The ANALYST

A Monthly Publication
dealing with all branches
of Analytical Chemistry:
the Journal of the Society
for Analytical Chemistry

Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE

Volume 84

No. 996, Pages 125-196

March 1959

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

14 BELGRAVE SQUARE, LONDON, S.W.1
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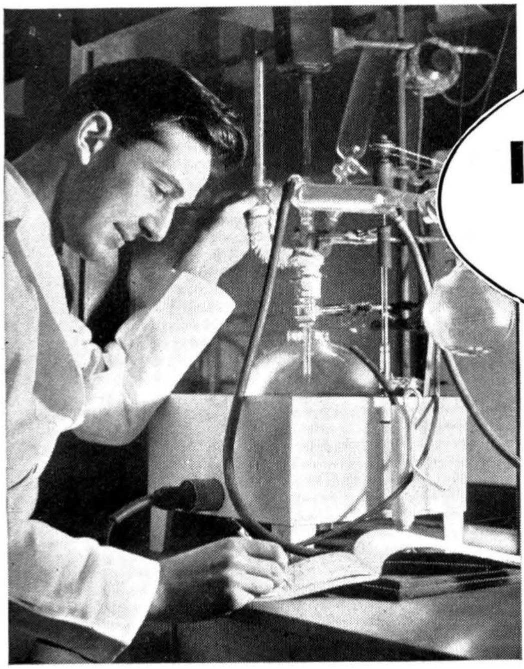
Volume 84, No. 996 March 1959



Incidental information

No. 12

items of interest from our laboratory notebooks

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Nitrosoresorcinol monomethyl ether is a comparatively new colorimetric reagent for iron and cobalt. There are others, of course, but S. M. Peach (**Analyst,** 1956, **81,** 371) considers that this one has some particular advantages. For instance, the Co complex can be extracted into an organic solvent. The reagent will appear as an addition (H. & W. Code 6303) in our next catalogue amendment.

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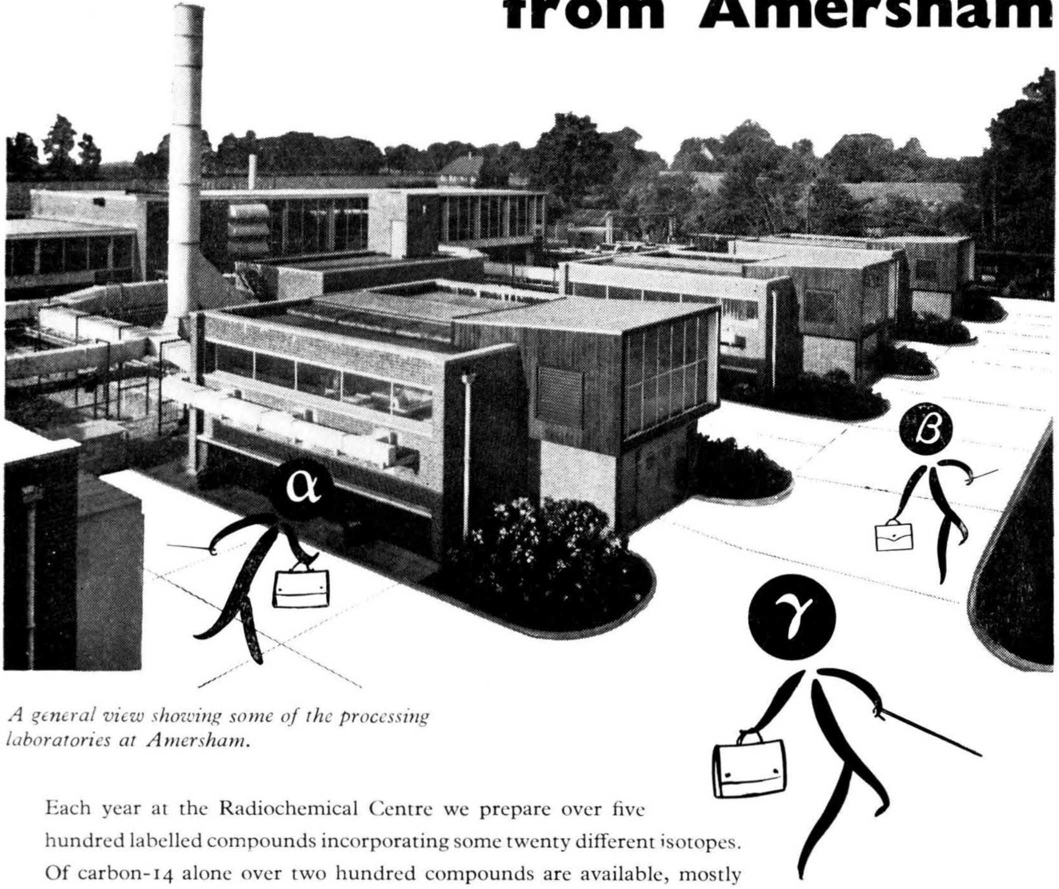

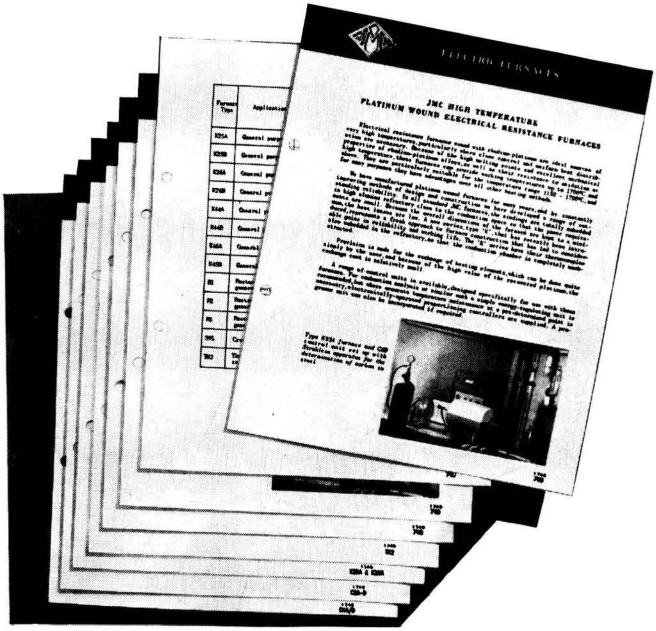
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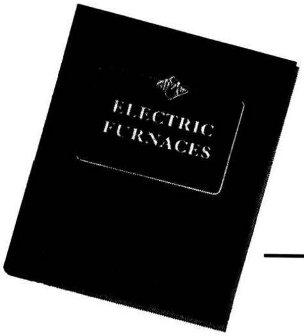
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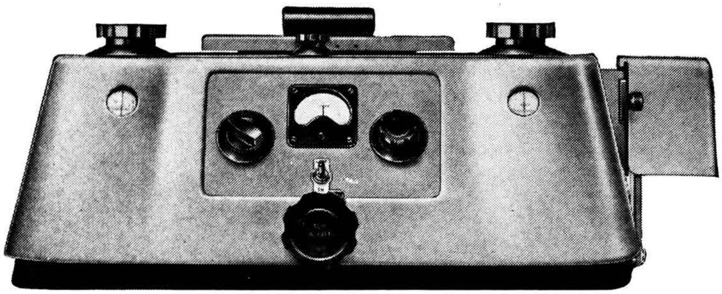

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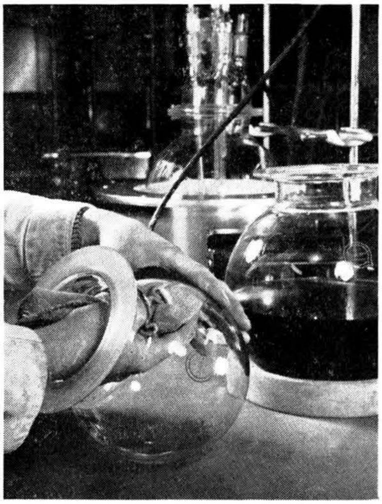

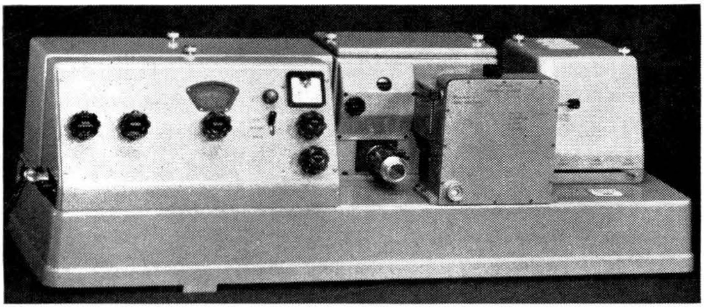

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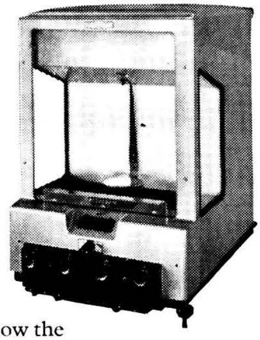

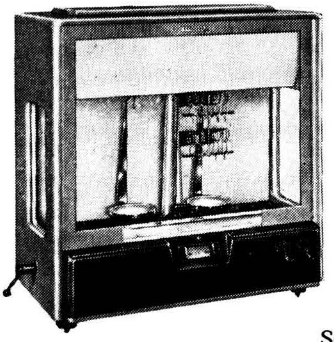

MARCH, 1959 Vol. 84, No. 996

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

ORDINARY MEMBERS

Peter Frederick Gordon Boon, B.Sc. (Lond.), A.R.I.C.; J. C. Burger, jun., M.S. (Tennessee); John Marie Jackson, B.Sc. (Glasgow); Ronald Victor Jeanes, B.Sc. (Lond.); Axel Bernhard Johansson, Ph.D.; Keith Leyshon; Kenneth Edward Parrott; William Hugh Roberts, B.Sc. (Liv.); Wolfgang Schöniger, Dr.ing.

DEATHS

WE record with regret the deaths of

Geoffrey Charles Matthews
George Rodney Simpson.

NORTH OF ENGLAND SECTION

THE Thirty-fourth Annual General Meeting of the Section was held at 2.15 p.m. on Saturday, January 31st 1959, at the Nag's Head Hotel, Lloyd Street, Manchester. The Chairman of the Section, Mr. A. N. Leather, B.Sc., F.R.I.C., presided. The following appointments were made for the ensuing year:—*Chairman*—Dr. J. R. Edisbury. *Vice-Chairman*—Mr. J. Markland. *Hon. Secretary and Treasurer*—Mr. B. Hulme, Ch. Goldrei, Foucard & Son Ltd., Brookfield Drive, Liverpool 9. *Members of Committee*—Messrs. J. F. Clark, W. Cule Davies, G. J. W. Ferrey, L. R. Flynn, A. O. Jones and H. Pritchard. Messrs. F. Dixon and T. W. Lovett were re-appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which a paper entitled "Synthetic Foodstuff Colours—Control of Quality" was given by H. E. Stagg, B.Sc., F.R.I.C.

SCOTTISH SECTION

THE twenty-fourth Annual General Meeting of the Section was held at 1.30 p.m. on Friday, January 30th, 1959, at the James Craig Restaurant, 10 Woodlands Road, Glasgow, C.3. The Chairman of the Section, Dr. Magnus Pyke, F.R.I.C., F.R.S.E., presided. The following office bearers were elected for the forthcoming year:—*Chairman*—Mr. A. N. Harrow. *Vice-Chairman*—Mr. A. F. Williams. *Hon. Secretary and Treasurer*—Mr. J. Brooks, Analytical Research Section, Nobel Division, Imperial Chemical Industries Ltd., Stevenston, Ayrshire. *Members of Committee*—Messrs. D. M. W. Anderson, A. L. Cochrane, J. C. Jack, A. O. Pearson, A. D. Walsh and R. S. Watson. Messrs. W. J. Murray and C. B. Hackett were appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which a paper entitled "The Identification of Artificial Colouring Matter in Food" was given by P. S. Hall, B.Sc., F.R.I.C.

WESTERN SECTION

THE fourth Annual General Meeting was held at 2.30 p.m. on Saturday, January 24th, 1959, at the Queen's Hotel, St. Mary Street, Cardiff. The Chairman of the Section, Mr. S. Dixon, M.Sc., F.R.I.C., presided. The following appointments were made for the ensuing year:—*Chairman*—Mr. S. Dixon. *Vice-Chairman*—Dr. G. V. James. *Hon. Secretary and Treasurer*—Dr. T. G. Morris, Brockleigh, Clevedon Avenue, Sully, Glamorgan. *Members of Committee*—Messrs. P. J. C. Haywood, C. H. Manley, B. W. E. Minifie, F. H. Pollard, H. K. B. Rout and R. F. Stephens. Messrs. R. E. Coulson and E. A. Hontoir were appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which T. G. Morris, B.Sc., Ph.D., A.R.C.S., D.I.C., A.R.I.C., gave a general account of the work of the Pneumoconiosis Research Unit of Llandough Hospital.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Wednesday, February 11th, 1959, in the Mason Theatre, The University, Edmund Street, Birmingham 3. The Chair was taken by the Vice-Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The subject of the meeting was "Recent Advances in the Analytical Chemistry of Polymers" and the following papers were presented and discussed: "Infra-red and Near Infra-red Spectroscopy in the Analysis of Elastomers," by P. J. Corish, M.Sc.; "Ultra-violet Spectroscopy in the Analysis of Raw Materials and Compounded Rubbers," by D. W. Huke, B.Sc., and C. E. Kendall, B.Sc., A.R.I.C.

MICROCHEMISTRY GROUP

THE eighteenth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, January 28th, 1959, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Dr. G. F. Hodsman, A.Inst.P.

A discussion on "The Determination of Small Quantities of Toxic Substances in the Atmosphere" was opened by J. C. Gage, Ph.D., F.R.I.C.

BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Biological Methods Group with the Biometric Society was held at 6.30 p.m. on Tuesday, February 3rd, 1959, in the Small Lecture Theatre, London School of Hygiene, Keppel Street, London, W.C.1. The Chair was taken by the Chairman of the Biological Methods Group, Dr. J. I. M. Jones, F.R.I.C.

The following papers were presented and discussed: "Factors Affecting χ^2 (Slope) in Insulin Assays Using the Mouse Convulsion Method," by J. V. Smart, B.Sc., and G. A. Stewart, B.Sc.; "The Use of a Range Method in Estimating Variance in Biological Assays," by V. J. Birkinshaw, F.P.S.; "Comparison of the Approximate with the More Refined Methods for Treating (2 + 2) Quantal Response Assay," by K. L. Smith, M.P.S.

Analytical Methods Committee

THE STABILITY OF RESCINNAMINE IN SOLUTION

THE Joint Committee of The Pharmaceutical Society of Great Britain and The Society for Analytical Chemistry on Methods of Assay of Crude Drugs has published a paper prepared by its Rauwolfia Panel on "The Stability of Rescinnamine in Solution" in *J. Pharm. Pharmacol.*, 1959, **11**, 211.

A summary of the paper is as follows—

> The ultra-violet absorption characteristics of solutions of rescinnamine in various solvents change when the solutions are exposed to daylight. The change, which is thought to be due to a *cis* - *trans* isomerism, is not accompanied by any detectable alteration in pharmacological properties.

Analytical Methods Committee

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

The Determination of Lead

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

In 1954, the Analytical Methods Committee published a method for the determination of lead in foodstuffs, developed by the Lead Panel of the Metallic Impurities in Foodstuffs Sub-Committee.[1] After the re-organisation of the Sub-Committee as the Metallic Impurities in Organic Matter Sub-Committee under the Chairmanship of Mr. T. McLachlan, it was decided to review this method, particularly with regard to the wider terms of reference of the Sub-Committee, which is not now restricted to the study of foodstuffs. The other members of the Sub-Committee were Messrs. L. Brealey, C. L. Hinton, E. I. Johnson, W. C. Johnson, I. MacIntyre, R. F. Milton, G. Taylor and G. E. Willis. The Sub-Committee has also had the advantage of the assistance of Mr. T. T. Gorsuch, the Society's Research Scholar, working at Harwell under the direction of Mr. A. A. Smales.

INTRODUCTION—

The method now recommended by the Sub-Committee is based on the method of the Lead Panel, but incorporates modifications of the methods of Johnson and Polhill[2] and Snyder.[3]

Usually, after suitable destruction of organic matter, preliminary extraction of the lead from acid solution with a carbamate reagent, as in the earlier method, has been replaced by extraction with dithizone from an alkaline citrate solution, hexametaphosphate being added, as in the method of Johnson and Polhill.[2] The addition of hexametaphosphate extends the applicability of the dithizone extraction of lead from alkaline solution by preventing or delaying the precipitation of ammonium magnesium phosphate or calcium phosphate.

When concentrations of calcium, magnesium and phosphates are so high that the recommended additions of sodium hexametaphosphate and ammonium citrate are insufficient to produce a clear solution, it has been considered advisable to retain the procedure of the earlier method, *viz.*, extraction from acid solution with the carbamate reagent. The alternative of increasing the concentration of the hexametaphosphate and citrate is not recommended, as the extraction of lead may then be incomplete.

Finally, details are given of a modified procedure for use when considerable interference from bismuth is indicated.

EXPERIMENTAL WORK—

Experimental work on the method was carried out by members of the Sub-Committee, and by Dr. J. Haslam and Dr. A. G. Lipscomb.

In preliminary experiments on the method, samples of bone meal and poly(vinyl chloride) were distributed for comparative analyses. The results were unsatisfactory; these materials were admittedly exceptional, and it seemed probable that the lack of precision in the results was due to difficulties in the destruction of the organic matter and isolation of the lead, rather than to the determination of the lead.

Further tests were carried out on more normal material, powdered tobacco being used for the purpose, since it usually has an appreciable lead content and problems due to inefficient distribution of any added lead are avoided. The results of these tests again pointed to the procedure for destruction of the organic matter as the source of incomplete recoveries and of variation between separate determinations, even when a standardised procedure was used. Since tobacco contains calcium, wet decomposition in the presence of sulphuric acid was less satisfactory than dry ashing, because lead was retained by the insoluble calcium sulphate remaining after the digestion.

Because of these difficulties, it was thought desirable to restrict this Report to procedures that are believed to be satisfactory for determining lead. A separate Report will be issued dealing with methods for the destruction of organic matter in general, with recommendations for appropriate procedures in particular instances. However, a few suggestions as to the method to be used for destruction of organic matter, with indications as to the avoidance of sources of error that may sometimes be met, are included in this Report.

Experiments with lead-212 as a radioactive tracer have been carried out at all stages of the work in order to investigate recoveries and losses. It has been shown that recoveries after dry oxidation are greatly influenced by the material being oxidised, as well as by the temperature of oxidation and the ashing aids used. It has been found, for example, that lead is lost from poly(vinyl chloride) at 450° C, whereas complete recoveries can be obtained from bone meal and dried milk at temperatures up to 650° C.

Wet oxidations with the appropriate reagents have always given good results. The use of sulphuric acid on materials high in calcium is to be avoided, and the retention of lead on calcium sulphate precipitates was clearly shown during the radiochemical investigation.

RESULTS OF COLLABORATIVE TESTS—

Several series of collaborative tests on powdered tobacco were undertaken in five different laboratories, but the results of only one of these are given. Dry combustion for destroying the organic matter and then extraction by Method A (see p. 132) was the method used for these tests. The results are as follows—

Laboratory	Lead found, p.p.m.
A	8·5, 8·8, 8·9, 9·6, 11·0
B	8·9, 9·0, 9·7, 11·1, 11·7
C	10·7, 11·2, 15·4
D	7·5, 10·2
E	8·0, 9·0

Although these results are not as satisfactory as might be desired, even when those from the same laboratory are examined, the Sub-Committee thought that sufficient work had been carried out to justify publication of this Report. Every precaution was taken to obtain a completely uniform powder, but it seems probable that the lead was not evenly distributed throughout the sample.

The results obtained on the same sample when the organic matter had been destroyed by wet combustion with nitric and sulphuric acids are as follows—

Laboratory	Lead found, p.p.m.
A	3·5, 4·4, 4·8, 5·0, 5·4, 6·0
B	6·4, 7·0
C	6·2, 7·8
D	2·9, 3·6
E	8·2, 11·0

It can be seen that results similar to those by dry ashing were obtained only in Laboratory E, where special precautions were adopted to bring precipitated calcium sulphate into solution. Variations of the wet-digestion procedure used in other laboratories were not found to give significantly higher lead yields, and, as results for lead are accurate if sufficient inorganic matter is present to provide an appreciable amount of ash, the Sub-Committee was of the opinion that nothing would be gained by adopting a procedure yielding a precipitate with adsorbed lead that could be removed only with difficulty.

Determinations of the lead content of this sample of tobacco were carried out by square-wave polarography both after dry ashing and after wet oxidation with nitric and perchloric acids. The results of both indicated a lead content of 9·9 p.p.m.

Since the destruction of organic matter is now to be treated as a separate problem, it was thought that the results shown above tended to obscure the purpose of this Report. Accordingly, some determinations were carried out in five different laboratories on a simple solution of a lead salt containing 100 p.p.m. of lead. The results obtained (*a*) after treatment by Method A, and (*b*) by direct extraction with dithizone, as described under "Determination of Lead," p. 133, are shown in Table I. These results indicate that the method is capable of reasonable accuracy after organic matter has been removed from the sample.

TABLE I

RECOVERIES OF LEAD FROM A PURE SOLUTION

Laboratory	Lead taken in aliquot, μg	No. of determinations	Range of results, p.p.m.	Mean, p.p.m.
(*a*) *Method A*—				
A	20	7	95 to 100	97·8
B	10	1	115	115·0
	20	1	95	95·0
	8	1	100	100·0
C	30	2	96·3, 97	96·7
D	—	3	99 to 102	100·7
E	10	6	84 to 101	90·2
	20	6	92 to 97	95·0
(*b*) *Direct extraction*—				
A	20	6	100·5 to 102	101·3
B	10	1	100	100·0
	20	1	102·5	102·5
C	10	2	97·8, 98·5	98·2
	30	1	100·3	100·3
	40	2	97·6, 99·2	98·4
D	5	1	102	102·0
	20	1	105	105·0
E	10	6	92 to 105	99·8
	20	6	100 to 108	104·8
	30	5	101 to 105	103·2

Appendix I

RECOMMENDED METHOD FOR THE DETERMINATION OF LEAD

Precautions against contamination—All the precautions usual in this type of work must be observed.

(*a*) Reagents as supplied for ordinary analytical work may contain up to 1 or 2 p.p.m. of lead. This difficulty has been largely overcome by the enterprise of one or two manufacturers, who can now supply the mineral acids and ammonium hydroxide containing not more than 0·005 p.p.m. of lead, and potassium cyanide and citric acid containing not more than 0·5 p.p.m. of lead. Some reagents can be freed from lead by washing with dithizone solutions. All reagents should be stored in borosilicate-glass or polythene bottles.

(*b*) The glass of the apparatus may yield lead in the course of usage; borosilicate glass or silica should therefore be used.

(*c*) Atmospheric dust normally contains substantial amounts of lead, often to the extent of several thousand parts per million. Particular attention must therefore be paid to the possibility of dust contamination during all stages of a determination.

(*d*) All water must be distilled or de-ionised and be free from lead.

PRINCIPLE OF METHOD—

After removal of interfering substances, lead is extracted with dithizone at pH 9 to 9·5 and determined absorptiometrically.

For the preliminary separation of lead, two methods are given. In Method A, lead is extracted with dithizone from an alkaline citrate and hexametaphosphate solution.

Method B requires additional manipulative work and should be used only when Method A will not suffice, *i.e.*, for samples that have so high a content of calcium, magnesium and phosphate that sodium hexametaphosphate and ammonium citrate in the stated amounts will not allow the quantitative extraction of lead with dithizone under the alkaline conditions of Method A; a preliminary extraction of lead from acid solution with a solution of diethylammonium diethyldithiocarbamate in chloroform is therefore substituted for the hexametaphosphate procedure.

RANGE—

For lead contents up to 5 p.p.m., but the range can be extended by using a suitable amount of sample. The calibration graph, based on the use of an absorptiometer and 1-cm cells, covers the range 0 to 40 μg of lead (approximately) in 10 ml of solution.

APPLICABILITY—

The method is of wide applicability, although bismuth is liable to interfere. An optical-density measurement at 490 mμ as well as at 520 mμ may be useful in deciding whether bismuth interference is considerable. It has been found that, with a solution of pure lead dithizonate, the optical density at 490 mμ is approximately 0·84 times that at 520 mμ; with a solution of pure bismuth dithizonate, the optical density at 490 mμ is approximately 1·20 times that at 520 mμ. When considerable bismuth interference is indicated, the modified procedure[4,5] given under "Interference of Bismuth" should be used.

APPARATUS—

Lead-free borosilicate glass or silica should be used throughout.

REAGENTS—

All reagents, including water, must be lead-free, either as purchased or by special preparation (see Appendix II).

Hydrochloric acid, 5 M.

Nitric acid, dilute—One volume of nitric acid, sp.gr. 1·42, diluted to 100 volumes with water.

Ammonium hydroxide, sp.gr. 0·880.

Ammonium citrate solution—A 25 per cent. w/v solution in water.

Sodium hexametaphosphate solution—A 10 per cent. w/v solution in water.

Potassium cyanide solution—A 10 per cent. w/v solution in water. This solution should be at least 2 days old, so that traces of sulphide may become oxidised.

Hydroxylamine hydrochloride solution—A 20 per cent. w/v solution in water.

Chloroform—Shake 250 ml of chloroform with 25 ml of water containing 1 ml of 10 per cent. w/v potassium cyanide solution and about 20 drops of 5 *M* ammonium hydroxide, separate and reject the aqueous layer, wash the chloroform with water, and filter.

Dithizone, stock solution—A 0·1 per cent. w/v solution of diphenylthiocarbazone (dithizone) in chloroform. Filter, and store in a refrigerator.

Dithizone, working solution—Shake 6 ml of the dithizone stock solution with 9 ml of water and 1 ml of 5 *M* ammonium hydroxide. Separate and reject the lower layer and spin the aqueous layer in a centrifuge until clear. Prepare this solution freshly on the day of use.

Ammoniacal sulphite - cyanide solution—Mix 340 ml of ammonium hydroxide, sp.gr. 0·880, 75 ml of 2 per cent. w/v sodium sulphite, Na_2SO_3, solution, 30 ml of 10 per cent. w/v potassium cyanide solution and 605 ml of water. (The concentrations of these reagents are critical.)

Standard lead solution—(*a*) Dissolve 1·60 g of lead nitrate in water, add 10 ml of concentrated nitric acid, and dilute to 1 litre.

(*b*) Dilute 1 volume of (*a*) to 100 volumes with water. Prepare solution (*b*) freshly as required.

$$1\ \text{ml} \equiv 10\ \mu\text{g of lead.}$$

Thymol blue indicator solution, 0·04 per cent. w/v—Warm 0·1 g of thymol blue with 4·3 ml of 0·05 *N* sodium hydroxide and 5 ml of 90 per cent. ethanol; when dissolution is complete, add sufficient 20 per cent. ethanol to produce 250 ml of solution.

The following additional reagents are required when Method B is to be used—

Sulphuric acid, diluted (1 + 1).

Perchloric acid, sp.gr. 1·54.

Sodium iodide solution—A 20 per cent. w/v solution in water.

Sodium metabisulphite solution—A 1·25 per cent. w/v solution in water. Prepare this solution freshly as required, and filter before use.

Diethylammonium diethyldithiocarbamate solution, 1 per cent. (*carbamate reagent*)—Dissolve 1 g of the pure crystalline reagent in 100 ml of redistilled chloroform, and store in an amber-coloured bottle. This solution is not stable and should be discarded after 1 week.

Methyl red indicator solution, 0·01 per cent. w/v—Warm 25 mg of methyl red with 0·95 ml of 0·05 *N* sodium hydroxide and 5 ml of 90 per cent. ethanol; when dissolution is complete, add sufficient 50 per cent. ethanol to produce 250 ml of solution.

The following additional reagents are required for extraction of bismuth—

Hydrochloric acid, sp.gr. 1·18.

Diethylammonium diethyldithiocarbamate solution, 1 per cent. (*carbamate reagent*)—Prepared as described above.

PROCEDURE

REAGENT BLANK—

Carry out a blank test by the entire procedure on all the reagents, omitting only the sample.

DESTRUCTION OF ORGANIC MATTER—

Destroy the organic matter in a measured amount of sample (containing not more than 40 μg of lead) by a suitable procedure, such as—

(1) Dry ashing with or without an ashing aid at a temperature not exceeding 500° C.

(2) Oxidation with nitric and perchloric acids.

(3) Oxidation with nitric and sulphuric acids.

It should be noted, for example, that the use of sulphuric acid is to be avoided when appreciable amounts of calcium are present, and dry ashing should be avoided in the presence of large amounts of chloride.

If Method B is used, an ashing aid in the form of a 10 per cent. solution of magnesium nitrate, $Mg(NO_3)_2.6H_2O$, may be used for dry ashing.

PRELIMINARY TREATMENT OF SAMPLE—

(*a*) *If the organic matter has been destroyed by wet decomposition*—Allow the contents of the Kjeldahl flask to cool, and add 5 ml of water.

If the solution is free from insoluble matter, transfer it to a 100-ml conical flask, rinsing with two 1-ml portions of water. Place 10 ml of 5 *M* hydrochloric acid in the Kjeldahl flask, boil gently for 5 minutes, swirl vigorously to wash the sides of the flask, and drain the acid into the conical flask. Finally, wash out the Kjeldahl flask with two 1-ml portions of water.

If the contents of the Kjeldahl flask contain insoluble deposit or suspended matter, however small in amount, filter the solution and washings through a 7-cm Whatman No. 1 filter-paper. If possible, retain any deposit in the Kjeldahl flask until it has been boiled with 5 *M* hydrochloric acid, and pass the hot acid also through the filter.

If the organic matter has been destroyed by an appropriate method, the amount of insoluble matter remaining should not be so great as to cause significant loss of lead through adsorption or occlusion. Any such difficulty is also minimised by restricting the amount of the sample.

(*b*) *If the organic matter has been destroyed by dry ashing*—Add 5 ml of water and 10 ml of 5 *M* hydrochloric acid to the ash in the silica or platinum basin, and boil gently for 5 minutes. Transfer the solution to a 100-ml conical flask, and filter if insoluble matter is present, as described in (*a*) above.

SEPARATION OF LEAD—

Method A

(*For samples in which the concentrations of calcium, magnesium and phosphate are not high*)

Cool the solution, and add 5 ml of ammonium citrate solution and 10 ml of sodium hexametaphosphate solution. (For certain samples, *e.g.*, cheese, the high calcium and phosphate concentrations may be held up by the addition of 10 ml of ammonium citrate solution, otherwise Method B must be used.)

