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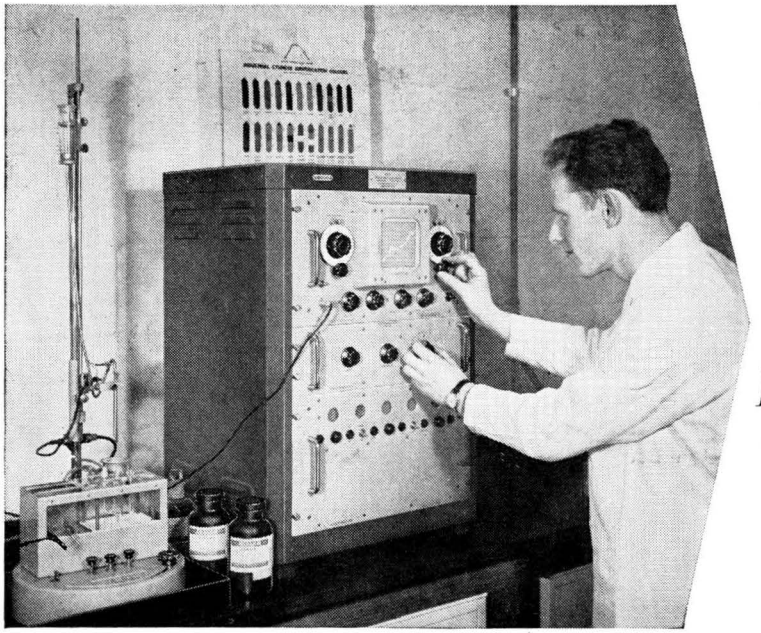

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, November 1st, 1961, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. A. J. Amos, F.R.I.C.

The following papers were presented and discussed: "Precipitation from Homogeneous Solution by Cation Release at Constant pH," by P. F. S. Cartwright, M.Sc., F.R.I.C.; "The Application of Atomic Absorption to the Rapid Determination of Magnesium in Electronic Nickel and Nickel Alloys," by T. R. Andrew, B.Sc., F.R.I.C., and P. N. R. Nichols; "Rapid Identification and Determination of Residues of Chlorinated Pesticides in Crops by Gas - Liquid Chromatography," by E. S. Goodwin, A.R.I.C., R. Goulden, F.R.I.C., and J. G. Reynolds, F.R.I.C.

NEW MEMBERS

Ordinary Members

Richard Hampton Biddulph, M.A., B.Sc. (Oxon.), Ph.D. (Manc.); Cyril Aaron Blau; Jillian Mary Bond, B.Sc., Ph.D. (Bris.); Harold Burnham, B.Sc. (Lond.), A.R.I.C.; William Allan Cregeen, A.M.C.T., F.R.I.C.; James Andrew Walter Dalziel, B.Sc., Ph.D., A.R.C.S., D.I.C., F.R.I.C.; Adrian John Eve, M.Sc. (Rhodes), A.R.I.C.; John Esam Fairbrother, B.Sc. (Hull); Eric Minshall, M.Sc. (Lond.), F.R.I.C.; James Arnold Palgrave, B.Sc. (Dunelm.), F.R.I.C.; Derrick George Porter, B.Sc. (Lond.); Khawaja Salah-ud-din, B.A., M.Sc. (Lond.), A.M.I. Chem.E., A.R.I.C.; Rudolf Schacherl; Edward Alexander Simpson, B.Sc., Ph.D. (Lond.); Elizabeth Margaret Speed, B.Sc. (Southampton); Robert P. L. V. Taubinger, A.R.I.C.; Walter Henry Walker, F.R.I.C.; Raymond Waspe, B.Sc. (Lond.).

Junior Members

John Harvey; John Michael Ottaway, B.Sc. (Exeter); Derek Robinson, M.Sc. (Manc.).

DEATHS

We record with regret the deaths of

Claude Leopold Leszynski Claremont

Alan French.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 2·15 p.m. on Saturday, September 30th, 1961, at the City Laboratories, Mount Pleasant, Liverpool, 3. The Chair was taken by the Chairman of the Section, Mr. J. Markland, B.Sc., F.R.I.C.

The following paper was presented and discussed: "Some Analytical Problems in the Baking Industry," by R. A. Knight, B.Sc., F.R.I.C.

NORTH OF ENGLAND SECTION and PHYSICAL METHODS GROUP

A Joint Meeting of the North of England Section and Physical Methods Group of the Society and the Modern Methods of Analysis Group of the Sheffield Metallurgical Association was held at 7 p.m. on Tuesday, October 10th, 1961, in the Conference Room of the British Iron

and Steel Research Association, Hoyle Street, Sheffield, 3. The Chair was taken by the Chairman of the Modern Methods of Analysis Group, Mr. F. Goodbold.

The following papers were presented and discussed: "Application of Atomic-absorption Spectrophotometry to Metallurgical Analysis," by W. T. Elwell, F.R.I.C.; "Some Interferences in Flame Photometry," by M. S. W. Webb, B.Pharm., F.P.S., F.R.I.C., and P. C. Wildy, B.Sc. (see summaries below).

The meeting was preceded by a visit to the United Steel Companies Research Laboratories, Swinden House.

Application of atomic-absorption spectrophotometry to metallurgical analysis

Mr. W. T. Elwell said that a major problem in metallurgical analysis was the determination of an element in the presence of an overwhelming excess of another, usually the matrix element of the sample. Chemical procedures frequently involved either removal of the major constituent from the element to be determined, or concentration of the latter. Both procedures were often tedious and time-consuming, and not free from inherent shortcomings. Determinations of trace constituents made directly in the presence of the major constituent had advantages, and atomic-absorption spectrophotometric methods were usually applied in this way.

After outlining a description of the equipment used in his laboratory, Mr. Elwell gave details of fundamental considerations in the development of analytical procedures involving this relatively new analytical technique.

He gave examples to illustrate the relative simplicity and reliability of atomic-absorption procedures when applied to the examination of ferrous and non-ferrous materials. Results obtained in this way were then compared with comparable values obtained by conventional analytical procedures, particularly in relation to speed and accuracy.

Some interferences in flame photometry

Mr. M. S. W. Webb said that flame photometry was the familiar technique in which the solution to be analysed was introduced under carefully controlled conditions into a flame possessing sufficient thermal energy to excite the spectra of the elements of interest. The required radiation was isolated, either by a monochromator or a filter system, and the intensity measured by means of a photo-electric detector and recorded on a meter. After careful calibration the intensity of the element line could be interpreted in terms of concentration. Good precision and sensitivity could be attained for many elements provided that care was taken in standardisation.

The chief factor affecting accuracy was the composition of the basic matrix and this could affect the apparent concentration of an element in a sample in many ways.

Optical interference—(*a*) Direct spectral interference was due to incomplete resolution of the analytical and interference lines or bands. (*b*) Interference could be due to a continuous background arising from an interfering element.

Background radiation was normally "backed off" by injecting a suitable d.c. signal into the measuring circuit—the so-called zero control. A far superior method was to use a photomultiplier to monitor the background on either side of the spectral line of interest and to inject the output from this into the measuring circuit. This automatically corrected any background fluctuation and improved stability.

Chemical interference effects—Incomplete dissociation due to the formation of refractory compounds gave rise to serious suppression (*e.g.*, aluminium, phosphate).

Ionic interference effects—Although a sample could be evaporated and vaporised in a reproducible manner, the atoms might be distributed in a variable manner between the ionised and un-ionised state (*e.g.*, mutual enhancement of the alkali metals).

Effect of solution properties—Acids and salts hindered the evaporation of the spray droplets. Surface tension, density and viscosity all affected the mean size of droplets produced by atomisation (Nukiyama and Tanasawa).

Techniques for minimising interference—

(*a*) Standards might be prepared in the same basic matrix as the samples.

(*b*) "Self standardisation" in which the desired element was determined in an aliquot of the solution and further aliquots were "spiked" with known amounts of the element to be determined.
(*c*) Radiation buffers might be used to overcome some ionic interferences.
(*d*) Internal standards might be used to overcome differences in solution properties.
(*e*) The interfering element could be removed completely.
(*f*) In the case of aluminium or phosphate suppression, for example, various cations (*e.g.*, lanthanum, iron) might be added to compete with the suppressing agent. An excess of EDTA or sucrose, for example, which probably caused disruptive decomposition of the dried spray, reduced particle size and resulted in an increased rate of evaporation, thereby relieving suppression.

