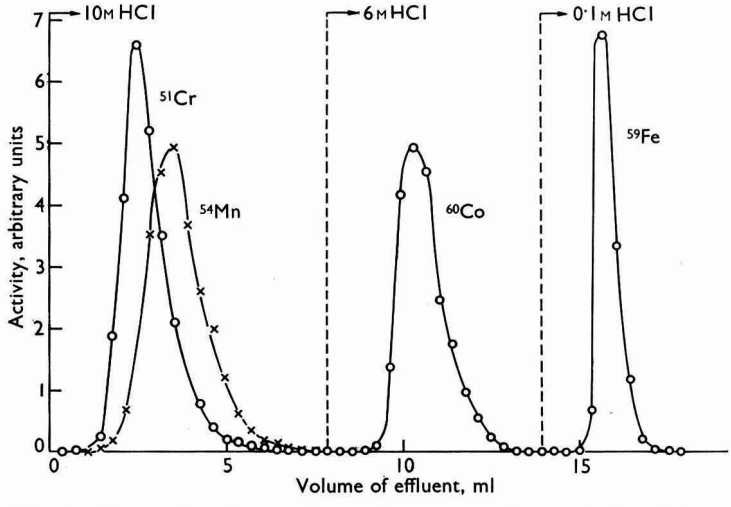

Fig. 1. Separation of chromium and manganese from cobalt and iron

Each isotope was then individually adsorbed on 12 M hydrochloric acid conditioned Deacidite FF columns, and eluted with the selected strength of hydrochloric acid. In all instances more than 99 per cent. of the isotope was removed from the resin.

BEHAVIOUR OF IRRADIATED STEEL—

A 100-mg sample of stainless steel that had been irradiated in the high flux region of the Harwell pile, BEPO, for several hours was dissolved in concentrated hydrochloric acid, with small additions of concentrated nitric acid and 100-volume hydrogen peroxide solution. After the addition of about 5 mg of a cobalt carrier, the solution was made up to 3 ml with concentrated hydrochloric acid. A 1-ml aliquot of this solution was placed on a conditioned Deacidite FF resin column and the activation products eluted according to the scheme shown in Fig. 1. Examination of the fractions by γ-ray spectrometry showed that a satisfactory separation of the isotopes into 10 M, 6 M and 0·1 M hydrochloric acid fractions had been achieved. The undissolved residue in the steel solution was then removed by centrifugation, washed several times with water and examined by γ-ray spectrometry; tantalum-182 was the only detectable γ-ray emitter present.

METHOD

APPARATUS—

Resin—Deacidite FF type SRA-66 with a 3 to 5 per cent. cross-linking and a particle size of 52 to 100 mesh.

Glass columns—Approximately 12-cm long by 0·5-cm diameter were filled to a depth of 7 cm with the resin and conditioned by washing with ten column-volumes of 12 M hydrochloric acid.

Polythene weighing bottles—Capacity 30 ml (3 inches × 1 inch diameter), to collect the liquid fractions for counting.

Spectrometer—A 400-channel γ-ray spectrometer with a 2-inch × $1\frac{3}{4}$-inch sodium iodide thallium-activated crystal.

REAGENTS—

Hydrochloric acid, sp.gr. 1·18.
Nitric acid, sp.gr. 1·42.
Hydrogen peroxide—100-volume solution.
Eluent solutions—Prepare solutions 10 M, 6 M and 0·1 M in hydrochloric acid.
Cobalt carrier solution—Dissolve 4·1 g of hydrated cobalt chloride, $CoCl_2.6H_2O$, in 100 ml of hydrochloric acid, sp.gr. 1·18. This solution contains approximately 10 mg of cobalt per ml.

PROCEDURE—

Accurately weigh 0·1 to 1·0 g of the clean, dry steel and dissolve it in hydrochloric acid, sp.gr. 1·18, containing several drops of the cobalt carrier solution. Dissolve any insoluble carbides by adding small amounts of nitric acid and hydrogen peroxide at the boiling-point. Cool, and make up to a known volume with hydrochloric acid, sp.gr. 1·18, such that the final iron concentration is about 40 mg per ml. Remove any insolubles by centrifugation. Place 1 ml of the solution on a conditioned Deacidite FF column and elute the chromium and manganese fraction with 7 ml of 10 M hydrochloric acid at a flow-rate not greater than 1·5 ml per minute per sq. cm. Elute the cobalt fraction with 6 ml of 6 M hydrochloric acid and finally elute the iron-59 fraction with 4 ml of 0·1 M hydrochloric acid. Measure the activity of each fraction by γ-ray spectrometry under standard counting conditions and determine the activities by comparing the photopeak areas (corrected for background and dead time) with the corrected photopeak areas of calibrated standards. A correction for the contribution of manganese-54 and cobalt-60 to the photopeaks of chromium-51 and cobalt-58, respectively, may also be necessary. This can be achieved mathematically or by using the subtraction facility in the γ-ray spectrometer.

RESULTS AND DISCUSSION

The precision of the proposed method was determined by the analysis of 100-mg samples of stainless steel that had been irradiated in a nominal fast neutron flux of 2×10^{11} neutrons per sq. cm per second and a thermal neutron flux of $1{\cdot}2 \times 10^{12}$ neutrons per sq. cm per second for a period of 8 hours. The steel used had the composition—

Carbon, 0·08 per cent.; manganese, 1·22 per cent.; nickel, 12·48 per cent.; chromium, 17·60 per cent.; copper, 0·10 per cent.; titanium, less than 0·05 per cent.

TABLE II

ANALYSIS OF IRRADIATED STAINLESS-STEEL SOLUTIONS

Sample	Chromium-51, μc per g of steel	Manganese-54, μc per g of steel	Cobalt-58, μc per g of steel	Cobalt-60, μc per g of steel	Iron-59, μc per g of steel
1	345·5	$7{\cdot}71 \times 10^{-2}$	1·080	0·495	4·25
2	347·6	$7{\cdot}63 \times 10^{-2}$	1·095	0·504	4·19
3	346·2	$7{\cdot}46 \times 10^{-2}$	1·079	0·494	4·26
4	344·2	$7{\cdot}76 \times 10^{-2}$	1·085	0·506	4·16
5	352·9	$7{\cdot}72 \times 10^{-2}$	1·115	0·513	4·12
6	349·9	$7{\cdot}69 \times 10^{-2}$	1·078	0·516	4·31
Mean	347·7	$7{\cdot}66 \times 10^{-2}$	1·089	0·505	4·22
Standard deviation ..	3·2	$0{\cdot}11 \times 10^{-2}$	0·015	0·009	0·07
Coefficient of variation, per cent.	0·92	1·4	1·4	1·8	1·7

The results are detailed in Table II and show that the precision for the determination of each nuclide is better than 2 per cent. at the 1σ level. By using the nuclear data in Table I and the measured iron-59 and cobalt-60 activities, the cobalt content of the steel was calculated to be 309 p.p.m. The value obtained by a spectrophotometric determination of cobalt by using nitroso-R salt was 332 p.p.m. Although it is more accurate to perform neutron-activation analyses by irradiating a standard with the sample, thereby eliminating errors due to neutron flux variation and cross-sectional data, the similarity between the two cobalt values demonstrates the potential of this method for determining cobalt in steel.

Conclusions

A method has been described for the separation of the principal γ-active nuclides in irradiated steels. It involves the selective elution of chromium-51, manganese-54, cobalt-58 and -60 and iron-59 isotopes from a strongly basic anion-exchange resin column in hydrochloric acid media. The separated fractions are determined by γ-ray scintillation spectrometry, and the method has a precision better than 2 per cent. at the 1σ level for each nuclide determined. The separation scheme also affords a method for determining cobalt in steels by neutron-activation analysis.

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The Polarographic Determination of Trace Amounts of Lead in Stainless and Other Steels

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A method is described for determining lead in stainless steels by successive extractions from a single phase, which is retained in the separating funnel throughout the procedure. An initial extraction as iodide and then one as diethyldithiocarbamate from a complexing solution gives effective separation from the rest of the sample.

When a differential cathode-ray polarograph is used, detection limits of 0·05 p.p.m. or better are possible with a 1-g sample, but reagent blank values generally limit the sensitivity to about 2 p.p.m.

The determination of traces of lead in ferrous materials has been shown to be of increasing importance in recent years, but the most recent British Standard method has a lower limit of applicability of only 0·05 per cent. This is inadequate at the levels now known to be of importance, and an earlier attempt to extend these limits[1] involved a solvent extraction separation and polarographic finish that was found to be satisfactory for low alloy material. When this method was applied to stainless steel and other high alloy materials, it was found that the very large amounts of complexing agents required to retain such elements as chromium and nickel in the aqueous phase led to difficulties caused by low solubility. It became apparent that some other technique for extracting lead that was not dependent upon masking of nickel and chromium must be used, and the iodide extraction[2] was therefore investigated.

Experimental work

The extraction of lead from pure solutions was investigated by an experiment of the factorial design described by Pantony.[3] Equal volumes (50 ml) of aqueous phase and extractant were used, and all extractions were carried out at room temperature, shaking by hand for 1 minute. The hydrogen ion concentration was varied from 0·1 to 5 M, and the iodide concentration from 0·05 to 2·5 M. It was found that the most efficient extraction was obtained with iodide and hydrogen ion concentrations of 0·25 M and 1 M respectively, and that both of these concentrations could be varied by $\pm$20 to 30 per cent. without deleterious results. The extractant chosen was isobutyl methyl ketone (hexone) and it was therefore desirable to use a single stage extraction since the solvent is lighter than the aqueous phase. This was possible since recovery in the iodide extraction was 97 to 100 per cent.

Initial experiments with a sample of stainless steel (12 per cent. nickel, 18 per cent. chromium, 2·5 per cent. molybdenum) were carried out in which the iron was removed with butyl acetate[4] with subsequent lead extraction as iodide; the hexone extract was wet oxidised, and attempts made to determine the extracted lead polarographically. The results were not satisfactory due to the formation of iodate during the oxidation stage; iodate gives waves that interfere with the lead determination. A second stage in which lead was extracted with chloroform as the diethyldithiocarbamate complex, with subsequent wet oxidation of the chloroform extract and polarography, gave satisfactory results, showing quantitative recovery and adequate precision. By using this combined technique, however, a single determination required 3 to 5 hours.

Further investigational work aimed at reducing the time required showed that lead could be extracted as iodide from a solution containing all of the iron present in the sample provided it was in the ferrous condition; the butyl acetate extraction can therefore be omitted if iron is retained in the reduced form. It was also found possible to dispense with the wet oxidation of the iodide extract by carrying out an exchange reaction in the non-aqueous solution such that the lead was converted to its diethyldithiocarbamate complex. Conditions are chosen so that the free iodine, iodide ion and all co-extracted metal ions are returned to the aqueous phase. This final method is described under "Procedure."

RECOVERY AND PRECISION—

A sample of stainless steel was processed through the entire procedure and was found to contain 0·0023 per cent. of lead. Standard additions of lead were made to solutions of this sample, and these solutions processed as before. The results obtained are given in Table I and are thought to show satisfactory recovery up to about 0·05 per cent. of lead. The fall off in recovery at the higher levels could be overcome by taking a smaller sample, since recovery of lead up to 200 to 400 μg is quantitative in the presence of the ions present from the stainless-steel matrix. The fall off in extraction efficiency has been shown to take place at the iodide extraction stage, and is attributed to the lowering of the iodide to lead ratio which has by then decreased by two orders from the level at which the initial investigations were carried out.

TABLE I

RECOVERY OF ADDED LEAD

Lead added,		Lead found,	Lead recovered,
μg	percentage of lead	μg	μg
Nil	Nil	23	—
20	0·0020	45	22
50	0·0050	73	50
100	0·010	123	100
500	0·050	475	450
1000	0·100	900	880
5000	0·500	4400	4400

TABLE II

PRECISION OF DETERMINATION

Lead content, per cent.	Blank value, per cent. of lead
0·0030	0·0005
0·0025	0·0006
0·0027	0·0004
0·0028	0·0004
0·0027	0·0004
0·0030	0·0004
0·0025	0·0003
0·0027	0·0003

The same sample was processed eight times, together with eight blank solutions. The results are shown in Table II. The mean content was found to be 0·0023 per cent. of lead and the standard deviation was 0·00021 per cent. Much of this variation must be ascribed to actual variation in the 1-g samples taken. The mean blank value corresponded to 0·00041 per cent. of lead with a standard deviation of 0·0001 per cent. This, with the reagents used, would therefore give a 2σ detection limit of 0·0002 per cent. By using higher quality reagents so that the blank becomes smaller, it should be possible to lower the detection limit; some experiments have been carried out with the equipment used in this work and it has been shown that the 2σ detection limit for lead in the supporting electrolyte used is usually between 0·005 and 0·01 μg per ml, *i.e.*, 0·025 and 0·05 p.p.m. of lead in the sample if a 1-g sample and a final volume of 5 ml are used.

The original lead method[1] was also suitable for the simultaneous determination of bismuth. The recovery of bismuth in the present method was investigated and found to be low, due to poor recovery at the iodide extraction stage. The results obtained indicated a recovery of only 60 to 65 per cent. of the added bismuth, so that the proposed method cannot be recommended for determining this element.

The method has been applied to several samples of stainless steel, and is now in routine use. Some typical results are shown in Table III. No stainless-steel standard samples suitable for proving the method were available; Tables II and III are therefore taken as indicating the precision of the method and Table I the accuracy.

TABLE III

TYPICAL RESULTS

Sample	Lead, per cent.
1·5% Ni, 0·2% Cr, Mo, Cu	0·0002, 0·0002
Hematite iron	0·0005, 0·0006
18/8 Stainless steel—	
Spectrographic Pb 0·005	0·0041, 0·0044
Spectrographic Pb 0·003	0·0031, 0·0032
Spectrographic Pb 0·001	0·0021, 0·0018
18 Cr/12 Ni/2·5 Mo	0·0052, 0·0050
18 Cr/12 Ni/2·5 Mo	0·0010, 0·0012
EN 57	0·0007, 0·0009
EN 58A	0·0023, 0·0027
EN 58B	0·0017, 0·0019

METHOD

REAGENTS—

Hydrochloric acid, sp.gr. 1·18.