Add a few drops of thymol blue indicator solution and sufficient ammonium hydroxide to give the blue-green colour indicating pH 9·0 to 9·5. Cool, add 1 ml of potassium cyanide solution, and, if much iron is present, add 1 ml of hydroxylamine hydrochloride solution. Transfer the solution to a 100-ml separating funnel containing 10 ml of chloroform, and rinse with a few millilitres of water. The volume of the aqueous layer at this stage should be approximately 50 ml. Add 0·5 ml of dithizone working solution, shake vigorously for 1 minute, and allow to separate. If the lower layer is red, add dithizone working solution until, after shaking, a purple, blue or green colour is obtained. Run the chloroform layer into a second separating funnel, and wash through with 1 or 2 ml of chloroform. Add to the liquid in the first separating funnel 3 ml of chloroform and 0·2 ml of dithizone working solution. Shake vigorously for 30 seconds, allow the chloroform layer to separate, and add it to the main chloroform extract. This last chloroform extract should be green. If it is not, further extractions with chloroform and dithizone must be made until the green colour of the final extract indicates that all the lead has been extracted. Reject the aqueous layer. Add 10 ml of dilute nitric acid to the combined chloroform extracts, and shake vigorously for 1 minute. Allow to separate, and reject the chloroform layer as completely as possible.

Method B

(*For samples with a high content of calcium, magnesium and phosphate*)

To the solution obtained by one of the methods described under "Preliminary Treatment of the Sample" add 2 drops of methyl red indicator solution, and make just alkaline with ammonium hydroxide, sp.gr. 0·880. Make the solution just acid with 5 *M* hydrochloric acid, and add a further 10 ml. Warm the solution to 50° to 70° C, add 2 ml of sodium iodide solution, and reduce any liberated iodine with 2 ml of sodium metabisulphite solution. Cool the solution, transfer it to a separating funnel, and adjust the volume to 50 to 75 ml in order to bring the acid concentration to *N* with respect to hydrochloric acid. Add 10 ml of carbamate reagent by pipette, and shake the funnel vigorously for 30 seconds. Allow the layers to separate, and transfer the chloroform layer to a 100-ml flask. Wash the aqueous layer twice with small amounts of chloroform without mixing, and add these washings to the flask. Repeat the extraction with 10 ml of carbamate reagent, and add the second extract to the main extract. Reject the aqueous layer.

To the combined extracts add 2·0 ml of diluted sulphuric acid, and evaporate the chloroform. Add 0·5 ml of perchloric acid to the residual solution, and heat until fumes are evolved and the fuming solution is clear and colourless. Cool the solution, add 10 ml of water and 5 ml of 5 *M* hydrochloric acid, boil for 1 minute, cool, and then add 2 ml of ammonium citrate solution.

Continue as in Method A, beginning at paragraph 2, "Add a few drops of thymol blue indicator solution. . . ."

DETERMINATION OF LEAD—

To the nitric acid layer left in the separating funnel add 30 ml of ammoniacal sulphite - cyanide solution, exactly 10 ml of chloroform and 0·5 ml of dithizone working solution, shake vigorously for 1 minute, and allow to settle. Run off a little of the chloroform layer. Insert a plug of cotton-wool into the dry stem of the funnel, and, after rejecting the first runnings, fill a 1-cm spectrophotometer cell with the chloroform solution.

Measure the optical densities of the test and blank solutions against chloroform (all in 1-cm cells) with a photoelectric absorptiometer fitted with filters that possess a maximum transmission at or near 5200 A with a band width of 230 A at 50 per cent. transmission (*e.g.*, Ilford No. 604 gelatin filters) or with a spectrophotometer at 5200 A. Read the number of micrograms of lead equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of lead in the sample.

Prepare the calibration graph as follows—

> Measure 0, 1·0, 2·0, 3·0 and 4·0 ml of standard lead solution (*b*) into separating funnels, and dilute each to 10 ml with dilute nitric acid. Proceed as described under "Determination of Lead." Measure the optical densities with chloroform in the comparison cell. Construct a graph relating the optical densities to the number of micrograms of lead.

INTERFERENCE OF BISMUTH

Prepare the digest from the wet decomposition or the ash in the silica or platinum basin, as described under "Preliminary Treatment of Sample."

If the organic matter has been destroyed by wet decomposition—Add to the contents of the flask 6·0 ml of hydrochloric acid, sp.gr. 1·18, and transfer the solution to a 50-ml graduated separating funnel. Rinse the conical flask with several 1-ml portions of water, and add the rinsings to the separating funnel. The volume of the contents of the separating funnel must not exceed 35 ml in order that the hydrochloric acid concentration may be not less than 3 N (see Note).

Extract the acid solution directly in the cold first with 10 ml and then with 5 ml of carbamate reagent, shaking for 30 seconds each time; separate and discard the lower (chloroform) layer. Finally, shake the acid layer with 5 ml of chloroform for 10 to 15 seconds, and discard the chloroform layer. Transfer the acid layer to a 100-ml conical flask, rinse the separating funnel with a few millilitres of water, and add the rinsings to the conical flask.

Proceed as in Method A or Method B.

If the organic matter has been destroyed by dry ashing—Add 15 ml of hydrochloric acid, sp.gr. 1·18, transfer to a 50-ml graduated separating funnel, and adjust the volume of the solution to a maximum of 35 ml in order that the hydrochloric acid concentration may be approximately 6 N (see Note). Continue as described above from "Extract the acid solution. . . ."

NOTE—After wet decomposition, the extraction solution consists of the residual sulphuric acid to which hydrochloric acid has been added; the acidity of the solution should not be less than 3 N in sulphuric acid and 3 N in hydrochloric acid.

When the organic matter has been destroyed by dry ashing, the extraction solution consists of hydrochloric acid alone, and the acidity must be raised to about 6 N in hydrochloric acid to effect quantitative separation of bismuth and other elements from lead.

Appendix II

PREPARATION OF LEAD-FREE REAGENTS

Hydrochloric, sulphuric, perchloric and nitric acids and ammonium hydroxide can be purchased lead-free to an extent that lead contamination does not exceed 0·005 p.p.m. Potassium cyanide and citric acid can be obtained with lead contents not exceeding 0·5 p.p.m.

If these are not available, ordinary laboratory reagents must be rendered lead-free as follows—

Sulphuric, nitric and hydrochloric acids—Distil from all-borosilicate-glass apparatus.

Ammonium hydroxide—Distil 400 ml of ammonium hydroxide, sp.gr. 0·880, from a borosilicate-glass flask fitted with a safety trap, into 250 ml of water kept cold by means of a bath of ice, and control the pressure of the liberated ammonia gas by adjusting the rate of heating. Subsequently determine the concentration of the distillate by titration with *N* hydrochloric acid.

Ammonium citrate solution—Dissolve 125 g of analytical-reagent grade ammonium citrate in 400 to 450 ml of water, make faintly alkaline to litmus paper with lead-free 5 *M* ammonium hydroxide, and extract with chloroform and appropriate additions of the stock dithizone solution. Continue extraction until all metals have been removed and the extract is faintly green, and then make the solution just acid by adding lead-free 5 *M* hydrochloric acid, and extract with further portions of chloroform until the final extract is colourless.

Potassium cyanide solution, 10 per cent. w/v—Dissolve 50 g of analytical-reagent grade potassium cyanide in water, and dilute to 100 ml. Extract this solution with chloroform and 1 or 2 drops of dithizone solution until the extract is no longer red, but has a greenish shade. (Use as small an excess of dithizone as possible, because the excess is not readily removed.) Extract the excess of dithizone by shaking the solution with successive portions of chloroform. Dilute the extracted cyanide solution to 500 ml with water, warm to remove chloroform, and then cool.

Sodium hexametaphosphate solution—Adjust to pH 9 with thymol blue indicator solution by adding lead-free ammonium hydroxide, and extract with dithizone in chloroform until free from lead. Make the solution just acid, and remove the dithizone traces by extraction with chloroform. Finally, adjust to the maximum blue colour of the indicator.

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Radiochemical Investigations on the Recovery for Analysis of Trace Elements in Organic and Biological Materials

Report to the Analytical Methods Committee by the Society's First Analytical Chemistry Research Scholar

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I. INTRODUCTION

1. PREVIOUS APPROACHES TO THE PROBLEM—

The increasing importance of determining trace elements in organic materials has led to the publication of many analytical methods. Most of these involve preliminary destruction of organic matter by wet or dry oxidation, but little emphasis has been placed on the efficiency of the oxidation methods used. As with any other analytical procedure, it is vital to know that this stage does not introduce appreciable errors, yet most workers assume that the recoveries obtained are 100 per cent.

Some attempts have been made to evaluate the oxidation methods, but, until recently, only one technique has been available, *i.e.*, the recovery of known additions of the particular element. Although this could provide useful information, it suffered from many drawbacks.

(*i*) Additions were usually made with the element in a chemical form differing from that occurring in the organic material.

(*ii*) It was not possible to isolate the oxidation stage and treat it separately. The methods used for determining the element usually involved some manipulation, and errors introduced during this stage were included in the final results.

(*iii*) In all such experiments, two opposing factors were involved; losses of trace element by volatilisation, fixation or other mechanisms, on the one hand, and accretions from the reagents or apparatus, on the other. As these tended to offset each other, the final view of the effect of the oxidation could be subject to much uncertainty.

2. RADIOCHEMICAL APPROACHES—

In the past few years, developments in radiochemistry have greatly improved the position for investigations of this kind, and it is now sometimes possible to overcome all the above-mentioned difficulties.

All the radiochemical methods used in these investigations have involved the final determination of radioactive isotopes, thereby avoiding the confusion likely to be caused by accretion during the investigation of oxidation methods. Any material picked up from apparatus or reagents was inactive and could not affect the final determination. This isolates errors introduced by the *loss* of trace elements and permits their evaluation. The separate problem of blank values during the analysis of samples is common to all conventional analytical methods and is not readily amenable to radiochemical investigation. It is not, therefore, considered further in this Report.

Three techniques have been used, each with its own advantages and disadvantages.

(*a*) *Recovery experiments with additions of radioactive tracer*—This is similar in essence to the inactive recovery experiments mentioned above, and as such is subject to the first drawback (*i*); however, it offers many advantages over the inactive method. Owing to the penetrating nature of nuclear radiation, determinations can be carried out directly on the oxidation products, and the introduction of errors during further manipulation is minimised. A further result of the penetrating power of nuclear radiation is that it is possible to determine the amount of material fixed to the apparatus or other solid material; this would not be possible by the more traditional methods of analysis. This is of great importance and permits adequate balance sheets to be drawn for each experiment. As stated above, accretion problems are avoided; moreover, the method is rapid. This method has been extensively used.

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(*b*) *Irradiation of samples in a nuclear reactor*—When organic material is irradiated in a nuclear reactor, the carbon, hydrogen, oxygen and nitrogen that make up the bulk of the sample are largely unaffected, whereas many of the other constituents, such as trace metals, sodium, chlorine or sulphur, absorb neutrons to form radioactive products. By comparing the activities produced for different elements with the activities of standards irradiated at the same time, measures of the concentrations of those elements can be obtained. This is the basis of activation analysis, and, for the determination of a given element, it is necessary that the radioactivity due to that element shall be measured specifically. This can be achieved in one of two ways.

(*i*) *Radiochemical separation*—This involves the separation, in a pure state, but not necessarily quantitatively, of the element or some compound of it; application of this method to the present problem would involve such separations, in the presence of a carrier, from all the fractions produced during the destruction of organic matter. It has the advantage that the tracer is not added in the form of foreign ions, but is produced *in situ* from the trace element originally present. The disadvantages are that it is time-consuming and there is no guarantee that the proportion of the element fixed to the apparatus is quantitatively recovered.

Both objections can be overcome in favourable circumstances by the use of the second method.

(*ii*) *Gamma-ray spectroscopy*—The gamma rays produced during the decay of many radioactive nuclides are emitted with characteristic energies; it is possible, by electronic means, to count separately those radiations falling within certain energy limits. In this manner and under favourable conditions, the gamma rays from particular nuclides can be isolated. As gamma rays are highly penetrating, a measure of the concentration of the particular element can be obtained by gamma-ray spectroscopy of the intact sample. A further determination after destruction of the organic material allows a direct comparison to be made; similarly, any activity retained by the apparatus can be easily measured.

The advantages of this method are apparent—it is comparatively rapid, it does not require the addition of tracer, with the doubts that this could raise, it allows total recoveries to be calculated and non-homogeneity of the sample is not a serious problem. Unfortunately, it is not universally applicable, its application being critically dependent on the level of occurrence of the element, its activation cross-section and the half-life, gamma energy and branching ratios of the activation product, all with reference to the properties of the other constituents of the sample. For this reason its application in these investigations has been limited.

These new approaches to an old problem have been made possible by the development over the last decade of powerful new analytical techniques by workers at Harwell and elsewhere. Radiochemical analysis is now an established technique with an extensive literature, and the work described in this Report is the specialised application of already well developed methods.

II. OXIDATION METHODS AND ORGANIC MATERIALS USED

1. METHODS FOR DESTROYING ORGANIC MATTER—

In the 114 years since Fresenius and von Babo[1] published their method for destroying animal tissue before trace-element determinations, many procedures have been described for this purpose. However, despite the many variations, almost all the methods fall into one of two main classes, *i.e.*, dry ashing and wet digestion. In the former, oxidation is accomplished by heating the sample to a relatively high temperature, usually between 400° and 700° C, when atmospheric oxygen serves as the oxidising agency. Chemical compounds may sometimes be added to aid the process; when a bomb technique is used, oxygen under pressure takes the place of air. In wet digestions, the temperature is much lower, liquid conditions are maintained throughout and the oxidation is carried out by oxidising agents in solution.

Within this framework possible variations are legion, and it was essential to select a few methods for more detailed study. To aid this selection, an analysis was carried out of the methods used in approximately 250 investigations reported in the literature. In 51 per cent., wet-digestion procedures were used; in the remaining 49 per cent., ashing was used.

The commonest single method was direct dry ashing (20 per cent.); then came wet digestion with nitric and sulphuric acids (14 per cent.), wet digestion with nitric, sulphuric and perchloric acids (12 per cent.), dry ashing with sulphuric acid (7 per cent.), dry ashing with nitric acid (6 per cent.), wet digestion with nitric and perchloric acids (5 per cent.) and dry ashing with magnesium compounds (5 per cent.). No other single method was used in more than 2 per cent. of the investigations, but, in 5 per cent., various combinations containing hydrogen peroxide were used. One worker used a mixture of sulphuric acid, nitric acid, perchloric acid and hydrogen peroxide.

For the purposes of this investigation, the first seven methods mentioned above were selected, together with Middleton and Stuckey's method,[2] for which considerable advantages have been claimed.

2. ORGANIC MATERIALS USED FOR EXPERIMENTS—

The organic material most commonly used in these experiments was cocoa. This was selected for a number of reasons. It is readily available in large amounts and samples are likely to be homogeneous. It contains several types of organic material, including fat, which is known to be resistant to wet oxidation. The trace-element content, which might influence the recoveries of added tracer, was believed to be low, as one sample had been shown to contain very little lead,[3] and irradiation and inspection by gamma-ray spectrometry did not reveal significant amounts of other trace elements.

In addition, a number of other materials were used for specific purposes. Powdered poly(vinyl chloride) has a low ash and high chlorine content; dried milk contains much calcium, as also does bone meal. An inter-laboratory comparison sample of tobacco was also used in some experiments. Human hair was used in zinc experiments because it satisfied the criteria necessary for direct determination by gamma-ray spectrometry.

III. RECOVERY OF TRACE ELEMENTS AFTER DESTRUCTION OF ORGANIC MATTER

1. Lead

The significance of lead as a trace element made it desirable to obtain as much information as possible about its behaviour during the destruction of organic matter. Unfortunately, ^{208}Pb, the common inactive isotope of lead, has an extremely low cross-section for neutrons, and pile irradiation is not a practicable means of obtaining an active tracer. However, an active isotope of lead, ^{212}Pb, does occur naturally as a decay product of ^{232}Th and can be separated in a pure state. Details of the separations are given in Appendix IV, p. 170.

A large number of experiments was carried out with this tracer, and investigations were made of both dry and wet oxidations.

(*a*) WET OXIDATIONS—

Oxidations of 2-g portions of cocoa were carried out with the acid mixtures described in Appendix I, p. 165, and the recoveries of lead, added at the 10-p.p.m. level, were determined. The results are shown in Table I.

TABLE I

RECOVERY OF LEAD AFTER WET OXIDATION

Oxidation mixture	Lead recovered, %
Nitric and perchloric	100, 100
Nitric, perchloric and sulphuric	99, 93
Nitric and sulphuric	90, 93
Middleton and Stuckey's method[2]	99, 101

From these results it appears that the presence of sulphuric acid in the oxidising mixture can cause low recoveries, even from a material, such as cocoa, having a comparatively low calcium content; it has long been suggested that, in the presence of much calcium, such losses can be greatly increased. To try to show that the losses were in fact due to adsorption on or co-precipitation with the heavy precipitate of calcium sulphate that formed during the oxidation of such materials, some oxidations of 2-g portions of dried milk with lead tracer added at the 1-p.p.m. level were carried out. In each test the precipitated calcium sulphate was separated either by filtration or centrifugation, washed with several portions of hot water and dissolved in sodium thiosulphate solution; a solution of the sodium salt of ethylenediaminetetra-acetic acid (EDTA) would probably be a valuable alternative solvent. The activities were then measured, and the percentages of lead retained were determined. The results are shown in Table II.

TABLE II

RETENTION OF LEAD ON CALCIUM SULPHATE PRECIPITATES

Oxidation mixture	Lead found in solution, %	Lead found on precipitate, %	Method of separation
Nitric, perchloric and sulphuric	68	23	Filtration
	76	21	
Nitric and sulphuric	52	38	
	77	15	
Nitric, perchloric and sulphuric	30	69	Centrifugation
	30	54*	
Nitric and sulphuric	55	49	
	71	32	

* Result should probably have been higher, as some precipitate was lost.

The results show clearly that serious losses of lead can occur by retention on calcium sulphate precipitates. The fact that the losses are higher after centrifugation than after filtration is probably due to the difficulty of washing the close-packed calcium sulphate precipitate obtained by centrifugation.

A modified procedure has been investigated by the Impurities in Organic Matter Sub-Committee of the Analytical Methods Committee of the Society for Analytical Chemistry, in which the oxidised solution containing the precipitate of calcium sulphate is boiled with 10 ml of dilute hydrochloric acid to recover any lead held by the precipitate. To check the efficiency of such a procedure, four more experiments were performed on dried milk, the oxidation being carried out with nitric and sulphuric acids; the results were as follows—

Amount of lead in solution, %	97	82	83	74
Amount of lead on precipitate, % ..	2	11	18	18

The modified procedure does not succeed in avoiding these losses, but it may be noted that, when the amount of retained lead was extremely low, the precipitate formed very slowly; in fact, it formed in the solution after the hydrochloric acid had been added. These are conditions that would be expected to reduce co-precipitation losses.

(*b*) DRY OXIDATIONS—

A study of the literature indicated that losses of lead during dry ashing were critically dependent on the temperature used. For this reason, all the oxidations were carried out in a muffle furnace, in which the temperature could be varied and controlled. To permit a large number of experiments to be carried out, open silica crucibles were used to contain the materials; this prevented the direct determination of lead lost by volatilisation, although a measure of it could be obtained by difference.

Many variables were studied, including temperature, ashing aid, the organic material and crucible material. All were found to affect the results.

(*i*) *Effect of variations of temperature and ashing aid*—The organic material, tracer form and crucible material were the same in all tests. Duplicate oxidations were carried out on 2-g portions of cocoa in silica crucibles at 450°, 550° and 650° C. Direct oxidation and the use of four ashing aids—nitric acid, sulphuric acid, magnesium nitrate and magnesium acetate—were employed. The time of heating was 16 hours, and the lead tracer was added at the 1-p.p.m. level in the nitrate form. The results are shown in Table III.

(*ii*) *Effect of chemical form of lead tracer*—The end product of the separation procedures described in Appendix IV (see p. 170) is a solution of lead in hydrochloric acid. As it seemed probable that the chemical form would affect the behaviour of the tracer, a comparison of

TABLE III

RECOVERY OF LEAD FROM COCOA UNDER VARIOUS CONDITIONS

		Temperature		
Ashing aid		450° C	550° C	650° C
None	Recovered,* %	100, 98, 97, 99	94, 95	71, 83
	Retained,† %	0, 1, 1, 1	3, 3	28, 15
Sulphuric acid	Recovered,* %	99, 99	95, 96	96, 90
	Retained,† %	0, 0	0, 0·5	2, 2
Nitric acid	Recovered,* %	99, 97	97, 99	83, 69
	Retained,† %	0·5, 1	2, 0	12, 19
Magnesium nitrate	Recovered,* %	98, 98	92, 94	91, 96
	Retained,† %	0·5, 0	3, 2	4, 2
Magnesium acetate	Recovered,* %	97, 98	98, 95	93, 93
	Retained,† %	1, 1	1, 2	4, 1

* Lead in solution after completion of oxidation.

† Lead on silica crucible after dissolution of the ash.

this solution with one containing lead in nitric acid was carried out. To reduce complicating factors to a minimum, the tracers were heated in silica crucibles for 16 hours at 650° C without organic matter. The results are shown in Table IV.

TABLE IV

EFFECT OF CHEMICAL FORM OF TRACER ON THE RECOVERY OF LEAD

Form of tracer	Lead recovered, %	Lead retained, %
Nitrate	78, 70	14, 22
Nitrate and magnesium acetate solution	96, 100	0, -0
Chloride	18, 23	2, 2
Chloride and magnesium acetate solution	55, 57	0, 0

This is further emphasised by Table V, in which some of the results from Table III are compared with similar results with a chloride tracer.

TABLE V

COMPARISON OF RECOVERIES OF NITRATE AND CHLORIDE TRACERS ADDED TO COCOA

Magnesium nitrate was used as ashing aid

	Amount of tracer recovered		Amount of tracer retained	
Temperature, °C	Nitrate, %	Chloride, %	Nitrate, %	Chloride, %
450	98	95	0·5	0
550	93	92	3	0
650	94	46	3	0

The selection of such vigorous conditions as 16 hours' heating at 650° C was based on the need to make some reaction take place. If less severe conditions had been used, and, after heating at, say, 450° C for 3 hours, no reaction had occurred, then no information would have been obtained.

(*iii*) *Effect of the organic material used as a base*—In Table VI is shown the effect of the organic base material on the recovery of lead. In each test 2 g of material were heated in silica crucibles for 16 hours. Lead tracer in the chloride form was added to give a level of 10 p.p.m., and 5 ml of 5 *N* sulphuric acid were added as ashing aid.

TABLE VI

RECOVERY OF LEAD WITH VARIATION IN THE ORGANIC BASE

	Lead recovered after dry ashing at—			Lead retained after dry ashing at—		
Organic base	450° C, %	550° C, %	650° C, %	450° C, %	550° C, %	650° C, %
Cocoa	97	98	98	0	0	3
Poly(vinyl chloride)	59	18	8	2	1	0

(*iv*) *Effect of variations in the crucibles*—As losses by retention of trace elements on the crucibles must be due to combination of the crucible material with the trace element, it is apparent that variations in the one are likely to affect the other.

Some recovery experiments were carried out with used platinum and either used or new silica crucibles. In each test, lead tracer was added as the nitrate without any organic matter and the crucibles were heated at 630° C for 16 hours. Two used platinum crucibles retained 1 and 2 per cent. of lead, eight used silica crucibles retained 20, 24, 19, 22, 13, 8, 8, and 10 per cent. and two new silica crucibles retained 69 and 74 per cent., showing how variations both of material and of the history of the crucibles can alter the amount of lead retained.

(*v*) *Effect of ashing aids*—Because of the marked effect of all these factors on the recoveries of lead, it is obviously desirable to study as simple a system as possible. Some duplicate experiments were carried out with 2 μg of lead nitrate tracer in used silica crucibles together with the additions of various ashing aids. These mixtures were heated at 650° C for 16 hours and the recoveries and retentions determined. No organic material was used in these experiments. The results are shown in Table VII.

TABLE VII

EFFECT OF VARIOUS ASHING AIDS ON THE RECOVERY OF LEAD

Ashing aid	Amount of ashing aid used, mg	Amount of lead recovered, %	Amount of lead retained, %
None	—	70, 67	22, 23
H_2SO_4	10	80, 61	23, 34
H_3BO_3	10	100, 101	0, 0
H_3PO_4	10	34, 46	46, 38
NaH_2PO_4	10 ($NaPO_3$)	98, 95	2, 2
Na_2HPO_4	10 ($Na_4P_2O_7$)	98, 97	2, 2
Na_3PO_4	10	82, 82	14, 16
$Al(NO_3)_3$	10 (Al_2O_3)	99, 99	0, 0
$Mg(NO_3)_2$	10 (MgO)	101, 98	0, 0·5
$(NH_4)_2S$	10	85, 92	4, 7
NaCl	10	4, 6	62, 56
NaCl + $Mg(NO_3)_2$ ..	10 (each)	48, 67	40, 21

(*c*) THE VOLATILITY OF LEAD NITRATE, CHLORIDE AND SULPHATE—

There has been much discussion on the volatility of lead under the conditions used for the destruction of organic matter, but until now no direct determination had been made. Such a determination has now been carried out with the separated lead tracer, and a comparison made between the volatilities of the chloride, nitrate and sulphate. Six silica crucibles were prepared, each containing 2 μg of lead tracer, two as lead nitrate, two as lead chloride and two as lead sulphate. The crucibles were dried under an infra-red lamp, and the activities were determined by counting on a gamma-scintillation counter; they were then heated for 4 hours at 650° C. At the end of this time, the crucibles were cooled and the activities re-determined. After allowing for radioactive decay, any loss due to volatilisation could be found. The results were as follows—

Crucible No. ..	1	2	3	4	5	6
Anion	Nitrate	Nitrate	Chloride	Chloride	Sulphate	Sulphate
Recovery, % ..	100	100	34	35	94	96

These results indicate that, under conditions more severe than are usually employed for trace-element determinations, lead is not volatile as sulphate or nitrate, but is as chloride.

(*d*) DISCUSSION—

The relative merits of wet and dry oxidations for the destruction of organic matter before the determination of lead have been discussed extensively, but much conflict of opinion is still evident. Dry ashing has been recommended for its simplicity and freedom from blank corrections and because large samples can be handled; wet digestion is considered superior in terms of speed of oxidation, the low temperature required and the freedom from loss by retention of trace elements on solid material in the system. Both methods have their devotees, and each, under the right conditions, can give excellent results. The potential errors in dry oxidation are two-fold; loss by volatilisation and loss by retention. For many years the former was considered to be the more important, and Fairhall[4] specified a temperature well below red heat to avoid this loss. More recently, Piper[5] has drawn attention to the possibilities of loss by retention, and he suggests that such losses are more widespread than is sometimes realised. Both types of loss increase with increasing temperature; about 500° C is usually recommended. However, in one paper[6] not more than 800° C is specified. With wet digestion the likelihood of loss by both these mechanisms is reduced and the main drawback is co-precipitation of sparingly soluble lead compounds, such as the sulphate, with precipitates formed during the digestion.

The results in this Report cast some light on the losses of lead that can occur during the preliminary oxidation of organic matter, but they more strikingly indicate the complexity of the problem, particularly for dry oxidations.

(*i*) *Dry oxidations*—The extreme conditions used in most of the experiments were designed to emphasise trends. It was essential that reactions should be made to occur, so that conclusions could be drawn on the mechanisms involved. The conditions customarily employed by analysts are intended to prevent reaction and would be useless for this purpose.

From the results in Tables III to VII, some general conclusions can be drawn on the behaviour of lead.

1. The temperature of oxidation strongly affects the recovery of lead; above 550° C, losses by retention on the crucible (at least with silica) increase sharply.
2. The chemical and physical nature of the organic material and its constituents exercises a considerable effect on the behaviour of the lead.
3. The crucible material has a direct bearing on the amount retained.
4. The previous history of the crucible appears to influence the amount retained.
5. The nature of any inorganic material added to the sample as an ashing aid can affect the results.
6. Losses by volatilisation are not important when lead is present as the nitrate or the sulphate, but can become important when it is present as the chloride. This suggests that all dry oxidations must be regarded as potentially dangerous when chloride ion or covalently bound chlorine is present. When chloride ion is present, the addition of sulphuric acid can be expected to improve the position, but the results in Table VI indicate that in presence of organically bound chlorine the addition of sulphuric acid has no effect.

Some of these findings are widely accepted, others have not been previously put forward.

The effect of temperature is well known and has led to the widespread acceptance of a temperature of about 500° C. The question of the volatility of lead, at least in the three chemical forms investigated, would seem to be settled; the conclusion is fully in accord with the known facts about macro amounts of the compounds. The effect of the crucible in determining the amount of lead retained is not widely appreciated; in fact, the importance of losses by retention does not seem to be fully realised.

The reduced losses in platinum crucibles can be readily explained by the low solubility of lead oxide in platinum, as contrasted with the ready formation of lead silicates by direct combination of lead oxide and silica. A more surprising result is the high retention of lead after heating in new silica crucibles. The use of new crucibles with unattacked surfaces is frequently recommended for trace-element determinations, yet these few results suggest that such crucibles give rise to the biggest losses.

The great variety of results obtainable by the addition of small amounts of ashing aids is amply shown in Table VII. Recoveries range from 4 to 100 per cent., and the amounts of lead retained range from 0 to more than 60 per cent. Perhaps the most surprising result is that obtained after the addition of sodium chloride. The volatility of lead chloride indicated by the results in Tables IV and V and those on p. 140 suggest that the main loss under these conditions is by volatilisation, whereas, in the event, a huge retention loss is found. The results with the series of phosphates are important, in view of the common occurrence of this radicle in biological materials. The results in Table III indicate that the commonly used ashing aids magnesium nitrate and sulphuric acid are reasonably satisfactory and do reduce retention losses at high temperatures. Nitric acid is less efficient in this respect, but does give a clean white ash at low temperatures.

The great variety of results, together with the variations between apparently similar experiments, underlines the difficulties inherent in tracer studies of this kind. It is possible to indicate the nature of the errors that are important in analysis, but it is not possible to translate the results under one set of conditions directly to those of a completely different experiment. However, by means of studies of this kind, an understanding of the mechanisms involved can be obtained, and, as the data available for reference increase, increasingly accurate predictions of the results obtainable under novel conditions should be possible.

(*ii*) *Wet oxidations*—The characteristic feature of tracer investigations involving wet digestion is that the relatively enormous amounts of oxidising acids added tend to swamp individual differences of sample and tracer. This renders the results much less liable to variation and greatly increases their applicability. The recoveries of lead after wet oxidation with mixtures containing sulphuric acid are less than 100 per cent., and this could possibly be due to deposition of lead sulphate. Losses of this kind were greatly increased when the organic material contained much calcium, and co-precipitation of lead with the calcium sulphate was clearly shown. In the samples high in calcium, the use of nitric and perchloric acids without added sulphuric acid gave a clear solution and complete recovery.