Woolwich Flame Photometer—This was based on a commercial monochromator and had a burner using premixed oxygen – propane fuel with direct oxygen atomisation of the sample. A filtered air jacket round the burner virtually eliminated atmospheric contamination and improved flame stability.

Both direct reading and integration recording were available. The incorporation of automatic background correction contributed markedly to the sensitivity and stability of the instrument and provision was made for an internal-standard channel. This flame photometer was demonstrated after the lecture; a full description has been given in A.E.R.E. report C/R 2659: "A general purpose flame photometer," by H. M. Davis, G. P. Fox, R. J. Webb and P. C. Wildy.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section of the Society and the Glasgow Section of the Society of Chemical Industry was held at 7.15 p.m. on Friday, October 6th, 1961, in the Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Glasgow Section, Professor J. Monteath Robertson, M.A., Ph.D., D.Sc., F.R.S.

The subject of the meeting was "Pentaerythritol" and the following papers were presented and discussed: "The Applications of Pentaerythritol," by A. W. E. Staddon, B.Sc. (read on his behalf by R. S. McKee, B.Sc., A.R.I.C., A.R.C.S.T.); "Analysis of Pentaerythritol," by A. F. Williams, B.Sc., F.R.I.C.

MIDLANDS SECTION—ELWELL AWARD, 1961

THE Elwell Award for 1961 was presented to M. L. Richardson, A.C.T., A.R.I.C., of J. & E. Sturge Ltd., Birmingham, for his paper entitled "The Determination of Manganese in High Quality Calcium Carbonate by Means of Tetraphenylarsonium Chloride," at a meeting of the Section held at 6.30 p.m. on Thursday, September 14th, 1961, at Regent House, St. Philip's Place, Birmingham, 3. The Chair was taken by Professor R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F.

The following papers, which had been submitted for the award, were also presented: "A Simple Chromatographic Gas Analysis Apparatus," by G. Blakemore; "The Determination of Trace Amounts of Cobalt in Titanium," by J. S. Caslaw.

MIDLANDS SECTION

A JOINT Meeting of the Midlands Section of the Society and the Birmingham and Midlands Section of the Royal Institute of Chemistry was held at 7 p.m. on Tuesday, October 10th, 1961, at the University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Birmingham and Midlands Section, Mr. G. King, M.B.E., M.Sc., F.R.I.C.

A lecture on "Research Work in Analytical Chemistry at the Technical University of Budapest" was given by Professor L. Erdey.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 7 p.m. on Wednesday, October 18th, 1961, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Honorary Secretary of the Group, Mr. K. L. Smith, M.P.S.

The following paper was presented and discussed: "The Use of Bacteriophages in Epidemiology," by Miss E. Maureen Wilson, B.Sc.

Studies in Precipitation from Homogeneous Solution by Cation Release at Constant pH

Part I. Oxidation of EDTA Solution by Hydrogen Peroxide*

By P. F. S. CARTWRIGHT

(Department of Chemistry, Sir John Cass College, London, E.C.3)

Precipitation from homogeneous solution by cation release at constant pH may be achieved by the controlled release of cations from their EDTA complexes by oxidation with hydrogen peroxide. A study has been made of the effects of variations in concentration of hydrogen peroxide and pH of solution on the rate of oxidation of EDTA solution. The presence of certain precipitates has been found to be detrimental, causing rapid break-down of the hydrogen peroxide, thus rendering the method ineffective. This may be overcome by the addition of phosphate ions and the precipitation of metal phosphates.

The object of precipitation from homogeneous solution is to bring about precipitation at a slow, controllable rate from an initially unsaturated solution, resulting in the formation of larger, more perfect crystals, with fewer errors owing to adsorption and co-precipitation. This is achieved by generating the necessary ions by chemical reaction in the solution. The technique has been widely applied to the precipitation of many compounds; much of the earlier work has been collected and described by Gordon, Salutsky and Willard.[1]

The methods employed may be broadly classified as pH increase, anion release, cation release and synthesis of the precipitant *in situ*. Methods of pH increase and anion release have been studied by earlier workers,[1] and methods involving the synthesis of the precipitant are restricted to use with certain organic reagents.[2,3,4] Comparatively few instances have been reported of the use of cation release, in which the cation to be precipitated is first held in solution as a complex and subsequently released by the slow, controlled destruction of the complex in the presence of a suitable anion.

Cation release may be brought about either by changing the pH of the solution to a value at which the complex is no longer stable or by chemical attack of the complex at constant pH. The former has been used for precipitating barium sulphate[5] and silver chloride[6,7] and the latter has been described by MacNevin and Dunton[8] for precipitating hydrated iron oxide and by Gordon, Salutsky and Willard for precipitating hydrous thorium oxide. MacNevin and Dunton obtained dense precipitates of hydrated iron oxide by oxidising an EDTA - iron complex with hydrogen peroxide, but Gordon reported that the precipitates of hydrous thorium oxide obtained by oxidising an EDTA - thorium complex were unsatisfactory from the analytical viewpoint.

MacNevin and Dunton's paper was short and contained few details of experimental work. Thus, although the rate of oxidation of EDTA solution by hydrogen peroxide was studied at various pH values, the effects of variations in pH were not reported. The rate of oxidation of EDTA was said to be slower in the presence of ferric iron and was accompanied by the evolution of oxygen.

It was considered that a more thorough investigation of the method of cation release by oxidation of metal complexes at constant pH would allow the value of the technique to be more fully assessed. The use of EDTA as the complexing agent was of particular interest, since it forms water-soluble complexes with many cations and since the conditions of complex formation and factors influencing complex stability have been widely investigated. Hydrogen peroxide was a suitable oxidising agent, since the products of its break-down—water and oxygen—were not likely to interfere in any precipitation reaction.

To obtain a fuller understanding of the reactions involved it was first necessary to investigate the break-down of EDTA in solution by hydrogen peroxide. This paper describes

* The substance of this paper and other parts of the series was presented at the meeting of the Society on Wednesday, November 1st, 1961.

experiments carried out to determine the effects of variations in pH and in concentration of hydrogen peroxide and the effect of the presence of precipitate particles.

Experimental

Reagents—

EDTA—Disodium ethylenediaminetetra-acetate dihydrate.
Hydrogen peroxide, 100-volume—Analytical-reagent grade.
All other reagents were of analytical grade.

Apparatus—

All volumetric glassware was calibrated before use. During precipitation from homogeneous solution it was essential to use unscratched beakers to avoid losses owing to adherence of the precipitates to the walls of the vessels. All pH measurements were made to the nearest 0·05 unit of pH with a Pye meter having a glass electrode and a calomel reference cell.

Method

1. Variations in pH and concentration of hydrogen peroxide

Reaction solutions consisted of 2 g of EDTA, different amounts of 100-volume hydrogen peroxide and water to a total volume of 300 ml. The pH of each solution was adjusted to the required value by the addition of nitric acid or ammonia solution.

Reactions were carried out in a flask fitted with a reflux condenser and a thermometer to measure the solution temperature. During the reaction the flask was placed in a boiling-water bath to maintain a reaction temperature of 98° to 100° C.

EDTA and the necessary amount of water were placed in the flask, and were brought up to the required temperature; the hydrogen peroxide was then added. Timing of the reaction began when the solution temperature again reached 98° to 100° C. Samples of reaction solution were withdrawn at intervals and cooled, and the amounts of EDTA remaining in solution were determined by titrating with standard bismuth solution, pyrocatechol violet being used as indicator. The effects of variations in pH and concentration of hydrogen peroxide were studied; a blank determination was carried out in the absence of hydrogen peroxide. The results were expressed graphically by plotting the logarithm of the concentration of EDTA remaining in solution against time; straight-line graphs were obtained from which values for K, a constant for the rate of reaction under the conditions of the experiments, were calculated. The variation of K with changes in concentration of hydrogen peroxide and pH is shown in Figs. 1 and 2.