Ascorbic acid—Prepare a solution to contain 100 g per litre in cold water.

Potassium iodide, 2·5 M—Prepare a solution to contain 415 g per litre in cold water.

iso-Butyl methyl ketone.

Iodide wash solution—Mix 100 ml of hydrochloric acid, 100 ml of 2·5 M potassium iodide solution and 50 g of ascorbic acid and dilute the solution to 1 litre.

Tartaric acid wash solution—Dissolve 45 g of tartaric acid and 50 g of ascorbic acid in cold water and dilute the solution to 1 litre.

Cyanide-diethyldithiocarbamate wash solution—Mix 5 ml of ammonia solution, sp.gr. 0·88, 5 g of potassium cyanide and 1 g of sodium diethyldithiocarbamate in 100 ml of water. This solution should be purified by shaking it with two 20-ml portions of chloroform, rejecting the chloroform layers. The solution should be prepared fresh daily.

Nitric acid, sp.gr. 1·42.

Perchloric acid, sp.gr. 1·54.

For lead contents down to about 0·002 per cent., AnalaR or equivalent grade acids can be used. For levels lower than this, higher purity acids are essential, and "lead free," "electronic" or "transistor" grades are recommended.

Distilled water should be used for the final dilution to volume; it has been found that de-mineralised water is not suitable for high sensitivity polarography.[1,5]

APPARATUS—

All glassware, including cover glasses, should be of Pyrex or similar grade glass. All the precautions usual for trace analysis should be taken.

The sensitivity of the method will depend upon the polarograph used, when the blank values are sufficiently low; the equipment used in this work was the Southern Analytical A1660 Davis differential cathode ray polarograph, giving an instrumental detection limit of 0·025 to 0·05 p.p.m. in the sample. By using an A1700 Southern-Harwell pulse polarograph this limit may be lowered to 0·005 to 0·01 p.p.m., whereas the A1670 Davis Southern single cell cathode ray polarograph gave a detection limit of 0·10 to 0·2 p.p.m.

PROCEDURE—

Dissolve 1 g of sample by warming it gently in 15 ml of hydrochloric acid, avoiding oxidation, the presence of ferric ion being undesirable. When it has dissolved, transfer the solution to a 150-ml separating funnel marked at 50 ml and add 10 ml of ascorbic acid solution. Shake the funnel, add 5 ml of potassium iodide solution, dilute to 50 ml, and extract by shaking for 1 minute with 50 ml of iso-butyl methyl ketone (hexone). Allow the two layers to separate, run off and reject the lower (aqueous) layer, and wash the hexone layer by shaking for 30 seconds with 10 ml of the iodide wash solution. Reject the aqueous layer again.

To the hexone layer add 5 ml of tartaric acid wash solution, shake for 30 seconds and add 25 ml of cyanide-diethyldithiocarbamate wash solution. Re-extract lead by shaking until the hexone layer is colourless, run off and reject the aqueous layer; wash the hexone layer with 10 ml of water and reject the washings. Transfer the hexone layer to a 50-ml beaker, washing

in with chloroform, and evaporate to dryness on a flash-proof hot-plate. To the dry residue add 2 ml of each of nitric and perchloric acids, and wet oxidise by heating first to fumes and finally to dryness. Re-dissolve the residue in 0·5 ml of hydrochloric acid, make up to 5 ml and transfer the solution to a polarograph cell. De-oxygenate, and measure the peak due to lead at −0·5 volt; calibrate either by standard addition or by constructing a calibration graph.

At least one reagent blank solution should be processed through the entire procedure, and, where the lead contents of the samples are sufficiently low for the blank value to be significant, it is preferable to process two or three blank solutions. Correct the results obtained for the samples by using the mean blank value.

The author thanks the Directors of Southern Analytical Limited for permission to publish this work, and Mr. J. N. Rawlings of Spartan Steels and Alloys Limited, who supplied most of the samples.

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The Determination of Copper and Magnesium in Blood Serum by High-resolution Flame Spectrophotometry

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The determination of copper and magnesium in human blood serum is used for demonstrating the potential of high-resolution flame spectrophotometry. A high-resolution monochromator provides the maximum differentiation between the spectrum line and the flame background and allows practical limits of detection to be obtained that approach the theoretical maximum as defined by spectrum line width. An automatic wavelength scanning and recording technique is used to obtained the value of the peak line intensity above the background in a single recording. Recordings of copper and magnesium lines illustrate the method and show the resolution required for separating the analytical line from adjacent lines or bands in the spectrum. Applications of the instrument to other analyses are also indicated.

THE wider application of flame spectrophotometry is mainly limited by the lack of sensitivity of the types of instrument in current use, which, except for sodium and potassium, usually require preliminary chemical separation or concentration of the sample to render it suitable for analysis; even then the concentration of element required for all but a few more easily determined metals is such that other methods of analysis are usually employed.

The principal cause of lack of sensitivity is to be found in the use of inadequate means of spectral isolation. The function of the monochromator is to provide the maximum differentiation between the spectrum line and the flame background, and to ensure that other lines or bands are sufficiently separated to minimise the spectral interference caused by overlapping of the line relevant to the analysis. The extent to which both these functions can be fulfilled in practice is ultimately limited by the finite width of the spectrum line.

When fully resolved, the line is still superimposed on a bandwidth of background radiation equal to the line width. Under these conditions the ratio of the intensity of the line to that of the flame background will be a maximum, and any reduction in resolving power will reduce this ratio by increasing the bandwidth of background radiation transmitted with the line. In the quest for higher sensitivity, as element concentration is reduced and line intensity diminishes, the problem of spectral interference will increase, since the overlapping of extremely weak bands becomes even more probable and significant; although complete separation cannot always be achieved, the effect is minimised when the line is fully resolved.

At low concentrations of the element, the half-intensity width of the spectrum lines produced in conventional flames is about 0·1 Å, and resolution of this order is required to provide the above conditions. Practical limits of detection can then be obtained that approach the theoretical maximum in terms of the limitations imposed by line width.

Instrument and method

The complete instrument used in this work and the method employed for measuring line intensities have already been described.[1,2]

Essentially, the instrument consists of a burner - atomizer unit and a scanning and recording spectrophotometer, whose monochromator was specially constructed for these investigations by the author and is of the Littrow type with a large 60° quartz prism.

Briefly, the method involves scanning and recording a small interval of wavelength in which the analytical line is centred. The recording is made automatically, the cycle being initiated by introducing the sample, whereupon a wavelength-drive motor and the chart drive of the recorder are simultaneously switched on. When the recording has been made, the sample is removed, the recorder stops and the wavelength drive reverses, finally stopping at the pre-selected wavelength from which the recording began. During a scanning time of 15 to 20 seconds the interval recorded is of the order of 1·0 Å with a sample consumption of about 0·3 ml.

The appropriate lines for the sample and a standard are recorded alternately, and triplicate pairs of such recordings are usually made (see Figs. 2 and 4).

This method of measuring line intensity ensures that the wavelength at which the measurement is made coincides reproducibly with the peak intensity of the line and avoids the difficulty of precise setting and re-setting of the line and background wavelengths required for direct reading at static wavelengths. It is also economical of time and sample volume since a single recording provides the value of the peak line intensity and shows the background level on both sides of the line.

When a linear relation between element concentration and line intensity is obtained, the concentration of the sample can be calculated directly from the ratio of the line intensities produced by a standard and the sample solution. The concentrations of the standard and sample should be of similar magnitude (this is essential if the range of linearity is small, when it is usually preferable to prepare a calibration curve from which the sample concentration can be read).

The determinations referred to in Figs. 2 and 4 were selected from recordings made during the routine analysis of human-blood serum for copper and magnesium, and these examples will be used for demonstrating the potential of high-resolution flame spectrophotometry.

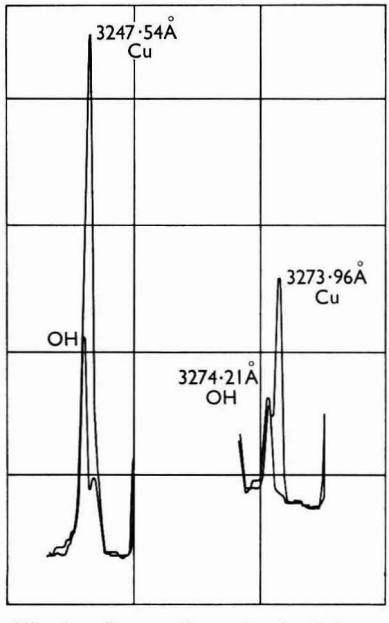

Fig. 1. Copper lines obtained from an aqueous solution containing 0·5 μg of copper per ml showing overlapping and adjacent hydroxyl bands. Linear dispersion, 12 Å per mm; slit width, 10 μ; amplifier gain, 50 per cent.

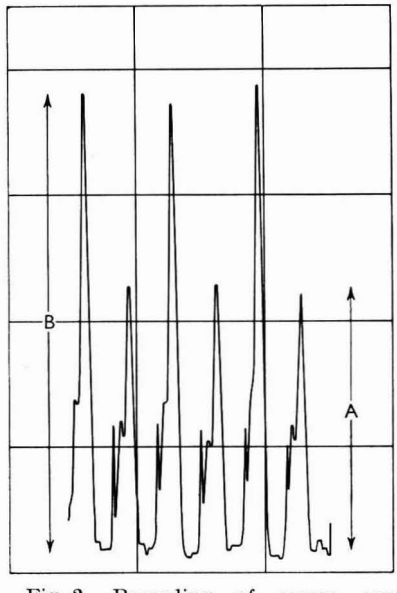

Fig. 2. Recording of serum copper determination on: solution A, 1 + 2 dilution of serum; solution B, 1 + 2 dilution of serum *plus* 0·33$_3$ μg of copper per ml. Wavelength, 3273·96 Å; slit width, 10 μ; maximum amplifier gain

COPPER—

The copper lines at 3247·54 and 3273·96 Å are the most intense. In Fig. 1 are shown the recordings of the two lines given by an aqueous solution containing 0·5 μg of copper per ml and 1200 μg of sodium per ml superimposed on recordings made when a solution containing the same concentration of sodium alone was substituted for the copper solution. Sodium was used in these solutions to simulate the composition of diluted blood serum. Although the 3247·54 Å line is of approximately twice the intensity of the other copper line it cannot be resolved from the overlapping O–H bands, whereas the 3273·96 Å line, although extremely close to the O–H band at 3274·21 Å, is sufficiently separated for satisfactory intensity measurements to be made at the concentration shown.

At full instrument sensitivity 0·5 μg of copper per ml produces a recorder deflection of about 60 per cent. full-scale deflector at the peak of the 3273·96 Å line. This corresponds to about one-third of the copper concentration in blood serum. If serum is diluted by only 1 + 2, the viscosity compared to that of an aqueous standard is such that the different rates of consumption by the atomizer would invalidate any direct comparison of the lines produced by the standard and sample solutions. Although sufficient dilution of the serum to overcome this viscosity difference would render the copper concentration too low for measurement, the problem can be avoided by using the method of standard addition, in which the sample is compared with itself *plus* a known added amount of copper; irrespective of the dilution, the viscosities of the two solutions are then the same. In practice, 1 ml of serum was added to 2 ml of copper-free distilled water to give solution A, and 1 ml of the same serum was added to 2 ml of a solution containing 0·5 μg of copper per ml to give solution B. These solutions were aspirated alternately to produce a recording (shown in Fig. 2), for which the serum copper concentration was found to be 1·35 μg per ml.

The application of this method depends on the linear relationship between copper concentration and net line intensity and on the same relationship applying for different additions of copper made to the same serum sample. Within the concentration range used (0·25 to 2·5 μg per ml) self-absorption is negligible and line intensity is linear with concentration. Repetitive comparisons of two test solutions containing 0·5 and 1 μg of copper per ml, in which the 0·5 μg per ml solution was taken as X μg per ml and the 1 μg per ml solution as X + 0·5 μg per ml, gave a mean value for X of 0·515 μg per ml with a coefficient of variation of 4·6 per cent. calculated from eleven sets of triplicate recordings of the two solutions.

For serum it was verified that the increase in line intensity corresponding to the addition of copper was also linearly proportional to the amount added by comparing a 1 + 2 dilution of serum without added copper to similar dilutions of the same serum with solutions containing 0·5 and 1 μg of copper per ml.

The mean value of the original serum copper concentration from eleven triplicate recordings with the 0·5 μg per ml addition was 1·32 μg per ml and the mean value obtained for the 1·0 μg per ml addition was 1·35 μg per ml, the coefficients of variation being 4·9 and 4·7 per cent., respectively.

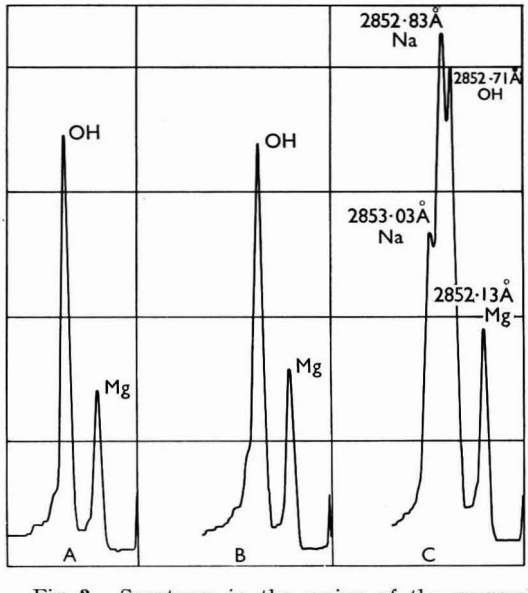

Fig. 3. Spectrum in the region of the magnesium line showing the effect of the presence of sodium. Solution contained 1 μg of magnesium per ml and: A, no sodium; B, 175 μg of sodium per ml; C, 2500 μg of sodium per ml. Linear dispersion, 8 Å per mm; slit width, 10 μ; amplifier gain, 30 per cent.