(*e*) CONCLUSIONS—

The choice of a method for destroying organic matter before determining trace amounts of lead is still largely a matter of personal taste, but it is possible to raise some relevant points.

Dry ashing at 500° C has been used with apparent success on many occasions and mostly appears to be satisfactory. However, the results with poly(vinyl chloride) at 450° C and with added sodium chloride at 650° C, and the results of the experiments in which lead was added as the chloride, must raise some doubts about such a procedure.

The use of ashing aids does not seem to be necessary unless the ash content of the sample is low. The use of nitric acid gives a clean ash readily soluble in dilute hydrochloric acid, but if added when much carbon is present it can cause deflagration and much loss. The use of sulphuric acid tends to retard the oxidation of the organic matter, as also does the use of magnesium acetate, but sulphuric acid will convert lead to lead sulphate, a form in which the risk of loss by volatilisation is negligible. The results in Table III do not indicate any difficulty in achieving dissolution of the lead sulphate produced, but in the presence of much calcium greater difficulty would be experienced.

Silica or platinum crucibles are usually satisfactory, but platinum is slightly preferable.

For the wet digestion, the most satisfactory mixture is nitric and perchloric acids. The use of sulphuric acid is suspect at all times and must be avoided in presence of much calcium.

2. Mercury

Because of the increasing use of mercury in agricultural sprays and the toxicity of some of its compounds, an extensive study was made of the behaviour of mercury during the destruction of organic materials.

The results of oxidations with mixtures of nitric and sulphuric acids, nitric, sulphuric and perchloric acids and nitric and perchloric acids, and of dry oxidation, the methods described in Appendix I being used, are shown in Table VIII.

TABLE VIII

RECOVERY OF MERCURY FROM ORGANIC MATTER

Method of oxidation	Oxidation mixture	Amount of mercury recovered, %	Amount of mercury distilled and collected, %
Wet	Nitric and perchloric	78, 80	12, 10
	Nitric, perchloric and sulphuric ..	87, 90	12, 11
	Nitric and sulphuric	93, 92	5, 7
Dry	—	0	7

In the dry oxidation, the mercury passed through the traps and into the water pump; no further experiments were carried out by this method.

To obtain more easily controlled conditions, several further series of experiments were carried out with use of a small distillation assembly. By using irradiated mercury and gamma-counting all the fractions obtained, it was possible to determine the behaviour of the element under a variety of conditions.

(*a*) COMPARISON OF OXIDISING MIXTURES—

Three mixtures were considered, similar to those used in the preliminary experiments above; the compositions are shown in Table IX.

TABLE IX

COMPOSITION OF WET-OXIDATION MIXTURES

Mixture	Amount of nitric acid, sp.gr. 1·42, ml	Amount of sulphuric acid, sp.gr. 1·84, ml	Amount of perchloric acid, sp.gr. 1·54, ml	Amount of distilled water, ml
Nitric and perchloric	15	—	10	5
Nitric, perchloric and sulphuric ..	15	10	10	5
Nitric and sulphuric	25	10	—	5

The proportions of the various acids were selected to allow approximately the same volumes to distil between room temperature and 200° C. The temperature - volume distilled relationships of the three systems when heated under similar conditions are shown in Fig. 1.

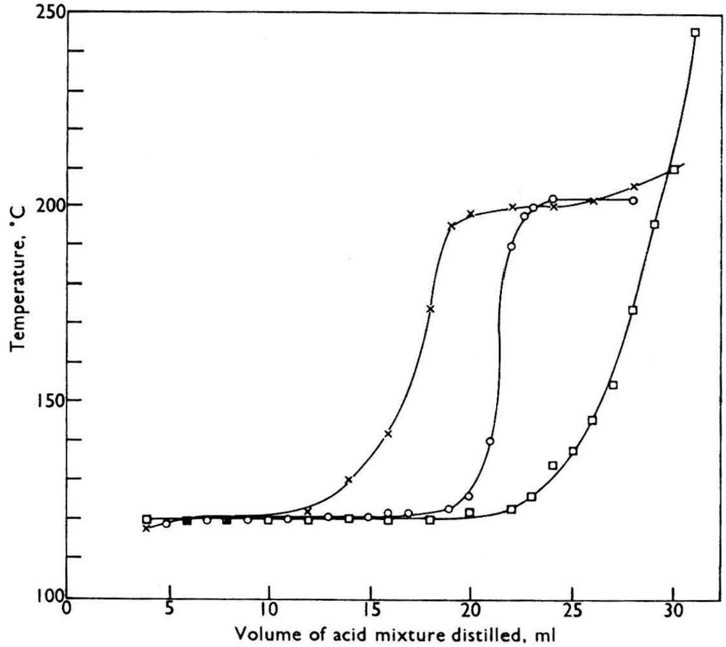


Fig. 1. Relationship between temperature and volume of different acid mixtures distilled: ×, 15 ml of concentrated nitric acid, 10 ml of 60 per cent. perchloric acid, 10 ml of concentrated sulphuric acid and 5 ml of distilled water; ○, 15 ml of concentrated nitric acid, 10 ml of 60 per cent. perchloric acid and 5 ml of distilled water; □, 25 ml of concentrated nitric acid, 10 ml of concentrated sulphuric acid and 5 ml of distilled water

It was not possible to consider all three systems in full detail and a choice was necessary at this stage. The presence of perchloric acid increased the oxidation potential and also set an easily maintained upper limit to the temperature; the presence of sulphuric acid in addition to the perchloric acid raised the temperature during the distillation of any nitric - perchloric acid mixture (see Fig. 1) and so enhanced the oxidising effect of the nitric acid. For these reasons, the nitric - perchloric - sulphuric acid system was chosen for further study.

(*b*) THE RECOVERY OF MERCURY DURING DISTILLATION—

For these experiments the 5 ml of distilled water in the nitric - perchloric - sulphuric acid mixture were replaced by 5 ml of a solution containing 1 μg of irradiated mercury per ml.

Experiments were carried out to determine the amounts of mercury distilling over a wide range of conditions. Fig. 2 shows the results when—

(*i*) the acid mixture was heated alone,
(*ii*) the acid mixture with 1 ml of concentrated hydrochloric acid was heated,
(*iii*) the acid mixture was heated in a stream of carbon dioxide, and
(*iv*) the acid mixture with 0·5 g of glycine was heated.

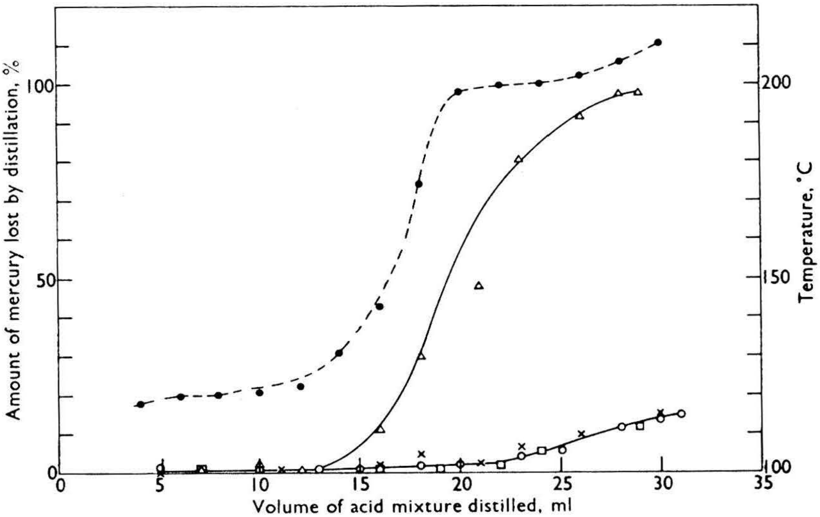


Fig. 2. Loss of mercury by distillation when acid mixture was heated under different conditions: ×, acid mixture alone; ○, acid mixture with 1 ml of concentrated hydrochloric acid; □, acid mixture in stream of carbon dioxide; △, acid mixture with 0·5 g of glycine. The broken line curve, ●, is the temperature - volume distilled curve for the nitric - perchloric - sulphuric acid mixture

The low loss of mercury from the mixed acids alone shows that mercury is not inherently volatile under such conditions. Similarly, the presence of chloride and of a stream of carbon dioxide did not affect the results, although both have been suggested as the cause of loss. However, in the presence of glycine, the loss rose to virtually 100 per cent., indicating that some interaction with the organic matter was responsible.

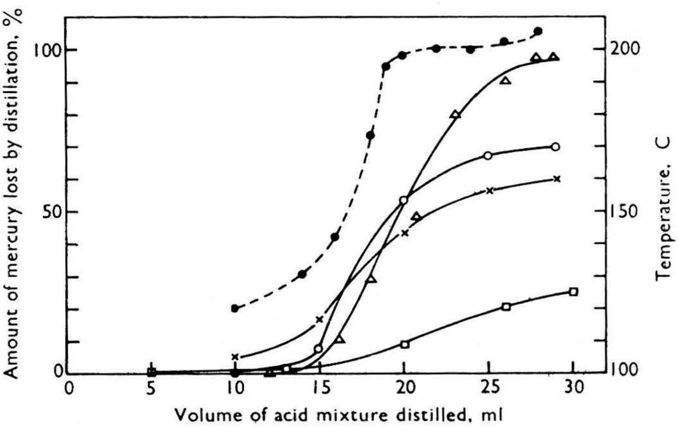


Fig. 3. Loss of mercury by distillation when heated in presence of various compounds: △, glycine; ○, alanine; ×, phenylalanine; □, chloroacetic acid. The broken line curve, ●, is the temperature - volume distilled curve for the nitric - perchloric - sulphuric acid mixture

To obtain further information, distillations were carried out with compounds related to glycine (see Fig. 3), compounds containing a benzene ring (see Fig. 4) and compounds related to ethane (see Fig. 5). These show that the amount of mercury lost under identical conditions is closely connected with the type of organic material being oxidised. The biggest losses occurred during the oxidation of compounds containing nitrogen, particularly when it was connected to a short carbon chain. At this point it was suggested that the mechanism involved was not a direct interaction between the organic material and the mercury, but a reaction between the organic material and the perchloric acid to produce hydrochloric acid, which then reacted with the mercury to give a volatile chloro compound or complex. To test this hypothesis, three further distillations were carried out, the oxidising mixtures being—

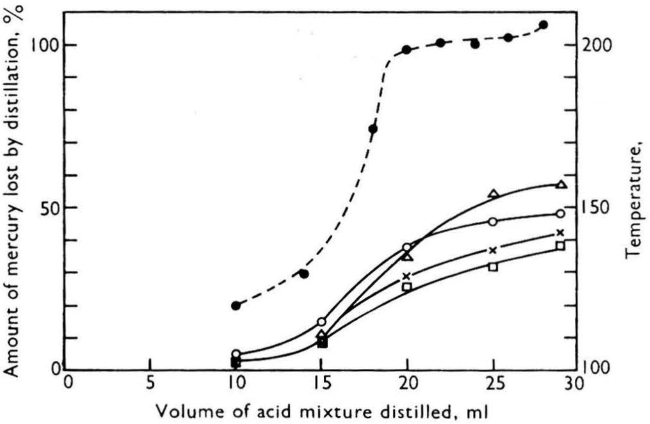


Fig. 4. Loss of mercury by distillation when heated in presence of various compounds: ×, aniline; ○, phenol; □, benzaldehyde; △, anisaldehyde. The broken line curve, ●, is the temperature - volume distilled curve for the nitric - perchloric - sulphuric acid mixture

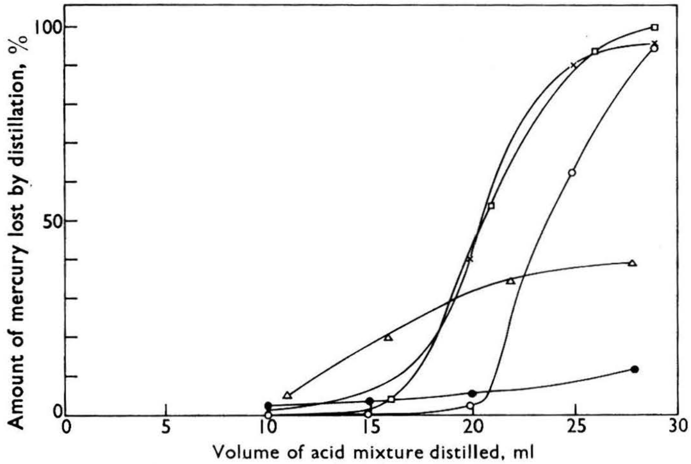


Fig. 5. Loss of mercury by distillation when heated in presence of various compounds: ×, ethanolamine; ○, ethylamine; □, ethylenediamine; △, ethylene glycol; ● ethylene chlorhydrin

(*v*) nitric and sulphuric acid with 0·5 g of ethylenediamine,

(*vi*) nitric and sulphuric acids with 0·5 g of ethylenediamine and a stream of hydrochloric acid gas, and

(*vii*) nitric and sulphuric acids with a stream of hydrochloric acid gas.

The results are shown in Fig. 6 and fully confirm the proposed mechanism. It would therefore seem that the variations observed during the oxidation of various organic materials are due to the comparative ease or difficulty with which they are oxidised. If they are readily oxidised, breakdown is virtually complete under the attack of the nitric acid at temperatures at which perchloric acid has little or no oxidising power. As the temperature rises, any remaining organic material progressively reduces the perchloric acid with the production of hydrochloric acid and the formation of a compound or complex with the mercury, which seems in general to be appreciably volatile at temperatures above about 140° C. The explanation of the extremely small loss that occurred when concentrated hydrochloric acid was added to the original oxidation mixture is probably that all the hydrochloric acid distilled over in the first few millilitres, below the temperature at which the mercury compound or complex is volatile.

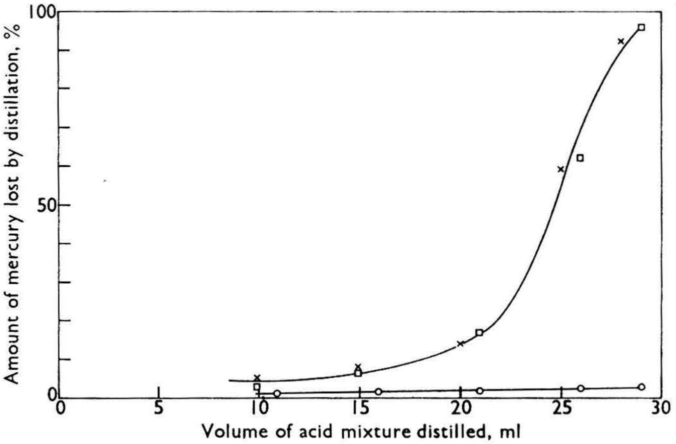


Fig. 6. Loss of mercury by distillation when heated in nitric - sulphuric acid mixture under different conditions: ○, acid mixture with 0·5 g of ethylenediamine; ×, acid mixture with 0·5 g of ethylenediamine in stream of hydrochloric acid gas; □, acid mixture in stream of hydrochloric acid gas

(*c*) METHOD FOR THE DESTRUCTION OF ORGANIC MATTER—

Despite the fact that loss of mercury by the formation of volatile chloro compounds or complexes seemed to be peculiar to oxidation methods involving perchloric acid, it was still thought that these methods offered notable advantages. Accordingly, an attempt was made to apply the information already obtained to the development of a method for the destruction of organic matter and the subsequent recovery of mercury.

As mercury has been shown to distil from systems containing perchloric acid and organic matter, it is obviously necessary to make some provision for the collection of the distillate. This is achieved by using the apparatus shown in Fig. 7. With this apparatus, a liquid heated in the flask would reflux through tap A, reservoir B and the condenser. If the tap were then turned through 90°, the distillate would rise up the side connection D, condense, and fall into the reservoir, where it would be retained. A further 90° turn of the tap would allow the condensate to run out through side-arm C. If a diluted mixture of nitric, perchloric and sulphuric acids were heated, with the tap in the second position, nitric acid and water would distil into B; this would cause the temperature of the solution remaining in the flask to rise. The whole system can be stabilised at any particular temperature by running the condensate out of B and turning the tap to the reflux position. The oxidation potential can thus be steadily increased or held constant.

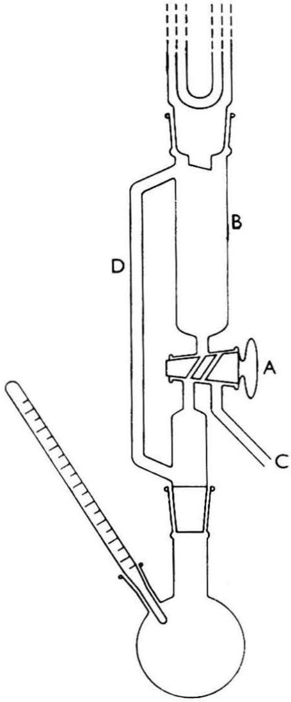


Fig 7. Apparatus for controlled decomposition of organic material

With this apparatus, which is similar to the modified Bethge apparatus described by Smith,[7] several oxidations were carried out and the recoveries of mercury determined. The general procedure is described below.

> Four grams of cocoa, 5 ml of active mercury solution (1 μg of irradiated mercury per ml), 15 ml of concentrated nitric acid, 10 ml of 72 per cent. perchloric acid and 5 or 10 ml of sulphuric acid were mixed in the flask and set aside for at least 30 minutes. Heat was then carefully applied and gradually increased until the solution was boiling under reflux. After 3 or 6 hours (see below), tap A was closed and the temperature was allowed to rise to 140° C. The condensate in B was then run off, and refluxing was continued for 1 hour. The temperature was raised similarly to 180° C for 30 minutes and then to 200° C for 30 minutes. The condensates were combined, and the apparatus was allowed to cool. When cold, the contents of the flask (residue) were transferred to a beaker and the combined distillates introduced into the flask. Five millilitres of concentrated sulphuric acid were added, and the mixture was refluxed for 1 hour. The tap was then closed and the temperature allowed to rise to 130° to 140° C, and heating was then stopped. The concentrations of mercury in the three cold fractions—residue, distillate residue and distillate distillate—were determined by gamma-counting.

Within this framework method, some modifications were investigated. These were—

(*i*) the effect of the addition of chloride or bromide,

(*ii*) variations in the amount of sulphuric acid originally added, and

(*iii*) variations in the time of refluxing at the first stage.

The results are shown in Table X.

It can be seen that the amounts of mercury distilling during the oxidation of the organic matter ranged up to 30 per cent., but that, by combining the oxidation residue and the residue from the second distillation, recoveries of 95 per cent. or more were consistently obtained. The presence of the added halides did not seem to affect the final recovery.

TABLE X

EFFECT OF CONDITIONS ON THE RECOVERY OF MERCURY

Distillation conditions				Amount of mercury found in—			
Additive	Amount of additive, mg	Sulphuric acid added, ml	Reflux time, hours	residue, %	distillate residue, %	distillate distillate %	total, %
None	—	10	6	91 82	6 17	3 3	100 102
Sodium chloride..	400	10	6	84 79	16 19	2 3	102 101
Sodium bromide..	400	10	6	67 69	28 27	3 3	98 99
None	—	10	3	73 80	27 19	2 1	102 100
None	—	5	3	71 71	26 24	2 3	99 98

(*d*) DISCUSSION—

The loss of mercury during the wet oxidation of organic materials is widely known and is attributed to the high volatility of the element and its compounds. Many special oxidation procedures have been suggested for overcoming the difficulties, and some workers have preferred to avoid the issue by not attempting to achieve complete destruction of the organic matter. This course is undesirable, as the readiness with which mercury will combine with organic radicles may lead to incomplete extraction at a later stage. The importance of complete oxidation is specifically mentioned in one publication.[8]

The Association of Official Agricultural Chemists' method for the destruction of organic matter involves oxidation with nitric and sulphuric acids at a "simmering" temperature, presumably close to the boiling-point of sulphuric acid. These conditions will not effect the oxidation of fat, which must be removed by filtration. A similar method involving filtration has been proposed by Barrett,[9] who, however, used potassium permanganate to complete the oxidation. For mercury in urine[10] and trade effluents,[11] the use of nitric acid and potassium permanganate at about 85° C under slight pressure is recommended. This is a specialised case, the amount of organic matter being relatively small, and its extension to other types of sample is unlikely.

The use of 50 per cent. hydrogen peroxide as the oxidising agent for organic material has been described by Polley and Miller.[12] This would seem to have a big advantage in that much of the oxidation is conducted without the application of external heat. No investigation of this method was carried out.

The method described in (*c*) of this Section is another attempt to solve this problem. The use of perchloric acid under conditions of controlled oxidation potential ensured the complete destruction of organic matter at comparatively low temperatures, but the breakdown of the perchloric acid introduced new difficulties, which required the introduction of an extra stage to overcome them.

All these methods and many more described in the literature are designed to minimise the effects of the volatility of mercury and its compounds. Few attempts have been made to turn this volatility to useful account, and there would seem to be considerable scope for development in this direction. Sulphur is readily determined in organic compounds by oxidation to sulphuric acid, the boiling-point of which is near to that of metallic mercury; it seems probable that some modification of a sulphur train and furnace would permit mercury to be similarly determined. Methods involving the distillation of mercury from samples are in use for mineral analysis and their application to organic samples would seem desirable.

3. Zinc

Preliminary survey experiments at the 10-p.p.m. level of zinc were carried out with ^{65}Zn; the methods are described in Appendix I. The results are shown in Table XI.

The results do not indicate any significant loss, but much discussion on the volatility of zinc and its compounds at temperatures above 500 °C has taken place. This problem can be easily investigated by means of direct gamma-ray spectrometry after neutron irradiation, human hair being used as the organic material. In the hair sample used, zinc had been found to occur at a level of approximately 250 p.p.m., and this abundance combined with the nuclear properties of the zinc isotopes rendered it suitable for this technique.

TABLE XI

BEHAVIOUR OF ZINC DURING WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of zinc recovered, %	Amount of zinc retained, %	Amount of zinc distilled, %
Wet	Nitric and perchloric	—	98, 100	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	101, 94	0, 0	0, 0
	Nitric and sulphuric	—	101, 99	0, 0	0, 0
Middleton and Stuckey	—	—	101, 97	0, 0	0, 0
Dry	—	None	96	2	1·5
		Nitric acid	97	0·5	1·5
		Sulphuric acid	100	0	0·5
		Magnesium nitrate	99	0	0·5

The radioactive isotope of zinc, ^{65}Zn, produced by pile irradiation emits a gamma ray of energy 1·12 MeV. This can be distinguished electronically from other gamma rays emitted from the irradiated hair sample, and the number of gamma photons determined. The activity is proportional to the amount of zinc present; by determining it before and after treatment of the sample, any change in the amount of zinc present can be detected. Accordingly, some samples of hair were irradiated and, after a suitable delay period, transferred to silica crucibles. The zinc activity was determined with a gamma-ray spectrometer and the samples were then ashed at 500° C for 16 hours. The zinc activity remaining in the ash was then determined. The crucibles were returned to the furnace for 3 hours at 600° C and re-counted. The counting was repeated after further 3-hour periods at 700°, 800°, 900° and 1000° C. The ash was dissolved by heating with 10 ml of 50 per cent. hydrochloric acid and 10 ml of 50 per cent. hydrochloric - nitric acid mixture, and the activity retained by the crucibles was determined. The results are shown in Table XII.

TABLE XII

BEHAVIOUR OF ZINC ON ASHING HUMAN HAIR

	Zinc activity found in ash of—	
Temperature, °C	sample A, %	sample B, %
Intact sample	101	94
500	100	100
600	100	100
700	100	100
800	102	95
900	100	96
1000	97	97
Activity retained on crucible	36	37

The results at 500° C were taken as 100 per cent. The low figure on the intact sample B is almost certainly due to geometrical variations, the small ash being nearer to the crystal than the bulky sample.

In view of the large retention of zinc on the crucibles, further experiments were carried out to determine the temperature at which such retention became serious.

Seven irradiated hair samples were ashed in silica crucibles at 500° C for 16 hours and the zinc activities determined on the gamma-ray spectrometer. One received no further heat treatment, one was heated at 600° C for 3 hours, two at 700° C for 3 hours and one each at 800°, 900° and 1000° C for periods of 3 hours. The zinc activities were re-determined, the ashes dissolved as before and the activities retained on the crucibles were measured. The results were as follows—

Temperature of additional heating, °C ..	None	600	700	700	800	900	1000
Activity retained on crucibles, % ..	0	0·3	0·7	2·4	5·9	6·6	7·1

(*a*) DISCUSSION—

The behaviour of zinc during dry ashing has been the subject of much discussion. Gottsch and Grodman[13] state that at "too high a temperature" zinc tends to sublime, and Monier-Williams[14] refers to the belief that zinc compounds are reduced to the metal by carbon and the zinc is lost by volatilisation; probably for this reason Reed and Cummings[15] ashed plant material below 450° C. On the other hand, Allport and Moon[16] found no appreciable volatilisation below 900° C.

The melting-point of zinc metal is 419° C and its boiling-point is 907° C; thus, if reduction of the oxide should occur, losses owing to volatilisation would be expected. However, in a paper by Ellingham[17] on the reducibility of oxides, it is shown that zinc oxide is not reduced by carbon below 950° C, so that this mechanism cannot be operative. The results in Table XII fully support Allport and Moon's[16] statement that zinc is not lost below 900° C, and it seems probable that this is generally true.

The retention of zinc on the silica of the crucibles seems to be a more serious problem, although losses were negligible at 600° C. However, there seems to be no reason to expect loss of zinc during dry ashing at about 500 °C, even with samples of low zinc content. This opinion is borne out by work reported by Thiers,[18] in which additions of zinc in the microgram and millimicrogram ranges were quantitatively recovered after ashing biological materials at temperatures between 450° and 550° C.

4. Selenium

Preliminary experiments were carried out by some of the methods described in Appendix I. The recoveries of selenium tracer at the 1-p.p.m. level after the oxidation of cocoa are shown in Table XIII.

TABLE XIII

BEHAVIOUR OF SELENIUM DURING THE OXIDATION OF ORGANIC MATTER

Oxidation mixture	Amount of selenium recovered, %	Amount of selenium distilled, %
Nitric and perchloric	100, 101	0·5, 0
Nitric, perchloric and sulphuric ..	100, 101	0, 0
Nitric and sulphuric	78, 80	0*, 0
Middleton and Stuckey's method ..	1, 0·5	0, 0

* Selenium was lost in a form too volatile to be retained in the distillate.

Although no appreciable activity was found in the distillates from the nitric and sulphuric acid oxidations, it is evident that the selenium was lost by volatilisation. No dry-ashing experiments were carried out in view of the virtually complete loss of selenium by Middleton and Stuckey's method.

To investigate the reasons for the losses in the wet oxidation with nitric and sulphuric acids, four oxidations were carried out in a distillation apparatus instead of in open flasks. The delivery tubes from the condensers dipped into water in an attempt to trap the volatile product. The oxidation mixture, organic material and selenium tracer were exactly as before. In two experiments the mixture was heated very rapidly, and the bulk of the nitric acid quickly distilled over. The organic material became heavily charred. In the other two experiments the heating was gradual and the oxidation period extended over several hours. At no stage did the organic matter char. The results of the four oxidations are shown in Table XIV.

The extremely small amount of selenium collected in the trap suggests that the volatile product has a low boiling-point. In an endeavour to characterise this compound, several oxidations were carried out in a small distillation apparatus connected to a train of traps. The first trap, T_1, was empty to collect liquid distilling over, and the compositions of the solutions in the second and third traps, T_2 and T_3, were varied in different experiments. In

TABLE XIV

EFFECT OF VARIATIONS IN OXIDATION TECHNIQUE ON THE RECOVERY OF 1 p.p.m. OF SELENIUM

Oxidation conditions	Amount of selenium recovered, %	Amount of selenium distilled, %
Very slow oxidation with no charring ..	95	0
	98	0
Rapid heating with heavy charring ..	4	3
	3	1

each test a mixture of 1 g of glycine, 15 ml of concentrated nitric acid, 10 ml of concentrated sulphuric acid, 5 ml of distilled water and 10 μg of irradiated selenium was heated in the flask in a stream of air until heavy charring occurred, and then for a further 5 minutes. After the flask had been cooled, the activities in traps T_1, T_2 and T_3 together with the activity remaining in the flask were determined by gamma-counting. The results and the solutions in T_2 and T_3 are shown in Table XV.

TABLE XV

COMPOSITION OF SOLUTIONS USED TO TRAP VOLATILE SELENIUM COMPOUNDS AND DISTRIBUTION OF SELENIUM AFTER OXIDATION OF GLYCINE

Traps T_2 and T_3 each contained 40 ml of solution

		Selenium found in—			
Solution in T_2	Solution in T_3	flask, %	T_1, %	T_2, %	T_3, %
Distilled water	Distilled water	3	1	0·5	0
2*N* NaOH	2*N* NaOH	3	1	25	18
2*N* HCl	2*N* HCl	2	1	3	0·5
Alkaline $KMnO_4$	Alkaline $KMnO_4$	4	2	35	27
50% NH_4OH	Neutral 10% $Pb(Ac)_2$	3	2	37	27

(*a*) DISCUSSION—

Difficulties in the recovery of selenium after the destruction of organic matter have been reported, and Klein[19] recommends a partial oxidation with the use of mercuric oxide to "fix" the selenium. Fogg and Wilkinson[20] reported losses of selenium when nitric and sulphuric acids were used to destroy organic matter, although in the presence of perchloric acid complete recoveries were obtainable. They explained this by suggesting that ". . . nitric acid is not a strong enough oxidising agent to prevent the loss of selenium. . . ." The results reported here fully confirm the losses of selenium, but not the explanation advanced to account for them. The results in Table XIV show that it is possible to achieve complete recoveries of selenium after destroying the organic matter with nitric and sulphuric acids alone, but that, if the nitric acid is rapidly driven off and the organic material allowed to char, virtually complete loss can occur. It seems, therefore, that the loss occurs after the nitric acid has been evaporated and reducing conditions have been established; the efficiency of perchloric acid in preventing such loss is probably due to its much higher boiling-point, which prevents its rapid evaporation and allows oxidising conditions to be maintained throughout.

The information in Table XV is insufficient to allow any definite conclusions to be drawn. However, the inefficiency of water and 2 *N* hydrochloric acid as trap solutions, coupled with the effectiveness of 2 *N* sodium hydroxide, suggest that the volatile compound is an acidic substance of low boiling-point. The surprising efficiency of the lead acetate solution further suggests that it is capable of forming a non-volatile lead salt. Further work would be required before a firm opinion could be advanced.