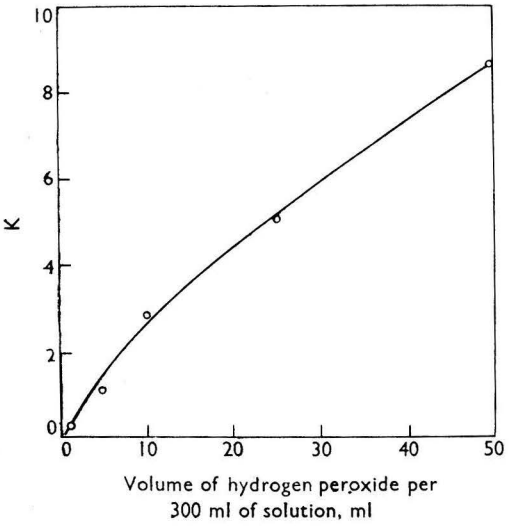

Fig. 1. Effect of variation in hydrogen peroxide concentration

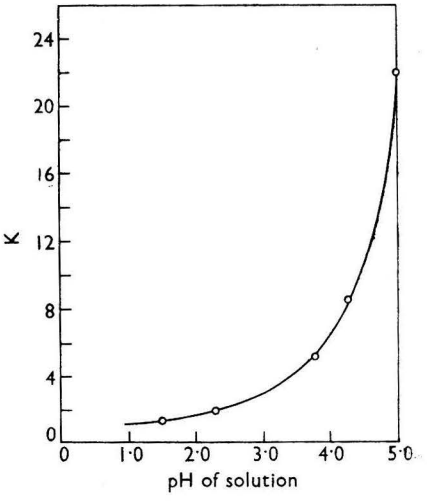

Fig. 2. Effect of variation in pH

2. The effect of precipitate particles

(*a*) HYDRATED OXIDES—

Experiments were carried out to determine the effect on the break-down of hydrogen peroxide solution of particles of precipitated hydrated oxides; precipitates of iron and bismuth were used.

Preparation of precipitates—Hydrated iron oxide was precipitated from homogeneous solution by MacNevin and Dunton's method. Hydrated bismuth oxide was prepared by boiling a solution containing bismuth - EDTA complex (500 mg of bismuth) with 50 ml of hydrogen peroxide at pH 4·0. The precipitates were collected in sintered porcelain filter crucibles, washed with water and dried at 105° to 110° C.

Reaction with hydrogen peroxide—Reactions were carried out under reflux with gentle boiling. Reaction solutions were prepared by diluting 25 ml of hydrogen peroxide to 500 ml with water and adjusting the pH values to 4·5 with nitric acid or ammonia solution. The solutions were gently boiled, and a sample was removed from each to determine the initial concentration of hydrogen peroxide. The required weights of precipitate were then added, and boiling was continued; samples were withdrawn at intervals, and the amounts of hydrogen peroxide remaining in solution were determined by titrating with standard potassium permanganate solution. A blank determination was carried out in the absence of precipitate particles. The results were expressed graphically by plotting the percentage of hydrogen peroxide remaining in solution against reaction time; from the graphs, the times for 90 per cent. break-down of hydrogen peroxide were calculated, as shown in Table I.

TABLE I

TIME FOR 90 per cent. BREAKDOWN OF HYDROGEN PEROXIDE

Cation present	Weight of precipitate, mg	Time for 90 per cent. breakdown, seconds
Blank	—	2470
Bismuth	7·0	1400
	28·0	750
	100·0	280
Iron	14·0	1820
	32·0	1330

(*b*) BISMUTH PHOSPHATE—

The experiments were repeated in solutions containing bismuth phosphate precipitate at various pH values. A study was also made of the reaction in solutions containing phosphate ions at various pH values in the absence of precipitate, and the effect of variation in concentration of phosphate at one pH value was investigated.

Preparation of bismuth phosphate—Bismuth phosphate was precipitated by boiling a solution containing EDTA - bismuth complex (500 mg of bismuth) and 10 ml of phosphoric acid with 25 ml of hydrogen peroxide at pH 1·0. The precipitate was filtered, washed, and dried at 105° to 110° C.

TABLE II

DECOMPOSITION OF HYDROGEN PEROXIDE IN THE PRESENCE OF BISMUTH PHOSPHATE PRECIPITATE

pH of solution	Weight of precipitate, mg	Break-down after 3000 seconds, %
1·5	100	3·5
1·5	Nil	67·0
4·5	100	5·0
4·5	Nil	63·5

The results were expressed graphically by plotting the percentage of hydrogen peroxide remaining in solution against reaction time, and the percentage break-down of hydrogen peroxide after 3000 seconds was calculated from the graphs. These results are shown in Tables II, III and IV.

TABLE III

DECOMPOSITION OF HYDROGEN PEROXIDE IN THE PRESENCE OF PHOSPHATE ION

pH of solution	Phosphoric acid present, ml	Break-down after 3000 seconds, %
1·2	5·0	5·0
1·2	Nil	>90·0
4·5	5·0	30·0
4·5	Nil	>95·0
6·2	5·0	65·0
6·2	Nil	n.d.

TABLE IV

VARIATION IN CONCENTRATION OF PHOSPHORIC ACID AT CONSTANT pH

Phosphoric acid present, ml	Break-down after 3000 seconds, %
Nil	45·0
0·25	25·0
0·50	23·0
1·00	23·0
2·50	20·0
5·00	3·0

DISCUSSION OF EXPERIMENTAL WORK

OXIDATION OF EDTA SOLUTION—

No attempt was made to derive true specific reaction constants for the reactions, since these were not necessary for this investigation and because insufficient was known about the mechanism of the reaction. The values of K shown in Figs. 1 and 2 provide a convenient method of expressing the rate of reaction, but apply only to the experimental conditions prevailing at the time.

Certain of the results obtained served to confirm the expected behaviour; the investigations were necessary, however, to provide a clearer understanding of subsequent experiments. Thus, the rate of oxidation of EDTA at constant pH was found to increase with increase in concentration of hydrogen peroxide. This agrees with the findings of MacNevin and Dunton, who reported that the rate of oxidation of an iron - EDTA complex could be increased by increasing the amount of hydrogen peroxide used.

The variation in the rate of oxidation of EDTA with change in pH is interesting; it can be seen from Fig. 2 that the rate is little changed over the pH range 1 to 4, but increases rapidly at higher pH values. This fact was found to have an important bearing on the ease of precipitation of certain metals; it will be referred to again at a later stage. During these oxidation reactions there were no signs of vigorous effervescence; some bubbles appeared to originate at imperfections in the wall of the flask, but these only served to prevent the solution from bumping during boiling.

EFFECT OF PRECIPITATE PARTICLES—

The addition of hydrated oxide precipitates caused an increase in the rate of break-down of hydrogen peroxide (see Table I), bismuth being more effective than iron; this was subsequently found to have a serious detrimental effect. In the precipitation of hydrated bismuth oxide, for example, all the peroxide present was destroyed by the first-formed precipitate particles, and further oxidation of the bismuth - EDTA complex ceased.

To study the precipitation of various metals it was necessary to find a method of overcoming the effect of the precipitate on the hydrogen peroxide. Phosphate ions were known to exert a stabilising action on hydrogen peroxide,[9] and consequently the effects of phosphate ion and phosphate precipitate were investigated. The results shown in Tables II, III and IV confirm the stabilising action of phosphate ions and indicate that bismuth phosphate precipitate is not only inert, but also has a slight stabilising power. In the absence of precipitate,

the stabilising action of phosphate ions was found to be more effective in acid solution, whereas at constant pH the effect increased with increasing phosphate concentration.

Thus, by carrying out the reactions in the presence of phosphate ions it should be possible to avoid undue decomposition of the hydrogen peroxide by the precipitate particles, and so to study the effects of complex stability and concentration of reagents on the ease of precipitation of metals. The method was then applied to the precipitation of a number of metals, as phosphates, to determine the effect of change of cation. This work is described in Part II.