That the coefficients of variation for the serum analyses are in close agreement with the variation for the test made with pure copper solutions suggests that no significant decrease in accuracy was incurred with the serum, despite the high concentration of salt and protein. The complete series of measurements, equal to 22 separate serum determinations, caused no fouling of the atomizer, and microscopic inspection of its nozzle showed a complete absence of salt incrustation.

MAGNESIUM—

The most intense magnesium line at 2852·13 Å is adjacent to a hydroxyl band at 2852·71 Å. Satisfactory measurement of the line intensity produced at the magnesium level encountered in diluted blood serum is only possible when the line is free from spectral interference due to overlapping by the O–H band. Similar interference could also arise from the presence of appreciable amounts of sodium in the sample by overlapping with the 2852·83 and 2853·03 Å sodium doublet if this is not adequately resolved.

The effect of sodium on the magnesium line is shown in Fig. 3. The principal features of these recordings are the small difference in total background for the three different solutions, the presence of the sodium doublet only at the higher sodium concentrations and an enhancement of the magnesium line with sodium at concentrations of 175 and 2500 μg per ml by 10 and 30 per cent., respectively. This was not due to the presence of magnesium as an impurity in the sodium salt used. At sodium concentrations of between 50 and 500 μg per ml, the degree of enhancement of the magnesium line was substantially constant. No enhancement was found for similar additions of potassium or calcium, either alone or together, nor did such additions modify the influence of sodium.

The sodium effect was observed only in an oxygen - butane flame. Although the effect was found to be absent in an oxy-hydrogen flame, butane is preferred, since it not only gives approximately 50 per cent. greater line intensity but cylinder changing is reduced to about once every 2 months instead of the daily changing of hydrogen cylinders required during a normal 4- to 6-hour period of operation.

At maximum instrument sensitivity, 1 μg of magnesium per ml produces a recorder deflection of 80 per cent. of the full scale. This permits a serum dilution of 1 + 19, and direct comparison of the standard and sample solutions is possible. To compensate for the sodium enhancement, the magnesium standard is made to contain one-twentieth of the physiological sodium concentration. A typical recording of the determination of magnesium in serum is shown in Fig. 4.

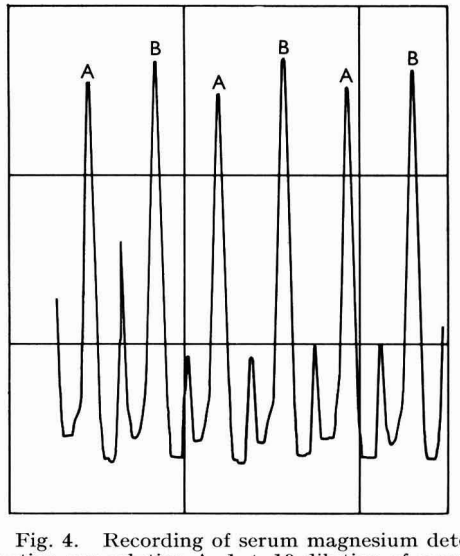

Fig. 4. Recording of serum magnesium determination on: solution A, 1 + 19 dilution of serum; solution B, 1 μg of magnesium per ml *plus* 175 μg of sodium per ml. Wavelength, 2852·13 Å; slit width, 10 μ; amplifier gain, 50 per cent.

At concentrations above 2 μg per ml, the relative increase in intensity of the magnesium line becomes less, owing to self-absorption, but is linear in the range 0·6 to 1·8 μg per ml. Based on eleven triplicate determinations on the same serum sample the coefficient of variation was 2·0 per cent. No interference effects other than that described for sodium have so far been encountered. Numerous recovery experiments with magnesium added to serum revealed no depression or enhancement of the added magnesium.

Limits of detection

A detailed discussion of limits of detection is outside the scope of this paper, although reference has been made above to theoretical detection limits. The discussion will be limited to one example to illustrate the manner in which spectral interference can be the principal factor in determining the minimum detectable concentration of an element.

If the flame background intensity produces a measurable signal when the analytical line is resolved, the minimum detectable line energy above the background (and, thus, element concentration) will be determined solely by the magnitude of the random fluctuations in the flame and recording systems. It is customary to express these fluctuations in terms of the standard deviation of a measured amount of element at a low concentration, and, by convention, some multiple of the standard deviation is accepted as the limit of detection.

The standard deviation obtained by direct comparison of replicate pairs of recordings of the 3273·96 Å copper line produced at a concentration of 0·5 μg of copper per ml was 0·012 μg per ml. The statistical probability that a copper concentration equal to three times the standard deviation could go undetected is 1 in 400. Recordings made with a solution containing 0·036 μg of copper per ml produced only a small inflection on the side of the 3274·21 Å O–H band, owing to the overlapping profiles of this band and the copper line. Repetitive comparison of the copper solution and distilled water revealed a difference that was consistent with the quoted probability and, in that sense, represented a detectable level of copper. The absence of a distinct and separate peak for the copper line at this concentration was of no consequence, since the test solution was known to contain copper. For an unknown solution, these results would not represent strict qualitative evidence of the existence of copper, since the precise wavelength of the emission cannot be determined. Positive confirmation of the presence of copper can only be obtained when a separate peak at 3273·96 Å is produced. This requires a copper concentration of approximately 0·2 μg per ml. No other substance is known to cause a similar inflection on the O–H band to that produced by copper, and its occurrence may be accepted as evidence of the presence of the element, although this is not equivalent to the example in which the precise wavelength can be identified.

The limit of detection deduced from the value of the standard deviation is therefore not applicable when overlapping of the line at extremely low concentrations becomes significant or when the limit of detection is also meant to imply the lowest concentration at which the element is positively identifiable.

Other applications

The applications described in this paper are confined to copper and magnesium, but the potential of the method is, naturally, much wider. Other investigations with this instrument include the determination of magnesium in various different biological samples[3,4] and the direct measurement of iron and lithium in serum and of strontium in drinking water. The sensitivity obtained with aqueous solutions of chromium, nickel, cobalt, manganese, silver and palladium is such that concentrations of these metals below 1 μg per ml can conveniently be measured.

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Determination of Barium, Strontium and Calcium in Barium Peroxide

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Improved methods have been developed for the determination of barium, strontium and calcium in barium peroxide. The barium is determined as barium chromate by using the technique of precipitation from homogeneous solution. The filtrate from the barium chromate is treated with ammonium oxalate and ethanol to precipitate the strontium and calcium oxalates, which are then converted to the nitrates. The calcium and strontium nitrates are separated by use of acetone, and the calcium and strontium are finally precipitated as the sulphates. The methods are applicable to the determination of barium, strontium and calcium in general.

BARIUM peroxide is an important chemical that is used in explosives and catalysts, and in the manufacture of hydrogen peroxide.

The methods described for the determination of barium, strontium and calcium in current specifications for barium peroxide[1,2] are unsatisfactory. This is not surprising considering that the determination of barium, strontium and calcium in the presence of each other is an extremely difficult problem.[3] In the specifications,[1,2] barium is determined by precipitation as the sulphate; this is inaccurate because most of the strontium and part of the calcium are co-precipitated with the barium sulphate.[4,5] In the methods for strontium and calcium described in the specifications,[1,2] the sample is fused with sodium carbonate and the barium, strontium and calcium carbonates are filtered off and converted to the nitrates. n-Pentanol is added, the barium and strontium nitrates are filtered off, and the calcium is determined in the filtrate by precipitation as the oxalate. The barium and strontium are separated by a double chromate precipitation, the barium chromate is discarded, and the strontium is precipitated as the carbonate and then as the sulphate. These methods are very erratic and unreliable, according to the experiences of this and other laboratories. "Reagent Chemicals and Standards" by Rosin[6] does not give a method for determining barium in barium peroxide, but describes a method for the determination of calcium in barium peroxide that involves precipitation of the barium as barium sulphate and determination of calcium in the filtrate. "Reagent Chemicals" by the Committee on Analytical Reagents of the American Chemical Society[7] does not give requirements for barium peroxide or a method for determining barium in barium compounds; however, it does describe a flame-photometric method for determining strontium and calcium in barium compounds that involves comparison of the solution of the sample with a standard prepared by adding the maximum permissible amount of strontium or calcium (according to the specification) to a solution of the sample. The exact determination of strontium and calcium in the presence of large amounts of barium by use of the flame photometer is difficult because of interferences.[8,9]

In 1961, this Arsenal proposed an improved method for the determination of barium in barium compounds by precipitation from homogeneous solution of barium chromate.[10] In this method, potassium dichromate, ammonium acetate and urea are added to an acidic solution of the compound, and the solution is boiled to hydrolyse the urea gradually to ammonia. By this technique, large, easily filterable crystals of barium chromate are formed with considerably less occlusion of salts than occurs with the classical dichromate method.

This Arsenal undertook an investigation to develop procedures for the determination of barium, strontium and calcium in barium peroxide that would involve precipitation from homogeneous solution of the barium and determination of strontium and calcium in the filtrate from the barium.

EXPERIMENTAL

DETERMINATION OF BARIUM—

In the method of precipitation from homogeneous solution previously proposed for barium salts,[10] directions are given for the determination of barium in barium peroxide by dissolution in hydrochloric acid, boiling to destroy the peroxide, filtering off the insoluble

matter, fusion of the insoluble matter with sodium carbonate and combination of the melt with the main filtrate. In specification procedures for barium peroxide, it is customary to filter off the insoluble matter (and in some grades to determine it) and to analyse for barium in the filtrate. According to tests in this laboratory, the insoluble matter consists almost entirely of barium sulphate with a trace of silica.

In the analysis of a barium compound, one precipitation of barium chromate from homogeneous solution suffices to separate the barium from up to approximately 3 per cent. of the strontium compound and a double precipitation will separate the barium from any percentage of strontium compound.[10] According to experiences in this Arsenal, there is less than 1·5 per cent. of strontium in commercial barium peroxide (as strontium), so a single precipitation of the barium will usually suffice. However, to allow for all possible eventualities, a double-precipitation method will be included.

An interesting fact about the co-precipitation of strontium by barium chromate in the method of precipitation from homogeneous solution is that the amount of strontium chromate co-precipitated levels off to approximately 10 to 13 mg after one precipitation, as judged by experiments with high-purity barium and strontium nitrates (see Table I). This levelling

TABLE I

CO-PRECIPITATION OF STRONTIUM WITH BARIUM AFTER ONE PRECIPITATION OF BARIUM

Weight of barium nitrate taken was 0·5 g

Strontium nitrate added, g	Barium chromate found, g	Strontium chromate found, mg*	Barium found, per cent.
0·000	0·4840	0·00	52·48
0·005	0·4839	0·00	52·46
0·015	0·4842	0·02	52·50
0·025	0·4867	2·7	52·77
0·050	0·4913	7·3	53·27
0·100	0·4942	10·2	53·58
0·200	0·4974	13·4	53·93
0·500	0·4956	11·6	53·73

* Calculated by deducting the amount of barium chromate found when strontium was absent (0·4840 g) from the amount of barium chromate found when strontium was present.

off is important, because it indicates an equilibrium involving the barium chromate and strontium chromate. Also, it shows that the maximum error that would be encountered in determining barium in barium compounds by one precipitation would not be expected to exceed approximately 1·5 per cent. of barium, no matter how much strontium were present in the compound.

SEPARATION OF STRONTIUM AND CALCIUM FROM THE BARIUM CHROMATE FILTRATE—

The separation of strontium and calcium from the potassium dichromate, ammonium acetate, ammonium chloride and urea contained in the filtrate from the barium determination proved difficult. Attempts were made to eliminate the ammonium salts and chromium by repeated evaporation with nitric acid, with subsequent volatilisation of the chromium as chromium oxide chloride from a perchloric acid solution. This did not prove feasible, although many variations of the technique were tried. In working with this method, it was observed that the decomposition of the ammonium nitrate was very vigorous, and on one occasion a violent reaction occurred that blew the watch-glass off the beaker. The vigorous reaction was probably caused by the fact that chromium is a catalyst for the rapid decomposition of ammonium nitrate.[11]

It was found necessary, therefore, to precipitate the strontium and calcium from the barium chromate filtrate. It has been proposed[3,5] that strontium and calcium be precipitated from the barium chromate filtrate as the carbonates by adding ammonium carbonate and ammonium hydroxide, after a little nitric acid has been added and the solution evaporated to a small volume if necessary. This method was found to give poor recovery of strontium and calcium, whether urea was present or not. These findings confirmed the observations of Wilkinson, Gibson and Headlee,[12] who found that as much as 8 mg of strontium carbonate remained unprecipitated by this method. In another procedure, sulphuric acid and alcohol

were added to the barium chromate filtrate to precipitate the strontium and calcium sulphates.[12] This method was troublesome, since much chromium always precipitated with the sulphate.[12] Another method of approach was to add alcohol and hydrochloric acid, heat the solution to reduce the chromium to the tervalent state, make an ammoniacal separation to remove the chromium, and precipitate the strontium and calcium as the sulphates.[13] This technique did not give satisfactory results in the laboratory, probably because some strontium was occluded by the chromium. In a qualitative scheme,[14,15] alcohol and ammonium hydroxide were added to the barium chromate filtrate to precipitate the strontium as strontium chromate. This method could not be made quantitative. In another qualitative scheme,[16] ammonium phosphate and ammonium hydroxide were added to the barium chromate filtrate to precipitate the strontium, calcium and magnesium as phosphates. Experiences in this laboratory showed that the precipitation of the strontium, calcium and magnesium as phosphates was quantitative. However, attempts to separate the strontium, calcium and magnesium from each other quantitatively were not successful because of the interference of the phosphate.