5. Arsenic

Preliminary experiments were carried out with cocoa as the organic material, the oxidation methods described in Appendix I being used. Irradiated arsenic was added to the cocoa to give a level of 1 p.p.m. The results are shown in Table XVI.

In discussions on the recovery of arsenic after wet digestion, the statement is often made that it is necessary to maintain oxidising conditions throughout the experiment, particularly in the presence of chloride, to prevent loss by volatilisation. As the results in Table XVI gave no information about the effect of chloride, further experiments were performed.

TABLE XVI

RECOVERY OF ARSENIC AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of arsenic recovered, %	Amount of arsenic distilled, %
Wet	Nitric and perchloric	—	99, 99	0, 0
	Nitric, perchloric and sulphuric	—	98, 100	0, 0
	Nitric and sulphuric	—	98, 97	0, 0
Middleton and Stuckey ..	—	—	91, 98	0, 0
Dry	—	None	88	4
		Nitric acid	84	7
		Sulphuric acid	96	1·5
		Magnesium nitrate	99	3

Oxidations were carried out on 2-g portions of cocoa, to each of which had been added 0·5 g of sodium chloride. Duplicate oxidations were carried out with nitric and perchloric acids and with nitric, perchloric and sulphuric acids, and four oxidations with nitric and sulphuric acids. The recoveries of arsenic from the oxidations with nitric and perchloric acids were 99 and 100 per cent., with nitric, perchloric and sulphuric acids, 99 and 99 per cent. and with nitric and sulphuric acids, 101, 100, 101 and 101 per cent.

In view of the results for selenium, the nitric - sulphuric acid oxidations were carried out as two pairs, and the effect of charring the organic matter was investigated. The first two results were obtained after rapid heating with heavy charring of the organic material, the other two after gentle heating with no charring.

(*a*) DISCUSSION—

The destruction of organic matter before the determination of arsenic is most commonly accomplished by wet oxidation, although Evans and Bandemer[21] dry ashed biological material at 600° C with magnesium nitrate as ashing aid. Kingsley and Schaffert[22] found complete oxidation unnecessary and considered a simple digestion with dilute hydrochloric acid sufficient. On the other hand, Gross[23] found that fuming nitric and sulphuric acids failed to destroy heterocyclic compounds in tobacco leaves and that it was necessary to destroy them by ignition. Allcroft and Green[24] compared several methods and found them all unsatisfactory; they state that nitric and sulphuric acids gave recoveries of 57 to 81 per cent., nitric and perchloric acids, 63 to 97 per cent. and dry ashing with magnesium nitrate, 47 to 90 per cent. The results when active tracers are used do not confirm these findings, but show virtually complete recoveries by each of these three methods. A probable explanation is that the loss occurred not during the oxidation, but during the subsequent steps of the determination and could not be differentiated. This difficulty is avoided by the use of radioactive tracers.

The results of experiments with added sodium chloride do not suggest that chloride is likely to cause serious loss of arsenic. Even when heavy charring occurred and reducing conditions were rapidly induced, recoveries of arsenic were complete. It seems probable that, provided sufficient nitric acid is initially present, all the chloride is removed long before there is any chance of the arsenic being reduced to the tervalent form.

6. Copper

Preliminary experiments, the result of which are shown in Table XVII, were carried out with 10 p.p.m. of irradiated copper added to 2-g portions of cocoa, the oxidation methods described in Appendix I being used.

TABLE XVII

THE BEHAVIOUR OF COPPER DURING WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of copper recovered, %	Amount of copper retained, %	Amount of copper distilled, %
Wet ..	Nitric and perchloric	—	99, 100	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	99, 99	0, 0	0, 0
	Nitric and sulphuric	—	101, 99	0, 0	0, 0
Middleton and Stuckey	—	—	100, 99	0, 0	0, 0
Dry ..	—	None	86	14	0
		Nitric acid	94	2	3
		Sulphuric acid	96	0·5	0·5
		Magnesium nitrate	98	0·5	0

The wet oxidation gave satisfactory results, and no further experiments were carried out. The "retained" figure for dry ashing without ashing aids was high, and further experiments were planned to investigate the mechanism. All subsequent experiments were in open silica crucibles in a muffle furnace. The effects of various ashing aids on the recoveries of copper when heated in silica crucibles without organic matter were investigated. Twenty micrograms of copper, as nitrate, were heated at 630° C for 16 hours in the presence of different ashing aids; the results are shown in Table XVIII. In each test the amount of ashing aid used was 10 mg or sufficient to give 10 mg of the non-volatile residue.

TABLE XVIII

EFFECT OF ASHING AIDS ON THE RECOVERY OF COPPER

Ashing aid	Amount of copper recovered, %	Amount of copper retained, %
None	98, 100	1, 0·5
H_2SO_4	99	1
$Mg(Ac)_2$	97, 97	0, 0·5
$Mg(NO_3)_2$	81, 84	1, 1
H_3PO_4	14, 5	60, 62
NaH_2PO_4	93, 92	4, 5
Na_2HPO_4	—, 97	6, 2
Na_3PO_4	91, 96	2, 4

The major cause of loss of copper in dry ashing organic material appears to be retention of the copper on the silica of the crucible, and two experiments designed to determine the effect of organic matter on this loss were carried out. In each experiment, 20 µg of irradiated copper, as nitrate, were heated in silica crucibles for 16 hours at 630° C. Eight of the crucibles contained 1 g each of organic matter in the form of carbonised sugar, the other seven being without organic matter. The ashes were extracted with A, two 5-ml portions of 50 per cent. hydrochloric acid and B, two 5-ml portions of 50 per cent. hydrochloric acid - 50 per cent. nitric acid mixture (2 + 1).[25] The activities remaining on the crucibles were then determined; the results were—

Extraction mixture	A	B	B	A	A	B	B	A	
Activity retained (no organic matter), % ..	0·5	0·5	0·5	0·5	0·5	1	0·5	–	Mean, 0·5
Activity retained (with organic matter), % ..	7	7	8	5	7	8	5	4	Mean, 6·5

(*a*) DISCUSSION—

The results in Table XVII suggest that wet oxidation will give complete recoveries of copper, and this confirms work already reported in the literature. Many workers have used dry ashing for the destruction of organic matter before the determination of copper, but several objections have been raised. Ashing temperatures of up to 850° C have been reported,[26] but 500° to 550° C is more common. In at least one paper[27] a maximum temperature of 400° C is specified.

Losses of copper on dry ashing were reported by Comrie,[28] who used silica basins. He found that the use of old basins and sulphuric acid as an ashing aid always led to some loss between 400° and 700° C. Substitution of magnesium nitrate as the ashing aid reduced or avoided the loss, as also did the use of new silica basins. In the same year, Sylvester and Lampitt[29] described the apparently successful determination of copper in milk after dry ashing at 500° to 550° C in silica basins with sulphuric acid as the ashing aid. In this test the ash would presumably be very bulky, owing to the high calcium content, and losses would not be expected. Bailey and McHargue[30] dismissed dry ashing procedures altogether, considering silica, porcelain and platinum dishes all to be unsuitable. High[25] made the important suggestion that losses of copper on dry ashing at more than 500° C are not due to volatilisation, but to retention on the crucible. He also contended that the copper could be recovered by the use of a mixture of hydrochloric and nitric acids as the extracting agent. With this mixture he claimed recoveries after dry ashing at 650° to 800° C similar to those by wet oxidation, whereas the use of hydrochloric acid as the extractant gave results 15 to 60 per cent. low. This is not borne out by the results on p. 153.

A number of comparisons of recoveries after the use of different oxidation methods can be found in the literature. Reed and Cummings[31] compared (*a*) oxidation with nitric, perchloric and sulphuric acids, (*b*) ashing at 450° C for 12 to 16 hours, (*c*) ashing at 450° C for 12 to 16 hours with nitric acid as ashing aid, and (*d*) ashing at 650° C for 12 to 16 hours. Method (*a*) gave quantitative recovery, (*c*) was rather worse than (*b*), and (*d*) gave less than 50 per cent. recovery. Two other papers[32,33] compare ordinary ashing of oils with sulphated ashing; both found the sulphated ashing to be superior.

The results reported here strongly support High's contention[25] that retention is the most important cause of loss on dry ashing; volatilisation does not appear to be an important factor. The most important results are those on p. 153 showing the effect of organic matter. The fact that retention on the crucible is higher in the presence of organic matter than in its absence is unexpected. It would normally be supposed that the organic matter would serve to separate the copper and the surface of the crucible, at least in the early stages, and so reduce the combination between them. The use of carbonised sugar was designed to give a virtually ashless material and so to reduce the possibility of attack on the silica by the ash constituents. The whole question of the retention of trace metals on silica is discussed in more detail in Section IV of this Report (see p 159), and the mechanism suggested to account for the copper results is reduction of the oxide to the metal and diffusion, in this form, into the silica of the crucibles. This suggestion can only be tentative, but it does explain logically why copper should be particularly susceptible to retention losses.

The effects of various ashing aids on the recovery of copper (see Table XVIII) are of interest when they increase the retention. The effect of phosphoric acid is predictable and is discussed later; it agrees with the results for lead (see Table VII).

7. Cobalt

Preliminary experiments by the methods described in Appendix I were carried out with cobalt tracer added to cocoa at both 10-p.p.m. and 1-p.p.m. levels. The results are shown in Table XIX.

Some further oxidations were carried out in silica crucibles to obtain information about the effect of organic matter on retention of cobalt. Twenty-microgram amounts of cobalt, as nitrate, were heated in silica crucibles at 630° C for 16 hours, with or without the presence of 1 g of carbonised sugar, and then extracted with High's nitric - hydrochloric acid mixture.[25] These experiments are exactly comparable with those carried out with copper. The results were—

Activity retained (no organic matter), %	21	3	15	3	9	9	42	6	13	12	12	Mean, 13
Activity retained (1 g of carbonised sugar), % ..	4	3	4	4	4	5	5	3	0	0	0	Mean, 4

(*a*) DISCUSSION—

Although the results in Table XIX indicate complete recovery of cobalt by all the methods tried, some workers have reported difficulties. Ambler and Griggs[34] said that cobalt was lost on dry ashing at 500° C, and Kidson, Askew and Dixon[35] found the use of calcium acetate, calcium hydroxide, magnesium acetate and magnesium nitrate as ashing aids all to be unsatifactory when ashing liver for cobalt determinations. Gottsch and Grodman[13] state that, at too high a temperature, cobalt tends to combine with the glaze of porcelain basins, although Saltzmann[36] used temperatures of up to 700° C. Other workers have used dry ashing successfully, including Thiers, Williams and Yoe,[37] who recovered millimicrogram amounts of cobalt from blood after ashing at 450° C for 18 hours.

TABLE XIX

RECOVERY OF COBALT AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of cobalt recovered, %	Amount of cobalt retained, %	Amount of cobalt distilled, %
For addition of tracer at 10-*p.p.m. level—*					
Wet ..	Nitric and perchloric	—	98, 98	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	99, 98	0, 0	0, 0
	Nitric and sulphuric	—	100, 101	0, 0	0, 0
Middleton and Stuckey	—	—	100, 99	0, 0	0, 0
Dry ..	—	None	99	0·5	0
		Nitric acid	96	1·5	0
		Sulphuric acid	98	0	0
		Magnesium nitrate	99	0	0
For addition of tracer at 1-*p.p.m. level—*					
Wet ..	Nitric and perchloric	—	98, 98	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	100, 99	0, 0	0, 0
	Nitric and sulphuric	—	99, 99	0, 0	0, 0
Middleton and Stuckey	—	—	100, 99	0, 0	0, 0
Dry ..	—	None	99	0	0
		Nitric acid	99	0·5	0·5
		Sulphuric acid	99	0	0
		Magnesium nitrate	101	0	0

8. Silver

A few recovery experiments were carried out with 10 p.p.m. of silver added to cocoa, the oxidation methods described in Appendix I being used. The results are shown in Table XX.

TABLE XX

RECOVERY OF SILVER AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of silver recovered, %	Amount of silver retained, %	Amount of silver distilled, %
Wet ..	Nitric and perchloric	—	98, 95	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	99, 99	0, 0	0, 0
	Nitric and sulphuric	—	100, 100	0, 0	0, 0
Middleton and Stuckey	—	—	94, 96	3, 2	0, 0
Dry ..	—	None	93, 99	8, 1	0, 1·5
		Nitric acid	87	7	5
		Sulphuric acid	97	1	2
		Magnesium nitrate	100	0·5	0·5

(*a*) DISCUSSION—

There are comparatively few references in the literature to the recovery of silver after the destruction of organic matter, and little trouble seems to have been experienced. Cheong, Perri and Sharpe[38] used wet oxidation with sulphuric acid and hydrogen peroxide for the destruction of biological materials, but Meeker and Pomatti[39] and Parks and Lykken[40] used dry oxidation in porcelain or platinum, respectively. No reference is made to loss of silver by retention on the crucible. In the few results shown in Table XX, losses by retention occur on several occasions and it is noteworthy that silver is the only element for which appreciable retention loss has occurred during the Middleton and Stuckey oxidations.

9. Cadmium

The results of a limited series of experiments in which 10 p.p.m. of cadmium tracer were added to cocoa before oxidation by the methods described in Appendix I are shown in Table XXI.

TABLE XXI

RECOVERY OF CADMIUM AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of cadmium recovered, %	Amount of cadmium retained, %	Amount of cadmium distilled, %
Wet ..	Nitric and perchloric	—	100, 101	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	102, 101	0, 0	0, 0
	Nitric and sulphuric	—	103, 100	0, 0	0, 0
Middleton and Stuckey	—	—	99, 98	0, 0	0, 0
Dry ..	—	None	91	6	6
		Nitric acid	76	6	13
		Sulphuric acid	92	2	5
		Magnesium nitrate	78	2	20

(*a*) DISCUSSION—

Although dry ashing has been described[41] as a preliminary to the determination of cadmium, its use has not, in general, been recommended. Klein and Wichman[42] preferred wet oxidation and stated that dry oxidation, even at 500° C, caused some loss of cadmium by volatilisation. Cholak and Hubbard[43] also preferred wet oxidation and laid particular stress on the losses occurring if nitric acid were used in dry ashing. The results in Table XXI amply confirm the dangers of dry ashing, and the high "distilled" figures when either nitric acid or magnesium nitrate was used as ashing aid fully corroborate the findings of Cholak and Hubbard.[43]

10. Antimony

The results of recovery experiments with 1 p.p.m. of antimony tracer added to cocoa are shown in Table XXII. The oxidation methods were those described in Appendix I.

TABLE XXII

RECOVERY OF ANTIMONY AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of antimony recovered, %	Amount of antimony retained, %	Amount of antimony distilled, %
Wet ..	Nitric and perchloric	—	99, 94	0, 0	0, 0
	Nitric, perchloric and sulphuric	—	100, 100	0, 0	0, 0
	Nitric and sulphuric	—	100, 99	0, 0	0, 0
Middleton and Stuckey	—	—	95, 98	0, 0	0, 0
Dry ..	—	None	96	1·5	2
		Nitric acid	92	1	1
		Sulphuric acid	94	0·5	3
		Magnesium nitrate	97	0	0

(*a*) DISCUSSION—

Comparatively few papers dealing with the recovery of antimony have been published, but both wet[44] and dry[45] methods have been used, apparently with success. The tracer results are reasonably in accord, although the recoveries after dry ashing with nitric or sulphuric acid tend to be low; the use of magnesium nitrate seems to be the most successful.

11. Chromium

Recovery experiments were carried out with 10 p.p.m. of chromium tracer added to cocoa, the oxidation methods used being those described in Appendix I. The results are shown in Table XXIII.

TABLE XXIII

RECOVERY OF CHROMIUM AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of chromium recovered, %	Amount of chromium retained, %	Amount of chromium distilled, %
Wet ..	Nitric and perchloric	—	100, 100	0, 0	2, 1·5
	Nitric, perchloric and sulphuric	—	101, 100	0, 0	1, 1
	Nitric and sulphuric	—	99, 100	0, 0	0·5, 0·5
Middleton and Stuckey	—	—	100, 101	0, 0	0, 0
Dry ..	—	None	98	0·5	1·5
		Nitric acid	99	0	2
		Sulphuric acid	99	0	0·5
		Magnesium nitrate	92	0	4

(*a*) DISCUSSION—

No difficulties seem to have been experienced in the recovery of chromium after the destruction of organic matter. Dry ashing has been used by some workers,[46,47] although Smith[48] insistently recommends wet oxidation. A combination method has been used by Urone and Anders.[49] With the exception of dry ashing in the presence of magnesium nitrate, good recoveries were obtained in all the tracer experiments. The amounts of the element distilling, particularly in the wet digestions, are rather higher than usual, and this is most marked in the presence of perchloric acid. It is possible that the formation of a volatile chlorine compound, such as chromyl chloride, might be a factor, its production during perchloric acid oxidations being well known.

12. Molybdenum

Recovery experiments were carried out with 1 p.p.m. of irradiated molybdenum added to cocoa. The oxidation methods described in Appendix I were used; the results are shown in Table XXIV.

TABLE XXIV

RECOVERY OF MOLYBDENUM AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of molybdenum recovered, %	Amount of molybdenum distilled, %
Wet ..	Nitric and perchloric	—	96, 98	<1, <1·5
	Nitric, perchloric and sulphuric	—	99, 97	<1·5, <1
	Nitric and sulphuric	—	101, 101	<1, <1
Middleton and Stuckey	—	—	97, 99	0, 0
Dry ..	—	None	99	<5
		Nitric acid	98	<1·5
		Sulphuric acid	100	<1
		Magnesium nitrate	98	<2

(*a*) DISCUSSION—

Little work seems to have been done on molybdenum and losses do not seem to be serious. Evans, Purvis and Bear[50] successfully used nitric and perchloric acids for the oxidation of plant material, and Johnson and Arkley[51] found both wet and dry oxidations satisfactory. The results in Table XXIV indicate good recoveries by each method.

After irradiation of ^{98}Mo to produce ^{99}Mo, the decay product, ^{99}Tc, begins to grow towards equilibrium with its parent, and it is necessary to make allowance for the chemical differences between the two nuclides. All counts were made 24 hours after the oxidations to give time for equilibrium to be largely re-established and to nullify the effects of any separation that may have occurred. The "distilled" figures are given as maxima, as it is possible that they may be due, in part, to technetium that had distilled over during the oxidation. Even after 24 hours, some 6 per cent. of such distilled activity would remain.

13. Strontium

For strontium, the cross-section for activation of ^{84}Sr is so low that the specific activity of the tracer was also very low. For this reason, results were obtained only for percentage recovery with 10 p.p.m. of tracer added to cocoa. The oxidation methods used were those described in Appendix I, and the results are shown in Table XXV.

TABLE XXV

RECOVERY OF STRONTIUM AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of strontium recovered, %
Wet	Nitric and perchloric	—	101, 98
	Nitric, perchloric and sulphuric	—	97, 96
	Nitric and sulphuric	—	96, 100
Middleton and Stuckey	—	—	96, 94
Dry	—	None	97
		Nitric acid	97
		Sulphuric acid	100
		Magnesium nitrate	100

The recoveries are reasonably satisfactory by each method and no difficulties seem to have been reported in the literature.

14. Iron

For iron also, only recoveries have been reported, owing to the low activity of the tracer Iron was added to cocoa at the 10-p.p.m. level. The results are shown in Table XXVI.

TABLE XXVI

RECOVERY OF IRON AFTER WET OR DRY OXIDATION

Method of oxidation	Oxidation mixture	Ashing aid	Amount of iron recovered, %
Wet	Nitric and perchloric	—	97, 100
	Nitric, perchloric and sulphuric	—	99, 99
	Nitric and sulphuric	—	102, 101
Middleton and Stuckey	—	—	98, 95
Dry	—	None	99
		Nitric acid	101
		Sulphuric acid	100
		Magnesium nitrate	100

(*a*) DISCUSSION—

Although the literature contains many references, *e.g.*, see Thompson[52] and Jaycox,[53] to the use of both wet and dry ashing without apparent loss of iron, several workers have reported difficulties. Jackson[54] compared recoveries of iron after wet oxidation with nitric, sulphuric and perchloric acids and after dry oxidation with sodium carbonate, with calcium carbonate and with sulphuric acid. He found that only the wet digestion gave complete recovery. Hoffman, Schweitzer and Dalby[55] found that recoveries of iron after dry ashing bread were low unless sodium hydroxide was used as ashing aid. Borda[56] specified a temperature of 550° C and an ashing time of 5 hours for the determination of iron in foods. He claimed that any deviation from these conditions caused losses. The possibility of loss of iron at temperatures above 600° C is noted by Peterson,[57] who suggests two mechanisms; the formation of glassy phosphates and attack on the crucible by alkaline ash constituents. He states that porcelain is more resistant to this attack than is silica or Vycor. Sandell[58] quotes a paper by Wijkstrom indicating the retention of iron on the residue of silica left after dry ashing. One other paper is relevant, although it does not deal with the destruction of organic matter. Shell[59] has shown that a sodium carbonate fusion carried out in platinum over a gas flame causes reduction of iron oxide to the metal and loss by reaction with the crucible. In a muffle furnace with air circulation this does not occur. Ellingham[17] showed that ferrous oxide is reducible to the metal by carbon at about 700° C; it would seem, therefore that the danger of loss by alloying of iron with platinum is slight under the conditions most commonly used for dry ashing, but is another warning against the use of very high temperatures. The active-tracer results in Table XXVI do not indicate any losses of iron during wet or dry oxidation.

IV. THE RETENTION OF TRACE ELEMENTS ON CRUCIBLES DURING DRY ASHING

In the results reported in Section III, one of the types of loss recognised was that caused by retention of the tracer on the reaction vessel. Losses of this kind have tended to be overlooked, although High,[25] Piper[5] and others have commented on them. An indication of their importance was obtained during the experiments with copper, for which a loss of 14 per cent. was attributed to this cause (see Table XVII).

It is apparent that the magnitude of these losses will be affected by the material of the reaction vessel, which, for this work, was nearly always vitreous silica.

Vitreous silica is a true glass made up of SiO_4 tetrahedra linked at the corners to give an isotropic solid with an infinitely large unit cell. Losses occur when some chemical or physical reaction takes place between the element being determined and the silica. The most obvious reaction is the formation of complex silicates, and this theory has been advanced by Piper[5] to account for the loss of copper during dry ashing.

For such losses to occur, two conditions must be fulfilled, (1) the reaction between the components must take place at the temperatures used, and (2) the product of the reaction must be stable to the reagents used to dissolve the ash.

1. FORMATION OF REACTION PRODUCT—

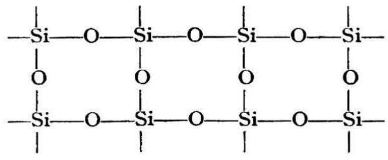
Most heavy-metal compounds are comparatively easily converted to the oxides; for the sake of simplicity these will be considered first.

The temperatures used for the ashing of organic matter seldom reach the melting-points of the heavy-metal oxides, and it is probable that any reactions occurring take place in the solid state.

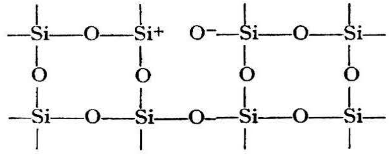
A considerable amount of work has been carried out, particularly by Tammann and by Hedvall, on the solid-state reactions occurring when mixtures of oxides are heated. Tammann has suggested that a connection exists between the melting-points of the components and the temperature at which reaction between them takes place; for most binary mixtures of inorganic compounds, excluding the metals, he found that reaction began at a temperature approximately equal to half the melting temperature, in degrees absolute, of the lower-melting component. This has also been shown to be the temperature at which bulk diffusion within the solid becomes significant. This simple picture is, however, not the whole story. Hedvall has shown that reaction is particularly rapid when molecular rearrangement takes place within one of the components, as, for example, at the quartz - tridymite transition temperature for silica; about 870° C. Similarly, Tammann and Kalsing[60] have shown that the vitreous form of silica reacts more readily with magnesium oxide and lead oxide than does quartz when heated for 1 minute at 600° C.

To continue the simplest picture of a reaction between silica and a metal oxide, MO, it is instructive to examine the situations arising when a mixture of the two components is heated. At absolute zero, the atoms are at rest and reaction is impossible; as the temperature increases, the atoms vibrate within the lattices until at some sufficiently high temperature the energies of individual atoms are sufficient to break the bonds connecting them to their neighbours. If we consider the silica giant molecule, the conditions found in the cold and at this unspecified high temperature are shown, respectively, in (*a*) and (*b*) below.

(*a*)

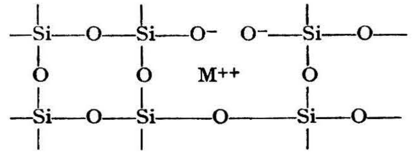


(*b*)



If a molecule of the oxide MO is nearby and has sufficient energy to approach the broken bond, the oxygen of the oxide may be captured by the silicon to give the situation in (*c*)—

(*c*)



This condition will arise if the oxide has sufficient energy to overcome the repulsion due to the charges on the broken bonds. This repulsion arises because all oxides have some degree of ionic character owing to the resonance between the forms M=O and M^{2+} O^{2-} and suggests that reaction will occur most readily when the oxide is only weakly ionic. This brings forward a point frequently made by Fajans, *e.g.*, see Fajans and Kreidl,[61] that reaction is facilitated if the ions in the metal oxide are strongly polarised, *i.e.*, the ionic character of the oxide is reduced.

It can be seen, therefore, that even the simple reaction considered here is governed by many factors, and the situation occurring in analytical practice is much more complicated. Consideration must be given to the effect of the organic matter, the effect of anions present other than the oxide and the reactions of other substances present with the silica.

2. Stability of the reaction product—

If it is assumed that a reaction has taken place between the metal oxide and the silica of the crucible, it cannot be said to have caused a loss of trace element until it has been shown that the product is not susceptible to hydrolytic attack and can resist the hot acids normally used for dissolution of the ash.

3. Results and discussion—

(*a*) *Reaction of metal oxides with silica*—Most references in the literature to losses of trace elements by retention on the crucible are concerned with copper and lead, but the results in Section III also indicate silver and cadmium as serious offenders.

Consideration of these four elements suggested that they fell into two groups. The oxides of lead and cadmium are strongly polarised, the other two are not. The oxides of copper and silver are readily reduced to the metals; those of lead and cadmium are much more stable. Silver and copper silicates are unstable and readily hydrolysed by acids, those of lead and cadmium are much more resistant. These points suggested that different mechanisms were possibly involved and further experiments were carried out to extend this view. The results in Table XXVII show the amounts of retention occurring when compounds of copper, silver, gold, lead and cobalt were heated in silica crucibles for 16 hours at 500° C in the absence of organic matter.

The results in Table XXVII support the division into two groups; the comparatively easily reducible metals copper, silver and gold and the others. Silver and gold can be reduced to the metal by heat alone at temperatures above 300° C, and this reduction would occur in the experiment described above. Copper, however, is not reduced by heat alone below 1000° C, but is readily reduced by carbon. The effect of organic matter is shown by the results on p. 153, which indicate that the retention of copper is increased by the presence of organic matter. The control experiments with cobalt on p. 154 show the opposite effect, but again support the idea of different mechanisms. In view of these results, it is suggested that, for lead, cadmium, cobalt and most elements with positive electrode potentials, the losses that occur are due to the formation of silicate glasses by reaction of the metal oxides with the silica crucibles, these glasses being more or less resistant to the action of dilute acids. The ease of reaction is governed to some extent by the polarisability of the oxide, as suggested by Fajans, and it should be noted that lead oxide, the oxide most frequently reported to be lost, has an exceptionally high polarisability. On the other hand, for copper, silver and gold, and probably for other metals of negative electrode potential, it is suggested that the loss involves reduction to the metal and diffusion into the silica of the crucible. In addition to the evidence given above, this is supported by the fact that diffusion is known to occur if metallic copper, silver or gold is heated in contact with glass at a temperature well below the melting-point, and there occurs in a paper by Forland and Weyl[62] the following passage, which is directly relevant. "There exists a general trend which increases from copper to the noble elements gold and platinum to form the metallic phase rather than a compound. Copper in ionic form is still very stable in silicate melts. Silver can be retained in ionic form in limited quantities only and is known to be in equilibrium with metallic silver in silicate glasses. With gold and platinum the equilibrium must be expected to be shifted far to the side of the metal."

TABLE XXVII

RETENTION OF METALS ON SILICA

Metal	Anion	Amount of metal retained, %
Copper	Sulphate	4, 3
Silver	Nitrate	47, 32
Gold	Chloride	17, 21
Lead	Nitrate	0, 0·5
Cobalt	Sulphate	0, 0

If these opinions are correct, it can be predicted that a transfer from silica to platinum crucibles would reduce the losses of those elements that form silicates, but should not prevent those owing to diffusion. The results on p. 140 show that the retention of lead is considerably less on platinum than on silica, but no experiments were carried out with the other group. However, duplicate determinations of the retention of silver on different types of crucibles were carried out after heating silver nitrate in the crucible for 16 hours at 515° C. The retentions on silica were 55 and 95 per cent., on nickel, 43 and 51 per cent. and on aluminium, 5 and 4 per cent.

The results with nickel and aluminium are in the order of the known solubility of silver in these metals; the analagous information for vitreous silica is not available.

(*b*) *Effects of ashing aids*—The use of ashing aids is widespread in the dry oxidation of organic matter; the results in Tables VII and XVIII show the influence of various materials on the recoveries of lead and copper and suggest that the effects can be rather complex. Several points are unexpected. It must, however, be remembered that the number of experiments is small, and it is not possible to draw firm conclusions from them. With both copper and lead, the addition of phosphoric acid caused large losses; this was most probably due to the formation of a phosphosilicate glass in which the metal ions were trapped. Phosphorus pentoxide is one of the few glass-forming oxides that is four co-ordinated and could be expected to form a stable compound. On the other hand, boric acid caused no loss; in fact, it acted as an efficient ashing aid. Boric oxide is also a glass-forming oxide, but is only three co-ordinated. This means that glasses formed from it are comparatively weak and would be susceptible to acid attack. The common ashing aids magnesium nitrate and magnesium acetate both appear to be adequate, but the behaviour of the sodium phosphates is more complex; they seem to have different effects on the recoveries of the two metals. In the experiments with copper, all three phosphates seemed to increase the rentention, but no significant differences appeared between the three forms. With lead, on the other hand, the loss of lead, relative to the loss occurring with no ashing aid, decreased, although trisodium orthophosphate was much less efficient than the other two. It is difficult to advance an explanation for this behaviour, but it seems to offer further support for the idea of two different mechanisms.