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Received *June 12th*, 1961

Studies in Precipitation from Homogeneous Solution by Cation Release at Constant pH

Part II.* Precipitation of Various Metal Phosphates†

By P. F. S. CARTWRIGHT

(*Department of Chemistry, Sir John Cass College, London, E.C.*3)

Previous studies in this field have been extended to cover the precipitation of some metal phosphates by the controlled release of the cations from their EDTA complexes by oxidation with hydrogen peroxide. The effect of metal-complex stability has been investigated. The method has been applied to the determination of bismuth, as bismuth phosphate, but was not found to be suitable for the separation of bismuth from lead.

When cation release is brought about by oxidation of metal - EDTA complexes with hydrogen peroxide, certain precipitates cause excessive break-down of the peroxide, resulting in a slowing down of the reaction and sometimes in failure to obtain complete precipitation.[1] The effect of the precipitates may be overcome by carrying out the reactions in the presence of phosphate ions. This paper describes the application of the method to the precipitation of some metal phosphates to determine the effect of the cation on the ease of precipitation. Subsequently, the method was applied to the determination of bismuth as phosphate.

A number of metals were chosen to cover a range of metal-complex stabilities—

Metal	Bismuth	Iron	Lead	Calcium	Barium
Log k absolute	27·81	25·10	18·04	10·70	7·76

Experimental

Reagents and apparatus—

As described in Part I.[1] Stock solutions were prepared containing approximately 10 g of the individual metals per litre and were used in all precipitation reactions. A solution containing 10 g of EDTA per litre was used when necessary to complex the metal ions.

* For details of Part I of this series, see reference list, p. 697.

† The substance of this paper and other parts of the series was presented at the meeting of the Society on Wednesday, November 1st, 1961.

PROCEDURE—

Aliquots of the stock solutions were transferred to beakers, and sufficient EDTA solution was added to complex the metal ions. Phosphoric acid (10 ml) was added, the solutions were diluted to about 250 ml, and their pH values were adjusted to the required levels. After the addition of 25 ml of hydrogen peroxide, the solutions were gently boiled for 2 hours

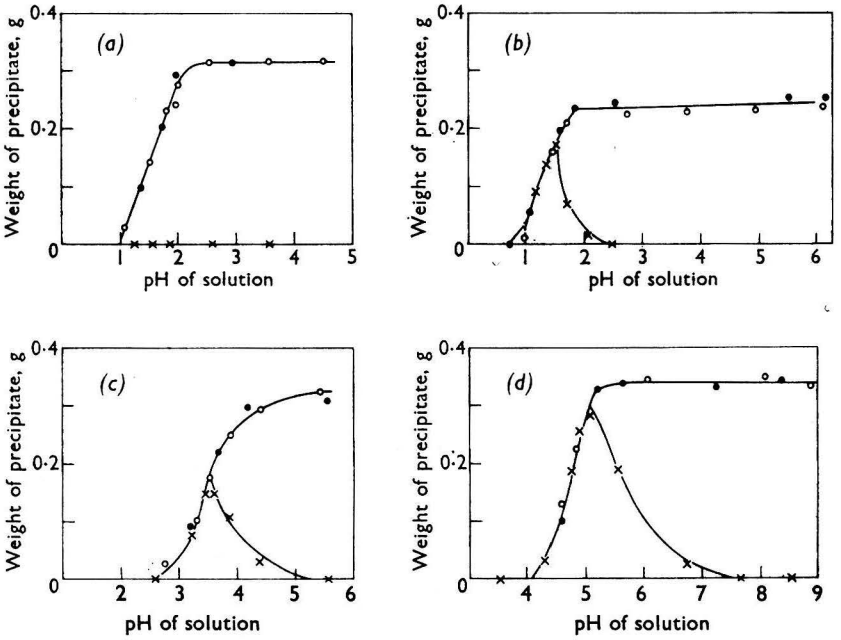

Fig. 1. Precipitation of various metal phosphates: (*a*) iron; (*b*) lead; (*c*) calcium; (*d*) barium. ○, in presence of 25 ml of hydrogen peroxide; ×, without hydrogen peroxide; ●, without EDTA

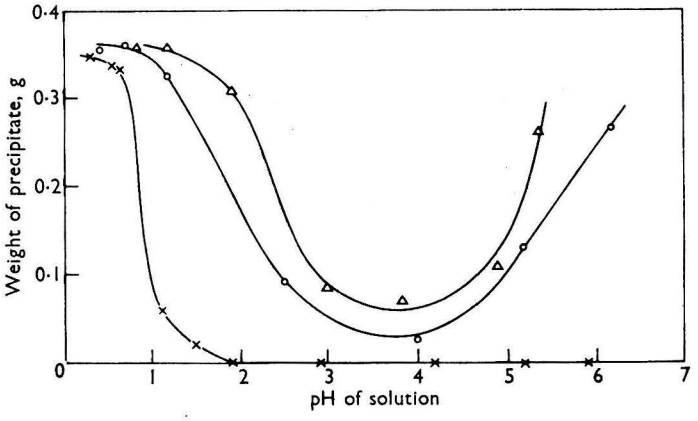

Fig. 2. Precipitation of bismuth phosphate: ○, in presence of 25 ml of hydrogen peroxide; △, in presence of 50 ml of hydrogen peroxide; ×, blank solution without hydrogen peroxide

on a hot-plate, water being added occasionally to make up evaporation losses. The precipitates were collected on weighed, sintered porcelain filter crucibles, washed, and then dried to constant weight at approximately 500° C. Blank determinations were carried out in

which solutions containing metal - EDTA complexes were boiled in the absence of hydrogen peroxide. A second series of blank determinations was performed in which precipitation was carried out in the absence of EDTA to determine the amounts of precipitate obtainable under the conditions of the reactions. The results of these experiments are shown in Figs. 1 and 2, in which weight of precipitate obtained is plotted against pH of solution. For bismuth, the effects of varying the concentration of hydrogen peroxide and prolonging the reaction time were investigated. The results are shown in Fig. 2 and Table I, respectively.

TABLE I

PRECIPITATION OF BISMUTH PHOSPHATE; VARIATION OF REACTION TIME

Complete precipitation ($BiPO_4$) = 363·0 mg

Time, hours	Weight of bismuth phosphate precipitated at— pH 1·9, mg	pH 4·0, mg	pH 5·8, mg
2	308·9	72·1	264·7
4	347·8	166·1	353·4
6	360·8	212·7	359·2
8	n.d.	270·5	n.d.

DISCUSSION OF THE METHOD—

In all instances dense precipitates were obtained, and reactions were not accompanied by undue effervescence. The results obtained with iron, lead, calcium and barium were in agreement with the expected behaviour and will be discussed first. The results with bismuth were anomalous and are discussed later in the paper.

PRECIPITATION BY OXIDATION WITH HYDROGEN PEROXIDE

The results for iron, lead, calcium and barium (see Fig. 1) show that at all pH values investigated the amounts of precipitates obtained by oxidation of metal - EDTA complexes were in good agreement with those obtained by the direct addition of phosphate ion in the absence of EDTA. There appeared to be no difference in the ease with which precipitation occurred, the amounts of precipitate obtained in acid solution being limited by the solubilities of the precipitates under the conditions of the reaction.

PRECIPITATION IN THE ABSENCE OF HYDROGEN PEROXIDE

When solutions containing EDTA complexes of lead, calcium and barium were boiled without the addition of hydrogen peroxide, some precipitation occurred over narrow pH ranges. This was considered to be due to the stabilities of the metal - EDTA complexes being decreased in acid solution to values too low to prevent some precipitation from occurring. This is the basis of methods of cation release in which the complex is broken up by increasing acidity. In more acid solution the weights of precipitate obtained were again limited by their solubilities in the reaction solutions.

The absence of such behaviour for iron was attributed to the iron - EDTA complex still being stable in solutions too acid to permit the formation of iron phosphate precipitate.

PRECIPITATION OF BISMUTH PHOSPHATE

Anomalous results were obtained with bismuth, since, as can be seen from Fig. 2, the weights of precipitate were greatly decreased between about pH 1 to 5. Increasing the amount of hydrogen peroxide resulted in only a slight increase in the weights of precipitate obtained during 2 hours' boiling. At the end of this time, the reaction solutions still contained considerable amounts of undecomposed hydrogen peroxide, so that failuré to precipitate could not be ascribed to lack of oxidising agent. When the time of boiling was extended, more precipitate was obtained, but the results in Table I show that the reaction was not complete at pH 4, even after 8 hours.