In another proposed method for utilising the barium chromate filtrate, the pH of the solution was adjusted to between 3·5 and 4·0 in the presence of EDTA, and ammonium oxalate was added to precipitate the calcium and strontium oxalates.[17]

The idea of precipitating the calcium and strontium as oxalates seemed to be the best method of approach to the problem. Good recoveries were obtained for calcium on adding ammonium oxalate directly to the filtrate obtained from the separation of barium chromate (the pH of which was about 5·5) and allowing the solution to stand overnight. However, the results for strontium were satisfactory only when a large amount of that element (more than 0·1 g) was present or when a large excess calcium was present. In the absence of calcium, the recoveries for smaller amounts of strontium were very poor. For example, for 10 mg of strontium, the recovery was 42 per cent.; for 5 mg, 22 per cent. Experiments were conducted on the effect of changing the pH of the filtrate obtained from the separation of barium chromate before the addition of the ammonium oxalate. There was no significant difference in the recovery of strontium at pH values of 3·5, 5·5, 7·5 and 9·0. Temperature also had no appreciable effect.

It was found that quantitative precipitation of strontium oxalate was obtained if 100 ml of 95 per cent. ethanol were added with the ammonium oxalate. For smaller amounts of strontium (single precipitation of the barium chromate) this was equivalent to about one volume of alcohol to three volumes of solution; for larger amounts of strontium (double precipitation of the barium chromate) this was equivalent to about one volume of alcohol to five volumes of solution. The use of alcohol to obtain quantitative precipitation of strontium oxalate was proposed by Peters in 1901,[18] but seems to have been little used. A large excess of ammonium oxalate (50 ml of a 6·5 per cent. solution of $(NH_4)_2C_2O_4.H_2O$) was also necessary to obtain quantitative precipitation of the strontium. The addition of the alcohol or a large excess of ammonium oxalate does not affect the subsequent determination of the magnesium as ammonium magnesium phosphate. According to Kolthoff and Sandell,[19] a large excess of ammonium oxalate keeps magnesium in solution when calcium is precipitated as the oxalate.

Separation of the strontium from the calcium—

The next problem to be resolved was the separation of the strontium and calcium (after the oxalates had been converted into a soluble salt). At first thought, it seemed that a precipitation method, whereby the strontium was precipitated as the insoluble salt while the calcium was kept in solution, would be more desirable than an extraction procedure, whereby the calcium was extracted from the strontium. The precipitation procedures listed here have been recommended for separating strontium from calcium (and magnesium): addition of fuming nitric acid to an aqueous solution of the nitrates to precipitate the strontium as strontium nitrate[20,21]; treatment of an aqueous solution of the nitrates with either n-pentanol[5,22,23,24] or butyl Cellosolve,[25] with subsequent boiling off of the water to precipitate the strontium nitrate; addition of n-butanol containing hydrogen chloride to an n-butanolic solution of the perchlorates of strontium and calcium, to precipitate strontium chloride[4,26]; precipitation of strontium rhodizonate[27]; precipitation of strontium salt of *p*-bromobenzoic acid (qualitative)[28]; precipitation of strontium salt of *p*-toluic acid (qualitative).[29] All but the last two of the above procedures were tested in this laboratory and were found to be less desirable for the problem at hand than the extraction procedures.

All the useful extraction procedures involve the nitrates. The solvents listed below have been used for extracting calcium (and magnesium) nitrate from strontium nitrate: nitric acid of specific gravity 1·42 to 1·47 at room temperature[3,13]; nitric acid of specific gravity 1·45 at 80° to 82° C[30]; absolute ethanol[31,32]; 2-propanol[31]; mixture of absolute ethanol and isobutanol[33]; n-pentanol[5]; butyl Cellosolve[34]; mixture of absolute ethanol and ether[3,5,35]; acetone[36,37,38]; glacial acetic acid.[17,39]

All the above extraction solvents were tested, and the absolute ethanol, absolute ethanol - ether mixture, and the acetone solvents were found to be the most reliable. Acetone was selected for the problem at hand because it was less hygroscopic, more conveniently handled, more readily obtainable and less expensive than absolute ethanol or the absolute ethanol - ether mixture. The procedures in which n-pentanol is used (which appear in many specifications for compounds of alkaline-earth metals) gave extremely poor results and should be abandoned.

In working with any extraction or precipitation method for separating strontium and calcium, corrections must sometimes be made to compensate for the solubility of the strontium salt and incomplete solubility of the calcium salt. If the strontium and calcium are present in approximately equal amounts, then the necessity of making corrections is not always apparent, since the errors tend to cancel each other. It has been suggested that for the n-pentanol precipitation method, 0·0008 g of strontium nitrate be added to the result for strontium for every 10 ml of alcohol used, and that 0·0005 g of calcium sulphate be deducted from the result for calcium for every 10 ml of alcohol used.[24] For the n-butanol - hydrogen chloride separation of barium from strontium, it has been recommended that 1 mg of barium chloride be added to the result for barium, and 1 mg of strontium chloride be deducted from the result for calcium.[4,26] In applying the acetone and butyl Cellosolve extraction procedures to large amounts of nitrates (total of approximately 1 to 2 g of mixed nitrates), the use of correction curves has been advocated.[34,37] Spectrographic analysis of precipitates obtained in alkaline-earth separation procedures has been suggested as a means for making appropriate corrections.[12]

It was found in this laboratory that good results for the acetone separation procedure were obtained, without the necessity of making corrections, if the total of the strontium and calcium did not exceed 0·15 g. For less than a total of 20 mg of strontium and calcium, 10 ml of acetone and a standing time of 1 hour were used; for a total of more than 20 mg of strontium and calcium, 30 ml of acetone and a 3-hour standing time were used.

To convert the oxalates into anhydrous nitrates, the oxalates were ignited for a few minutes, the resultant oxides were dissolved in nitric acid, the solutions evaporated to dryness, and the beakers heated in an oven at 150° to 160° C for 30 to 45 minutes (when less than 10 mg of strontium and calcium are present, the oven treatment can be omitted if the hot plate is properly regulated). At first, attempts were made to dissolve the oxalates directly in diluted (1 + 1) nitric acid and to evaporate the solution to dryness. However, erratic results were obtained, since the oxalates were not completely converted to the nitrates by this means, although they dissolved readily in the nitric acid. Proof that the oxalates were not converted to nitrates was the observation that the salts would not completely dissolve in water after the evaporation.

ACTUAL DETERMINATION OF STRONTIUM AND CALCIUM—

The final problem to be resolved was the actual determination of the strontium and calcium. Some investigators have suggested that the strontium and calcium be weighed as nitrates. However, this practice is not to be recommended in ordinary analytical work, since impurities (both inorganic and organic) may be present. Calcium should not be determined as the nitrate, because calcium nitrate is extremely hygroscopic.

The sulphate method was found to be the best means for determining the strontium and calcium. For the determination of the strontium, the filter-paper and precipitate from the acetone separation were treated with a mixture of 15 ml of nitric acid, 3 ml of sulphuric acid and 2 ml of perchloric acid, and the solution was evaporated to a volume of about 2 ml to destroy the filter-paper and volatilise the perchloric acid. Forty millilitres of water were added, the solution was evaporated to a volume of 20 ml to dissolve any traces of chromium (which would be precipitated with the strontium, not the calcium) and other impurities, and 50 ml of 95 per cent. ethanol were added. For the determination of calcium, the acetone filtrate was evaporated to dryness, a mixture of 5 ml of nitric acid, 2 ml of sulphuric acid

and 0·5 ml of perchloric acid was added, and the solution was evaporated to a volume of about 1 ml. Ten millilitres of water were added, the volume was reduced by evaporation to about 5 ml, and 50 ml of 95 per cent. ethanol were added. The strontium and calcium sulphates were both washed with the same wash solution (9 parts of 95 per cent. ethanol to 1 part of water containing 0·1 per cent. of sulphuric acid). The strontium and calcium sulphates were both ignited by placing the sintered-porcelain crucibles into regular porcelain crucibles and heating them over a Meker burner for 15 minutes. The temperature obtained at the bottom of the sintered-porcelain crucible was about 500° C. The ignition was not critical, since the same result was obtained whether the crucibles were heated for 10 minutes or 1 hour. An alternative method for determining the calcium was to evaporate off the acetone, add a mixture of nitric and perchloric acids, evaporate to fumes of perchloric acid, precipitate the calcium as calcium oxalate, and ignite to calcium oxide. This method was less accurate than the sulphate method.

The accuracy of the methods for strontium and calcium as applied to barium peroxide was limited somewhat by the fact that only a 0·5-g sample could be used for the precipitation of the barium chromate without there being some co-precipitation of salts and urea. However, the results obtained for a 0·5-g sample were still very satisfactory.

POSSIBLE DETERMINATION OF MAGNESIUM AS AMMONIUM MAGNESIUM PHOSPHATE—

Magnesium can be determined in the filtrate from the strontium and calcium oxalate precipitation by adding 20 ml of 20 per cent. w/v diammonium hydrogen phosphate solution and sufficient ammonia solution, sp.gr. 0·90, so that there is one volume of ammonia solution to nine volumes of solution, allowing the mixture to stand for 3 or more hours, filtering off the precipitate, washing it with 10 per cent. v/v ammonia solution, and igniting the residue to magnesium pyrophosphate. This will subsequently be shown in the results. However, since the 8-hydroxyquinoline precipitation method for magnesium recommended in the barium peroxide specifications[1,2] and also by Hillebrand, Lundell, Bright and Hoffman,[3] who used a separate sample, was satisfactory, it was not considered necessary to determine magnesium in barium peroxide by utilising the filtrate from the strontium and calcium oxalate precipitation.

Firsching[40] described a procedure for the precipitation from homogeneous solution of barium chromate at pH 8 to 10 that involved complexing the barium with EDTA and the dropwise addition of a magnesium chloride solution over a period of 1 hour at 90° to 95° C to break up the complex of barium with the EDTA. It was found in this laboratory that the methods for determining strontium and calcium described in the present paper gave low results when they were applied to the determination of strontium and calcium in the filtrate from Firsching's method for determining barium. The use of magnesium chloride by Firsching precludes the determination of magnesium.

METHOD

REAGENTS—

Hydrochloric acid, sp.gr. 1·18.

Nitric acid, sp.gr. 1·42.

Perchloric acid, sp.gr. 1·70.

Sulphuric acid, sp.gr. 1·84.

Ammonium acetate solution, 40 per cent. w/v, aqueous—Dissolve 400 g of ammonium acetate in water, and dilute the solution to 1 litre.

Potassium dichromate solution, 10 per cent. w/v, aqueous—Dissolve 100 g of potassium dichromate in about 950 ml of water by warming on a hot plate, cool the solution and dilute it to 1 litre.

Potassium dichromate wash solution—Add 50 ml of the 10 per cent. potassium dichromate solution to 1 litre of water.

Urea—Analytical-reagent or U.S.P. grade.

Ammonium oxalate solution, 6·5 per cent., aqueous—Dissolve 130 g of diammonium oxalate monohydrate in about 1950 ml of water at 80° to 90° C, allow the solution to cool, and dilute it to 2 litres. Disregard the small amount of salt that occasionally settles out on standing.

Ammonium oxalate wash solution—Add 35 ml of the 6·5 per cent. ammonium oxalate solution to 1 litre of water.

Alcoholic wash solution—Add 1 ml of sulphuric acid and 900 ml of 95 per cent. ethanol to 100 ml of water.

Acetone—Analytical-reagent grade.

Standard barium solution—Dissolve 3·8058 g of high-purity barium nitrate in water and dilute the solution to 1 litre in a calibrated flask.

1 ml of solution ≡ 2 mg of barium.

Standard strontium solution—Dissolve 4·8306 g of high-purity strontium nitrate in water and dilute the solution to 1 litre in a calibrated flask.

1 ml of solution ≡ 2 mg of strontium.

Standard calcium solution—Transfer 4·9946 g of high-purity calcium carbonate to a covered 600-ml beaker, add 100 ml of water and then add 10 ml of hydrochloric acid in small portions. Boil the solution for several minutes, cool it and dilute it to 1 litre in a calibrated flask.

1 ml of solution ≡ 2 mg of calcium.

PROCEDURE FOR THE DETERMINATION OF BARIUM—

For high-purity barium peroxide—Transfer a 0·5-g sample accurately weighed to 0·1 mg to a covered 400-ml beaker. Add 50 ml of water and 7 ml of hydrochloric acid, and boil the solution moderately for 12 minutes. This boiling is important to destroy the peroxide, which would reduce the chromate. If the solution is not clear, filter it and wash the residue with hot water. Dilute the filtrate and washings to 225 ml with water and heat the solution to about 80° C. Add with stirring 10 ml of 40 per cent. ammonium acetate solution, 25 ml of 10 per cent. potassium dichromate solution and 10 g of urea. Cover the beaker with the watch-glass, heat the solution to boiling and boil it moderately until a precipitate settles on the bottom of the beaker, and then continue boiling moderately for 60 to 65 minutes. Midway during the 60- to 65-minute boiling period, wash down the cover lid with water, and bring the volume to 225 to 250 ml by adding hot water. At the end of the heating period, filter the solution through a tared sintered-glass crucible of medium porosity, transfer the precipitate to the crucible with potassium dichromate wash solution, and finally wash the precipitate four times with water. Wash the filtrate into a 600-ml beaker and retain it for the determination of strontium and calcium. Dry the crucible at 120° C for 1 hour, cool it and weigh the residue as barium chromate.

Calculate the barium content as follows—

$$\text{Barium content, per cent.} = \frac{54{\cdot}21 \times \text{weight of barium chromate, g}}{\text{weight of sample, g}}$$

For low-purity barium peroxide—Determine the insoluble matter by treating a 2-g sample accurately weighed to 0·1 mg with 25 ml of 10 per cent. hydrochloric acid, evaporating the solution to dryness and baking the residue in an oven at 105° to 110° C for 2 hours. Add 2 ml of hydrochloric acid and 60 ml of water, heat the mixture on a steam-bath for 10 minutes, filter the solution through a Whatman No. 42 filter-paper, transfer and wash the precipitate with diluted (1 + 20) hydrochloric acid, and calcine the precipitate at about 700° C.