The most surprising result is that obtained after the addition of 10 mg of sodium chloride to the lead tracer. The expected behaviour was loss of lead by volatilisation (vapour pressure of $PbCl_2 = 2{\cdot}8$ mm of mercury at 600° C), not the huge loss by retention on the crucible. Singer[63] has described the effect of chloride and other univalent ions in weakening the silica structure by replacing some of the oxygen. It seems possible that some such effect had promoted sufficient weakening at the high temperatures used to increase the reaction between the silica and the lead oxide.

V. MISCELLANEOUS EXPERIMENTS

In addition to the experimental work on the main problem of the destruction of organic matter, some determinations of trace elements were carried out, to take advantage of the unique facilities available at Harwell for assisting the Metallic Impurities in Organic Matter Sub-Committee.

1. Radioactivation analyses for arsenic by the method of Smales and Pate[64] were carried out on samples of tobacco, dried milk and blood plasma circulated by the Sub-Committee. The results of these analyses are shown in Table XXVIII.

TABLE XXVIII

ARSENIC CONTENTS OF TOBACCO, DRIED MILK AND BLOOD PLASMA

Tobacco powder		Dried milk		Blood plasma	
Weight of sample, mg	Amount of arsenic found, p.p.m.	Weight of sample, mg	Amount of arsenic found, p.p.m.	Weight of sample, mg	Amount of arsenic found, p.p.m.
112	3·37, 3·44	149	0·045, 0·047	140	3·06, 3·07
145	3·28, 3·35	175	0·038, 0·040	116	2·73, 2·74
113	3·48, 3·65	1650	0·041, 0·042	66	2·66, 2·65
135	3·46, 3·64	1340	0·042, 0·043	79	2·88, 2·86
				110	2·99, 3·08
Mean,	3·46 ± 0·13	Mean,	0·042 ± 0·003	Mean,	2·87 ± 0·17

2. Determinations of the lead content of a sample of tobacco powder were carried out by square-wave polarography after destruction of the organic matter by dry and by wet oxidation. Tracer recovery experiments were also carried out on the tobacco sample with ^{212}Pb. The results were as follows—

Method of oxidation	Wet	Dry
Recovery of tracer, %	99, 99, 99	98, 95, 94
Lead found, p.p.m.	9·9	9·8

TABLE XXIX

MISCELLANEOUS DETERMINATIONS BY DIRECT GAMMA-RAY SPECTROMETRY

Organic material	Source or type	Trace element	Concentration of trace element, p.p.m.
Tobacco I	Pipe	Bromine	137
Tobacco II			47
Tobacco III			234
Tobacco IV	Cigarette		162
Tobacco V			241
Tobacco VI			230
Tobacco VII			129
Tobacco VIII			128
Tobacco IX			136
Hair I	Male	Gold	0·13
Hair II			0·08
Hair III			0·06
Hair IV			0·04
Hair V	Female		0·19
Hair VI			0·06
Tobacco		Manganese	100
Bone meal		Manganese	35

3. In the course of the search for suitable materials for the determination of trace elements by direct gamma-ray spectrometry after neutron irradiation, some unchecked determinations were carried out. These included bromine in nine brands of smoking tobacco, gold in six samples of human hair and manganese in tobacco and bone meal. The results are shown in Table XXIX.

VI. ASSESSMENT OF OXIDATION METHODS

In the course of the work described in this Report, eight oxidation methods were extensively applied to the destruction of organic matter as a preliminary to the recovery of a wide variety of trace elements. From the results, it is possible to put forward some opinions as to their relative merits.

Of the eight oxidation methods, three involved wet digestion, four were dry ashing procedures and one was hybrid.

The relative merits of wet and dry oxidations have been exhaustively discussed. In favour of the former are cited the low temperatures involved, the maintenance of liquid conditions, which reduce the chances of retention losses, the simple apparatus required and the speed of oxidation. On the debit side may be laid the large amounts of reagents added, with the consequent risk of increased blank values, the difficulty of handling large samples and the greater amount of operator's time required.

The advantages of dry-ashing methods are the lack of added reagents, the simplicity of operation and the ease with which large samples can be handled. Against this must be balanced the lack of knowledge of the interaction between sample constituents, the trace elements and the material of the receptacle, with the consequent risk of loss by volatilisation or retention; also the high temperatures required for such reactions, the need for relatively expensive equipment and the length of time required to complete the oxidation. This last point is to some extent offset by the convenience of an overnight ashing period.

As far as the results presented here are concerned, the use of radioactive tracers solved the problem of reagent contamination, and no weight has been given to freedom from such accretions.

1. WET OXIDATIONS—

The three oxidation mixtures investigated were nitric and perchloric acids; nitric, perchloric and sulphuric acids; and nitric and sulphuric acids.

Although comparatively few experiments were carried out with it, it seems probable that the best procedure for wet oxidations involves the use of the apparatus shown in Fig. 7; the operation of the apparatus is described on pp. 146 and 147.

By this means, oxidation can be accomplished with the minimum amount of reagents, thereby reducing the blank error, and, by control of the rate of increase of the oxidation potential, conditions can be varied to suit the material being destroyed. This could be of particular importance with oxidation mixtures containing nitric and perchloric acids, for which care over the rate of increase would avoid the risk of explosion.

When this apparatus was not used, the most suitable vessels for wet oxidations were found to be 500-ml conical flasks, in which the comparatively long sides and restricted openings gave some measure of refluxing. With all three oxidation mixtures, the vital point is that the temperature should be increased slowly. Most of the oxidation is carried out by the nitric acid, and it is pointless to place the flask on a very hot hot-plate so that the nitric acid rapidly distils away. When mixtures containing perchloric acid are used, this is also undesirable, as the material that enters into explosive combination with perchloric acid is also that most readily destroyed by nitric acid.

(*a*) *Nitric and perchloric acids*—In my experience, the use of this mixture has proved to be excellent and trouble-free; only with mercury were losses significant. This method is particularly suitable for the recovery of trace elements, such as lead, that form insoluble sulphates; this advantage is greatly increased if the material being oxidised contains much calcium.

(*b*) *Nitric, perchloric and sulphuric acids*—Again, this is an excellent oxidising system, particularly valuable in the oxidation of obdurate materials. A disadvantage is the presence of sulphuric acid during the recovery of elements such as lead. The variable recoveries of lead from cocoa by this mixture are shown in Table I; in Table II are shown the large losses that can occur when the sample contains a high percentage of calcium.

(*c*) *Nitric and sulphuric acids*—This mixture was usually satisfactory, although its action is slower than that of mixtures containing perchloric acid and care is required to prevent charring. The recoveries of selenium after the use of this mixture were generally low (see Tables XIII, XIV and XV), and its use for this purpose should be avoided. The presence of sulphuric acid imposes the limitations described above for the use of nitric, perchloric and sulphuric acids.

2. MIDDLETON AND STUCKEY'S METHOD—

This method gave reasonable results for most of the trace elements, although the recoveries obtained were often a little lower than those by the wet-oxidation methods. Selenium and mercury were lost completely, and in use it proved somewhat tedious and time-consuming.

3. DRY OXIDATIONS—

Dry-ashing methods are widely used and are hotly supported by their protagonists. The main outcome of the results in this Report is that it is unwise to generalise at all on this subject.

One thing, however, is clear; it is essential to maintain the temperature both of the furnace and of the sample at the lowest level consistent with completion of the oxidation in a reasonable time. Temperatures have been quoted up to 800° C and more, but the general opinion seems to favour 500° to 550° C. Another important point demonstrated by the results is that the other constituents of the sample, as well as the nature of the trace element being determined, can greatly influence the recoveries. The effects vary so widely from sample to sample and from element to element that it is unwise to try to draw general conclusions.

(*a*) *Dry ashing alone*—This is the most widely used single oxidation method and usually appears to be satisfactory provided the temperature is not too high. In the oxidations with cocoa as the organic material at a temperature of approximately 550° C, the recoveries of zinc, cobalt, antimony, chromium, molybdenum, strontium and iron were satisfactory; the recoveries of arsenic, copper, silver and cadmium were low at this temperature. Recoveries of lead were complete at 450° C and very low at 650° C; 550° C appeared to be marginal.

This method of destruction of organic matter is satisfactory only when experience has shown it to be so; its application to an unknown sample is inadvisable without first discovering its suitability.

(*b*) *Dry ashing with nitric acid as ashing aid*—The use of nitric acid seldom greatly affected the recoveries, although it was helpful in producing a clean ash readily soluble in dilute acid. With cadmium, it caused large volatilisation losses.

Nitric acid should not be added until the material is well ashed. If much carbon is still present, deflagration may well occur and can cause serious loss.

(*c*) *Dry ashing with sulphuric acid as ashing aid*—The beneficial effect of sulphuric acid is demonstrated by the high recoveries of lead even after ashing at 650° C. This was probably due to its conversion to the non-volatile and comparatively inert sulphate. One disadvantage of the use of sulphuric acid is that it slows down the rate of oxidation and increases the time required.

(*d*) *Dry ashing with magnesium nitrate or acetate as ashing aid*—The use of these ashing aids appeared to improve the recovery of some elements, but, unless the ash content of the sample is low, their use is probably unnecessary. The presence of magnesium nitrate caused a large loss of cadmium by volatilisation, similar to that occurring when nitric acid was used. As did sulphuric acid, magnesium acetate retarded the rate of oxidation.

(*e*) *Apparatus*—Little comparative work was carried out on the effects of different types of reaction vessel, but the results on pp. 140, 153 and 154 show that this is an important source of variation. Platinum is probably best, but vitreous silica ware, properly handled, will give similar results. No experiments were carried out with porcelain.

4. CONCLUSIONS—

It would indeed be pleasant, if, as a conclusion to this Report, it were possible to formulate a set of concise statements giving comprehensive instructions for the oxidation of any conceivable combination of trace element and organic sample. Such is, however, far from being possible, and the only confident generalisation that can be made is that wet oxidation of organic material with nitric and perchloric acids has proved most satisfactory for the recovery of all the trace elements investigated, with the single exception of mercury. As both these reagents can be purified by distillation, the problem of blank values should not be serious, although a study of this aspect of the problem was never part of the programme.

For dry oxidation, no such clear-cut recommendation is possible. Its occasional obvious advantages will no doubt ensure its continued use when it has been proved satisfactory, but it is hoped that the results in this Report will have served to show some of the difficulties that may arise and to sound a warning in certain specific instances.

It is further hoped that this demonstration of the application of radiochemistry to such analytical problems will encourage more people to use this relatively recent and very powerful tool.

It is with pleasure that I record my debt to those who made this work possible: to the Analytical Methods Committee of the Society for Analytical Chemistry, who initiated and supported the project; to the United Kingdom Atomic Energy Authority and the Director of the Atomic Energy Research Establishment, Harwell, for making available the specialist facilities required; and, particularly, to Mr. A. A. Smales, Head of the Analytical Chemistry Group at Harwell, under whose guidance the work was carried out.

APPENDIX I

1. APPARATUS AND OXIDATION METHODS—

For all the elements discussed in Section III, survey experiments were first carried out with cocoa as the organic material. In these experiments, eight oxidation systems were compared and the proportions of the trace elements lost, by volatilisation or retention, were determined. The eight systems were:

(*a*) Nitric and perchloric acids.
(*b*) Nitric, perchloric and sulphuric acids.
(*c*) Nitric and sulphuric acids.
(*d*) The method of Middleton and Stuckey.[2]
(*e*) Dry oxidation.
(*f*) Dry oxidation with nitric acid as ashing aid.
(*g*) Dry oxidation with sulphuric acid as ashing aid.
(*h*) Dry oxidation with magnesium nitrate as ashing aid.

2. WET OXIDATIONS—

(*a*) *Apparatus*—The apparatus consists of a 500-ml conical flask fitted with a splash head and adaptor; the end of the adaptor dips into a 150-ml beaker. This system is not completely closed, as it was necessary to be able to move the flasks rapidly during the oxidation, and a rigid construction was not considered advisable. In each experiment 2 g of cocoa were weighed into the flask and the appropriate mixture of acids was added. The three mixtures used are shown in Table XXX.

TABLE XXX

ACID MIXTURES USED IN WET-OXIDATION EXPERIMENTS

Acid mixture	Amount of nitric acid, sp.gr. 1·42, ml	Amount of sulphuric acid, sp.gr. 1·84, ml	Amount of perchloric acid, sp.gr. 1·54, ml
Nitric and perchloric	15	—	10
Nitric, perchloric and sulphuric . .	15	5	10
Nitric and sulphuric	15	10	—

(*b*) *Oxidations with nitric - perchloric and nitric - perchloric - sulphuric acid mixtures*—The flask containing the cocoa, tracer and oxidising mixture was placed on a cold hot-plate, which was then switched on to medium heat. Copious brown fumes were evolved and the cocoa dissolved. The splash head served to some extent as a condenser and some refluxing took place. As heating continued, liquid distilled over into the beaker and the volume in the flask decreased until a vigorous, but not violent, exothermic reaction started. At this stage, the flask was removed from the hot-plate until the reaction subsided and then returned for a further few minutes, after which it was again removed. When it was cool, 5 ml of fuming nitric acid were added, and the mixture was heated steadily until fumes were evolved. After it had been diluted with water and boiled for a few minutes, the solution was adjusted to volume.

(*c*) *Oxidations with nitric - sulphuric acid mixture*—The technique was similar to that used for the mixtures containing perchloric acid, except that no vigorous reaction occurred in the later stages of the oxidation, and it was not necessary to remove the flask from the hot-plate before heating to fumes. The addition of fuming nitric acid was the same.

(*d*) *Oxidations by Middleton and Stuckey's method*—The cocoa and tracer were introduced into a 1-litre tall beaker, together with 10 ml of distilled water and 10 ml of nitric acid containing 0·5 per cent. of sulphuric acid. The mixture was heated to dryness on a hot-plate and the residue moistened with concentrated nitric acid. After covering the beaker with a clock-glass, it was again heated to dryness. This was repeated several times, first with concentrated and then with fuming nitric acid, until a white ash was obtained. The ash was dissolved by heating with 20 ml of dilute sulphuric acid, and then adjusted to volume.

In the three genuine wet-oxidation methods, the liquid that had distilled into the 150-ml beakers was also made up to a known volume.

3. DRY OXIDATIONS—

(*a*) *Apparatus*—Most of the dry oxidations were carried out in an apparatus consisting of a 500-ml silica flask enclosed in an asbestos box and connected to a train of traps. Leading into the flask was a length of silica tubing that reached just into the bulb; from the flask, another piece of silica tubing connected with the first component of the train, T_1, an empty tube containing a plug of glass-wool, which served to collect any liquid distilling from the flask. T_2 and T_3, the other two vessels in the train, contained, respectively, dilute sulphuric acid and dilute sodium hydroxide and were intended to retain gaseous decomposition products. The inlet tubes were surrounded by glass-wool to break up the gas bubbles and to increase the time of contact. The use of sintered-glass discs had to be adandoned as they were rapidly clogged by decomposition products carried in the gas stream. A flow of air was maintained through the apparatus by means of a water pump.

(*b*) *Oxidation methods*—Two grams of cocoa were weighed into the flask and the tracer was added as 0·1 ml or 0·25 of solution. For simple dry ashing, the train of traps was then connected and heat applied by two bunsen burners, gently at first. Heating was continued until the ash in the bottom of the flask was white.

When nitric acid was used as the ashing aid, the preparations were the same, but heating was continued only until the cocoa was completely charred and reduced to small bulk. The flask was then allowed to cool, and a few drops of nitric acid were added through the inlet tube. Heating was then resumed.

For the experiments with sulphuric acid and magnesium nitrate, the ashing aids were added at the beginning, and gentle heating was maintained until the contents of the flasks had dried. Full heating was then applied until a suitable ash was obtained.

The amounts of ashing aid added were 5 ml of 5 *N* sulphuric acid or 10 ml of a 7 per cent. solution of magnesium nitrate, $Mg(NO_3)_2.6H_2O$.

When the oxidation was complete, a considerable amount of tar and soot remained on the upper parts of the silica flask. This was removed by direct local heating of the outside of the flask with a bunsen flame, causing the carbonaceous material to oxidise. Care was exercised to prevent the residue in the bottom of the flask from being directly heated. After this oxidation procedure has been completed, the ash was dissolved by heating with two 10-ml portions of diluted hydrochloric acid (1 + 1) and made up to a suitable volume for counting.

The contents of T_1 were treated with a few drops of concentrated sulphuric acid and made up to a known volume with acetone. The glass-wool plug, after it had been washed with acetone, was squeezed as dry as possible and transferred to a 150-ml beaker. The contents of T_2 and T_3 were made up to known volumes, and the glass-wool plugs, after having been squeezed free from excess of liquid, were transferred to the 150-ml beaker containing the plug from T_1. The glass-wool was then pressed down to form a tight uniform layer.

Although it was not possible, with the apparatus described, to obtain close control of the temperature, experiments were carried out to determine the temperature reached at the bottom of the flask. A number of pure chemicals of known temperature characteristics were heated, separately, in the flask under the standard conditions, and their behaviour was observed. A list of the chemicals used and the results obtained is shown in Table XXXI.

TABLE XXXI

EFFECT OF HEATING VARIOUS MATERIALS IN THE OXIDATION APPARATUS

Compound	Temperature (T), °C	Alteration occurring at temperature T	Behaviour of compound during test
Lead nitrate	470	Decomposes	Decomposed
Ammonium bromide	542	Sublimes	Sublimed
Calcium nitrate	561	Melts	Possibly slight melting
Barium nitrate	592	Melts	No change

The results indicate that the temperature attained is between 550° and 560° C.

APPENDIX II

NUCLEAR DATA

With the exception of the ^{212}Pb tracer, which was separated from thorium, the tracers used in this investigation were prepared by irradiation of the required element or its oxide or carbonate in BEPO at a flux of approximately 10^{12} neutrons per sq.-cm per second for periods of up to 4 weeks. The elements used and the relevant nuclear data are shown in Table XXXII.

TABLE XXXII

NUCLEAR DATA FOR ELEMENTS USED IN TRACER EXPERIMENTS

Element	Parent nuclide	Abundance, %	Cross-section, barns	Daughter nuclide	Half-life	Sensitivity, disintegrations per minute per μg
Copper	^{63}Cu	69·0	4·4	^{64}Cu	12·8 hours	860,000
Silver	^{109}Ag	48·6	2·0	^{110}Ag	270 days	14,500
Zinc	^{64}Zn	48·9	0·5	^{65}Zn	245 days	65,700
Cadmium	^{114}Cd	28·8	1·1	^{115}Cd	54 hours	50,400
Mercury	^{196}Hg	0·15	2500·0	^{197}Hg	65 hours	337,000
Iron	^{58}Fe	0·33	0·9	^{59}Fe	45 days	520
Cobalt	^{59}Co	100·0	37·0	^{60}Co	5·2 years	143,000
Chromium	^{50}Cr	4·4	16·0	^{51}Cr	27 days	216,000
Molybdenum	^{98}Mo	23·8	0·13	^{99}Mo	67 hours	5760*
Arsenic	^{75}As	100·0	4·3	^{76}As	26·7 hours	1,030,000
Antimony	^{121}Sb	57·0	7·0	^{122}Sb	2·8 days	590,000
	^{123}Sb	43·0	3·4	^{124}Sb	60 days	17,000†
Selenium	^{74}Se	0·93	40·0	^{75}Se	127 days	16,000
Strontium	^{84}Sr	0·55	1·0	^{85}Sr	65 days	420

* Activity due to nuclide alone. † Activity induced after irradiation for 2·8 days.

The sensitivity figure quoted is the activity induced after irradiation at 10^{12} neutrons per sq. cm per second for a period equal to 24 days or one half-life, whichever is the shorter. ^{123}Sb is an exception, the figure quoted being for an irradiation of 2·8 days; this gives the contribution of the longer lived activity after irradiation for one half-life of ^{122}Sb.

The activity quoted for ^{99}Mo is that due to this nuclide alone; it decays by beta-emission to ^{99m}Tc, which is also radioactive, and, when equilibrium has been established, each nuclide will undergo the same number of disintegrations per second, thereby roughly doubling the sensitivity.

Data are not reported for the irradiation of ^{88}Sr, the most abundant nuclide, because the daughter, ^{89}Sr, is a pure beta-emitter and was not counted by the gamma counter used in these experiments.

APPENDIX III

COUNTING METHODS, ERRORS AND STATISTICS

In almost all instances, the activity determinations were carried out by counting gamma rays, a thallium-activated sodium iodide crystal being used as the detector. The advantage gained by counting gamma rays rather than beta particles is to be found in the high penetrating power of the former. This permits counts to be obtained from active material retained on the insides of reaction vessels or dispersed on other solid matter, circumstances that would greatly reduce the efficiency of beta-particle counting; it also permits material in solution to be counted effectively without being too dependent on the energy of the emitted particle. The main disadvantage lies in the high background of the gamma counter compared with the normal Geiger - Muller tube used for beta-particle counting.

1. COUNTING LIQUIDS—

All liquids were counted by comparing the activity of the unknown solution, either the whole volume or an aliquot, with the activity of the same volume of a known standard solution under conditions of identical geometry.

2. COUNTING SOLIDS—

This includes the determination of the activity retained in the silica flask and on the plugs of glass-wool in the survey experiments, and the activities retained on the silica crucibles used in the experiments with lead, copper and cobalt. For this purpose, the object or container was placed on top of the detector, and the activity above the normal background was determined. A small volume of standard solution was then added and the activity re-determined. If C_1 is the count of the object itself, C_2 the count after the addition of the standard, v the volume of standard added and V the volume of standard containing the amount of activity originally used in the experiment, the percentage activity on the solid material is—

$$\frac{C_1 \times v \times 100}{(C_2 - C_1) \times V}$$

3. ACCURACY OF COUNTING AND POSSIBLE ERRORS—

(*a*) Because of the statistical nature of radioactive decay, all activity measurements are subject to certain random fluctuations. On any one count, the relative uncertainty falls as the number of observed disintegrations rises, because the standard deviation is equal to the square root of the total count. Thus, on 10,000 counts, the standard deviation is ± 100 counts, or a coefficient of variation of ± 1 per cent. Most counts were made to approximately this accuracy, and under these conditions there is a 99 per cent. chance of the observed value being within ± 3 per cent. of the true value. This is true of the total count of the sample *plus* the background; provided the former is large compared with the latter, it is approximately true of the net count. When, however, the activity is low, *i.e.*, of the same order as the background, the position is considerably worsened. With a sample count of 300 counts per minute, a background count of 300 counts per minute and total counts of 12,000, then—

$$\text{Background} = \frac{12{,}000 \pm \sqrt{12{,}000}}{40} = 300 \pm 2\tfrac{3}{4} \text{ counts per minute,}$$

$$\text{Sample } \textit{plus} \text{ background} = \frac{12{,}000 \pm \sqrt{12{,}000}}{20} = 600 \pm 5\tfrac{1}{2} \text{ counts per minute,}$$

which gives a coefficient of variation of approximately 1 per cent. for each. However, the net count of the sample alone is—

$$\begin{aligned} &300 \pm \sqrt{2\tfrac{3}{4}^2 + 5\tfrac{1}{2}^2} \\ = \ &300 \pm 6{\cdot}2 \end{aligned}$$

a coefficient of variation of approximately ± 2 per cent.

If the sample count were as low as one-tenth of the background count, the coefficient of variation would be approximately ± 12 per cent.

(*b*) In addition to these inherent statistical variations, there is a further important source of error due to geometrical considerations. The number of counts recorded when a source is near a detector is determined by the number of gamma rays that strike the detector, and this will depend upon the relative positions of the source and the detector. Variations in this relationship will lead to variations in the count. When solutions are being counted, no appreciable error is introduced, as the geometry can be standardised for both sample and standard by the use of special counting cups; with solid objects it becomes more serious. The addition of known volumes of standard solution to the reaction vessels or the glass-wool plugs, as described under "Counting Solids," above, was an attempt to overcome this difficulty, but it was by no means completely effective. Fig. 8 shows how the apparent activity of the standard in counts per millilitre varies with the volume of standard added.

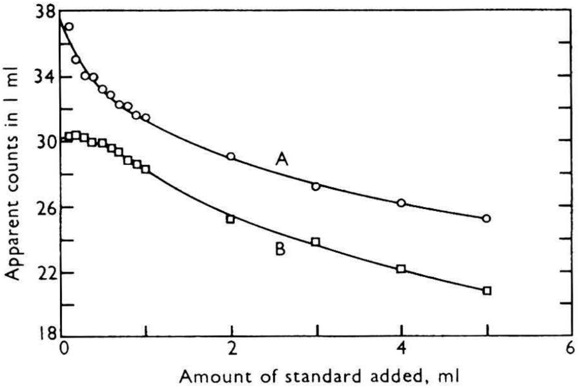


Fig. 8. Variation of counting efficiency with volume of standard added: curve A, in silica flask; curve B, in silica crucible

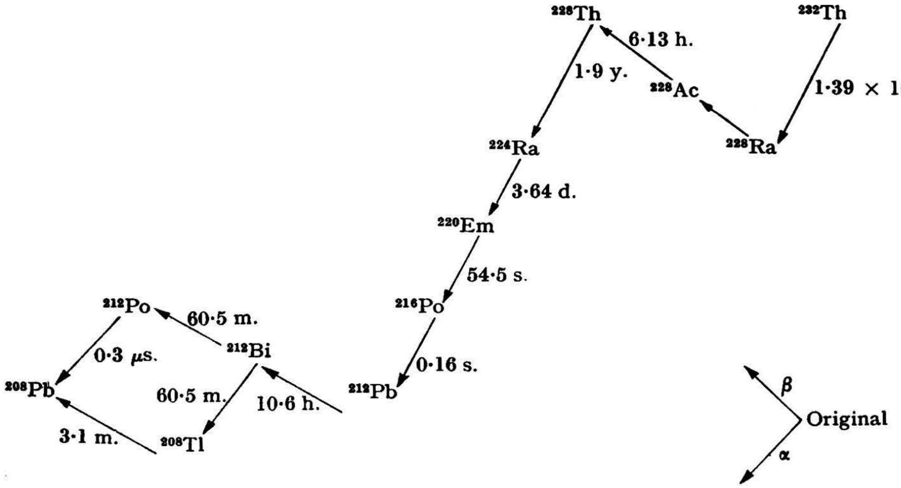
(*c*) A further difficulty can arise when the time required to count all the fractions from an experiment is an appreciable fraction of the half-life of the tracer. This will mean that in the time elapsing between counting the standards and counting the various fractions, sufficient decay could occur to cause significant errors. This situation occurred in the experiments with ^{64}Cu and ^{212}Pb and was overcome by counting the standards at intervals through the experiment and constructing a plot of standard count against time. From the straight line obtained by the use of semi-log paper, the standard count at any time can be obtained and used for the experimental counts.

(*d*) One final complication that arose during these experiments was the growth of a radioactive daughter from the nuclide used as a tracer. This occurred with ^{99}Mo, which gave rise to ^{99m}Tc, and with ^{212}Pb, which gave rise to a chain of decay products, the most important of which was ^{212}Bi. After a period of time equal to several half-lives of the daughter, a position of equilibrium is reached and the total activity of parent *plus* daughter decays with a constant half-life. With both ^{99}Mo and ^{212}Pb this situation obtained in the reference standard solutions, but with the experimental solutions there was the possibility that the two elements would behave differently under the reaction conditions, and this would lead to the state of equilibrium being disturbed. In these circumstances, the experimental fractions and the reference standards would not be comparable and the results would be inaccurate. To make allowance for this situation, all counting in these experiments was delayed for a period of approximately four half-lives of the daughter nuclide to allow the equilibrium to be largely re-established. At the end of this period, the parent had re-grown the daughter to about 95 per cent. of its equilibrium value, and any of the daughters that had become separated from the parent would have decayed to about 3 per cent. of the original total count. This would be the position after a complete initial separation of the elements, and any lesser degree of separation would give even more favourable results.

APPENDIX IV

SEPARATION OF LEAD FROM THORIUM

The occurrence of the active isotope of lead, ^{212}Pb, in thorium is the result of the long chain of nuclear decay of thorium, as follows—



At equilibrium, the amounts of the elements present are in inverse ratio to their half-lives. As the half-life of ^{232}Th is 1·39 × 10^{10} years and that of ^{212}Pb is 10·6 hours, 1 gram-molecule or 232 g of thorium will contain—

$$\frac{6{\cdot}02 \times 10^{23} \times 10{\cdot}6}{1{\cdot}39 \times 10^{10} \times 365 \times 24}$$
$$= 5{\cdot}2 \times 10^{10} \text{ atoms of } {}^{212}\text{Pb}.$$

Therefore 1 g of thorium contains—

$$\frac{5{\cdot}2 \times 10^{10} \times 212}{6{\cdot}02 \times 10^{23} \times 232} \text{ g of } {}^{212}\text{Pb}$$
$$= 7{\cdot}75 \times 10^{-14} \text{ g.}$$

Two methods were used to separate the lead, one by solvent extraction and the other by ion exchange based on the work of Kraus and Nelson.[65]

1. EXTRACTION WITH SODIUM DIETHYLDITHIOCARBAMATE—

Fifty grams of thorium metal were dissolved carefully in dilute nitric acid and the solution was diluted to 250 ml. A 50-ml portion of this solution diluted with 50 ml of distilled water was used for each experiment. The pH of the thorium nitrate solution was adjusted to 3·0 to 3·2 by the addition of ammonia solution. Thorium hydroxide was precipitated on the addition of ammonia, but could be re-dissolved by shaking and heating. To the solution at pH 3·0 to 3·2 were added 50 ml of a 0·25 per cent. solution of sodium diethyldithiocarbamate and then two 50-ml portions of *iso*amyl acetate. When shaken, the lead and bismuth complexes were extracted into the ester layer, which was then separated and washed twice with small volumes of water of pH 3·4. The *iso*amyl acetate was removed by evaporation, and the residue was dissolved in 40 ml of 2 *N* hydrochloric acid containing 0·08 per cent. of copper. Hydrogen sulphide was passed through the solution, which was then boiled, re-saturated with hydrogen sulphide and filtered after the addition of paper pulp. The filtrate contained the lead originally present in the thorium, but the bismuth was retained on the filter.

2. SEPARATION OF LEAD BY ION EXCHANGE—

In a paper read by Kraus and Nelson[65] at Geneva there appeared a figure summarising the behaviour of all the elements of the periodic table up to uranium when passed in hydrochloric acid solution through a column of strongly basic anion-exchange resin.

From this figure it appeared that, of the thorium decay products, lead, bismuth, thallium and polonium could be absorbed from 2 *N* hydrochloric acid, but that only lead would be eluted by 8 *N* hydrochloric acid. This offered promise of a rapid separation of lead, and the technique described below was evolved.

A sample of −100 to +200-mesh De-Acidite FF quaternary ammonium resin of nominal 8 per cent. divinylbenzene content was prepared by repeated washing to remove fines and packed into a column as a resin bed 15 cm × 1·5 cm. It was then converted to the chloride form by washing with several column volumes of 2 *N* hydrochloric acid.