It was known that bismuth formed a very stable complex in acid solution. The results obtained in the absence of hydrogen peroxide showed that although the complex broke

down completely below about pH 0·5, the amounts of precipitate decreased rapidly until, at about pH 2·0, no weighable amounts were formed. Over this pH range precipitation was considered to be due to the combined effects of the instability of the complex and the oxidising action of the peroxide when this reagent was present. Above pH 2·0, however, the bismuth complex is stable and any precipitation must therefore be due to the action of the hydrogen peroxide. It was previously shown[1] that the rate of oxidation of EDTA in the absence of metal ions was slow in the region of pH 1·5 and remained little altered until the pH was increased to about pH 4·0; above this value the rate of oxidation increased rapidly with decreasing acidity. Thus, in the precipitation of bismuth phosphate, the rate of liberation of bismuth due to oxidation of the bismuth - EDTA complex remained slow up to about pH 4·0, whereas precipitation due to complex instability decreased sharply with increasing pH; above pH 4·0 the rate of oxidation increased and larger amounts of precipitate were again obtained.

Thus, although most of the metals investigated gave satisfactory results when precipitated as phosphates, the method was somewhat restricted in its application to bismuth. Similar behaviour might be expected with other metals that form stable complexes in acid solution. The method was next applied to the determination of bismuth, as phosphate, and the possibility of separating bismuth from lead was examined.

Determination of bismuth as bismuth phosphate

The precipitation of bismuth phosphate has been studied by Silverman and Shideler,[2] who have reported that the optimum pH range is between 0·5 and 0·7. Below pH 0·5 the results are low, and from pH 0·8 to 2·0 the results are affected by the presence of basic bismuth salts. To test the accuracy and repeatability of the cation-release method, determinations were carried out at about pH 0·7 with solutions of known bismuth content.

Procedure—

A stock solution of bismuth was prepared by accurately weighing about 10 g of metal, dissolving it in 50 ml of nitric acid and diluting to 1 litre. The bismuth content of this solution was checked by precipitation of basic bismuth formate from homogeneous solution.[3]

Table II

Precipitation of bismuth in presence of EDTA

Amount of EDTA present	Bismuth taken, mg	Bismuth found, mg	Bismuth recovered, %
Sufficient to complex the metal	101·2	100·9	99·7
		100·9	99·7
		101·0	99·8
	254·2	254·1	100·0
		254·2	100·0
		254·3	100·0
	508·4	509·3	100·2
		507·6	99·8
		508·0	99·9
Three-fold excess	254·2	254·1	100·0
		251·6	99·0
		252·3	99·3

For each determination the required amount of bismuth solution was transferred to a 400-ml beaker, and sufficient EDTA solution was added to complex the metal. Phosphoric acid (15 ml) was added, the solution was diluted to about 250 ml, and the pH value was adjusted to about 0·7 by the addition of nitric acid. After the addition of 50 ml of hydrogen peroxide, the solution was gently boiled for 2 hours, water being added to make up for evaporation losses. The solution was filtered through a weighed, sintered porcelain filter crucible. The precipitate was washed with water, dried at 105° to 110° C and then heated to constant weight at 600° C. The precipitate was weighed as bismuth phosphate and the bismuth content was calculated.

Determinations were carried out at three levels of concentration, *i.e.*, about 100, 250 and 500 mg of bismuth. The experiments were repeated at the 250-mg level in the presence of a three-fold excess of EDTA. The results are shown in Table II.

DISCUSSION OF THE METHOD—

In the presence of sufficient EDTA to complex the bismuth, the results were in good agreement with those obtained by the basic bismuth formate method. In all experiments the precipitates were dense and readily filtered.

A precipitate containing 250 mg of bismuth settled to a bulk of 1·0 ml in a 100-ml cylinder in 60 minutes, leaving a clear solution, whereas a similar precipitate formed by direct addition of ammonium phosphate in the absence of EDTA settled incompletely to a bulk of 5·0 ml, leaving a cloudy solution.

When a three-fold excess of EDTA was used, the results were slightly low. Some darkening of the precipitates was noticed on heating, suggesting that some adsorbed organic material was present. It was thus evident that too great an excess of EDTA should be avoided.

DETERMINATION OF BISMUTH IN THE PRESENCE OF LEAD

Silverman and Shideler[2] have reported a separation of 0·5 g of bismuth from 0·5 g of lead in a three-stage procedure in which lead was first removed as chloride and bismuth precipitated, first as cupferride and finally as phosphate. More recently, Ross and Hahn[4] have described the precipitation of bismuth phosphate in the presence of lead by hydrolysis of metaphosphoric acid in boiling solution to liberate orthophosphate ions. A double precipitation was necessary to separate bismuth from lead completely.

To test the efficiency of the present method the determination of bismuth was repeated in solutions containing known amounts of bismuth and lead. Precipitation was carried out at pH 0·7 to 0·8; the total precipitate formed was weighed as bismuth phosphate, and the apparent bismuth content was calculated. The results are shown in Table III.

TABLE III

PRECIPITATION OF BISMUTH IN PRESENCE OF LEAD

Bismuth taken, mg	Lead taken, mg	Apparent bismuth found, mg	Apparent bismuth found, %
254·2	500·0	281·6	110·8
		285·5	112·3
254·2	250·0	291·9	114·8
		273·1	107·4

DISCUSSION OF THE METHOD—

The results illustrate a weakness of the technique. Hydrogen peroxide is not selective in its attack on metal - EDTA complexes, so that all metals present are released into solution. Under these conditions the EDTA is of no help in separating metals, but only serves to control the rate of cation release and improve the quality of the precipitates.

To obtain a separation of metals it is necessary that there should be no overlapping of precipitation curves. However, it can be seen from Fig. 1 that the curve for lead phosphate started at about pH 0·75. Since bismuth was precipitated at pH 0·7 to 0·8, some co-precipitation of lead might be expected. The results in Table III show that this did in fact occur. A better separation might have been achieved by precipitating bismuth at pH 0·5, but such a pH value would be difficult to achieve and measure accurately. Improved results would also be expected by carrying out a re-precipitation, but it was considered that no useful purpose would be served by introducing yet another multi-stage separation of the two metals.

CONCLUSIONS

The method has been found to be satisfactory for the precipitation of some metals, as phosphates, although the pH range over which complete precipitation can be achieved may be restricted for metals forming very stable complexes in acid solution.

Single metals may be precipitated provided that the particles cause no undue decomposition of the hydrogen peroxide, but the method was not selective for pairs of closely similar metals. The precipitates so far described have all been dense and readily filterable.

Attention was next given to the effect of the solubility of the precipitate on the ease of precipitation; this work will be described in Part III of this series.

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

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Rapid Identification and Determination of Residues of Chlorinated Pesticides in Crops by Gas-Liquid Chromatography*

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The development of a rapid "sorting test" for identifying traces of chlorinated pesticides in crops by means of gas - liquid chromatography with electron-capture ionisation detection is outlined. In this method, the crop is macerated with acetone and the extract partitioned into hexane before gas - liquid chromatography in nitrogen on a 2-foot column of 100- to 120-mesh kieselguhr supporting 2·5 per cent. by weight of E301 silicone elastomer and 0·25 per cent. by weight of Epikote 1001 maintained at 163° C. Only conventional gas - liquid chromatographic equipment is required, and neither preliminary "clean up" nor concentration of the extract solution is necessary. The seven insecticides lindane, heptachlor, aldrin, Telodrin (1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran), dieldrin, endrin and DDT can be identified when in admixture in fifteen varieties of crops representative of top fruit, leafy vegetables and root crops, the first six insecticides in concentrations generally down to 0·1 to 0·25 p.p.m. and the last generally down to 1 p.p.m. Chlordane, toxaphene and methocychlor can also be identified, but only in rather higher concentration. The procedure, which requires about 50 minutes for a single analysis and 30 minutes for serial analyses, can readily be made fully quantitative and improved in sensitivity for any particular insecticide by adjustment of sample volumes, operating conditions and/or the introduction of an extract "clean-up" stage. Interference present in extracts from grain samples can be resolved by the use of a polar gas - liquid chromatographic column or removed by liquid - solid chromatography. Some indication is given of the considerable potentialities of the technique in allied fields.