Dilute the filtrate to 200 ml in a calibrated flask and transfer by pipette a 50-ml aliquot (equivalent to about a 0·5-g sample) into a 400-ml beaker. Add 6 ml of hydrochloric acid, dilute the solution to 225 ml, heat it to 80° C and precipitate the barium chromate as for high-purity barium peroxide, but filter the solution through a Whatman No. 40 filter-paper into an 800-ml beaker. Wash the precipitate on to the filter-paper with potassium dichromate wash solution and then wash the filter-paper three times with the same solution. Transfer the filter-paper and precipitate back to the 400-ml beaker and add 15 ml of nitric acid and 6 ml of perchloric acid. Cover the mixture with a watch-glass, evaporate to fumes of perchloric acid at moderate heat, continue fuming at moderate heat until the solution is red, and then fume for 1 to 2 minutes more. Dilute the solution to 225 ml, add 2 ml of hydrochloric acid, heat to 80° C, and precipitate the barium chromate as described for high-purity barium peroxide, but use 15 ml of 10 per cent. potassium dichromate solution. Filter the solution through a tared sintered-glass crucible and combine the filtrates for the determination of strontium and calcium.

PROCEDURE FOR THE DETERMINATION OF STRONTIUM AND CALCIUM—

Add 50 ml of 6·5 per cent. ammonium oxalate solution and 100 ml of 95 per cent. ethanol to the filtrate from the barium determination, and allow the solution to stand overnight if less than 10 mg of strontium *plus* calcium are present, or 3 hours (or more) if more than 10 mg are present. Filter the solution through a Whatman No. 40 filter-paper, and transfer and wash the precipitate with ammonium oxalate wash solution. (Retain the filtrate for the determination of magnesium by the ammonium magnesium phosphate method if magnesium is to be determined by this means.)

Place the filter-paper and precipitate in a platinum crucible, char and burn off the filter-paper at low heat, and calcine the precipitate over a Meker burner for 15 minutes. Transfer most of the precipitate from the platinum crucible to a 400-ml beaker. Add 10 ml of nitric acid to the crucible, warm it to dissolve the salts (if necessary), and wash the solution into the 400-ml beaker with a little water. Evaporate the solution to dryness by heating on a hot plate at low heat without a watch-glass, and then heat the residue in an oven at 150° to 160° C for 30 to 45 minutes. (If the determination is interrupted at this stage, store the beaker in an oven at 105° C.) Cover the beaker with a dry watch-glass and allow it to cool to room temperature. Add 10 ml of acetone around the sides of the beaker if less than 20 mg of strontium *plus* calcium are present, or 30 ml of more than 20 mg are present. Rub the bottom of the beaker with a dry "policeman" and break up any clumps of salt. Wash down the "policeman" with a little acetone and remove it. Allow the salt to stand for 1 hour if less than 20 mg of strontium *plus* calcium are present, or 3 hours if more than 20 mg are present. Swirl frequently during the period. Filter the liquid through a Whatman No. 40 filter-paper; use a 6-cm filter-paper if less than 20 mg of strontium *plus* calcium are present, and a 9-cm filter-paper if more than 20 mg are present. Use an untorn filter-paper and set it into the funnel with acetone. Wash the precipitate into the filter-paper with acetone (contained in a wash bottle) and then wash the filter-paper and precipitate thoroughly four times with acetone. Collect the filtrate in a 250-ml beaker and retain it for the determination of calcium.

Transfer the filter-paper and precipitate back to the 400-ml beaker and add 15 ml of nitric acid, 3 ml of sulphuric acid, and 2 ml of perchloric acid. Cover the beaker with a watch-glass, reduce the volume of the solution by boiling on a hot plate at moderate heat until the organic matter has been destroyed and the solution is fuming, and then with the cover lid ajar, heat the beaker at the maximum temperature of the hot plate until the volume is reduced to about 2 ml. Cool, add 40 ml of water, cover the beaker with the watch-glass and boil the solution at moderate heat until the volume is about 20 ml. Remove the beaker from the hot plate and add 50 ml of 95 per cent. ethanol. Allow the solution to stand for 3 or more hours (overnight if less than 1 mg of strontium is present). Filter it through a tared Selas porcelain filtration crucible, transferring the precipitate and washing it with the alcoholic wash solution, and finally wash it with 10 ml of 95 per cent. ethanol. Place the sintered-porcelain crucible into a 30-ml porcelain crucible and heat it over a Meker burner for 15 minutes. Cool the residue and weigh it as strontium sulphate.

Calculate the strontium content as follows—

$$\text{Strontium content, per cent.} = \frac{47{\cdot}70 \times \text{weight of strontium sulphate, g}}{\text{weight of sample, g}}$$

For the determination of calcium, evaporate the acetone filtrate to dryness by heating it on an electric hot plate under a hood. Add 5 ml of nitric acid, 2 ml of sulphuric acid, and 0·5 ml of perchloric acid, and fume to a volume of about 1 ml. Cool the solution, add 10 ml of water, and reduce the volume of the solution to about 5 ml by boiling. Remove the solution from the hot plate and add 50 ml of 95 per cent. ethanol. Allow the solution to stand for 3 hours (overnight if less than 1 mg of calcium is present) and filter off and calcine the precipitate as described for strontium sulphate.

Calculate the calcium content as follows—

$$\text{Calcium content, per cent.} = \frac{29{\cdot}44 \times \text{weight of calcium sulphate, g}}{\text{weight of sample, in g}}$$

Results

Results obtained for the separation of strontium and calcium by use of the acetone extraction procedure are shown in Table II. Results obtained for mixtures of barium, strontium, calcium and magnesium carried through the entire recommended procedure are shown in Table III. Magnesium was determined by the pyrophosphate method for the

Table II

Results for strontium and calcium by acetone-extraction procedure

Present, g		Found, g	
strontium	calcium	strontium	calcium
0·1000	0·0000	0·0996	0·0001
0·1000	0·0500	0·1010	0·0494
0·1000	0·0100	0·1005	0·0095
0·0100	0·0100	0·0098	0·0099
0·0100	0·1000	0·0097	0·1003
0·0500	0·1000	0·0494	0·1004
0·0000	0·1000	0·0002	0·0993
0·0700	0·0700	0·0695	0·0698

Table III

Results for barium, strontium, calcium and magnesium in synthetic samples

Present, g				Found, g			
barium	strontium	calcium	magnesium	barium	strontium	calcium	magnesium
0·2000	0·1000	0·0500	0·0500	0·2002*	0·0995	0·0498	0·0503
0·2000	0·0500	0·1000	0·0500	0·1997*	0·0508	0·0996	0·0503
0·2000	0·0100	0·0100	0·0100	0·1998*	0·0108	0·0097	0·0102
0·2000	0·0050	0·0050	0·0050	0·2001†	0·0047	0·0048	0·0051
0·2000	0·0020	0·0010	—	0·2003†	0·0022	0·0008	—
0·1000	0·0700	0·0700	—	0·1000*	0·0705	0·0695	—
0·0200	0·1000	0·0500	0·0500	0·0197*	0·1011	0·0491	0·0502
0·0200	0·0500	0·1000	0·0500	0·0198*	0·0503	0·0992	0·0501
0·0200	0·0100	0·0100	0·0100	0·0201*	0·0097	0·0096	0·0103

* Double precipitation of barium chromate.
† Single precipitation of barium chromate.

Table IV

Results for barium, strontium and calcium, per cent., in typical samples of barium peroxide

Element	Sample 1 (high purity)	Sample 2 (high purity)	Sample 3* (low purity)	Sample 4† (low purity)
Barium	78·60	79·79	76·77‡	76·74‡
	78·68	79·75	76·87‡	76·70‡
	78·65	79·69	76·86§	76·65§
	78·60	79·71	76·84§	76·76§
Mean	*78·63*	*79·74*	*76·84*	*76·71*
Standard deviation ..	*0·040*	*0·045*	*0·045*	*0·049*
Strontium	0·82	0·52	0·84‡	0·86‡
	0·84	0·58	0·78‡	0·94‡
	0·78	0·54	0·76§	0·90§
	0·80	0·52	0·78§	0·90§
Mean	*0·81*	*0·54*	*0·79*	*0·90*
Standard deviation ..	*0·026*	*0·028*	*0·035*	*0·033*
Calcium	0·02	0·01	0·02‡	0·02‡
	0·01	0·01	0·03‡	0·01‡
	0·01	0·02	0·01§	0·01§
	0·02	0·02	0·01§	0·02§
Mean	*0·02*	*0·02*	*0·02*	*0·02*
Standard deviation ..	*0·008*	*0·008*	*0·010*	*0·008*

* Contained 1·42 per cent. of insoluble matter.
† Contained 0·87 per cent. of insoluble matter.
‡ Double precipitation of barium chromate.
§ Single precipitation of barium chromate.

sake of information, but as stated previously it is recommended that the magnesium in barium peroxide be determined by the 8-hydroxyquinoline procedure. The results obtained for barium, strontium and calcium in typical samples of barium peroxide are shown in Table IV. Recoveries obtained after strontium and calcium had been added to one of these samples are shown in Table V.

TABLE V

RECOVERY OF STRONTIUM AND CALCIUM ADDED TO BARIUM PEROXIDE

Synthetic sample	Total present, g		Total found, g		Recovered, g	
	strontium	calcium	strontium	calcium	strontium	calcium
0·5 g of sample 1 + 0·0050 g of Sr + 0·0050 g of Ca	0·0090	0·0051	0·0092†	0·0050	0·0052	0·0049
0·5 g of sample 1 + 0·0020 g of Sr + 0·0020 g of Ca	0·0060	0·0021	0·0059‡	0·0024	0·0019	0·0023
0·5 g of sample 1 + 0·0010 g of Sr + 0·0010 g of Ca	0·0050	0·0011	0·0047‡	0·0013	0·0007	0·0012
0·5 g of sample 1 + 0·0050 g of Sr + 0·0010 g of Ca	0·0090	0·0011	0·0089†	0·0009	0·0049	0·0008

* Contained 0·81 per cent. of strontium and 0·02 per cent. of calcium (see Table IV).
† Double precipitation of barium chromate.
‡ Single precipitation of barium chromate.

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Analytical Results for the Paper-chromatographic Zone-Strip Technique

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The use of paper chromatography for micro-scale quantitative analysis by the zone - strip technique gives accurate and reproducible results within certain concentration limits. Analytical results concerning these limits, obtained under specified experimental conditions, are given. Furthermore, some other conclusions concerning the mode of deviation outside these ranges are given.

Shimi and co-workers[1,2] have described a method for micro-scale quantitative analysis by partition - paper chromatography. They used narrow strips, and solutions to be assayed were loaded in 2-mm wide zones near the upper end of the strips. They derived the equation—

$$l = K \log c \log d$$

where l is the length, in cm, of the loaded zone in the developed strip,
c is the concentration of the substance, in μg, that occupies the zone and
d is the distance, in cm, travelled by the developing solvent.

K is a constant factor that determines the value of l with respect to the two variables, c and d. This constant factor, as well as the concentration limit, are specific for every compound under the experimental conditions adopted.

The present study was made to determine the range of concentrations over which application of the zone - strip technique gives accurate and reproducible results for the various compounds. The experimental conditions adopted are described.

Experimental

The experimental technique used was that described by Shimi and co-workers.[1,2] The classes of compounds used in this study were organic, amino- and keto-acids, 2,4-dinitrophenylhydrazones of keto-acids and acetone, sugars and penicillins.

Organic acids—

Isopropanolic or ethanolic solutions containing 10 mg of organic acid per ml were prepared from the organic acids. The strips were loaded with solutions of the different acids, separately and in various combinations, and then allowed to dry at room temprature. The strips were then hung in a chromatographic tank for 24 hours before development. The tank contained the aqueous phase of the developing solvents, which was freshly prepared from n-butanol - acetic acid - water (4 + 1 + 5) mixture. This solvent allowed a satisfactory resolution of the various acids,[3] if a development time of 10 to 15 hours was used. Subsequently, the strips were removed from the chromatographic tank and dried in air for 6 hours. The developed strips were then sprayed with—

(*a*) an alcoholic solution of bromophenol blue,[4] or
(*b*) an alcoholic solution of bromophenol blue and then with a 2 per cent. aqueous solution of copper sulphate,[4] or
(*c*) an alcoholic solution of bromophenol blue, a 2 per cent. aqueous solution of copper sulphate and then with 10 per cent. ammonia solution.[4]

2,4-Dinitrophenylhydrazones of keto-acids and acetone—

These were prepared and purified by crystallisation from ethyl acetate and light petroleum, boiling-range 40° to 60° C. The developing solvent suggested by Cavallini, Frontali and Toshi[5,6] was tried, but it was found unsatisfactory. The solvent used in this study comprised equal volumes of n-butanol and water. This solvent was found suitable for the formation of well defined zones. The strips loaded with 2,4-dinitrophenylhydrazones were left in a closed chromatographic tank containing water saturated with n-butanol for 4 hours, and the aqueous butanol was then added. The solvent was allowed to descend for 10 hours and the strips were subsequently removed, dried in air and finally subjected to ultraviolet light.[7]

AMINO-ACIDS—

Solutions of the amino-acids in 10 per cent. aqueous isopropanol were prepared. The experimental steps applied were those described above. The loaded strips were treated as described for organic acids. The developed strips were finally sprayed with ninhydrin reagent and then heated.[8]

SUGARS—

Solutions of sugars in aqueous isopropanol were prepared. The rest of the experimental technique was as described for organic and amino-acids. The developed strips were sprayed with silver nitrate reagent.[9]

PENICILLINS—

The various penicillins were kindly presented by the World Health Organisation. The penicillins were assayed as described by Shimi and co-workers.[1,2] The developed strips were sprayed with 0·01 per cent. iodine solution and then subjected to ultraviolet light.

During this work, several developing solvents and reagents[10,11] for locating the zones of the different compounds, other than those described above, were tried and were considered to be less satisfactory.

RESULTS AND DISCUSSION

ORGANIC ACIDS—

The bromophenol blue spray reagent was sensitive to concentrations above 30 μg per strip, but the sensitivity was increased, down to 2 μg per strip, by subsequently spraying the strips with copper sulphate solution.

If the strips that were sprayed with bromophenol blue and copper sulphate solutions were subsequently treated with dilute ammonia solution, the zones appeared as shiny golden-yellow areas and the sensitivity increased to 0·5 μg per strip. The values of K and the concentrations below which they remain constant are given in Table I; they were obtained by using bromophenol blue, copper sulphate and ammonia solutions to locate the zones on the developed strips.