Fifty grams of thorium were dissolved in hydrochloric acid and made up to 250 ml of solution 2 *N* in hydrochloric acid.

A 50-ml portion of the thorium solution was passed through the column and washed with 30 ml of 2 *N* hydrochloric acid. The lead was then eluted with 50 ml of 8 *N* hydrochloric acid.

The column was cleaned by washing with 50 ml of *N* sulphuric acid and re-equilibrated with 2 *N* hydrochloric acid.

3. PURITY OF THE SEPARATED TRACER—

When separated, the tracer should consist solely of lead, but with the passage of time the active daughters ^{212}Pb, ^{212}Po and ^{208}Tl should grow in. The purity of the product can be shown by plotting a decay curve of the separated solutions. The count rate should begin at a fairly low level, rise to a maximum after approximately 3 hours and then fall steadily with a slope of 10·6 hours achieved after about 7 hours. This was done for the solutions separated by the two methods, and the graphs are shown in Fig. 9.

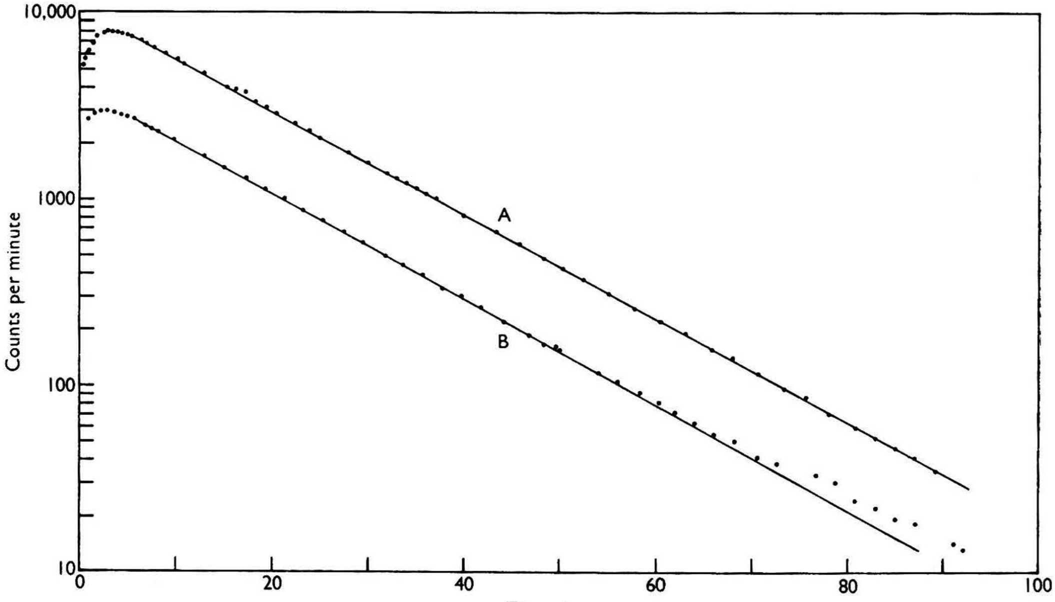


Fig. 9. Radiochemical purity of ^{212}Pb separated from thorium: curve A, ion-exchange product (half-life 10·8 hours); curve B, solvent-extraction product (half-life 10·6 hours)

It can be seen that both methods yield solutions of reasonable purity, but that the ion-exchange product is superior. After the ion-exchange separation, there is still no deviation from the straight line after 80 hours, whereas with the extraction method, deviations are apparent from about 63 hours onwards. This indicates the presence of some longer-lived impurity at a concentration such that it contributes less than 1 per cent. of the original activity.

The column method scores heavily also in terms of speed and convenience; it requires little attention. It has the further advantage that the solution passing straight through the column contains all the thorium and radium, and after standing for 2 days will have re-grown about 95 per cent. of the equilibrium concentration of ^{212}Pb. This means that the same solution can be repeatedly used without any re-processing.

4. PREPARATION OF TRACER SOLUTIONS FOR USE—

The products of both separation schemes were solutions of lead in hydrochloric acid. These solutions were not suitable for direct use as tracers, both on account of volume and of acid concentration, and evaporation to dryness was first carried out. Before the evaporation, sufficient inactive lead was added to bring the final concentration to the required level. After evaporation, the residue was dissolved directly in water containing a few drops of hydrochloric acid to give lead in the chloride form, or evaporated again with a little concentrated nitric acid and dissolved in water containing a few drops of nitric acid to give lead in the nitrate form. The solutions were diluted to an appropriate volume and were then ready for use.

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Radiochemical Tracer Analysis: A New Approach Resulting in Increased Accuracy

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It is shown that the errors inherent in radioactive tracer analysis may be reduced by measuring the unseparated rather than the separated fraction of the radioactivity.

In the traditional tracer method introduced by Hevesy, radioactive isotopes are used to determine the proportion of an element recovered after the analytical separations, the actual amount being determined by standard quantitative procedures. For example, Hevesy and Hobbie[1] in the determination of trace amounts of lead in rocks added a "weightless" amount of radium D, a radioactive isotope of lead, to the samples, and, after separating and weighing the lead as peroxide, measured its associated radioactivity, which was compared with the amount initially added. As isotopes are chemically inseparable, provided that they are in the same chemical form, this gave a measure of the recovery and permitted a correction to be made for losses during the analysis.

The method has since been applied to the determination of other elements, especially since the artificial production of radioactive isotopes has provided tracers for most naturally occurring elements.

The method is potentially of value when recovery of the element being determined is unlikely to be quantitative, but it is possible only when a suitable radioactive isotope is available in a state of radiochemical purity.

However, a weakness of the tracer method in its traditional form is the inherent lack of precision caused by unavoidable errors in radioactivity measurements. For example, owing to the random nature of radioactive decay, all measurements are subject to the possibility of a statistical error proportional to the square root of the number of disintegrations counted in the measurement (the standard deviation). In addition, failure to secure uniform counting "geometry" causes other errors in the usual comparative measurements. Cabell and Smales,[2] experienced workers in this field, give ± 2 to 5 per cent. as the range of errors. In consequence, tracer methods are subject to errors of up to at least ±5 per cent. This compares most unfavourably with conventional methods of analysis, which at best may have errors as low as 1 part in 1000.[3]

As a result, the use of tracer methods in inorganic analysis has been virtually restricted to the determination of trace constituents, for which, in the absence of more accurate methods, the imprecision is tolerated.

This paper describes a procedure by which this defect of tracer methods may be overcome, so opening the way to the use of such methods in the determination of major constituents without sacrifice of accuracy. It is not implied that tracer methods can be of universal utility; there are numerous instances, however, when quantitative separation of an element is difficult or tedious, but, when separation of the bulk of the element as a pure stoicheiometric compound is relatively simple, radioactive tracers make possible the use of such simplified methods for quantitative analysis by providing a means of determining the proportion of element recovered. I hope to describe applications of this technique in a series of papers, the first being to the determination of cobalt.

DESCRIPTION OF THE METHOD

As already stated, in the traditional tracer method the radioactive isotope recovered in the weighed precipitate is measured. Any error in this measurement will give an equal error in the final result. In the proposed method, which might well be called the "residue" method to distinguish it from the tracer or dilution method, the radioactivity is determined not in the weighed precipitate, but in the filtrate or combined filtrates from the precipitate.

If the unprecipitated or residual amount of the element and therefore the radioactivity is a small proportion of the whole, although it can be determined with no greater precision, the error in the final results will be much less than the error of measurement. This is most readily made clear by an example.

Let us assume that we are to determine a hypothetical element, X, which can be separated pure (this is essential) but not quite quantitatively and can be weighed in its elementary form. Both methods of analysis are initially the same. To the solution containing X, a measured volume of a solution of radio-X (the weight of which is negligible) is added, and, at the same time, an equal volume of the radio-X solution is set aside for subsequent use as a standard. The solution is treated as necessary to ensure isotopic equilibrium. Pure X, which will also contain an amount of radio-X depending on the proportion of X recovered, is separated and weighed. At this point, the methods diverge; in the traditional method, the radioactivity of the weighed precipitate is measured, but, in the proposed method, that remaining in solution is measured, comparison being made with the standard in both methods. The traditional method gives a measure of the element precipitated, which, of course, approaches 100 per cent., the other gives a measure of the element not precipitated, which approaches zero. If the error in radioactivity measurement is y per cent., the error in the first instance will be y per cent., but, in the second instance, the error will be y per cent. of the small percentage of the element in the filtrates.

Suppose that our solution contains 0·5000 g of X, that radio-X equivalent to 10,000 counts per minute is added and also that 0·4950 g of X is recovered and weighed. Radio-X will be equivalent to 9900 counts per minute in the precipitate and 100 counts per minute in the filtrate. Suppose now that our measurements of these amounts of radioactivity are in error, so that, instead of 10,000 counts per minute being recorded for the standard, the counting rate is 1 per cent. high and 10,100 counts per minute are recorded, and the count on the precipitate is 1 per cent. low, so that only 9801 counts per minute are recorded.

The traditional method will therefore indicate the presence of $\frac{0{\cdot}4950 \times 10{,}100}{9801} = 0{\cdot}5101$ g of X in the sample; this result is in error by + 0·0101 g (2 per cent.).

In the "residue" method, the precipitate again weighs 0·4950 g, the counting rate on the standard is 1 per cent. high, *i.e.*, 10,100 counts per minute, and the count rate on the filtrate is 1 per cent. low, *i.e.*, 99 counts per minute are recorded instead of 100. The result is obtained by adding to the main fraction the weight of X in the filtrate, which is calculated as follows.

If 10,100 counts per minute are equivalent to the total weight of X initially in solution and 99 counts per minute are equivalent to the total weight of X in the filtrate, 10,100 — 99 counts per minute are equivalent to 0·4950 g of X. Ninety-nine counts per minute are equivalent to $\frac{0{\cdot}4950 \times 99}{10{,}100 - 99} = 0{\cdot}0049$ g of X.

The total amount of X in the sample is therefore 0·4950 + 0·0049 g, *i.e.*, 0·4999 g; the error is thus −0·0001 g (0·02 per cent.).

As it would in practice be tedious and uneconomic to count long enough to attain a precision of ± 1 per cent. at a rate as low as 100 counts per minute, let us therefore assume an error of 5 per cent. in measuring the amount of radioactivity in the filtrate, *i.e.*, 95 counts per minute are recorded instead of 100. The corresponding weight of X in the filtrate is $\frac{0{\cdot}4950 \times 95}{10{,}005}$ g, *i.e.*, 0·0047 g, and the total weight of X is therefore 0·4950 + 0·0047 = 0·4997 g; the error is − 0·0003 g (0·06 per cent.). This is the error in the correction for losses during the chemical separations, and takes no account of errors in the final gravimetric determination.

EXPERIMENTAL

Although the principle of the method requires no experimental verification, it is thought that some results of a practical application will add weight to the argument. That chosen is the determination of cobalt in mixtures of nickel and cobalt by precipitation of ammonium cobalt phosphate and ignition of this to cobalt pyrophosphate. This method is not new; it was published by Dirvell[4] in 1879, and, with various modifications, by a number of other workers since. It is not now included in standard works on inorganic analysis, as conditions that favour complete precipitation of cobalt nearly always result in contamination of the precipitate with nickel and conditions that favour complete separation from nickel lead to loss of cobalt, which must be recovered from the filtrate.

Nevertheless, it is a simple method, which was favoured by the late W. R. Schoeller, and is an ideal subject for the proposed tracer method. It must be emphasised that this paper is not intended as a contribution to the analytical chemistry of cobalt; examination of the phosphate method will not be rigorous at this stage. For the present, the reader is asked to accept that the procedure used produces pure nickel-free cobalt pyrophosphate (precipitates were, in fact, tested for freedom from nickel).

Solutions of cobalt and nickel sulphates and a cobalt-60 tracer solution were prepared and standardised. Aliquots of the cobalt and nickel solutions were placed in 250-ml beakers, and a 10-ml portion of tracer solution was added, by pipette, to each (no special treatment was needed to establish isotopic equilibrium). At the same time, a further 10-ml portion of tracer solution was set aside as a standard in a 25-ml calibrated flask, to which approximately 10 ml of the cobalt solution (as carrier for the radiocobalt) and 2 ml of diluted hydrochloric acid (1 + 1) were added. The mixed solutions were treated successively with 5 ml of diluted hydrochloric acid (1 + 1), 10 ml of 20 per cent. ammonium chloride solution and amounts of 10 per cent. ammonium dihydrogen orthophosphate solution equivalent to five times the combined weights of nickel and cobalt. Each solution was diluted to between 50 and 60 ml with water and heated to between 70° and 80° C, and 9 N ammonium hydroxide was slowly added, with continuous stirring, until the blue flocculent precipitate initially produced re-dissolved to give a dark blue solution (approximately 15 ml of 9 N ammonium hydroxide were required). This solution was stirred and boiled gently for a few minutes until the cobalt separated as a purple crystalline precipitate, which was allowed to settle for about 1 minute. The solution was filtered through a 9-cm Whatman No. 41 filter-paper, the bulk of the precipitate (which was washed by decantation with hot water) being retained in the beaker. Filtration and washing were completed in a few minutes. The beakers containing the filtrates were placed on a hot-plate to evaporate.

The precipitates on the filter-papers were dissolved, *in situ*, with diluted hydrochloric acid (1 + 1), each solution being added to the beaker containing the bulk of the appropriate precipitate. The filter-papers were washed free from acid with hot water and were retained for the second filtration. An amount of 10 per cent. ammonium dihydrogen orthophosphate solution equivalent to five times the weight of cobalt was added to each beaker, and ammonium cobalt phosphate was re-precipitated as before. Each solution was again filtered, without delay, through the appropriate reserved filter-paper, the precipitate being quantitatively transferred to the filter-paper and thoroughly washed with hot water. The precipitates were ignited in weighed porcelain crucibles, gently at first until all carbon had been burnt off and then at red heat, and were weighed as $Co_2P_2O_7$. Meanwhile, the filtrates from the two precipitations were evaporated to remove excess of ammonia, acidified with hydrochloric acid and combined in a 50-ml calibrated flask; the solution was then diluted to volume and mixed. The standard was also diluted to volume and mixed. Ten-millilitre portions of the solutions from the filtrates and of the standard were counted with a gamma-scintillation liquid counter.

RESULTS

The results, which are shown in Table I and can be seen to give admirable support to the theory, were calculated as in the following example, which is from experiment No. 7.

The main cobalt fraction was 0·5066 g of $Co_2P_2O_7$, which is equivalent to 0·2046 g of cobalt.

A 10-ml portion of the standard (containing, in 25 ml, an amount of cobalt tracer equal to that added to each sample) was counted, and rates of 25,835 and 25,828 counts per minute were recorded. The background count was 818 counts in 5 minutes, *i.e.*, 163 counts per minute. The mean counting rate for the standard corrected for background was therefore 25,669 counts per minute.

A 10-ml portion of the filtrates, which were combined in a 50-ml calibrated flask, gave rates of 1870 and 1857 counts in 5 minutes, *i.e.*, a mean counting rate corrected for background of 210 counts per minute. Since the total volume of the filtrates was twice that of the standard, it was necessary to double the counting rate of the filtrates to make it comparable with that of the standard, *i.e.*, 420 counts per minute.

The counting rate proportional to the cobalt in the precipitate is therefore 25,669 − 420 counts per minute, *i.e.*, 25,249 counts per minute, which is equivalent to 0·2046 g of cobalt.

The amount of cobalt in the filtrate is therefore $\frac{0{\cdot}2046 \times 420}{25{,}249}$ g, *i.e.*, 0·0034 g; the total amount of cobalt is $(0{\cdot}2046 + 0{\cdot}0034)\ \text{g} = 0{\cdot}2080\ \text{g}$.

TABLE I

DETERMINATION OF COBALT IN PRESENCE OF NICKEL

Experiment No.	Amount of cobalt present, g	Amount of nickel present, g	Amount of cobalt found—		Total amount of cobalt found, g	Error, g	Error, %
			in filtrate, g	in precipitate, g			
1	0·1040	0·0000	0·0020	0·1017	0·1037	−0·0003	−0·3
2	0·1040	0·0000	0·0020	0·1017	0·1037	−0·0003	−0·3
3	0·1040	0·1065	0·0052	0·0979	0·1031	−0·0009	−0·8
4	0·1040	0·1065	0·0023	0·1016	0·1039	−0·0001	−0·1
5	0·1040	0·1065	0·0028	0·1014	0·1042	+0·0002	+0·2
6	0·1040	0·1065	0·0076	0·0964	0·1040	Nil	Nil
7	0·2080	0·0533	0·0034	0·2046	0·2080	Nil	Nil
8	0·2080	0·0533	0·0035	0·2046	0·2081	+0·0001	+0·05
9	0·0520	0·0000	0·0053	0·0489	0·0522	+0·0002	+0·4
10	0·0520	0·0000	0·0009	0·0512	0·0521	+0·0001	+0·2

CONCLUSIONS

The proposed method can be used with advantage only when (*a*) more than 50 per cent. of the element to be determined can be obtained as a pure precipitate for weighing or volumetric determination, (*b*) there is available a radioisotope of adequate purity and convenient half-life, which is suitable for measurement in solution and is associated with negligible carrier, and (*c*) it is more accurate than conventional methods, or, if no more accurate, more rapid and more easily carried out. In any instance, it would be of most value when many determinations are made as a routine; occasional analysis would hardly justify installation of the requisite equipment.

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First received *August 28th*, 1957
Amended, *October 15th*, 1958

Determination of Small Amounts of Tri*iso*octylamine in Aqueous Solution

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A rapid spectrophotometric method for the determination of small amounts of tri*iso*octylamine in aqueous solution is described. The method is based on the formation of a complex between the amine and cobalt thiocyanate; the complex is developed in dilute sulphuric acid and is extracted with carbon tetrachloride. The method is applicable to aqueous solutions containing tri*iso*octylamine, which are used in the treatment of uranium-bearing ores. Interference from iron is suppressed by adding sodium dihydrogen orthophosphate. The method is satisfactory for the determination of up to 5 mg of tri*iso*octylamine (standard deviation ±0·00012 per cent.).

TRI*iso*OCTYLAMINE dissolved in kerosene is used for the extraction of uranium from sulphuric acid leaches of uranium-bearing ores. Loss of the amine occurs by dissolution and entrainment in the aqueous phase, and, owing to the high cost of tri*iso*octylamine and the large volume involved, it was imperative to control the amine loss. Calculation of loss by inventory was found to be too slow and rather unreliable, and chemical testing of the waste effluent (barren) solutions appeared to be the only satisfactory method. A search of the available literature failed to disclose a suitable procedure.

Ziegler and Glemser[1] described a method for the detection of bivalent cobalt as its thiocyanate - tributylamine complex; this appeared to be suitable for our purpose.

EXPERIMENTAL

Known amounts of tri*iso*octylamine were added to 50-ml portions of water containing 5 g per litre of sulphuric acid in separating funnels. To each was added 2 g of cobalt nitrate and 2 g of sodium thiocyanate, and the solutions were shaken. The blue complexes were extracted with 10-ml portions of carbon tetrachloride. The colours of the extracts showed a direct relationship to the amounts of tri*iso*octylamine present.

When a barren solution was tested, it was found that iron, which was present in the sample to the extent of approximately 5 g per litre, interfered by forming the red thiocyanate. This interference was overcome by adding sodium dihydrogen orthophosphate to precipitate iron as ferric phosphate. Uranium did not interfere.

The optical density of the carbon tetrachloride extract was at a minimum in the 620-mμ region, and this wavelength was used throughout. No change in optical density of the extract was observed over a period of 1 hour.

DEVELOPMENT OF THE COMPLEX—

Different volumes of 10 per cent. w/v solutions of cobalt nitrate and sodium thiocyanate were added to known amounts of tri*iso*octylamine in separating funnels. Sufficient sulphuric acid was added to each separating funnel to give a 0·05 *N* solution after dilution to 100 ml. The amine complexes were extracted with 10-ml portions of carbon tetrachloride and the optical density of each extract was measured. The results are shown in Table I, from which it can be seen that 20 ml each of the cobalt nitrate and sodium thiocyanate solutions give full development of the complex from up to 5 mg of tri*iso*octylamine per 100 ml of solution.

In subsequent work, 20 ml of an aqueous solution containing 10 g each of cobalt nitrate and sodium thiocyanate per 100 ml were used.

EFFECT OF SULPHURIC ACID CONCENTRATION—

Concentrated sulphuric acid was added in different proportions to the sample solution up to a concentration of 5 per cent. v/v. Sodium dihydrogen orthophosphate (5 g) was then added, and the amine complex was developed by adding 20 ml of cobalt - thiocyanate reagent solution and was extracted with carbon tetrachloride. The results showed that sulphuric acid concentration had no adverse effect on complex formation. When iron was present, however, its interference increased, owing to breakdown of the phosphate complex. The optimum concentration of sulphuric acid was 0·25 per cent. v/v.

TABLE I

RECOVERY OF TRI*iso*OCTYLAMINE IN PRESENCE OF DIFFERENT AMOUNTS OF REAGENTS

Amount of 10 per cent. w/v sodium thiocyanate solution present, ml	Amount of 10 per cent. w/v cobalt nitrate solution present, ml	Amount of tri*iso*octylamine—		Amount of 10 per cent. w/v sodium thiocyanate solution present, ml	Amount of 10 per cent. w/v cobalt nitrate solution present, ml	Amount of tri*iso*octylamine—	
		added, mg	found, mg			added, mg	found, mg
1	1	2·0	0·3	20	30	2·0	2·0
3	3	2·0	1·1	5	20	2·0	1·1
5	5	2·0	1·3	10	20	2·0	1·6
7	7	2·0	1·4	20	20	2·0	2·1
10	10	2·0	1·5	30	20	2·0	2·0
15	15	2·0	1·9	20	5	4·0	3·4
20	20	2·0	2·0	20	10	4·0	3·9
25	25	2·0	2·0	20	20	4·0	4·1
30	30	2·0	1·9	20	30	4·0	4·0
20	5	2·0	1·5	5	20	4·0	2·4
20	10	2·0	1·8	10	20	4·0	3·2
20	20	2·0	1·9	30	20	4·0	4·0

INTERFERENCE BY IRON—

The effect of increasing the amount of sodium dihydrogen orthophosphate added to suppress interference by iron in presence of sulphuric acid concentrations greater than 0·25 per cent. v/v was studied. To samples containing 2·5 and 5 per cent. v/v of sulphuric acid, 0·5 per cent. w/v of iron and known amounts of tri*iso*octylamine, different amounts of sodium dihydrogen orthophosphate were added. The complex was developed and extracted as previously described. The results of these experiments are shown in Table II, from which it can be seen that tri*iso*octylamine can be determined in aqueous solutions containing up to 5 per cent. v/v of sulphuric acid in the presence of 0·5 per cent. w/v of iron if sufficient sodium dihydrogen orthophosphate is present to prevent formation of ferric thiocyanate.

TABLE II

AMOUNT OF SODIUM DIHYDROGEN ORTHOPHOSPHATE NEEDED TO INHIBIT INTERFERENCE BY IRON AT DIFFERENT SULPHURIC ACID CONCENTRATIONS

Each solution contained 0·5 per cent. w/v of iron

Amount of sulphuric acid present, % v/v	Amount of sodium dihydrogen orthophosphate added, g	Amount of tri*iso*octylamine found, mg
0·25	2	0·7
	3	1·1
	4	1·5
	5	1·5
	6	1·4
2·5	5	1·0
	7	1·3
	10	1·5
5·0	5	0·8
	7	1·0
	10	1·3
	15	1·6

METHOD

REAGENTS—

Cobalt - thiocyanate reagent solution—Dissolve 10 g each of cobalt nitrate and sodium thiocyanate in 100 ml of water.

Sodium dihydrogen orthophosphate—Analytical-reagent grade.

Sulphuric acid, concentrated.

Sulphuric acid, 2·5 *per cent. v/v.*
Carbon tetrachloride—Analytical-reagent grade.
Methanol—Analytical-reagent grade.

PROCEDURE—

By pipette, place a suitable volume of sample in a 125-ml separating funnel, add sufficient sodium dihydrogen orthophosphate to inhibit formation of ferric thiocyanate, and shake vigorously. Add 20 ml of cobalt - thiocyanate reagent solution, shake for 1 minute, and set aside for 2 minutes. Accurately add 10 ml of carbon tetrachloride, and shake vigorously for 1 minute. Allow the layers to separate, and filter the organic layer through a dry filter-paper. Measure the optical density of the solution in 1-cm cells at 620 mμ (set the spectrophotometer to zero against carbon tetrachloride). Calculate the amount of tri*iso*octylamine present from a previously prepared calibration graph.

PREPARATION OF CALIBRATION GRAPH—

Dissolve 0·500 g of tri*iso*octylamine in methanol, and dilute to 1 litre with methanol. Place volumes up to 10 ml of this solution in 125-ml separating funnels, add 10 ml of 2·5 per cent. v/v sulphuric acid to each, and dilute to 80 ml. Add 20 ml of cobalt - thiocyanate reagent solution to each, and continue as described under "Procedure." Plot a graph of optical density against weight of tri*iso*octylamine present.

RESULTS

Known added amounts of tri*iso*octylamine were recovered by the proposed method from aqueous solutions containing iron and sulphuric acid and from kerosene solutions (which are used in plant operation). The results, which are shown in Table III, indicate good recoveries.

TABLE III

RECOVERY OF ADDED TRI*iso*OCTYLAMINE FROM AQUEOUS AND NON-AQUEOUS SOLUTIONS

Amount of tri*iso*octylamine added, mg	Amount of tri*iso*octylamine found—		
	in aqueous solution, mg	in BAT-2 kerosene, mg	in Arctic diesel kerosene, mg
0·6	0·6	—	—
1·0	0·9	1·0	1·1
	1·0	1·1	1·1
2·0	2·0	1·9	1·8
	2·1	1·9	1·9
3·0	3·1	2·8	2·7
	—	2·7	2·8
4·0	—	3·7	3·7
	—	3·6	3·6
5·0	5·0	4·5	4·4
	5·0	4·5	4·5

Recovery from kerosene solutions is slightly lower than that from aqueous solution, as kerosene dissolves in carbon tetrachloride, thereby increasing the volume of the extract and decreasing its optical density.

When a routine sample of barren solution was analysed, the amounts of tri*iso*octylamine found were 0·048, 0·051, 0·051, 0·050, 0·050, 0·048, 0·049, 0·048, 0·05 and 0·049 g per litre.

I thank Eldorado Mining and Refining Ltd. for permission to publish this paper.

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Received *August* 18*th*, 1958

The Determination of Bismuth in Lead and Lead Cable-sheathing Alloys

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A modified method for the determination of small amounts of bismuth in lead and lead cable-sheathing alloys has been developed. Separation is based on extraction of the bismuth - diethyldithiocarbamate complex with chloroform, bismuth being finally determined absorptiometrically as its yellow thiourea complex. Results are good in the range 0·002 to 0·06 per cent. of bismuth.

METHODS for the determination of bismuth in lead and its alloys usually avoid the separation of lead as lead sulphate, owing to the tendency of the precipitate to retain some of the bismuth. Hall[1] developed a procedure in which bismuth was separated by co-precipitation with manganese dioxide from dilute nitric acid. After dissolution of the precipitate and evaporation of the resulting solution to dryness, bismuth was determined absorptiometrically as its coloured thiourea complex in dilute nitric acid. This method cannot, however, be applied to the analysis of lead cable-sheathing alloys, since antimony and tin are precipitated and some bismuth is carried down by the precipitate. Kinnunen and Wennerstrand[2] devised a procedure for the determination of bismuth in metallurgical products in which an absorptiometric finish was applied. Bismuth diethyldithiocarbamate was extracted from an ammoniacal solution with chloroform; cyanide, ethylenediaminetetra-acetic acid and tartaric acid were used as masking reagents. We found that antimony was co-extracted with bismuth, and this interfered with the subsequent photometric measurements. Attempts to overcome this difficulty by evaporating the extract until fumes of sulphur trioxide were evolved and then forming the complex with thiourea were unsuccessful, as lead sulphate was formed, which retained small amounts of bismuth. Ethylenediaminetetra-acetic acid, therefore, was not completely effective in complexing the lead, and, consequently, an alternative procedure was shown to be necessary. Strafford, Wyatt and Kershaw[3] used diethylammonium diethyldithiocarbamate in chloroform to separate bismuth and copper quantitatively from lead by extraction from 4 to 6 *N* hydrochloric acid; this procedure appeared to offer a solution to the problem.

EXPERIMENTAL

Preliminary experiments on the dissolution of the sample showed that diluted nitric acid (1 + 2) reacted rapidly, and this was preferred to a hydrochloric acid - bromine mixture, which was too slow. Excess of nitric acid was removed initially by evaporation and the final traces were removed from hydrochloric acid solutions by controlled addition of hydrobromic acid, liberated bromine being removed from solution by boiling. When appreciable amounts of tin are present, as in certain of the alloys, the action of nitric acid produces a precipitate of metastannic acid, which dissolves on subsequent treatment with hydrochloric acid. Unpublished work carried out in these laboratories by J. H. Thompson and C. A. B. Vincent has shown that antimony^{III} can be readily extracted from 6 *N* hydrochloric acid by diethylammonium diethyldithiocarbamate in chloroform, whereas antimony^{V} is only removed to a slight extent. In the final procedure, when antimony is present in the quinquivalent state, only a small proportion is concentrated in the combined extracts, and this is suppressed by tartaric acid. Any absorption caused by antimony at 470 mμ is then sufficiently small to be neglected. Before colour development of the bismuth - thiourea complex, the combined extracts were evaporated in the presence of oxidising agents until fumes of sulphur trioxide were evolved; this destroyed organic matter. Copper does not interfere with the photometric measurements.

METHOD

REAGENTS—

Nitric acid, diluted (1 + 2).
Nitric acid, dilute (1 + 15).

Hydrochloric acid, sp.gr. 1·18.

Hydrochloric acid, diluted (1 + 1).

Hydrobromic acid, sp.gr. 1·46.

Oxidising mixture—Mix 4 volumes of sulphuric acid, sp.gr. 1·84, 2 volumes of nitric acid, sp.gr. 1·42, and 1 volume of perchloric acid, sp.gr. 1·70.

Diethylammonium diethyldithiocarbamate solution—Prepare a 1·0 per cent. w/v solution of the reagent in chloroform.

Thiourea solution—Prepare a 10 per cent. w/v solution of thiourea in dilute nitric acid (1 + 15), and filter the solution before use. This solution should be freshly prepared as required.

Standard bismuth solution, 0·005 per cent. w/v—Dissolve pure bismuth in dilute nitric acid (1 + 15).

Carbon tetrachloride.

Tartaric acid.