THE continued growth in the use of pesticides on edible crops coupled with the increased attention being paid to consumer safety has produced a pressing need for rapid "sorting tests" by means of which residues of these chemicals can be identified and determined. The importance of this need was recognised by the Pesticide Residues in Foodstuffs Sub-Committee set up by the Analytical Methods Committee of the Society for Analytical Chemistry in the commissioning of an investigation by Needham[1] into the use and potentialities of bioassay methods for the determination of pesticide residues in foodstuffs.

As a complement to the bioassay approach, techniques based on the use of gas - liquid chromatography show much promise for this purpose. With katharometer detection, Coulson, Cavanagh and Stuart,[2] Zweig and Archer[3] and also Dimick *et el.*[4] were able to separate, identify and determine mixtures of pesticides or pesticide isomers on the milligram or decimilligram scale. The application of such gas - liquid chromatographic methods to residue analysis on the microgram scale with more sensitive means of detection, *e.g.*, argon ionisation, is made difficult by chromatographic interference resulting from material co-extracted from the crop, unless a "clean-up" stage is included. The need for such a "clean up" was overcome by

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Coulson *et al.*[5] by the use of a gas - liquid chromatographic - combustion - coulometric titration procedure applicable to both chlorinated and thiophosphate pesticide residues and by Zweig, Archer and Rubenstein,[6] who used a gas - liquid chromatographic - infra-red spectrophotometric method.

To avoid the necessity for these somewhat complex combination methods, a simpler means of detection is required that possesses not only great sensitivity but also a high degree of selectivity towards the pesticides to be identified and determined. These requirements are met to a considerable extent by the electron-capture ionisation detector of Lovelock and Lipsky,[7,8] which can be made to exhibit exceptional response to halogenated compounds. This selective response, which is such that nanogram (10^{-9} g) amounts of chlorinated compounds can readily be determined, permits the identification and determination of traces of chlorinated pesticides in crop extracts without the need either for prior "clean up" or for the preliminary concentration of the extract solution.

In an earlier note[9] we indicated that a readily obtainable argon-ionisation detector could be used for electron-capture ionisation detection and outlined the results of some preliminary work on its application to the analysis of crop extracts for traces of chlorinated pesticides. This paper reports the extension of this work, with particular emphasis on the development of a simple rapid "sorting test" for identifying and determining residues of chlorinated pesticides in crops. At the same time, the wide scope of the electron-capture gas - liquid chromatographic technique in the analysis of agricultural, atmospheric and industrial samples for traces of halogenated pesticides is indicated.

EXPERIMENTAL AND RESULTS

A. Development of the "sorting test"

1. Extraction procedure—

In order to make the "sorting test" as rapid as possible and to take full advantage of the speed of the gas - liquid chromatographic stage of the method it was considered essential to employ a quick simple extraction procedure. The use of non-polar - polar solvent mixtures, *e.g.*, hexane - isopropanol or hexane - acetone, was found unsatisfactory in that, with crops containing a high proportion of water, emulsification problems were often encountered and two phases obtained. This resulted in partition of the polar solvent between the aqueous and organic phases; in consequence, the latter had to be completely freed from polar solvent by washing with water before a quantitative aliquot could be taken for analysis. Further, since the extraction time was to be kept as short as possible, it was desirable to use the most effective solvent available. For this reason acetone was chosen as the crop-maceration solvent. The minimum volume consistent with obtaining a fairly fluid macerate was used, the mixture then being filtered and washed with acetone and the filtrate adjusted to volume. Direct gas - liquid chromatography of the acetone extract was, however, found to be impracticable, since the massive amounts of co-extracted crop material present resulted in swamping of the detector, despite its comparative insensitivity to non-halogenated compounds. The insecticide present in the acetone extract was therefore partitioned into hexane in the presence of an excess of 2 per cent. aqueous sodium sulphate solution, much of the interfering co-extracted material being left behind. This process took up little time, since separation of the phases was rapid and the aliquot for analysis could be taken directly from the supernatant hexane layer.

2. Gas - liquid chromatographic procedure—

(*a*) *Apparatus used*—The Shandon Universal Gas Chromatograph employed comprised a U-shaped chromatographic column heated by boiling liquid under reflux and surmounted by an argon-ionisation detector, the signal from which was fed via a d.c. amplifier to a 10-inch chart width recorder (full-scale deflection, 1 mV) having a pen-response time of 2 seconds. The detector was modified only to improve its insulation, this being achieved by substitution of the Sindanyo and silicone-rubber components by polytetrafluoroethylene. This effected a three-fold improvement in detector sensitivity. The range of potentials that could be applied to this detector was extended by the use of high-tension batteries to below the minimum of 300 volts available from the instrument.

(*b*) *Column packings*—In all of this work kieselguhr was used as supporting medium. Initially, columns up to 8 feet long of 60- to 100-mesh material packed in $\frac{5}{32}$-inch internal diameter copper tubing were employed, but the need to eliminate insecticide decomposition and to reduce retention times led to the use of 2-foot (and even shorter) columns of 100- to 120-mesh material. Kieselguhr from several sources appeared equally satisfactory and the use of specially acid-washed material effected no improvement. No advantage was obtained by the use of Ballotini glass micro beads, whether plain, acid or water-washed, in place of the kieselguhr.

The number of stationary phases that can be employed in gas - liquid chromatography at temperatures in excess of 200° C is limited. One of these, which is widely used, is E301 silicone elastomer (obtained from Imperial Chemical Industries Ltd.), which was employed in our earlier experiments at concentrations up to 10 per cent. of the weight of supporting medium. Under these conditions and at even lower temperatures, columns containing the stationary phase mentioned above gave satisfactory chromatography immediately for aldrin, but some conditioning with insecticide was needed (compare Coulson *et al.*[5]) before the chromatography of either lindane or dieldrin could be achieved. This effect was thought to be due to adsorption of these insecticides on to the kieselguhr, and it was for this reason that the glass micro beads referred to above were examined. Stable, involatile polar compounds have been used with success[10,11] for reducing the adsorptive properties of supporting media. The addition to the E301 silicone elastomer of 10 per cent. of its weight of Reoplex 400 (Geigy Co. Ltd.) or Epikote 1001 (Shell Chemical Co. Ltd.) was therefore studied and found to obviate the need for column conditioning. Further, the decomposition encountered during the chromatography of some of the insecticides examined (compare Goodwin *et al.*[9]) was either reduced or eliminated. Of the above two polar additives, Epikote 1001 was found preferable on account of its greater adsorption-suppressing efficiency and thermal stability under the conditions employed. Some tests were carried out in which the whole of the E301 silicone elastomer was replaced by Epikote 1001, but the insecticide separations obtained were much less satisfactory than with E301. As the work developed, the amount of stationary phase employed was progressively decreased. This resulted in shorter retention times and a reduction in the tendency of the stationary phase to "bleed," with its consequent adverse effect on the detector. In most of the later work, 2·5 per cent. of E301 silicone elastomer *plus* 0·25 per cent. of Epikote 1001 on plain 100- to 120-mesh kieselguhr was used.

(*c*) *Carrier gas*—Initially, argon was employed as carrier gas at flow rates of about 6 litres per hour and was dried before use by passage through indicating silica gel. Subsequently, argon was replaced by oxygen-free nitrogen, dried as above, in order to eliminate any residual argon-ionisation response from the detector still obtaining at the low potentials employed. In addition, flow rates were raised to 12 litres per hour, so as to reduce insecticide retention times and also effect some improvement in sensitivity.

(*d*) *Column temperature*—In conformity with earlier published work on the gas - liquid chromatography of chlorinated pesticides, column temperatures in the region of 230° C were employed at first. Under these conditions, some of the insecticides examined, when chromatographed in submicrogram amounts, decomposed with the production of multi-peak chromatograms. Further, decomposition of co-extracted crop material coupled with "bleeding" of the stationary phase resulted in the gradual de-sensitisation of the detector. Lowering of the column temperature helped to overcome these failings, but at the same time resulted in increased retention times, which were counteracted, as already described, by employing faster gas-flow rates and lower concentrations of stationary phase. Column temperatures as low as 163° C were used, this being practicable with compounds having such low vapour pressures as the chlorinated insecticides, provided that the loads injected do not exceed a few micrograms.