From Table I it is clear that each organic acid had a constant value of K within certain concentration limits, above which these values either increased or decreased. The values tended to rise for the aliphatic acids, whereas a drop was observed for the acids containing an aromatic group or unsaturated bonds. Constant values of K could be calculated for oxalic, succinic and phenylacetic acids at concentrations below 15 μg per strip. Most of the other acids showed constant values of K at concentrations of up to 30 or 45 μg per strip. For gluconic, maleic, phenylpyruvic, oxaloacetic, acetoacetic and aconitic acids the strips could be loaded with up to 60 μg per strip without the values of K being altered.

2,4-DINITROPHENYLHYDRAZONES OF KETO-ACIDS AND ACETONE—

Amounts down to 1 μg of derivative per strip could be detected by subjecting the chromatogram to ultraviolet light. The period required for saturating the loaded strips before they were developed (pre-development period), was found to influence the accuracy of the results. A period of 3 hours proved to be the most suitable. Shorter periods did not allow good resolution, and longer periods resulted in ill defined zones with predominant "tailing." The values of K and the concentrations below which they remain constant are given in Table I; they were obtained by using ultraviolet light. Constant values of K could be obtained at concentrations of below 30 μg per strip for acetone, 45 μg for acetoacetic acid, 60 μg for pyruvic and oxaloacetic acids and below 90 μg for α-ketoglutaric acid. It is worth mention that above the concentration limits below which the values of K were constant, decreasing values were found. This is in accordance with the previous conclusion that the presence of an aromatic group decreases the values of K calculated for concentrations outside the proper range.

AMINO-ACIDS—

Treating the loaded strips with the ninhydrin reagent proved adequate for the location of as little as 2 μg of amino-acid per strip. The reagent reacted instantaneously with aliphatic amino-acids containing primary or secondary amino groups. Aromatic amino-acids reacted at a slower rate.

The values of K and the concentrations below which they remain constant are given in Table I. Mixing of the different amino-acids gave no changes in the values of K. It can be seen from the results in Table I that concentrations of up to 45 μg of any of the amino-acids used per strip, except proline, tryptophan and threonine could be applied to the strips without fear of any deviation in the values of K being obtained. Most of the amino-acids could be safely assayed in concentrations of up to 60 μg per strip. This range is comparatively high when compared with that of the corresponding organic acids; this is probably due to the presence of the amino groups.

TABLE I

VALUES OF K AND CONCENTRATION LIMITS FOR VARIOUS COMPOUNDS

Compound	Value of K when constant	Concentration below which K is constant, μg per strip
Organic acids—		
Tartaric	1·51 ± 0·02	30*
α-Ketoglutaric	0·91 ± 0·01	30*
Malonic	0·81 ± 0·01	30*
Acetoacetic	0·80 ± 0·02	60*
Oxaloacetic	0·69 ± 0·01	60*
Lactic	1·20 ± 0·04	15*
Citric	1·58 ± 0·03	45*
Pyruvic	0·83 ± 0·02	60*
Oxalic	1·10 ± 0·03	15*
Malic	1·04 ± 0·04	45*
Gluconic	0·66 ± 0·03	60*
Succinic	0·70 ± 0·03	15*
Maleic	0·78 ± 0·01	60†
Aconitic	1·18 ± 0·03	60†
Fumaric	0·86 ± 0·02	30†
Phenylpyruvic	0·88 ± 0·03	75†
Benzoic	1·16 ± 0·03	20†
Phenylacetic	0·79 ± 0·02	15†
2,4-*Dinitrophenylhydrazones of*—		
Pyruvic acid	1·32 ± 0·01	60†
Oxaloacetic acid	0·89 ± 0·03	60†
Acetoacetic acid	0·81 ± 0·01	60†
α-Ketoglutaric acid	0·63 ± 0·03	90†
Acetone	1·00 ± 0·01	30†

Compound	Value of K when constant	Concentration below which K is constant, μg per strip
Amino-acids—		
Glycine	1·36 ± 0·04	45*
Glutamic	0·36 ± 0·01	45*
Aspartic	0·81 ± 0·01	75*
Lysine	1·21 ± 0·03	60*
Cystine	1·30 ± 0·02	75*
Arginine	0·88 ± 0·03	45*
Valine	1·12 ± 0·02	60*
Cysteine	0·40 ± 0·03	30*
Leucine	1·05 ± 0·03	75*
Isoleucine	1·69 ± 0·03	75*
Tyrosine	0·90 ± 0·02	45*
Serine	0·60 ± 0·03	60*
Methionine	1·41 ± 0·02	45*
Histidine	1·26 ± 0·03	60*
Alanine	2·23 ± 0·02	60†
Proline	1·18 ± 0·03	15†
Phenylalanine	1·51 ± 0·02	60†
Threonine	1·43 ± 0·03	30†
Tryptophan	1·29 ± 0·03	30†
Sugars—		
Glucose	0·31 ± 0·02	30*
Galactose	0·26 ± 0·02	30*
Lactose	0·14 ± 0·01	60*
Sucrose	0·19 ± 0·03	45*
Penicillin types—		
G	0·87 ± 0·03	45†
F and H_2F	0·36 ± 0·02	60†
K	0·76 ± 0·01	45†
V	0·56 ± 0·02	45†

* Above concentration value given, the value of K increases.
† Above concentration value given, the value of K decreases.

SUGARS—

Application of silver nitrate reagent for locating the zones was found to be more suitable than the use of alkaline permanganate. The results in Table I were obtained by using the former reagent. Up to 45 μg of each of the sugars per strip used gave reproducible and constant values of K.

PENICILLINS—

Leaving the loaded strips for 3 hours in a chromatographic tank saturated with saturated aqueous ether solution proved to be essential for satisfactory resolution of the different penicillins. Up to 45 μg of the penicillins per strip gave constant values of K. Above this limit the values of K decrease (see Table I).

Conclusions

From the above results it is concluded that—

(*i*) the value of the constant K and the concentration below which it remains unaltered is specific for each compound under strictly defined experimental conditions and

(*ii*) above this concentration limit, the values of K may decrease or increase. The decrease was only recorded for compounds containing an aromatic group or unsaturated bonds or having a heterocyclic nature.

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SHORT PAPERS

Determination of Cyclamates in Soft Drinks*

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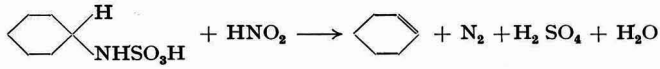
THE introduction of the new Soft Drinks Regulations,[1] which came into force in June, 1965, has brought about many important changes in the composition of soft drinks, one of these being the permitted use of cyclohexylsulphamic acid, and its sodium and calcium salts (cyclamates for short), as well as saccharin, as sweetening agents. In the U.S.A., cyclamates have in fact been permitted in dietetic foods for more than a decade. The various physical properties of cyclamates and their sweetening effect on foods have been described elsewhere.[2,3,4]

The present recommended method[5,6,7] for determining cyclamates in soft drinks is based on the fact that they react with nitrous acid in the following manner—



the sulphate produced being precipitated and weighed as barium sulphate. This method is, however, very tedious when applied to comminuted citrus drinks due to the large amount of fibrous matter present. Another modification is the direct titration of the acidified cyclamate solution with standard sodium nitrite solution with starch - iodide used as an external indicator.[8] This method has only proved suitable in our hands as a very rough check on the cyclamate content of comminuted drinks for quality-control purposes.

The method described below for determining cyclamates in soft drinks (although specifically for comminuted citrus drinks, it can be adapted for any food product), is based on the analysis of the cyclohexene produced, after reaction with nitrous acid, with a gas - liquid chromatographic technique. The method, which is both rapid and very specific, is suitable for analysing cyclamates in the range of 0 to 1 mg per ml of fruit drink.

In addition to cyclohexene, gas-chromatographic evidence indicated the presence of at least two other products of reaction, probably cyclohexananone and cyclohexanol, which had retention times of about 50 and 58 minutes under the conditions described. The presence of these compounds prevented the extension of the range above 1 mg per ml since they would have interfered unduly with subsequent chromatographic analyses. Three comminuted orange drinks containing the usual amounts of benzoic acid and saccharin, and also 0, 0·067 and 0·137 per cent. of sodium cyclamate were analysed by this technique. The amounts of sodium cyclamate found were 0, 0·069 and 0·134 per cent., respectively.

METHOD

APPARATUS—

Pye Argon chromatograph.

Column—4 ft. × 0·25 in.; 10 per cent. Apiezon L on acid-washed, siliconised Embacel, 100 to 115 mesh, at 50° C.

Detector voltage—1000 volts.

Amplifier sensitivity—× 10.

Argon inlet pressure—10 p.s.i.

Argon flow-rate—40 ml per minute.

Chart speed—6 inches per hour.

REAGENTS—

Sodium cyclamate solution, 0·20 per cent., aqueous.

Zinc acetate solution—Add 21·9 g of zinc acetate dihydrate and 3 ml of glacial acetic acid to water and dilute to 100 ml.

Potassium ferrocyanide solution, 10·6 per cent., aqueous.

* Presented at the meeting of the Society on Wednesday, March 31st, 1965.

Light petroleum—Boiling-range, 30° to 40° C. Analysis by the conditions given above should give no peaks corresponding to benzene or cyclohexene. If peaks are obtained, re-distil the light petroleum and collect fraction distilled at 30° to 35° C.

Light-petroleum solution—Add enough benzene (about 2 drops) to 50 ml of the light petroleum, such that on analysis of a 1-μl sample by the conditions given above, a benzene peak is obtained, 2 to 2½ inches high.

Sodium nitrite, 0·5 M.

PROCEDURE—

Construction of calibration curve—Transfer by pipettes, 5 ml of the cyclamate solution, 15 ml of water, 2 ml of concentrated hydrochloric acid, 1 ml of light-petroleum solution and 1 ml of sodium nitrite solution into a 25-ml calibrated flask. Stopper the flask, shake the contents for a few seconds and release the stopper very carefully. Continue this process for a further 3 minutes until there is no sign of effervescence on releasing the stopper. Add water, if required, so that the solvent enters the neck of the flask. By means of a 1-μl syringe, transfer a 1-μl sample of the light-petroleum solution to the top of the column and record a chromatogram.

Repeat the above process with 10 ml of cyclamate solution and 10 ml of water.

A straight-line relationship should be obtained on plotting a graph of the height of the cyclohexene peak (retention time of about 6·5 minutes) relative to that of benzene (retention time of about 5·5 minutes) against the concentration of sodium cyclamate.

Analysis of comminuted orange drinks—Transfer by pipettes, an aliquot of comminuted orange drink containing less than 50 mg of sodium cyclamate (preferably about 25 mg), 5 ml of concentrated hydrochloric acid, 1 ml of zinc acetate solution and 1 ml of potassium ferrocyanide solution into a 50-ml calibrated flask, shake well and dilute to the mark with water. Filter the solution through a fluted Whatman No. 41 filter-paper, and extract a portion of the filtrate with three 50-ml aliquots of chloroform and then twice with 25-ml aliquots of light petroleum. Transfer by pipette, 20 ml of the final aqueous solution, 1 ml of light-petroleum solution and 1 ml of sodium nitrite solution into a 25-ml calibrated flask, shake the contents and analyse the light petroleum solution as described in the paragraph above. Calculate the height of the cyclohexene peak relative to that of the benzene peak, and by means of the calibration curve, determine the concentration of sodium cyclamate in the original comminuted orange drink.

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Spectrophotometric Determination of Carbaryl in Insecticide Formulations

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SEVERAL carbamic acid esters have shown a high degree of activity against a wide range of insects as contact poisons. One of the best known compounds is carbaryl, 1-naphthyl *N*-methylcarbamate, introduced in 1957.[1] During laboratory experiments on the stability of carbaryl "col," a concentrated stable aqueous suspension of carbaryl containing dispersing agents, it became necessary to determine the active-ingredient contents. For macro-determination of carbaryl, the evolution method involving non-aqueous titration of the total base and distillation of methylamine after

alkaline hydrolysis of the insecticide,[2] the infrared spectroscopy at 5·75 μ[2] and the ultraviolet absorptiometry at 280 mμ[3] have been reported. These methods are not applicable to samples containing a high percentage of water or interfering constituents. Methods for determining carbaryl residues are usually based on alkaline hydrolysis of the insecticide to 1-naphthol, with subsequent coupling of this with *p*-nitrobenzenediazonium fluoroborate.[4,5,6] Hardon, Brunink and van der Pol[7] determined carbaryl in treated apples by coupling it with diazotised sulphanilamide to give a red colour, which was measured at 520 mμ.

The method described by Hardon, Brunink and van der Pol is attractive, because hydrolysis of the carbaryl is not required. It suffers, however, from the disadvantage that the coupling reaction must be carried out at 3° C, otherwise the reagent blank value is high. At the temperature specified, mist forms on the walls of optical cell, causing error in absorptiometry. It was found that coupling of carbaryl with either diazotised sulphanilic acid or sulphanilamide could be done at room temperature, provided that the final solution was made fairly alkaline. In this way, the result for a blank determination was negligible. The solubility of carbaryl in chloroform was found to be about 12 per cent. The rapid and specific method described below for analysing carbaryl "col," containing 40 to 50 per cent. w/w of active ingredient, depends on extraction of the insecticide into chloroform and formation of a red colour by coupling with diazotised sulphanilic acid in strong alkali. The maximum colour is developed in 10 minutes, and is stable for a further 10 minutes, permitting differential absorptiometry to be applied.

Method

Apparatus—

Boiling flasks—Round-bottomed 100-ml flasks, with B24 1-inch necks.

Distillation apparatus—An apparatus, similar to that shown in Strouts, Gilfillan and Wilson's book,[8] is suitable.

Spectrophotometer—A Unicam SP600 can be used.

Reagents—

Sodium nitrite solution, 0·3 per cent. w/v, aqueous—Prepare a fresh solution each day.