Lead—British Chemical Standard No. 210*b* was used.

PROCEDURE—

Place 5 g of finely divided sample in a 400-ml conical beaker, and add 30 ml of diluted nitric acid (1 + 2). Dissolve the sample by gentle heating, evaporate the solution as far as possible without loss, add 70 ml of hydrochloric acid, sp.gr. 1·18, and boil carefully. Add small (4 to 5 ml) portions of hydrobromic acid, sp.gr. 1·46, until evolution of bromine has ceased. (Care should be taken, as the reaction is vigorous while the liberated bromine is being evolved.) Add 50 ml of water, cool to room temperature, filter the solution through a Whatman No. 541 filter-paper, and wash the precipitated lead chloride with diluted hydrochloric acid (1 + 1). (The lead chloride can also be separated by decantation.) Discard the filter-paper and precipitate, adjust the normality of the filtrate to between 4 and 6 with hydrochloric acid, sp.gr. 1·18, transfer the solution to a 350-ml separating funnel, and extract any residual bromine with 10 ml of carbon tetrachloride. Extract the aqueous layer with four to six 10-ml portions of diethylammonium diethyldithiocarbamate solution, and combine the extracts in a 125-ml conical beaker. (To ensure that extraction is quantitative, it is essential to remove all the copper with the bismuth; consequently, more than six 10-ml portions of diethylammonium diethyldithiocarbamate solution may be needed if larger amounts of these metals are present.) Add 6 to 8 ml of oxidising mixture (depending on the amount of diethylammonium diethyldithiocarbamate solution used), and evaporate the solution on a hot-plate until fumes are evolved. Finally, heat over an open flame, with swirling, until copious fumes of sulphur trioxide are evolved. Cool, add 1 to 2 ml of water, 5 g of tartaric acid and 20 ml of dilute nitric acid (1 + 15), swirl to dissolve the tartaric acid, and add 20 ml of thiourea solution. Transfer the mixture to a 100-ml calibrated flask, and dilute to the mark at 20° C with thiourea solution.

Measure the optical density of the solution in 1-cm cells at 470 mμ with a spectrophotometer adjusted against water. Determine the amount of bismuth present from a previously prepared calibration graph.

PREPARATION OF CALIBRATION GRAPH—

Prepare a series of solutions to cover the range 0·002 to 0·06 per cent. of bismuth by dissolving 5-g samples of pure lead (British Chemical Standard No. 210*b*, which contains 0·0021 per cent. of bismuth, was used) in diluted nitric acid (1 + 2) and adding calculated amounts of standard bismuth solution. Follow the procedure described, and plot a graph of optical density against bismuth concentration.

DISCUSSION OF THE METHOD

The final solution often becomes turbid, owing to the separation of sulphur. Sandell[4] states that hydrazine sulphate will prevent this decomposition, but we were unable to substantiate the claim. However, it was found to be entirely satisfactory to remove the deposited sulphur by filtration through a dry Whatman No. 540 filter-paper immediately before optical-density measurements were made.

The range of bismuth concentrations covered by the calibration graph (0·002 to 0·06 per cent. of bismuth) was that likely to be encountered in the analysis of lead and lead cable-sheathing alloys. The graph is linear from approximately 0·002 to 0·06 per cent. of bismuth, but, between about 0·06 and 0·1 per cent. of bismuth, the slope decreases slightly. Sandell's report[4] that Beer's law is obeyed between 0·2 and 5·0 mg of bismuth per 100 ml was therefore not confirmed over the whole range when a wavelength of 470 mμ was used.

The effect of other elements present in lead cable-sheathing alloys on the determination of bismuth was examined; 1·3 per cent. of antimony, 0·8 per cent. of tin, 0·6 per cent. of cadmium, 0·1 per cent. of copper and 0·01 per cent. each of tellurium, silver, zinc and arsenic did not interfere.

RESULTS

Recoveries of known amounts of bismuth from synthetic lead cable-sheathing alloys are shown in Table I. Optical-density measurements were made with a Unicam SP500 spectrophotometer. The composition of the alloys included the approximate maximum amounts of the impurities and alloying elements listed in the British Standard,[5] *viz.*, 0·005 per cent. each of silver and tellurium, 0·06 per cent. of copper, 0·002 per cent. of zinc and 0·01 per cent. of other elements (including cadmium in alloys B and E). Alloy B contained 0·85 per cent. of antimony, alloy D contained 0·5 per cent. of antimony and 0·25 per cent. of cadmium and alloy E contained 0·4 per cent. of tin and 0·2 per cent. of antimony.

TABLE I

RECOVERY OF BISMUTH FROM LEAD CABLE-SHEATHING ALLOYS

Alloy B		Alloy D*		Alloy E	
Amount of bismuth present, %	Amount of bismuth found, %	Amount of bismuth present, %	Amount of bismuth found, %	Amount of bismuth present, %	Amount of bismuth found, %
0·002	0·002	0·002	0·002	0·002	0·002
0·004	0·004	0·006	0·006	0·006	0·007
0·006	0·007	0·010	0·011	0·010	0·010
0·008	0·009	0·020	0·021	0·012	0·013
0·010	0·010	0·030	0·031	0·020	0·021
0·015	0·016	0·032	0·032	0·022	0·023
0·020	0·021	0·050	0·051	0·030	0·031
0·030	0·029	0·052	0·051	0·032	0·032
0·040	0·041	—	—	0·050	0·051
0·050	0·051	—	—	0·052	0·054

* This alloy is encountered less frequently than are alloys B and E.

The range of concentrations used is not necessarily the maximum tolerable, but is the maximum likely to be encountered in the analysis of these alloys. Lead cable-sheathing in current production for Post Office use normally contains bismuth in the range 0·002 to 0·02 per cent. The proposed method gives good results for amounts of bismuth in the range 0·002 to 0·06 per cent., and, although this is the most useful range, it can be conveniently extended by reducing the weight of sample taken.

We thank Brigadier Sir Lionel H. Harris, K.B.E., T.D., Engineer-in-Chief of the General Post Office, for permission to publish this paper. We also acknowledge the assistance of Mr. P. F. Preston and Mr. R. P. Harrison, who carried out part of the experimental work.

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Received *July 25th*, 1958

The Determination of Lead Styphnate in Priming Compositions used in Explosives

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A method for determining lead styphnate based on extraction of the sample with a solution of ammonium acetate and measurement of the optical density of the resulting solution is described. The determination can be completed in less than 1 hour, as opposed to almost 2 days by the accepted lead sulphate procedure, and can be applied in the presence of other water-soluble constituents, such as lead nitrate, normally present in priming compositions. The method is applicable on a micro scale and can be used for determining lead styphnate in samples of priming composition weighing less than 30 mg.

LEAD styphnate, the lead salt of trinitroresorcinol, is used in priming compositions in the manufacture of service and sporting ammunition.

The two most reliable methods for determining this constituent are based on precipitation of styphnic acid with organic reagents[1] or precipitation of lead as sulphide, which is then converted to sulphate. In the sulphate procedure, which is the method usually preferred, lead styphnate is extracted from the priming composition with ammonium acetate solution; barium nitrate is often present in priming compositions and is also extracted by ammonium acetate solution, but precipitation of lead as sulphide serves to separate these two constituents.

This procedure is time-consuming and a determination may extend over 2 days; further, in the presence of other water-soluble lead compounds, such as nitrate, hypophosphite or thiocyanate, the method has serious limitations. Hypophosphite and thiocyanate can, however, be determined separately and the lead equivalent calculated, but, in the presence of lead nitrate, the procedure is vitiated because of the difficulty in differentiating between nitrate and styphnate.

Because this method is time-consuming and in some instances not entirely reliable, an alternative method, based on measurement of the bright yellow colour of lead styphnate in ammonium acetate solution, has been developed. This method has proved to be particularly useful in the examination of single cap charges weighing about 30 mg and for the regular control examination of compositions.

EXPERIMENTAL

PREPARATION OF CALIBRATION GRAPH—

A solution containing 6 mg of lead styphnate in 100 ml of 1 per cent. ammonium acetate solution was prepared, and the optical density of the bright yellow solution was measured at several wavelengths. It was established that a suitable wavelength for absorptiometric measurements is 4125 A and optical densities were subsequently determined by using a Spekker absorptiometer with Ilford No. 601 filters and a tungsten-filament lamp.

The optical densities of a series of solutions containing between 0 and 15 mg of lead styphnate in 1 per cent. ammonium acetate solution were measured against water (see Fig. 1, curve A).

AMMONIUM ACETATE CONCENTRATION—

Under the conditions described, a ten-fold increase in ammonium acetate concentration results in only a slight decrease in optical density (see Fig. 1, curve B), and, for economy reasons, the lower concentration was used in subsequent experiments.

EFFECTS OF TEMPERATURE AND TIME—

Optical-density measurements are usually made at a standard temperature of 20° C, but this introduces a practical difficulty when small cells must be used, as heat transfer during handling and from the source of illumination can readily introduce a significant rise in temperature. The effect of temperature was therefore investigated, and it was shown that the error in the determination caused by an increase of 2° C at 20° C is about 2 per cent. of the lead styphnate content. It was established that adequate temperature control can be achieved by setting the cells containing sample solution aside in a water bath maintained at 20° C before optical-density measurements are made.

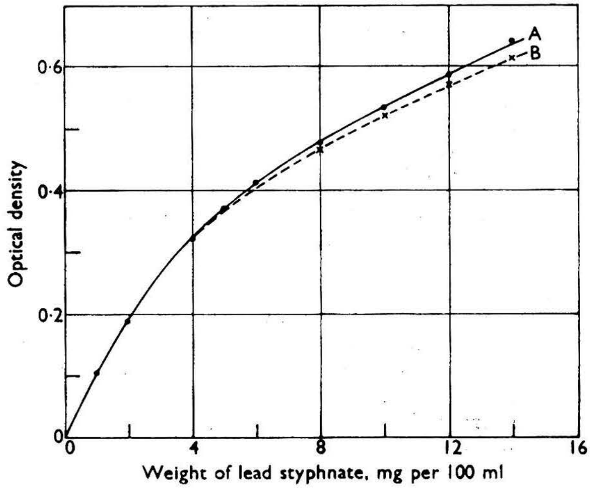

Fig. 1. Calibration graph for lead styphnate: curve A, in 1 per cent. ammonium acetate solution; curve B, in 10 per cent. ammonium acetate solution. The optical densities were measured at 20° C in 1-cm cells with a Spekker absorptiometer, a tungsten-filament lamp and Ilford No. 601 filters being used

The exterior of the cell must be dried with a minimum of handling and the optical density must be measured without delay. Even with these precautions, it is advisable to check the temperature of the solution in the cell immediately after the optical density has been measured, and, if necessary, a correction must be applied according to the following expression—

$$\text{Optical density at } 20^\circ\text{ C} = \frac{\text{Optical density at } (20 + t)^\circ\text{ C}}{1 + 0{\cdot}0107\, t}$$

where t is the difference in temperature from 20° C and takes the appropriate sign.

It was observed that optical densities remained constant over 24 hours.

TESTS ON PREPARED SAMPLES—

Small amounts of lead styphnate, representing the weight nominally present in a 30-mg sample of priming (a single cap), were weighed into separate micro extraction crucibles and extracted with 10 per cent. ammonium acetate solution. The extracts were diluted to 100 ml with water, and optical densities were determined as described under "Procedure," p. 185. The results were as follows—

Optical density	0·565	0·555	0·570
Amount of lead styphnate present, mg	11·35	10·87	11·55
Amount of lead styphnate found, mg	11·30	10·90	11·50

Recoveries were accurate to within less than 0·1 mg, which is equivalent to a deviation of ±0·2 per cent.

Experiments were also made with mixtures containing approximately equal amounts of lead styphnate and barium nitrate; the results were as follows—

Optical density	0·555	0·500	0·527	0·654	0·556	0·429
Amount of lead styphnate present, mg	10·97	8·97	9·85	14·88	10·89	6·70
Amount of lead styphnate found, mg ..	10·90	8·90	9·88	14·90	10·97	6·60

These results show that barium nitrate does not interfere in the proposed procedure.

Lead styphnate and barium nitrate are both soluble in ammonium acetate solution, so that, by determining the loss in weight and deducting the amount of lead styphnate found, the amount of barium nitrate can be calculated, provided that no other soluble compounds are present. When barium nitrate is replaced by lead nitrate in priming compositions, the amount of lead nitrate present can be calculated in the same way. Other water-soluble constituents likely to be present in this type of priming composition, *e.g.*, lead peroxide, tetrazene, calcium silicide and antimony sulphide, were shown not to interfere in the proposed procedure.

A Spekker absorptiometer was used in the following method for the analysis of single cap charges, the source of illumination being a tungsten-filament lamp.

METHOD

PREPARATION OF CALIBRATION GRAPH—

Dissolve 0·25 g of lead styphnate in 25 ml of 10 per cent. ammonium acetate solution. Transfer the solution to a 250-ml calibrated flask, and dilute to the mark with water; this solution contains 1·0 mg of lead styphnate per ml.

From a burette, add 2·0, 4·0, 6·0, 8·0, 10·0, 12·0 and 14·0 ml of the solution to separate 100-ml calibrated flasks, and dilute each to the mark with 1 per cent. ammonium acetate solution. Adjust the temperature to 20° C, and immediately measure the optical densities in the way described under "Procedure." (Confirm that the temperature of the solution has not altered.) Plot a graph of optical density at 20° C against weight of added lead styphnate.

PROCEDURE—

Transfer the sample to a weighed 1-ml porcelain micro extraction crucible having a porous base, and re-weigh. Attach the crucible to a micro extraction apparatus, and add 0·5 ml of 10 per cent. ammonium acetate solution. Stir gently with the flame-polished round end of a micro stirring rod; use gentle pressure to break up any lumps. Apply gentle suction, and slowly extract the soluble lead styphnate, together with barium nitrate and other water-soluble salts.

Continue to add 10 per cent. ammonium acetate solution in 0·5-ml increments, with alternate water washes, until the extract is colourless; use a total of 10 ml of ammonium acetate solution. Thoroughly wash the crucible with water, dry by suction, and place it in an air oven at 110° C for 30 minutes. Cool in a desiccator, without desiccant, for 15 minutes, and re-weigh.

Transfer the ammonium acetate extract and washings to a 100-ml calibrated flask, dilute to the mark, and immerse the flask in a water bath maintained at 20° C.

Fill a 1-cm cell with the solution, and place it in the water bath for a few minutes. Remove the cell, dry the exterior with the minimum amount of handling, and immediately measure the optical density of the solution. Use water in the compensating cell and Ilford No. 601 filters. Check the temperature of the solution in the cell after measuring the optical density, and, if necessary, correct the optical density by using the expression on p. 184.

Calculate the lead styphnate content from the calibration graph.

APPLICATION OF THE METHOD

CONTROL ANALYSIS OF PRIMING COMPOSITIONS—

Four separate 0·3-g samples from one batch of priming composition containing lead styphnate, barium nitrate and other water-insoluble constituents were weighed into tared sintered-glass crucibles, and each was slowly extracted in a Witt apparatus with 25 ml of 10 per cent. ammonium acetate solution, added in 2-ml portions with alternate water washes, as described above. The crucibles were dried at 110° C, and the losses in weight were used to calculate the combined lead styphnate and barium nitrate contents. Each extract was diluted with water to 250 ml, a 25-ml aliquot of each was diluted to 100 ml with 1 per cent. ammonium acetate solution and the optical densities of the final solutions were determined as before. The results are shown in Table I.

TABLE I

ANALYSIS OF PRIMING COMPOSITION

Weight of sample, g	Optical density	Amount of lead styphnate found— mg	%
0·3030	0·567	11·38	37·5
0·3006	0·565	11·34	37·7
0·3027	0·568	11·45	37·8
0·3108	0·574	11·66	37·5

An additional determination was made on a 1-g sample of the same material (sample A). A 50-ml portion of 10 per cent. ammonium acetate solution was used for extraction, and the extract was diluted to 500 ml. A 25-ml aliquot was diluted to 250 ml with 1 per cent. ammonium acetate solution and the optical density was measured as before. Lead styphnate was also determined in a 250-ml aliquot (0·5 g) by the gravimetric lead sulphate method previously described.

Comparison of both procedures was made in this way, and similarly with a second sample of priming, B, in which barium nitrate was also determined. The results, which are shown in Table II, are all in good agreement.

TABLE II

COMPARISON OF RESULTS BY PROPOSED AND GRAVIMETRIC PROCEDURES

	Amount of lead styphnate found by—		Amount of barium nitrate found by—	
Sample	proposed method, %	gravimetric method, %	proposed method, %	gravimetric method, %
A	37·8	37·9	—	—
B	40·1	40·4	37·0	36·7

With these larger sample weights, it is only necessary to dilute the ammonium acetate extract so that the lead styphnate concentration is within the range 0 to 15 mg per 100 ml and the ammonium acetate concentration is approximately 1 per cent.

CHOICE OF INSTRUMENT

A Spekker absorptiometer was used in the early experiments, as this instrument is readily available in most laboratories. However, because of the increasing tendency to use a spectrophotometer for optical-density measurements, the use of a Unicam SP500 instrument in the final stages of the procedure was investigated; the calibration graph was linear. The characteristics of a Unicam SP500 spectrophotometer, particularly in relation to its available narrow wavelength-band, necessitate the use of solutions more dilute than those used with a Spekker absorptiometer; this is shown by the results in Table III.

TABLE III

COMPARISON OF RESULTS OBTAINED BY USING DIFFERENT INSTRUMENTS

Instrument	Filter	Wavelength, A	Optical density	Equivalent of extracted lead styphnate in diluted solution	Total amount of lead styphnate found, g
Spekker absorptiometer	Ilford No. 601	—	0·578	$^1/_{10}$	0·1185
Unicam SP500 spectrophotometer	—	4125	0·392	$^1/_{100}$	0·1180

The method was adapted for use with a Unicam SP500 instrument fitted with a temperature-control attachment.

ADAPTATION OF METHOD FOR USE WITH UNICAM SP500 SPECTROPHOTOMETER

PREPARATION OF CALIBRATION GRAPH—

Dissolve 0·2 g of lead styphnate in 50 ml of 10 per cent. ammonium acetate solution. Transfer the solution to a 1-litre calibrated flask, add a further 50 ml of 10 per cent. ammonium acetate solution, and dilute to the mark with water; this solution contains 0·20 mg of lead styphnate per ml.

From a burette, add 1·0, 2·0, 3·0, 4·0, 5·0, 6·0 and 7·0 ml of the solution to separate 100-ml calibrated flasks, and dilute each to the mark with 1 per cent. ammonium acetate solution. Adjust the temperature to 20° C, measure the optical density as described under "Procedure," p. 185, and then deduct the blank value of the cell. Plot a graph of optical density at 20° C against weight of lead styphnate.

PROCEDURE FOR SINGLE CAP CHARGES—

Proceed as described for single cap charges. Transfer a 10-ml aliquot from the 100 ml of solution to a second 100-ml calibrated flask, and dilute to the mark with 1 per cent. ammonium acetate solution. Adjust the temperature to 20° C, as described previously. Measure the optical density of the solution in 1-cm cells at 4125 A against water. Take care not to touch the surfaces of the cells after they have been cleaned and dried; remove solutions from the cells by suction, and add all solutions carefully.

PROCEDURE FOR CONTROL ANALYSIS OF PRIMING COMPOSITIONS—

Weigh 0·3 g of sample into a sintered-glass crucible, and slowly extract with 50 ml of 10 per cent. ammonium acetate solution added in 2-ml portions with alternate water washes. Dilute the extract with water to 500 ml. Transfer a 10-ml aliquot to a 250-ml calibrated flask, and dilute to the mark with 1 per cent. ammonium acetate solution. Measure the optical density as described above.

DISCUSSION OF THE METHOD

Although there is no evidence to confirm that solutions of lead styphnate samples from different sources differ in optical density, it is advisable to check the calibration graph when new batches are brought into use.

All results for lead styphnate by the proposed method are within ±0·3 per cent. of the expected values, and, in consideration of the usual permissible tolerance, this accuracy is adequate. Results for barium nitrate are also in good agreement with expected values. When a second soluble lead salt is present in addition to barium nitrate, the sample is extracted with ammonium acetate solution, lead styphnate is determined by the proposed method and barium is determined as sulphate after removal of the total lead as sulphide. From the results, the amount of the second lead salt present can be calculated. The absorptiometric procedure is relatively simple, and a determination of lead styphnate can be completed in 15 minutes after extraction with ammonium acetate, as opposed to 2 days by the accepted gravimetric procedure.

Acknowledgment is made to Mr. W. T. Elwell, Division Chief Analyst, Imperial Chemical Industries Ltd., Metals Division, for his interest in this investigation and for assistance in the preparation of this paper.

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Received *July 3rd*, 1958

Notes

COLORIMETRIC DETERMINATION OF CYSTEINE

In a recent paper, Saville[1] has shown that the S-nitroso derivatives of several thiols can be hydrolysed to the equivalent nitrous acid in the presence of certain heavy-metal cations. By adding suitable reagents, it has been possible to cause this hydrolytically generated nitrous acid to result in the formation of an azo dye. These reactions indicated a general method for the colorimetric determination of thiols; a method for determining cysteine is described below.

METHOD

REAGENTS—

Solution A—Add 5 ml of a 0·01 *M* aqueous solution of analytical-reagent grade sodium nitrite (approximately 0·7 g of sodium nitrite per litre) to 40 ml of *N* sulphuric acid, and make up to 100 ml with water.

Solution B—Dissolve 5 g of ammonium sulphamate in 1 litre of water.

Solution C—Mix 4 volumes of a 3·44 per cent. solution of sulphanilamide in 0·4 *N* hydrochloric acid with 1 volume of a 1·0 per cent. solution of mercuric chloride in 0·4 *N* hydrochloric acid. Store in a closed bottle (see Note).

Solution D—Dissolve 10 g of N-1-naphthylethylenediamine dihydrochloride in boiling methanol containing a trace of concentrated hydrochloric acid, cool slightly, and add 2 g of activated charcoal. Maintain the mixture at 50° C for a few minutes, filter, allow to cool, and add about 400 ml of diethyl ether. Agitate the solution during the addition of ether. Separate the purified N-1-naphthylethylenediamine dihydrochloride, wash it with diethyl ether, and rapidly dry it at 40° to 50° C. Store the product in a dark-glass container. Add 1·0 g of purified N-1-naphthylethylenediamine dihydrochloride to 1 litre of 0·4 *N* hydrochloric acid; stir during the addition. Store the solution in a closed bottle (see Note).

> NOTE—No colour should develop when solutions C and D are mixed. If either is allowed free access to laboratory air for a prolonged period, atmospheric nitrous acid, although present in only minute amounts, will cause diazotisation of sulphanilamide and hence produce slight colours. Although these colours make no difference to the calibration results, they can be avoided by storing the solutions in narrow-necked stoppered bottles; solutions so stored are satisfactory for at least 14 days.

PREPARATION OF CALIBRATION GRAPH—

Prepare solutions of cysteine hydrochloride (in either L or DL form) in 0·01 *N* hydrochloric acid so as to contain from 1 to 70 µg of cysteine per ml.

Place 1·00 ml of each cysteine solution in a 25-ml calibrated flask, add 5 ml of solution A, and set aside for 4 to 5 minutes. Add 1 ml of solution B (use it to wash down the inside neck of the flask), insert the stopper, and shake vigorously for a few seconds. After 1 to 2 minutes, rapidly add approximately 10 ml of solution C from a pipette having an enlarged tip. Dilute to the mark with solution D, and, after 2 minutes, measure the intensity of the coloured solution against an appropriate blank solution containing water in place of cysteine solution. Use a Spekker absorptiometer and Ilford No. 605 filters. (Measure low colour intensities in 3·0-cm cells and higher intensities in 1·0-cm cells.) Plot a calibration graph from the results.

DISCUSSION OF THE METHOD

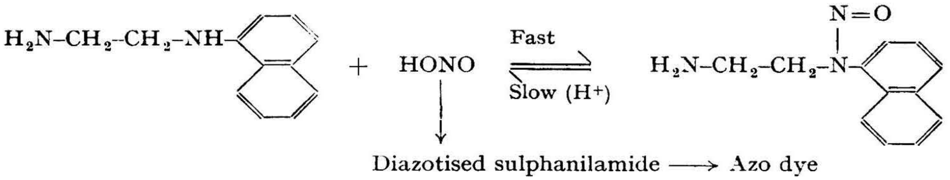
Experience has shown that, for the best results, the only critical points to be observed are (*i*) the initial measurement of the cysteine solution to be analysed, and (*ii*) the final dilution to the calibration mark with solution D. Provided that these operations are accurately performed, the timings and accuracy of the other reagent additions are of secondary importance. For example, if the concentration of nitrous acid in solution A is doubled, the calibration results never deviate by more than 2 per cent. Reaction times between 3 and 6 minutes with solution A and up to 5 minutes with solution B again cause little change. If the acid concentration in solutions C and D is increased ten times, the time for colour development is increased to 20 minutes, but the final results are almost unchanged. The accuracy and reproducibility of results by the proposed method depend on the order of cysteine concentration in the solutions to be analysed.

For cysteine concentrations of the order of 1 µg per ml (0·00001 *M*), the result is accurate to within ±10 per cent., whereas, at 10 µg per ml, the corresponding accuracy is to within ±2 per cent.; the accuracy improves to within about ±1 per cent. over the concentration range 30 to 60 µg of cysteine per ml.

SIMPLIFIED PROCEDURE FOR CYSTEINE DETERMINATION—

The procedure described for preparing a calibration graph can be further simplified by combining solutions C and D. When this is done, however, colour development is rapid at first and, after 10 minutes, slowly increases at about 0·001 absorptiometer unit per 2 to 3 minutes. Further, colour intensities after 20 minutes are only 95 per cent. of those previously found.

A possible explanation of this may be that the N-1-naphthylethylenediamine competes with sulphanilamide for the nitrous acid liberated from the S-nitrosocysteine and forms N-nitrosonaphthylethylenediamine, which slowly liberates nitrous acid on acid hydrolysis. This nitrous acid reacts with more sulphanilamide, and more dye is slowly formed according to the following equation—



A satisfactory procedure based on these results is described below—

> To a 1·00-ml aliquot of cysteine solution in a 25-ml calibrated flask add 5 ml of solution A, and set aside for 4 to 5 minutes. Add 1 ml of solution B, mix well, insert the stopper, and shake. After 1 to 2 minutes, make up to the mark with a mixture of 10 volumes of solution C and 4 volumes of solution D. Allow the colour to develop for 20 minutes at about 20° C, and measure its intensity with a Spekker absorptiometer as previously described.
>
> Specimen calibration results are shown in Table I.

TABLE I

DETERMINATION OF CYSTEINE BY SIMPLIFIED PROCEDURE

Cysteine concentration—		Absorptiometer reading (1-cm cell)
µg per ml	mole per litre	
12·1	0·0001	0·149, 0·147
24·2	0·0002	0·308, 0·312
36·3	0·0003	0·466, 0·473
48·4	0·0004	0·619, 0·620
60·5	0·0005	0·771, 0·773
72·6	0·0006	0·912, 0·909

This simplified technique can yield satisfactory results of only slightly inferior accuracy to those previously found.

SPECIFICITY—

The reactions leading to the azo dye appear to be specific for sulphydryl groups, hence thiols other than cysteine will also react. Glutathione has been determined as equivalent cysteine. The possibility of interference by secondary aliphatic amines was investigated, but no effect caused by 0·005 *M* dimethylamine was noticed. This is probably because nitrosation of secondary amines is not significant at high acidities. Primary and secondary aromatic amines will give different colours. Substances containing primary amino groups might interfere by competitively removing nitrous acid, but this difficulty could be overcome by using more nitrous acid than interfering substance, *e.g.*, urea. The effect of several amino acids not containing –SH groups on the recoveries of cysteine was studied quantitatively. There was positively no interference from about 1000-fold excesses of glycine, alanine, tyrosine, arginine, valine, lysine, leucine, *iso*-leucine, histidine, threonine, serine and proline. Ornithine reduced recoveries of cysteine to between 94 and 96 per cent. It seems that nitrosation of cysteine is complete before there is any de-amination of the excess of amino acids.

Iodide ion causes partial interference, as it rapidly destroys nitrous acid. Bromide and chloride ions do not affect the results. Extremely large amounts of mercuric, silver and cupric salts seriously interfere. Zinc salts do not, and hence cysteine produced from cystine by reduction with zinc and hydrochloric acid can be accurately determined.

Pure cystine neither reacts in the method nor interferes with cysteine determinations. Aldehydes, ketones and alcohols also have no effect.

SCOPE—

The proposed procedure seems to offer special advantages over the classical colorimetric methods for cysteine described by Snell and Snell.[2] It seems to be considerably more sensitive and specific than methods involving molybdenum blue formation and does not determine other reducing agents that normally interfere with the Folin reagent method. In accuracy and sensitivity, the proposed method compares favourably with modern techniques involving titration with silver nitrate, for which specially prepared electrodes are needed,[3] and with the polarographic procedures described by Coult.[4]

The method may find wide application in the determination of cysteine and cystine in protein hydrolysates and also in the determination of potential –SH groups in soluble biological material. In this connection, minute amounts of finger-nail chippings were digested in concentrated hydrochloric acid, the solution was diluted and reduced with zinc and the cysteine equivalent to the cystine content was determined by the proposed method. The chippings were found to contain about 12 per cent. of cystine, a figure in good agreement with values reported in the literature.

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CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT
PORTON DOWN
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Received *June 25th*, 1958

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ANOMALOUS ALKOXYL VALUES FOR *tert.*-BUTYL SUBSTITUTED PHENOLS

IN the analysis of a series of compounds isolated in studies of the metabolism of butylated hydroxyanisoles,[1] difficulty was experienced in interpreting high methoxyl values found for chromatographically pure samples. We have found that Zeisel's alkoxyl procedure, as modified by Vieböck and Schwappach[2] and adapted to the micro scale, gives high methoxyl values when applied to the analysis of *tert.*-butyl substituted phenols, although results are close to theoretical values for methyl, *sec.*-butyl and *iso*butyl substituted phenols.

Zeisel's method for determining alkoxyl groups has been successfully applied, with various modifications, to the analysis of a wide range of organic compounds; propoxyl and *n*-, *sec.*- and *iso*butoxyl groups can be determined quantitatively.[3] However, anomalous results have been reported for certain tetra-alkyl dibenzyls,[4,5] polyhydric alcohols[6] and erythroaphin.[7] The conditions described by Huang and his co-workers[4,5] for the analysis of tetra-alkyl dibenzyls are rather drastic and resemble the cracking procedure used for N-methyl determinations. In view of this, it would appear that the high results originate from a volatile fragment produced in this cracking process.