(*e*) *Detector characteristics*—The Shandon argon-ionisation detector is a metal-cased detector having a radium D source. When employed in the conventional manner at a potential of 1200 volts and with argon as the carrier gas, positive chromatographic peaks were obtained for both chlorinated and organo-phosphorus insecticides when injected in microgram amounts. As the potential was decreased, the peaks for the chlorinated insecticides became negative, indicating that the detector was exhibiting electron-capture ionisation response. This response increased steadily as the applied potential was decreased to 300 volts, the lowest potential obtainable from the power pack of the instrument. At this voltage

significant peaks were given by nanogram amounts of the chlorinated insecticides. It was under these conditions that much of the earlier work[9] was done, and it is probable that the lack of interference shown by co-extracted crop material on the injection of comparatively large volumes (100 μl) of extract was due in part to the balancing out of any negative peaks owing to electron-capture ionisation response for the non-chlorinated material by the residual positive argon-ionisation effect still obtaining at an applied potential of 300 volts. Subsequently, lower potentials were applied by the use of high-tension batteries, with consequent increase of sensitivity, until a point of maximum sensitivity was reached. After the detector had been cleaned with an abrasive and washed with solvent, the optimum potential for maximum sensitivity was still further reduced (to less than 40 volts), and at the same time an additional increase in sensitivity occurred. This fact, coupled with the replacement of argon by oxygen-free nitrogen, resulted in the elimination of any argon-ionisation effect and necessitated a reduction in the volume of extract injected in order to bring the crop background interference down to an acceptable level. When argon, a potential of 300 volts and injections of 100 μl volume were used, there was a tendency for premature contamination of the column and for drift in the balance point between electron-capture ionisation and argon-ionisation effects due to de-sensitisation of the detector, *e.g.*, by decomposition products coming from the column. Accordingly, it was considered preferable to employ electron-capture ionisation as the sole means of detection.

By using the Shandon detector for this purpose it was found that the amplifier gain control could be set at up to $\times 200$ before the "noise" level became significant. In the "sorting-test" procedure, therefore, the gain was normally set at $\times 50$, so as to give adequate sensitivity and yet leave some amplification in hand. The response of the Shandon detector when used under conditions of electron-capture ionisation showed reasonable linearity over a limited range only, which varied with the insecticide under test. For example, the response of the detector was fairly linear for aldrin in amounts up to about 0·0025 μg, for dieldrin up to about 0·006 μg and for DDT up to about 0·02 μg, equivalent in the present work to about 2, 5 and 20 p.p.m., respectively, on the crop. Under the standardised conditions employed for the "sorting test," loss of linearity became significant when the chromatographic peak due to the insecticide exceeded about 70 per cent. of the full-scale deflection of the recorder. Thus, by setting the gain at $\times 50$, the most effective use could be made of the recorder chart width under conditions of near-linear detector response.

The success of the Shandon argon-ionisation detector when employed as an electron-capture ionisation detector resulted in the construction and study of an argon-ionisation detector of the small diode pattern described by Lovelock.[8] When used at its optimum potential for electron-capture ionisation detection (about 100 volts), this detector, which contained a tritium source, exhibited a sensitivity to chlorinated compounds about ten times greater than that of the Shandon detector. By means of this Lovelock detector, chlorinated insecticides could be determined on the decinanogram scale. Despite its greater sensitivity and lower "noise" level, it was decided for the present work not to employ this detector in place of the Shandon model, since the latter had adequate sensitivity for the purpose in hand and, moreover, had the merit of being typical of detectors readily obtainable commercially.

A brief study was made of the behaviour of the Shandon detector towards organo-phosphorus insecticides when used at the optimum potential for maximum electron-capture ionisation response for the chlorinated insecticides. The sensitivity of the detector to the organo-phosphorus compounds was found to be very much lower than to the chlorinated compounds. For example, the response to parathion was fifteen times lower, to malathion forty times lower and to Phosdrin three hundred times lower than it was to aldrin.

B. Procedure recommended for "sorting test"

1. Instrument preparation—

(*a*) *Column packing*—Weigh out amounts of 100- to 120-mesh chromatography-grade kieselguhr, E301 silicone elastomer and Epikote 1001 to give 2·5 per cent. by weight of silicone and 0·25 per cent. by weight of Epikote on the weight of support. Dissolve the mixed stationary phases in AnalaR ethyl acetate, and add the kieselguhr to the solution. Remove the organic solvent on a bath of hot water, stirring the mixture throughout the evaporation. Sift the dried material, collecting the portion between 100 and 120 mesh. A yield of about 70 per cent. is generally obtained. Fill a 2 foot long $\frac{5}{32}$-inch internal diameter

copper gas - liquid chromatographic column with 4·0 to 4·5 g of the freshly prepared graded column filling, tapping the column repeatedly during the addition in order to achieve uniform and dense packing.

(*b*) *Column temperature*—Maintain a constant column temperature of 163° C by boiling cyclohexanol under reflux in the surrounding vapour jacket.

(*c*) *Carrier gas*—By the use of a soap-film flow meter, prepare a plot of the flow rate of oxygen-free nitrogen through the packed column against the column inlet pressure as indicated on the nitrogen cylinder reducing valve. By using this calibration, adjust the flow rate of nitrogen to a room temperature value of 12 litres per hour. The usual inlet pressure for this flow rate is about 8 lb per sq. inch gauge.

(*d*) *Column pre-treatment*—Condition the new column at 163° C by passing nitrogen through it at 12 litres per hour for some hours before fitting the detector in place. This process avoids contaminating detector parts during the high initial "bleed" of stationary phase.

(*e*) *Control settings*—Set amplifier and recorder zero pre-set controls so that the recorder pen is 2 inches from the normal full-scale deflection side of the chart. Arrange for the chart to run at 24 inches per hour, and set the gain control at ×50. Adjust "backing off" until the pen position coincides with that for amplifier and recorder zeros when the instrument is switched to "READ."

(*f*) *Detector potential*—Potentials are applied to the detector from a battery source connected with its positive terminal earthed. Record the detector response at applied potentials in the range 4 to 40 volts for a constant injection of a standard solution of lindane in hexane. By inspection, select the optimum potential for maximum detector response. In this connection it may be noted that the point of inflexion on the curve produced by plotting "backing-off" readings against applied potential gives an approximate value for the optimum potential for maximum detector response. Some slight drift from the optimum potential occurs with column use. For maximum sensitivity it is therefore advisable to check the optimum position by measurement of the detector response to lindane when the potential is varied a few volts either way.

2. SAMPLE PREPARATION—

From the field sample supplied, weigh a fully representative 50-g sub-sample of the crop into the 200-ml maceration jar of a high-speed top-drive macerator. Chop the crop material into small pieces with a long-bladed knife, and add sufficient redistilled AnalaR acetone just to cover the sample. The volume of solvent normally required is 60 ml, but for dry crops, such as tea and flour, and some bulky materials, such as cabbage, the volume may be increased to a maximum of 80 ml. Macerate the sample for 3 minutes, and then set aside for 5 minutes. Transfer the contents of the jar as completely as possible to a 7-cm diameter No. 3 sintered-glass funnel. Filter under reduced pressure directly into a 100-ml graduated cylinder, and press the crop material as dry as possible by means of a flattened glass rod or small beaker. Wash the crop residue on the filter with acetone so as to make the filtrate up to 100 ml. Remove the filter, insert the stopper in the cylinder, and shake well.

By pipette, place 5 ml of the acetone extract into another 100-ml graduated stoppered cylinder. Add 10 ml of redistilled laboratory-reagent grade n-hexane by pipette, and mix thoroughly by swirling. Measure 85 ml of a 2 per cent. aqueous solution of sodium sulphate into the cylinder, insert the stopper, and shake vigorously for 30 seconds. Allow the two phases to separate, then remove 5 μl of the supernatant hexane layer by means of a 10-μl fixed-needle Hamilton syringe pipette.