Sulphanilic acid solution, 0·2 per cent. w/v in N *hydrochloric acid*—Prepare the solution by heating 0·2 g of sulphanilic acid in 100 ml of N hydrochloric acid, and make up the cooled solution to 100 ml with water.

Sodium hydroxide solution, 4 N.

Standard carbaryl solution—Dissolve 0·050 g of pure carbaryl in methanol, and dilute to 500 ml with methanol. Then—

$$1 \text{ ml of solution} \equiv 0{\cdot}1 \text{ mg of carbaryl.}$$

Procedure—

Weigh accurately about 0·5 g of well mixed sample into a 250-ml separating funnel. Add 50 ml of water, and extract successively with two 100-ml and one 50-ml portions of chloroform by shaking vigorously for 1 minute. Collect the combined extract in a 250-ml calibrated flask, make up to the mark with chloroform, and mix. Filter the extract through a Whatman No. 40 filter-paper. Dilute 10·0 ml of the filtrate to 200 ml with chloroform, and call this solution *A*.

Transfer by pipette 10·0 ml of solution *A* to a 100-ml boiling flask, and evaporate off chloroform under reduced pressure, using moderate suction, on a water-bath at about 60° C. Dissolve the residue in 10 ml of methanol and transfer the solution to a 100-ml calibrated flask. Wash the flask twice with 5-ml portions of methanol and pass the washings into the calibrated flask. At the same time, dilute 2·0, 3·0, 4·0 and 5·0 ml of standard solution, equivalent to 0·2, 0·3, 0·4 and 0·5 mg of carbaryl, respectively, to 20 ml with methanol in four 100-ml calibrated flasks. Add to each flask 20 ml of water and 5 ml each of sodium nitrite and sulphanilic acid solutions, mix, then set the flasks aside for 10 minutes. Add, by a fast-running pipette, 10 ml of 4 N sodium hydroxide, dilute the contents to the mark with water, and mix. Measure the optical densities of solutions 10 minutes after mixing at 520 mμ in a 1-cm optical cell against the 0·2-mg standard as reference. Draw the calibration graph relating optical densities of standards to concentrations of carbaryl in milligrams, and read off the carbaryl content of the test solution; alternatively compute the carbaryl content by interpolation. Call this amount *Y* mg.

$$\text{Carbaryl content, per cent. w/w} = \frac{50 \times Y}{\text{Weight of sample in g.}}$$

Discussion of the method

In the formulated material, 1-naphthol is the principal contaminant, either introduced as an impurity from the technical sample or formed by decomposition of carbaryl during storage. Under the recommended conditions, 1-naphthol gives a yellow colour at the diazotisation stage, and this is intensified when the solution is rendered alkaline. This colour has an absorption maximum at 400 to 420 mμ, and the optical density, measured at 520 mμ, is about one-tenth of that obtained from an equivalent amount of carbaryl. Under normal conditions, separation of carbaryl from 1-naphthol is not required. If a substantial amount of 1-naphthol is suspected, indicated in the final solution by an orange-red instead of a red colour, a portion of solution *A* should be washed twice with dilute sodium hydroxide and then with water to remove 1-naphthol,[7] before the chloroform is evaporated off and the colour is developed.

The proposed method has been satisfactorily applied to the determination of the percentage suspensibility[9] of carbaryl "col," and of carbaryl in technical samples and other formulations.

Results

Recoveries of carbaryl were determined on 16 samples of laboratory-prepared "col," each containing 50 per cent. w/w of carbaryl and dispersing agents, together with varying amounts of 1-naphthol. Results obtained are shown in Table I. It can be seen that 1-naphthol in amounts up to 10 per cent., equivalent to 20 per cent. of the carbaryl content, had no appreciable effect on the determination. The average recovery, based on 12 carbaryl "col" containing 0 to 10 per cent. of 1-naphthol, was found to be 100·7 per cent. with a standard deviation of $\pm$1·3 per cent.

Table I

Recovery of carbaryl by the proposed method

1-Naphthol added, per cent. w/w	Carbaryl added, per cent. w/w	Carbaryl found, per cent. w/w
0	50·0	50·0, 50·5, 50·8, 49·6
2·5	50·0	50·9, 51·1
5·0	50·0	49·2, 49·7
7·5	50·0	50·8, 49·8
10·0	50·0	51·2, 50·4
12·5	50·0	51·4, 51·9
15·0	50·0	52·7, 53·7

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A Rapid Method for Quantitative Separation of Vitamin D from Vitamin A

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The greatest difficulty encountered in the determination of vitamin D in natural products is the quantitative elimination of vitamin A, which masks the absorption of vitmin D in the ultraviolet and also interferes with the colour tests. Some of the various methods that are proposed from time to time are referred to in an earlier paper by Barua and Rao,[1] in which a method has been given for determining vitamin D after removal of vitamin A as anhydrovitamin A.

It was shown by Ball, Goodwin and Morton[2] that retinene is formed when a solution of vitamin A in light petroleum is kept in contact with manganese dioxide for some days. In a very elegant and rapid method, Wald[3] has been successful in preparing retinene in 80 per cent. yield by passing a solution of vitamin A in light petroleum through a column of manganese dioxide. In the present paper a smooth and rapid method of eliminating vitamin A is proposed, based on the conversion of vitamin A to retinene on a column of precipitated manganese dioxide.[4] The separation of vitamin D from retinene is effected by chromatography on weakened alumina. Retinene, which is weakly adsorbed, will slowly pass through the column with light petroleum, while vitamin D is retained. The column is extruded and vitamin D is eluted and determined with the Zimmerli - Nield - Russell modified antimony trichloride reagent.[5]

Method

Apparatus—

The spectrophotometric determinations were carried out on a Beckman DK-2 spectrophotometer. For fluorescence observations, a mercury arc lamp fitted with a Wood's filter was used as an ultraviolet light source.

Reagents—

Manganese dioxide—Precipitated (obtainable from B.D.H. Ltd.).

Calciferol—B.P. grade.

Other reagents (alcohol, light petroleum, chloroform, diethyl ether, anhydrous sodium sulphate, antimony trichloride reagent, alcoholic potassium hydroxide and weakened alumina) should be purified or prepared by substantially the same procedures as were described in a previous paper by the authors.[1]

Saponification—

Carry out all operations in subdued light.

Saponify a convenient weight of the sample with freshly prepared 12 per cent. w/v alcoholic potassium hydroxide solution under a slow stream of nitrogen for 5 minutes on a boiling-water bath. Maintain a ratio of 2·5 g of potassium hydroxide to 1g of the sample. Cool and dilute the alcoholic soap solution obtained with an equal volume of water. Extract it four times with suitable volumes of light petroleum. Combine the extracts, and wash the solution with water until the washings are neutral to phenolphthalein. Dry the solution over sodium sulphate and reduce its volume to about 25 ml under reduced pressure.

Oxidation—

Prepare a column, 2 to 2·5 cm in height, with precipitated manganese dioxide powder. Use 0·5 g of manganese dioxide for every 10,000 i.u. of vitamin A. Transfer the solution of the unsaponifiable matter to the column and allow it to pass through in a quick succession of drops. When all the solution has passed through, wash the column with three 5-ml portions of light petroleum into the same receiver. Add successive portions of the solvent before the top of the column becomes dry. Reduce the volume of the solution to 1 to 2 ml, for use in chromatography.

Chromatography—

Transfer the above solution to a chromatographic column, 1 × 10 cm, packed with alumina (initially Brockman grade I) weakened by the addition of 8 per cent. v/w of water. Develop the chromatogram with light petroleum. Maintain the rate of elution at approximately 2 ml per minute. It will be found that retinene will slowly move down, and two distinct yellow bands gradually separate at the top of the column. Stop the development when about 175 ml of the solvent has passed through the column. At this stage, three yellow bands will be seen on the column. Described from the top of the column, they will be—

(*i*) A thin yellow band at the top of the column that will show brown fluorescence under ultraviolet light. This substance has a broad absorption maximum at 280 to 290 mμ; the colour with antimony trichloride reagent has absorption maximum at 560 mμ.

(*ii*) A thin yellow band about 0·5 cm below the first. This substance will show brown fluorescence under ultraviolet light and has broad absorption maxima at 290, 305 and 320 mμ. The colour with antimony trichloride reagent has absorption maximum at 608 mμ.

(*iii*) A colourless portion of about 4 cm below the second yellow band. This portion will show no fluorescence under ultraviolet light.

(*iv*) A wide yellow band of retinene below the colourless portion, which will show yellow fluorescence under ultraviolet light.

EXTRACTION AND DETERMINATION OF VITAMIN D—

Extrude the column, and cut a 2-cm length of the colourless portion below the second yellow band. Elute the cut piece with four 10-ml portions of diethyl ether. Evaporate the eluate to dryness under nitrogen. Dissolve the residue in 10 ml of light petroleum. Evaporate a suitable volume of the solution to dryness under nitrogen in a small dry flask. Dissolve the residue in 3 ml of Zimmerli - Nield - Russell's modified antimony trichloride reagent. Gently swirl the flask for 30 seconds and transfer the solution quickly to an absorptiometer cell. Measure the optical density at 500 mμ against antimony trichloride reagent as the blank solution. Calculate vitamin D, assuming the $E^{1\%}_{1cm}$ value at 500 mμ with pure calciferol to be 1800.

RESULTS

Recovery experiments were carried out with pure calciferol mixed with an oil that contains vitamin A and no vitamin D. Prepalin, a vegetable-oil medicinal preparation obtainable from Glaxo Ltd., which contains 75,240 i.u. of vitamin A per gram, was used in these experiments. To different weights of this oil measured volumes of an alcoholic solution of calciferol of known strength were added before saponification. The procedure described above was followed, to determine vitamin D. The results are shown in Table I.

TABLE I

RECOVERY OF VITAMIN D IN THE PRESENCE OF VITAMIN A

Weight of oil, g	Calciferol added, i.u.	Calciferol recovered, i.u.	Recovery of calciferol, per cent.	Ratio of vitamin A (i.u.) to vitamin D (i.u.)
0·0342	439	418	95	6
0·0317	439	427	97	
0·0537	380	356	94	11
0·0551	380	364	96	
0·1195	420	400	95	22
0·1264	420	400	95	
0·1700	461	453	98	27
0·1695	461	444	96	

By using the same procedure, vitamin D was determined in duplicate in some proprietary preparations. The results are shown in Table II.

TABLE II

DETERMINATION OF VITAMIN D IN PROPRIETARY PREPARATIONS

Multivitamin preparation	Potencies of vitamins as given, i.u. per tablet— vitamin A	vitamin D	Vitamin D found, i.u. per tablet
A	20,000	2000	2178
	20,000	2000	2133
B	25,000	1000	1044
	25,000	1000	1089
C	2500	250	235
	2500	250	241
*D	5000	1000	956
	5000	1000	978

* The potencies given are per 0·6 ml of the preparation.

The recovery experiments show (see Table I) that even with as little as 10 μg of added calciferol, the recovery of the vitamin is 94 to 98 per cent. Table II shows the values, for medicinal preparations, determined by this method against the potencies as stated by the manufacturers. Although no biological assay was attempted, there was substantial agreement between the stated potencies and the duplicate determinations. The determination of vitamin D by this method can be completed in about 3 hours, which is an advantage over the other methods. In these

determinations, only B.D.H. Ltd. manganese dioxide (precipitated) was used. It was found, from preliminary experiments, that by using this variety of manganese dioxide in the above stated proportion, *viz.*, about 0·5 g for every 10,000 i.u. of vitamin A, no unreacted vitamin A was left; and the quantitative separation of vitamin D from the oxidation products of vitamin A by chromatography on weakened alumina as described above, was reproducible. Further, it has been found that vitamin A is completely eliminated even in samples in which the ratio of vitamin A (i.u.) to vitamin D (i.u.) is as high as 27. As a rapid method for determining vitamin D in medicinal preparations, the proposed method has been tested with considerable success. Its applicability to natural products that contain vitamin D is being examined.

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Plastic Standard-taper Joints

BY G. M. LEET

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WITH the increased use of rigid plastic tube in the laboratory, the need arose for a suitable glass-to-plastic joint.

At the Rukuhia Soil Research Station, a method has been developed that involves the use of standard-taper ground-glass joints as moulds, and results in joints as good as, and in some instances better than, glass ones. This system has proved invaluable in such instances as acid evaporation, when the receptacle containing the sample is connected directly to a plastic fume-extraction manifold (see Fig. 1), and in freeze-drying units for connecting the vacuum pump to the ice trap (see Fig. 2).

To fabricate, for example, a B24 standard-taper cone in rigid poly(vinyl chloride) (PVC), the method described below was used.

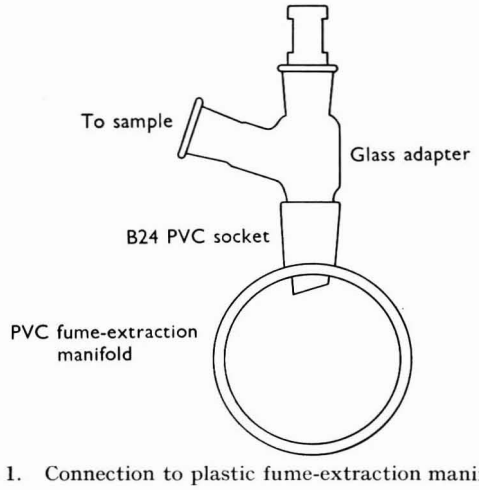

Fig. 1. Connection to plastic fume-extraction manifold

The portion of the plastic material to be moulded by heating, *e.g.*, a PVC tube, is lightly coated with petroleum jelly. Mild heat is then applied until the end 30 mm become soft and pliable. The B24 standard-taper glass socket is also lightly coated with petroleum jelly and slightly warmed. The softened PVC is then worked into the glass joint. On cooling, a perfect impression of the glass joint is formed.

To produce appropriate sockets, the plastic material is pushed over a glass cone in a similar manner.

A suitable size of plastic tubing must be selected for each joint size, *e.g.*, $\frac{3}{4}$-inch internal diameter for B24 cone and socket in plastic, $\frac{3}{4}$-inch internal diameter for B29 socket and 1-inch internal diameter for B29 cone in plastic.