EXPERIMENTAL

EFFECT OF SOLVENT—

In the published modifications of the micro Zeisel method, phenol is almost invariably used as solvent, but mixtures of phenol and either propionic[8,9,10] or acetic[11] anhydride have been used. A summary of experiments carried out with and without propionic anhydride in the solvent is shown in Table I; 25 per cent. w/v sodium acetate solution was used as the scrubbing liquid. It can be seen that, although results for *tert.*-butyl substituted phenols are anomalous under both sets of conditions, the error is greater in presence of propionic anhydride. The presence of more than one *tert.*-butyl group increases the anomaly still further. However, the presence of methyl, *sec.*-butyl or *iso*butyl groups did not give rise to anomalous results.

TABLE I

EFFECT OF SOLVENT ON ANOMALOUS METHOXYL VALUES

The scrubbing liquid used was 25 per cent. w/v sodium acetate solution

Compound	Theoretical methoxyl content, %	Amount of methoxyl found with— phenol *plus* propionic anhydride as solvent, %	phenol as solvent, %
4-*tert.*-Butyl phenol	0·0	2·07	1·35
2-*tert.*-Butyl-4-methoxyphenol	17·23	21·30, 20·56,* 22·56†	18·06
3-*tert.*-Butyl-4-methoxyphenol	17·23	20·74	—
2-*tert.*-Butylquinol	0·0	3·68	1·25
2 : 6-Di-*tert.*-butyl-4-methylphenol	0·0	7·30	1·68
2 : 5-Di-*tert.*-butyl-4-methoxyphenol	13·13	18·95	15·84
2 : 6-Di-*tert.*-butyl-4-methoxyphenol	13·13	24·17	15·62
2-Methyl-4-methoxyphenol	22·46	22·41	—
3-Methyl-4-methoxyphenol	22·46	22·71	—
2 : 6-Dimethyl-4-methoxyphenol	20·39	20·24	—
2-*iso*Butyl-4-methoxyphenol	17·23	17·22	—
2-*sec.*-Butyl-4-methoxyphenol	17·23	17·37	—
Methyl-3 : 5-di-*tert.*-butyl-4-hydroxybenzoate	11·74	14·54	13·22
2 : 5-Di-*tert.*-butylquinol	0·0	—	1·46
4-*tert.*-Butyl-2-hydroxyphenol	0·0	—	1·06

* Propionic acid was used instead of propionic anhydride.

† A 2-ml portion of hydriodic acid and a 0·5-ml portion of propionic anhydride were used.

EFFECT OF SCRUBBING LIQUID—

Two scrubbing liquids, (*a*) a 25 per cent. w/v aqueous solution of sodium acetate,[9] and (*b*) a mixture of equal amounts of 5 per cent. w/v solutions of cadmium sulphate and sodium thiosulphate,[8,11] were used to study the effect of the scrubbing liquid on the anomalous methoxyl values. The results (see Table II) show that, both in presence and absence of propionic anhydride, deviation from theoretical values is greater when 25 per cent. w/v sodium acetate solution is used. It is therefore obvious that this scrubbing liquid, which was found to be perfectly satisfactory for a wide range of compounds in our laboratory, should not be used in the analysis of *tert.*-butyl substituted phenols. With phenol alone as solvent and cadmium sulphate - sodium thiosulphate solution as scrubbing liquid, results are close to the theoretical methoxyl values.

TABLE II

EFFECT OF SCRUBBING LIQUID ON ANOMALOUS METHOXYL VALUES

Compound	Theoretical methoxyl content, %	Amount of methoxyl found with— 25 per cent. w/v sodium acetate solution as scrubbing liquid, %	(1 + 1) mixture of 5 per cent. w/v solutions of cadmium sulphate and sodium thiosulphate as scrubbing liquid, %
With phenol plus *propionic anhydride as solvent*—			
2 : 6-Di-*tert.*-butyl-4-methylphenol	0·0	4·02	3·64
2-*tert.*-Butyl-4-methoxyphenol	17·23	21·30	17·45
With phenol as solvent—			
2 : 6-Di-*tert.*-butyl-4-methylphenol	0·0	1·69	1·11, 1·55
2-*tert.*-Butyl-4-methoxyphenol	17·23	18·06	17·44
2 : 6-Di-*tert*-butyl-4-methoxyphenol	13·13	15·62	13·10
2 : 5-Di-*tert.*-butyl-4-methoxyphenol	13·13	15·84	12·88

METHOD

The apparatus used is similar to that described by Elek,[8] but the scrubber contains 2-mm diameter glass beads to break up the gas flow. A 30-mg portion of phenol is added to the sample (5 to 10 mg) in the reaction flask, and then about 0·02 ml of 30 per cent. hypophosphorous acid and 1 ml of M.A.R. hydriodic acid, sp.gr. 1·7, are added. The flask is quickly connected to the apparatus, and, at a carbon dioxide flow rate of about 4 ml per minute, the mixture is maintained at gentle reflux for 1 hour. The stationary water in the condenser warms to about 30° C. The scrubber contains 2 ml of a mixture of equal parts of 5 per cent. w/v cadmium sulphate solution and 5 per cent. w/v sodium thiosulphate solution,[8] and the absorber contains 5 ml of a solution prepared by adding 10 drops of bromine to glacial acetic acid containing potassium acetate.[9] The contents of the absorber are rinsed into a conical flask containing 2 ml of 25 per cent. w/v aqueous sodium acetate solution, excess of bromine is destroyed by the usual procedure with 98 per cent. formic acid and the iodine liberated on addition of 50 mg of potassium iodide and 5 ml of 10 per cent. sulphuric acid is titrated against 0·02 *N* sodium thiosulphate.

DISCUSSION OF THE METHOD

Bell[12] has shown that the *tert.*-butyl group is a relatively mobile substituent in aromatic systems. Its lability was further demonstrated by Dacre, Denz and Kennedy,[1] who isolated *tert.*-butylquinol, 4-methoxyphenol and quinol from the reaction products when 2-*tert.*-butyl-4-methoxyphenol was hydrolysed with dilute hydrochloric acid.

If then, under the acid conditions of the Zeisel determination, a *tert.*-butyl group splits from a *tert.*-butyl substituted phenol as a carbonium ion, it could combine with an iodide ion to form *tert.*-butyl iodide. *tert.*-Butyl iodide, however, boils at 103° C and is easily decomposed into *iso*-butylene and hydrogen iodide by heat, and one would expect such a decomposition to occur to some extent under the experimental conditions. In a time - recovery experiment, the distillation of the volatile iodides from 2 : 6-di-*tert.*-butyl-4-methoxyphenol was investigated by determining the volatile iodides after various intervals of time. The results are shown in Table III, from which it can be seen that the theoretical methoxyl value is exceeded after 5 minutes and that almost all the volatile iodides distil in the first 15 minutes, as in the distillation of methyl iodide from vanillin. This is consistent with the decomposition of most of the *tert.*-butyl iodide not distilled during the early part of the reaction. It also explains the low recovery (18·8 per cent.) of *tert.*-butyl iodide from *tert.*-butyl alcohol[3] under conditions that gave theoretical recoveries of *n*- and *sec.*-butyl iodides from the corresponding alcohols.

TABLE III

RECOVERY OF VOLATILE IODIDES AT VARIOUS TIMES

Phenol *plus* propionic anhydride was used as solvent and 25 per cent. w/v sodium acetate solution as scrubbing liquid

Compound	Theoretical methoxyl content, %	Amount of methoxyl found after—				
		5 minutes, %	10 minutes, %	15 minutes, %	30 minutes, %	60 minutes, %
Vanillin	20·36	11·66	19·13	19·95	20·28	—
2 : 6-Di-*tert.*-butyl-4-methoxyphenol	13·13	15·20	20·69	21·56	21·91	22·14

Undecomposed *tert.*-butyl iodide will pass into the scrubbing liquid. *tert*-Butyl iodide is hydrolysed to *tert.*-butyl alcohol and hydriodic acid by water, and hence any aqueous solution will decompose the iodide to some extent. From our experiments, it is apparent that the scrubbing liquid containing sodium thiosulphate is the more efficient of those tested. If a long spiral bubbler is used for the scrubbing liquid, as described by Heron, Reed, Stagg and Watson,[9] hydrolysis by water to form *tert.*-butyl alcohol may be complete.

We thank Dr. J. C. Dacre for his interest in the investigation and for supplying pure samples of *tert.*-butyl substituted phenols for analysis. We acknowledge grants from the Mellor Research Fund.

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THE DETERMINATION OF ANIONIC DETERGENTS IN RIVER WATER AND SEWAGE EFFLUENTS

LONGWELL and Maniece's method[1] for determining anionic detergents is time-consuming. Three extractions are needed and the separating funnel is rinsed with chloroform after each extraction. The time required for a determination can be reduced considerably by extracting with one 50-ml portion of chloroform.

METHOD

REAGENTS—

As described by Longwell and Maniece.[1]

PROCEDURE—

Prepare the sample and separating funnels as previously described.[1] To the first separating funnel, add exactly 50 ml of chloroform, and extract in the normal way. Run the chloroform layer (or as much of it as is conveniently possible) into the second separating funnel. Shake, and run the chloroform layer through a small funnel plugged with cotton-wool. Reject the first 5 ml of filtrate, and then collect a volume sufficient for optical-density measurement.

RESULTS

Solutions of Manoxol O.T. in distilled water were examined by both methods; the results were as follows—

Amount of Manoxol O.T. present, µg	0	20·2	50·5	101·0	151·5
Colorimeter reading (Longwell and Maniece's method) ..	2·5	7·7	—	29·5	43·0
Colorimeter reading (proposed method)	2·5	7·5	16·5	30·0	44·0

Samples of river water and sewage effluent were also examined; the results are shown in Table I.

TABLE I

DETERMINATION OF DETERGENT IN RIVER WATER AND SEWAGE EFFLUENT

	Amount of detergent found, as Manoxol O.T., by—	
Sample	Longwell and Maniece's method, p.p.m.	proposed method, p.p.m.
Effluent	5·0	4·8
	3·8	3·3
	3·7	3·8
River water	0·46	0·50
	0·35	0·34
	0·86	0·85
	0·39*	0·39*

* Sample of softened and filtered river water.

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Received *August* 11*th*, 1958

THE MOLISCH REACTION

THE Molisch test for carbohydrates is well known.[1] Generally, text-books describe a procedure in which the colour is developed at the interface between concentrated sulphuric acid and the test solution.

I have found that the utility of the test can be extended if it is applied in the following manner—

> Add 0·1 g of the sample to 0·1 g of 1-naphthol in a dry test-tube. Add 2 ml of concentrated sulphuric acid, and mix the contents.

The test is positive if a violet or red-violet colour slowly develops when the test-tube is set aside. It is essential to use pure 1-naphthol and concentrated sulphuric acid.

This procedure can be rationalised in the following way. There are at least three possible stages in the reaction, (*i*) hydrolysis, (*ii*) dehydration and ring closure, and (*iii*) condensation; these stages are necessary for opening the carbohydrate ring, possibly forming a furan nucleus and forming the violet condensation product. Condensation is rapid, as compounds containing an aldehyde group react rapidly under the conditions described. The proposed procedure, in which the slowness of the hydrolysis and dehydration stages is utilised, serves to distinguish between aldehyde compounds and carbohydrates.

A positive test indicates a compound having a cyclic ketol group, *e.g.*, a pentose, hexose, polysaccharide or glycoside. The test can be successfully used for glycosides; it occasionally appears to fail, as glycosides form coloured products with concentrated sulphuric acid, but the slow formation of the violet colour can often be detected against this background.

REFERENCE

1. Cheronis, N. D., and Entrikin, J. B., "Semimicro Qualitative Organic Analysis: The Systematic Identification of Organic Compounds," Second Edition, Interscience Publishers Inc., New York and London, 1957.

CHEMISTRY DEPARTMENT
UNIVERSITY OF AUCKLAND
AUCKLAND, NEW ZEALAND

T. A. TURNEY

First received *April* 30*th*, 1958
Amended, *November* 11*th*, 1958

Book Reviews

FLAVOR RESEARCH AND FOOD ACCEPTANCE. Sponsored by Arthur D. Little Inc. Pp. vi + 391. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1958. Price $10.00; 80s.

In 1956 and 1957 Arthur D. Little Inc. of Cambridge, Mass., sponsored four symposia to discuss the technical and practical aspects of flavour research for industry. Contributors came from universities, industrial laboratories and consultant companies; this book is based on their papers.

Part I (42 pages) deals with the physiological basis of flavour and the responses to taste and odour stimuli. From measurement of the electrical nerve activity of animals, interesting results have been obtained on the behaviour of the taste organs; on the more difficult study of olfactory mechanisms, the papers indicate that a great deal remains to be done.

Part II (79 pages) covers flavour testing in the laboratory. The subjects include panel selection and training, difference tests, scoring and ranking and the application of statistics to experimental testing. One article gives hints on the examination of wrapping materials liable to transfer "off" odours and flavours to food; industrial chemists, particularly those concerned with confectionery, will appreciate the importance of this problem.

The flavour profile method originally described in 1949 is reviewed in this book. It is used for recording the flavour of a food by tabulating such features as the intensity of character notes, order of perception, after taste and amplitude, the last-named based on the general flavour effect. It is claimed that the procedure is a useful tool for control work, in "trouble-shooting" and in research directed towards product development.

Part III (59 pages) gives information on new product development and on testing for consumer reaction. One chapter deals with statistical planning, emphasising the need for designing tests to make efficient use of the data from market research.

Part IV (64 pages) describes the methods of flavour and odour testing and of product development used by some prominent firms representing such industries as baking, brewing and canning.

Part V (120 pages) is entitled Physiochemical Research and refers to analytical methods for identifying the various components of a flavour. The importance of paper and gas chromatography is apparent. Separate articles deal with the application of the methods to determining the components of strawberry and citrus flavours and to the flavours of milk and its products.

A publication on the "Chemistry of Natural Food Flavours" (from the Quartermaster Food and Container Institute for the Armed Forces, Chicago) may be known to many chemists. "Flavor Research and Food Acceptance" covers a much wider range of subjects, as the above brief outline of the contents will indicate. Some chemists might consider market research to be outside the scope of a scientific book on flavour, but this would not be the view of those engaged on development work in foods. Thus mention is made of an obviously artificial strawberry essence used in a beverage being preferred by a consumer panel to a new flavour that closely resembled the fruit. Again, colour is considered to have a powerful influence on judgment of a flavour—as to identity, strength and quality. Indeed, the opinion is expressed that, provided the flavour is pleasantly acceptable, it is outweighed in importance by other properties, particularly by the appearance. Such facts as these emphasise the need to test consumer reaction when producing new flavours or products.

This book justifies the claim that it gives the modern views of some authorities on the many different aspects of flavour research. The method of presentation must give some overlapping of information, but this does not affect the usefulness of the book. Food chemists should find the articles stimulating, and they will undoubtedly be of special interest to those engaged in research and development.

S. BACK

WATER TREATMENT. By G. F. MUGELE, B.Sc. (Eng.), A.M.I.C.E., A.M.I.W.E., and A. WISEMAN, B.Sc., A.R.I.C. Pp. x + 141. London: George Newnes Ltd. 1958. Price 21s.

A book on "Water Treatment" containing only 132 pages of text, including numerous diagrams, and in which 46 pages are devoted to "Chemical and Physical Estimations," cannot be expected to deal comprehensively with either of these entirely separate subjects. Treatment must inevitably be superficial, and for this reason the book cannot be whole-heartedly recommended to industrial users, public health officials and swimming bath superintendents, for whom, in a preface, the authors state that it is intended. Busy men whose work is not primarily concerned with water treatment usually consult text-books on this subject for guidance on some individual problem; they are more likely to obtain it from specialised articles in a good dictionary of applied chemistry than from this book. This is not to say that the text is without merit; approximately 12 pages are concerned with disinfection of water by chlorine, for example, and most modern methods of applying chlorine to water receive mention. But even on this subject, which is the one accorded fullest description, the authors have reproduced several diagrams derived from commercial sources that really contribute nothing at all to an understanding of basic principles of chlorination, and are not likely to be of practical use to an engineer. Indeed, throughout the entire book the illustrations appear to have been included more with the object of filling out the pages than for their intrinsic value in elucidating the text; the reviewer at least can find no useful purpose in a full-page plate of a Lovibond Nessleriser or a photograph of a chlorine residual controller.

There seems to be a convention that any book on the subject of water must once again reproduce more or less adequate directions for determining free and saline ammonia, oxygen demand, total solids and the rest. This book is no exception, but the chapter on Chemical and Physical Examinations does not contain anything that has not already been published in specialised works, and the selection of some of the methods of analysis will not always be approved by analysts experienced in this work.

Perhaps it is the title of the book that is too ambitious; as a first introduction to the subject of water treatment it could be recommended.

J. G. SHERRATT

BIOLOGICAL METHODS FOR THE EVALUATION OF RODENTICIDES. By E. W. BENTLEY, B.Sc., Ph.D. *Technical Bulletin* No. 8. Pp. iv + 35. London: Her Majesty's Stationery Office. 1958. Price 3s.

The title accurately describes the contents of this booklet. No attempt is made to discuss or compare the relative merits of rodenticides. Essentially, it is a review of the relevant literature on the testing of rodenticides in the laboratory and the field and as such is a source of useful information. The author emphasises the need for constant care in watching the effects on palatability of such factors as particle size or staleness in the base used for the poison. He regrets the lack of interest in tests for the efficiency of poisons against house mice. There are some hints on handling wild rats and a cogent warning on the dangers of infection from the bites of these animals. An alternative explanation for so called bait shyness is offered.

However, a technical bulletin of this kind should contain more precise recommendations for procedures to be used. As it comes from an acknowledged authority in this field, many readers would probably like to know such things as: the statistical method Dr. Bentley finds most convenient; how long does he watch his rats in an acute test (p. 9); what is his idea of a "toxicity of the right order" (p. 11); what impresses him in a test for palatability (p. 13). He criticises a technique suggested in a World Health Organisation specification, but does not provide evidence that the double test he recommends in its place is any more efficient. To the reviewer there seemed to be comparatively little on the technique of field trials compared to their obvious importance in evaluating rodenticides. The photographs could be much better.

The scientific detachment with which this booklet is written together with the 40 references to the scientific literature will certainly commend it to those seeking ways of improving methods currently in use for testing rodenticides. J. M. BARNES

AN INTRODUCTION TO ELECTRONIC THEORIES OF ORGANIC CHEMISTRY. By G. I. BROWN, B.A., B.Sc. Pp. viii + 209. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 15s.

Chemists who started their professional career before terms like σ and π bonds, mesomerism, bond order and resonance energy came into everyday usage will welcome this very simple but up-to-date introduction to such topics as covalent and ionic bonds, the structure of benzene, substitution in the benzene ring, and the mechanism of sundry organic reactions.

H. IRVING

TABLES OF INTERATOMIC DISTANCES AND CONFIGURATION IN MOLECULES AND IONS. Scientific Editor: L. E. SUTTON, M.A., D.Phil., F.R.S. Special Publication No. 11. Pp. 391. London: The Chemical Society. 1958. Price 42s.; $6.00.

This profusely illustrated and documented monograph details the "vital statistics" of more than thirteen hundred inorganic and organic compounds. At 42s., anyone who needs to know about the size and shapes of molecules can readily afford a personal copy of this scholarly work.

H. IRVING

Publications Received

ANALYSE DER FETTE UND FETTPRODUKTE EINSCHLIESSLICH DER WACHSE, HARZE UND VERWANDTER STOFFE. Volume I: Allgemeiner Teil. Volume II: Spezieller Teil. By Professor-Dr.Dr.h.c. H.P. KAUFMANN. Pp. xx + 1104 (Volume I); xii + 1105 to 1816 (Volume II). Berlin, Göttingen and Heidelberg: Springer-Verlag. 1958. Price DM 248.

THE CHEMICAL ANALYSIS OF FOODS AND FOOD PRODUCTS. By MORRIS B. JACOBS, Ph.D. Third Edition. Pp. xxiv + 970. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1958. Price $13.75; 103s. 6d.

ADVANCED ANALYTICAL CHEMISTRY. By LOUIS MEITES and HENRY C. THOMAS with a chapter on The Absorption of Infrared Radiation by ROBERT P. BAUMAN. Pp. xii + 540. New York, Toronto and London: McGraw-Hill Book Co. Inc. 1958. Price $8.90; 69s.

ANALYTICAL APPLICATIONS OF DIAMINO-ETHANE-TETRA-ACETIC ACID. By T. S. WEST and A. S. SYKES. Pp. 106. Poole, Dorset: The British Drug Houses Ltd. 1959. Price 4s. 6d.

A SHORT GUIDE TO CHEMICAL LITERATURE. By G. MALCOLM DYSON, M.A., D.Sc., Ph.D., F.R.I.C., M.I.Chem.E. Second Edition. Pp. viii + 157. London, New York and Toronto: Longmans, Green and Co. Ltd. 1958. Price 15s.

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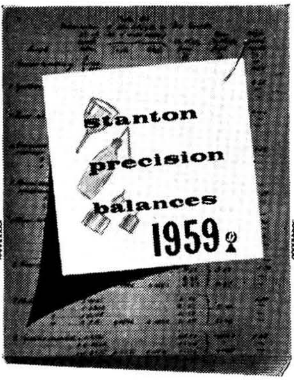
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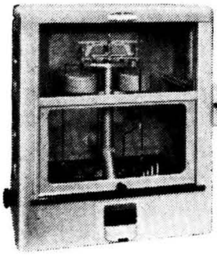

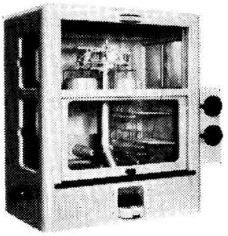

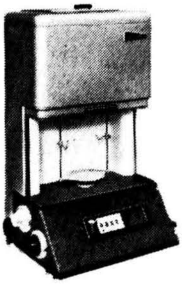

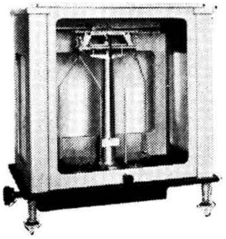

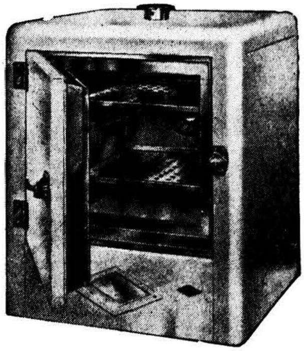

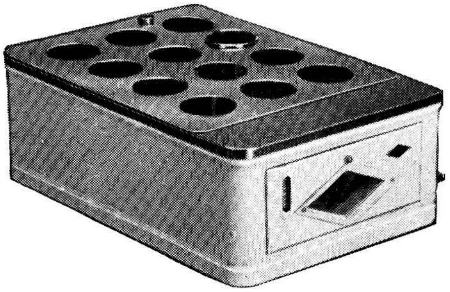

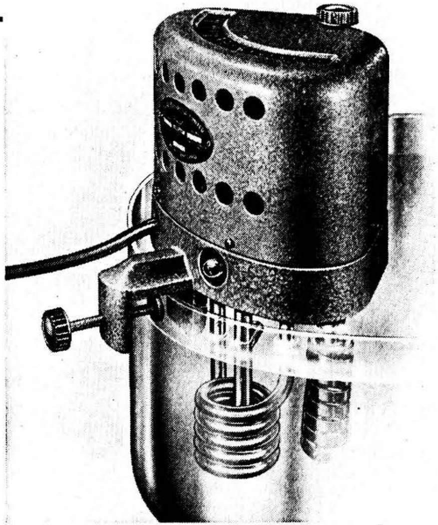

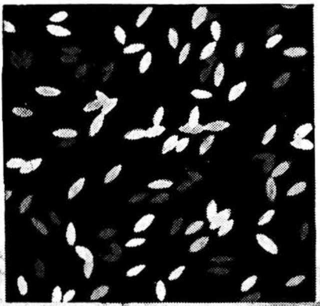

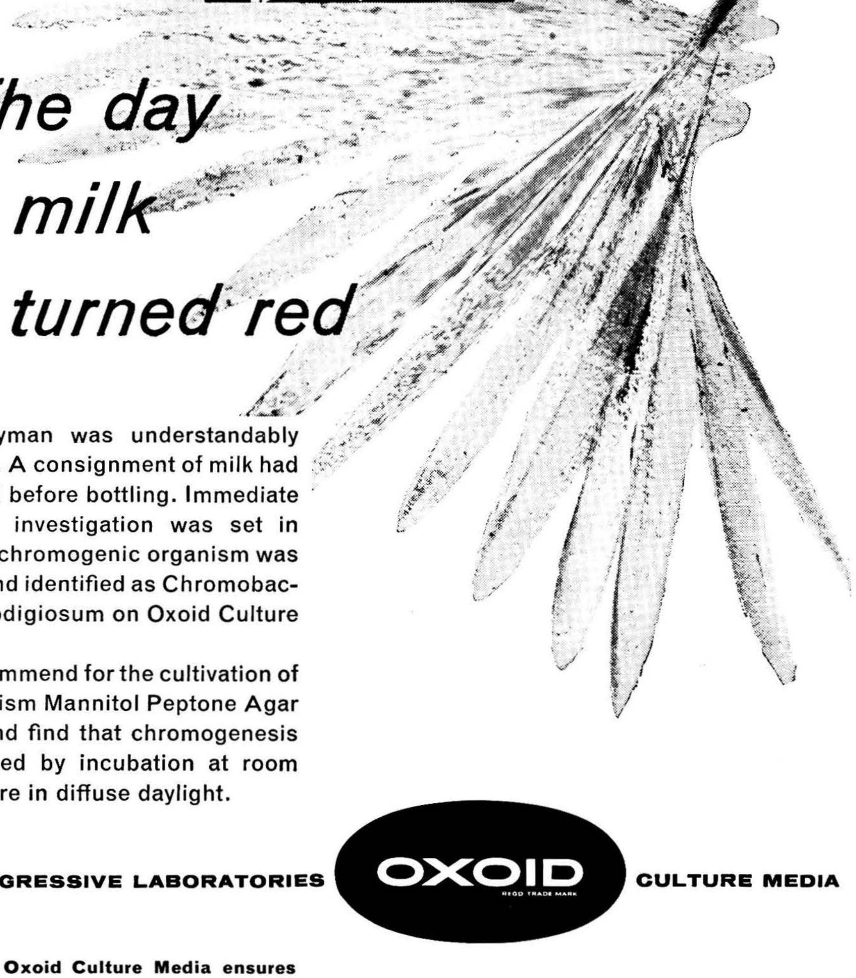

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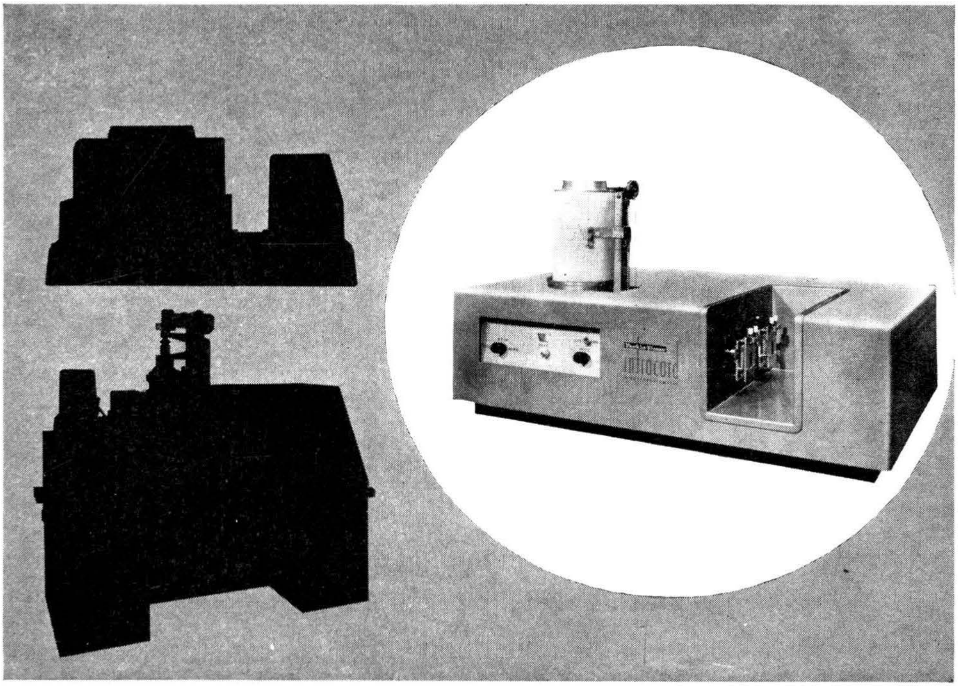

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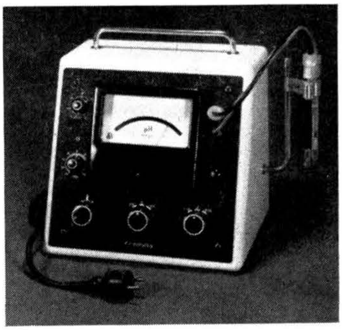

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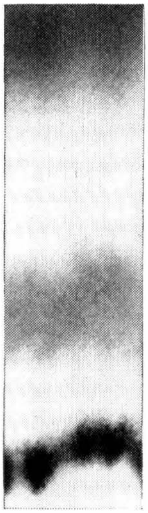

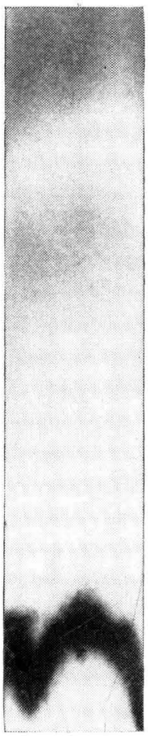

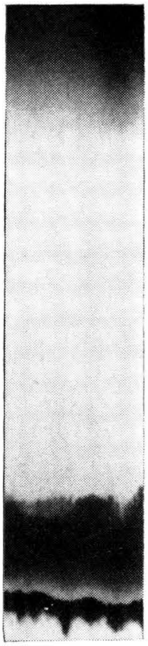

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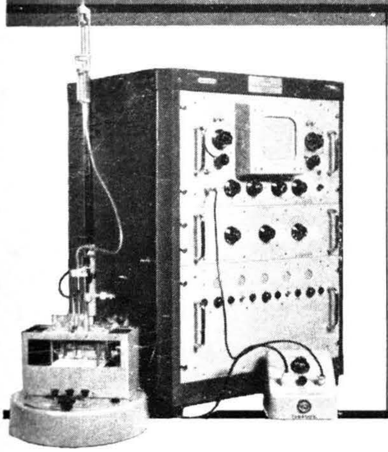

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Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd. Cambridge, England.
Communications to be addressed to the Editor, J. B. Attrill, 14 Belgrave Square, London, S.W.1. Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office