As plastics, *e.g.*, polypropylene, polythene, Perspex and poly(vinyl chloride), are now available in a wide range of sizes, no difficulty should be found in selecting suitable sizes for almost all standard-taper joints.

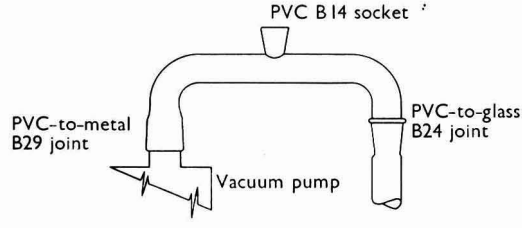

Fig. 2. Connection in freeze-drying units between vacuum pump and ice trap

To fit the finished plastic joint to its glass partner, it has been found better to twist slightly upon insertion and removal. A small amount of suitable grease may be smeared on the joint if desired.

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Book Reviews

METHODS IN MICROANALYSIS. Volume 1. SIMULTANEOUS RAPID COMBUSTION: MICROCHEMICAL PAPERS OF M. O. KORSHUN. Edited by J. A. KUCK. Translated by PHYLLIS L. BOLTON and KURT GINGOLD. Pp. xiv + 560. New York and London: Gordon & Breach. **1964**. Price $27.50.

This volume consists of 56 research papers, most of which were published from the laboratory of the late Miss M. O. Korshun during the period 1941–60; the remainder are papers from other Russian and Czech laboratories. Methods are described for all the organic elements, including phosphorus, and certain other elements (mercury and silicon). The most significant contributions are the simultaneous determination of several elements (C, H and S; C, H and F; C, H, S and Si; C, H and P; O and halogens; N and F; C, H, S and halogens, etc.) and the extension of the original empty-tube method to cope with all classes of compound by pyrolysing the sample in a silica capsule according to Friedrich (*Mikrochemie*, **1931**, **9**, 27; **1932**, **10**, 329) and to Marek (*Archiv. Kem. Farm.*, **1927**, **1**, 188). Several other methods are described, based on hydrogenation, bomb-fusion, magnesium fusion, etc. The determination of C and H by conductivity measurement is also considered.

The translation and collection of a series of research papers spread over such a long period is rarely of any value, except to the historian. It is inevitable that changes and improvements have to be made to methods, as new compounds are encountered and as the originators gain greater experience. If there is to be a published volume, then this is best done either by the originators or by their co-workers; the most recent forms of the methods can then be presented and the earlier forms and their weaknesses can be referred to briefly in the preliminary discussion.

Several papers describe the same determination twice or even more; the latest paper could then be considered to represent the latest recommendation, but this is not always clear. And it is certainly not possible in one instance, when two papers were published in different journals in the same year on the same topic. Different reagents are advocated to achieve the same purpose and there are no cross-references.

These observations are not intended to reflect on the authors of these papers, but rather on the American editors, who would have been better advised to have translated the book of Korshun and Gel'man (1949) and to have supplemented it with papers published after that date, or better still not to have translated at all, for many of the papers are completely out of date and the later papers are nowadays well known when it is a fairly simple matter to have Russian or Czech papers translated. Večeřa's work on the determination of C and H (the most recent work to be described in the book) has been known in this country for some years and studies on its applications have been reported.

There are some notable discrepancies, and it is difficult to assess whether these are the fault of the original authors or of the translators. The titrimetric method of Unterzaucher, for the direct determination of oxygen, is called a *gravimetric* method (page 3). Vieböck's mercuric oxycyanide reagent is called mercuric cyanate. Credit for the introduction of manganese dioxide and of sulphuric acid containing oxidising agents, for the absorption of nitrogen oxides, is incorrectly ascribed to investigators who used the reagents much later than the originators. In the final paper entitled "Advances in Organic Elemental Analysis," by A. P. Terent'ev and E. A. Terent'eva, the following statement is made: "At the beginning of the 1940's, Korshun in the U.S.S.R. and Unterzaucher in Germany, *simultaneously and independently* formulated the principle of a micro method for direct determination of oxygen, based upon decomposition of the sample in a stream of N_2, quantitative conversion of all O-containing decomposition products to CO by means of carbon heated to 1150°, and then to CO_2 over I_2O_5." Perhaps it may be permitted to recall the facts. It is known that this principle was used as early as 1904, but it was not described in the literature. It was re-discovered independently by Schütze, who described the gravimetric macro method in 1939. A gravimetric micro method was described simultaneously by Zimmermann, based on Schütze's investigations. Unterzaucher (1940) modified the micro method by raising the temperature for reaction and completing the determination titrimetrically. The first paper by Korshun on this determination appeared in 1941, and she herself properly acknowledges the earlier investigators. The quibble might be raised that the first methods had too many shortcomings. But this is true of most new methods in their early form and does not deny the originators the right of having discovered the principle. In any case, after the passage of a generation, the later methods referred to above are now known to have their shortcomings. As a matter of interest, it may be recorded that in modern practice, the tendency has been to return to the Schütze - Zimmermann gravimetric method. Korshun's important contribution to this determination was the use of heated carbon to scavenge the nitrogen.

There are other factual errors in the same paper, but probably more than enough has been said on this topic. There is no doubt that these papers contain valuable information but, as indicated earlier, these contributions are now well known and have been studied outside Russia. Certainly this particular Russian laboratory was ahead in coping with difficult compounds containing P, Si, etc.; and, although simultaneous determinations were known in the last century, the massive contribution made in this field by Korshun and her co-workers stands apart. Nevertheless, there are many analysts who prefer not to put all their eggs in one basket. Accurate simultaneous determinations require that the combustion has proceeded faultlessly, and it may be preferable to split the various determinations into at least two groups, as a check.

There are some statements with which the reviewer cannot agree. In the determination of oxygen, CO_2 and I_2 are both determined (sometimes a third check is made by weighing the loss in weight of the I_2O_5 tube); it is said ". . . which eliminates the necessity of carrying out two parallel determinations." In fact, all this procedure does is check the amount of CO produced. If all the oxygen has not been quantitatively converted to CO, the gravimetric and titrimetric figures might agree, but they would not represent the true oxygen content of the compound. Although useful, this procedure cannot replace a duplicate determination if there is doubt.

When the oxygen-flask is used, it is fitted with a side-arm to which a stop-cock is attached. It is claimed that "A flask without a side-arm (as used by Schöniger) is not suitable to work with. It is sometimes impossible to remove the stopper on account of the vacuum which develops. . . ." Few analysts would support this statement.

It is difficult to suggest a group of chemists, apart from the historian, to whom this book can be recommended. Those who are interested in the simultaneous determinations and have not had access to the various translations might find it useful, but it would probably be necessary to write to the original authors to discover which of the alternative recommendations to follow or if, in fact, these are still used.

R. BELCHER

CHIMIE ANALYTIQUE. I. GÉNÉRALITÉS. By BERNARD TREMILLON. Pp. 198. Paris: Librairie Armand Colin. 1965. Price 5.70 F.

The aim of this book may be summarised by an extract from the author's preface. "The object of this account is to communicate to the non-specialised reader the spirit of modern analytical chemistry by describing briefly the tools used by the analyst. For this purpose we divide analytical chemistry into two parts. . . . (1) Methods of reasoning, which constitute a systematisation of reactions, particularly in solution. This is the mental tool of the analyst. (2) Methods of measurement, whose application allows us to invent methods of determination. . . ." The volume under review deals with the first of these two parts, but it should be noted that it only treats inorganic analysis.

I think it is fair to call this little book, which is about the size of a Penguin paperback, a typical product of the distinguished Paris school of analytical chemistry. It is logically worked out, and though for non-specialised readers, would be heavy going to anyone whose physical chemistry of reactions in solution is not in good repair. There is nothing vague about the "généralités" discussed here; a much better English equivalent than "generalities" would be "general theory." A typical chapter heading is "Prévision des réactions en solution," *i.e.*, calculating or forecasting the course of reactions in solution. Other chapters deal with displacement of equilibria by chemical action, reactions in solvents other than water, reactions involving two phases and reactions at electrodes. This last is somewhat cursory compared with the others. The treatment is purely didactic, fundamental equations are stated, but not derived, there is no historical treatment, there are no references and no index. Some of the diagrams would be clearer if they were given a few more lines of explanation; this is a defect in a book for general readers. As far as possible, reactions are dealt with in general terms, there are no practical details and not many examples. Some of the examples are over-simplified, particularly the treatment of the precipitation of aluminium hydroxide, as the real reaction is or can be so much more complicated than the author's description that the account is almost misleading.

Where this book would fit in to the usual British course of students' reading I do not know. It has the great merits of being logical, quite self-consistent, and—so to speak—homogeneous. Mostly it is very clearly written and a chemist with a moderate knowledge of French would find it stimulating and enjoyable reading. An analyst with a "purely practical" mind—if such people exist—would get little or nothing out of it. H. N. WILSON

THERMAL METHODS OF ANALYSIS. By WESLEY WM. WENDLANDT. Pp. x + 424. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons. 1964. Price 124s.

"The purpose of this monograph is to acquaint chemists and other investigators with the relatively new series of instrumental techniques which are broadly classified as 'thermal methods'."

Professor Wendlandt's book is to be welcomed as the first comprehensive book in this field. After a brief opening chapter, in which thermal methods are defined and the physical parameters that have been studied as a function of temperature, are listed, three chapters each are devoted to thermogravimetry and differential thermal analysis, covering in each instance, the technique, instrumentation and applications. The remaining chapters deal with other thermal methods such as thermometric titrimetry, pyrolytic techniques, dynamic reflectance spectroscopy and thermal analysis.

The author claims that the book is intended as a critical review rather than a comprehensive survey and that the subject matter is biased towards the analytical applications of the techniques. However, it is questionable as to whether he has fully achieved this. The chapters devoted to the technique of thermogravimetric and differential thermal analysis are a good survey of the instrumental factors and the sample characteristics, an understanding of which is essential to intepreting results and comparing with the results of other workers. One would like to have seen an attempt to suggest the results that should be recorded when any thermogravimetric or differential thermal analysis work is reported. The chapters dealing with instrumentation provide a brief survey of some of the commercial instruments as well as a discussion of some of the more interesting non-commercial instruments. The selection is necessarily somewhat arbitrary. Unfortunately, the presentation is spoilt by the fact that some of the information reported is very much out of date; for example, Chevenard have for a long time had several additional models on the market, the photograph of the Stanton thermobalance is about seven years out of date and the description

of the thermobalance as an AD-2 is wrong. There have been, for some time, several models, including one capable of operating under vacuum and others up to 1550° C. Both the thermogravimetric and differential thermal analysis chapters on applications discuss many analytical applications. The author eschews the element-to-element approach of Duval in his book, "Inorganic Thermogravimetric Analysis," and brings together a selection of some of the more recent contributions in these fields. The applications of these two techniques are, of course, far wider, and one feels that the author is somewhat inhibited in restricting himself to analytical applications.

Of the other techniques treated in the book, several are the complements of thermogravimetric and differential thermal analysis, but the chapter on thermometric titrations, which is a good review on the subject, is out of place where it is and one even questions its appearance in the book as a whole. The chapter on pyrolytic techniques, which contains a discussion on gas evolution analysis should follow those on differential thermal analysis, since gas evolution analysis is often, as the author is at pains to point out, associated with differential thermal analysis.

It is unfortunate that there is ample evidence that this book has been written in a hurry. The English is somewhat quaint in places. Two examples will suffice: page 4, "A great amount of exactness . . ." and page 205, "while the sample may be either a powder, solid, or liquid of any shape." There are several typographical errors: for example: on page 18 "brucine" should be "brucite"; on page 81, the words appearing in quotes "The circuits are discussed in the text". are an interpolation; page 111, Fig. IV 14, is described as Fig. IV. 1.4.; page 218, Fig. 11. 26, should be Fig. VI. 26, and the index (page 423) provides "straing-gage".

The indiscriminate use of the word thermogram, to describe both a weight-loss curve (or thermogravimetric curve) and the differential thermal analysis curve is misleading; thermogram should be reserved for the latter.

In conclusion, this is a valuable book, since there are, at present, so few available on this topic, but it seemed desirable to acquaint prospective purchasers with some of its limitations—one of which they may well consider to be its price. J. P. REDFERN

COLLABORATIVE TESTS. Pp. 58. London: The Pharmaceutical Press. 1965. Price 12s. 6d.

The principles on which collaborative tests are based are adequately described and discussed in this inexpensive little book that summarises the proceedings of a Symposium held at Brighton last year. The preface contains a fitting extract taken from a leading article in the January, 1964, issue of *The Pharmaceutical Journal*.

"A panel takes a method of analysis that has been published or that has gained currency by continual use, and puts it to the test in a number of laboratories and with a number of samples. Having done this, it evaluates the results and prepares a report setting out the recommended method in detail. In practice there are many pitfalls but, by dogged persistence, improvements are effected one by one—some seeming so insignificant as to be unworthy of consideration, others representing major advances—until gradually a method is fashioned that can be relied upon to give results of the required degree of precision and accuracy when translated from the relative calm of the investigational laboratory to the hurly-burly of everyday routine."

Very few analytical laboratories have never taken part in collaborative tests involving the application of a tentative procedure and if, by the very nature of the problem, the homogeneity of the circulated samples cannot be guaranteed, a reliable assessment of the method becomes increasingly difficult.

It is interesting to read how the pharmaceutical industry tackles these problems, but the approach is by no means restricted to this industry, and anyone engaged, or likely to be engaged in similar tests should read this compact publication, because it highlights the problems of analytical exercises carried out on a co-operative basis. W. T. ELWELL

Erratum

MARCH (1963) ISSUE, p. 166, formula in centre of page. *For the term*

$$\left[\frac{\mu u_0 (1-\epsilon)}{g(\rho-\rho_0)\epsilon^3}\right]^{\frac{1}{2}} \quad \textit{read} \quad \left[\frac{\mu u_0 (1-\epsilon)^2}{g(\rho-\rho_0)\epsilon^3}\right]^{\frac{1}{2}}$$

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