3. GAS - LIQUID CHROMATOGRAPHY OF SAMPLE—

Check and, if necessary, adjust the gas-flow rate, column temperature and instrument control settings. Start the chart drive, and inject the 5-μl sample via the silicone-rubber septum at the inlet to the column. After about 22 minutes, stop the chart drive. Examine the chromatogram in comparison with one obtained from a control crop (if available) processed under identical conditions. When peaks are obtained compare their retention times with those obtained for mixtures of chlorinated technical insecticides run under identical chromatographic conditions.

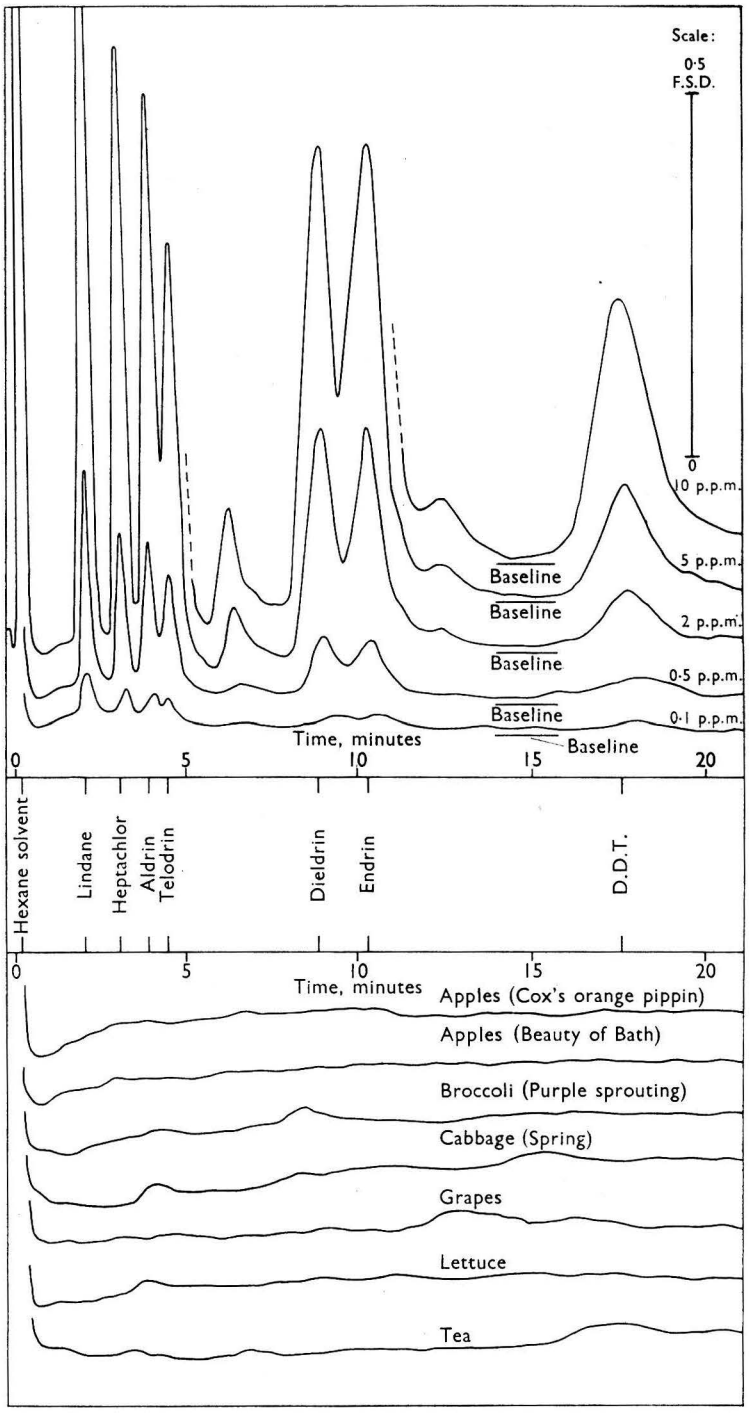


Fig. 1. Chromatograms of technical-grade insecticide mixtures and control-crop extracts

C. Results obtainable by "sorting test"

1. Chromatograms of insecticides against control crops—

Control samples of the crops listed below, taken as being representative of top fruit, leafy vegetables and root crops, were processed according to the above procedure and in all instances gave chromatograms in which the level of background interference was acceptably low—

Apples (Blenheim)	Cabbage (spring)	Lettuce
Apples (Cox's orange pippin)	Cabbage (winter)	Potatoes
Apples (Beauty of Bath)	Carrots (English)	Swedes
Broccoli (purple sprouting)	Carrots (Italian)	Tea
Broccoli (spring heading)	Grapes	Tomatoes.

Examples of these control chromatograms are shown in Fig. 1, together with the chromatograms produced by a mixture of the technical insecticides lindane, heptachlor, aldrin, Telodrin,* dieldrin, endrin and DDT in amounts equivalent to 0·1, 0·5, 2·0, 5·0 and 10·0 p.p.m. on the weight of the crops. As would be expected, the peak heights given by the insecticides decrease as their retention times increase. With lindane, for example, 0·1 p.p.m. of insecticide is readily detectable in the crops shown, full-scale deflection of the recorder being obtained when about 1·5 p.p.m. are present. With dieldrin, on the other hand, the minimum amount generally detectable in the "sorting test" is about 0·25 p.p.m., full-scale deflection of the recorder being obtained when about 8 p.p.m. are present. The general limits of detection for the seven insecticides in the above-mentioned fifteen crop varieties are given in Table I. Sometimes, however, the control chromatograms are such that the limit of detection for one of the insecticides may be less satisfactory, *e.g.*, aldrin in spring cabbage and DDT in tea.

Table I

General limits of detection of seven technical insecticides in apples, broccoli, cabbage, carrots, grapes, lettuce, potatoes, swedes, tea and tomatoes, by the standard "sorting-test" procedure

Insecticide	Lindane	Heptachlor	Aldrin	Telodrin	Dieldrin	Endrin	DDT
General limit of detection, p.p.m.	0·1	0·1	0·1	0·2	0·25	0·25	1·0

In the insecticide chromatograms shown in Fig. 1 a further two small peaks are evident, particularly so at the higher concentrations. The first of these peaks, which has a retention time of 6·3 minutes, is due to heptachlor epoxide. This epoxide, which is not the biological metabolite, was present in the technical heptachlor used in this work and could be isolated from it by liquid - solid chromatography on Florisil. The second peak, having a retention time of 12·2 minutes, is due to *op'*-DDT present in the technical DDT employed.

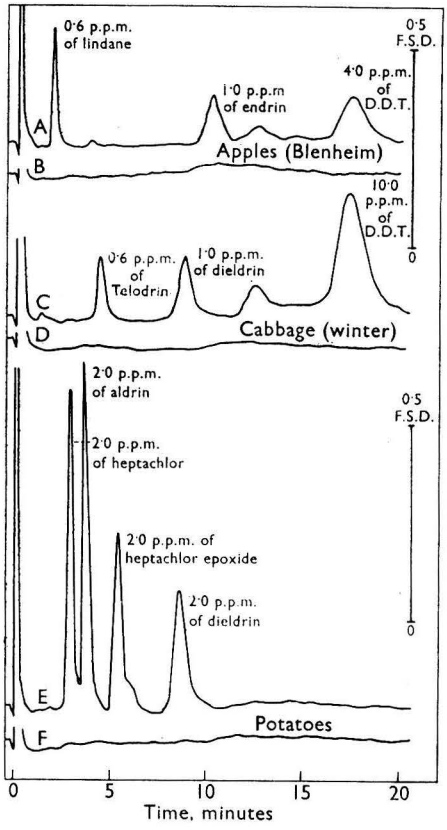
In addition to the seven insecticides referred to above, chromatograms were run on technical samples of chlordane, methoxychlor and toxaphene. The first of these gave a well defined chromatogram having eight peaks with retention times ranging from 1·6 to 12·8 minutes. Two of the peaks had retention times not far removed from that of heptachlor, but of only about one-tenth the sensitivity of the latter. Methoxychlor produced only one major peak of 35-minutes retention time and one minor peak of retention time between that of lindane and heptaclor, but had a peak sensitivity of only about one fifth that of chlordane. Toxaphene gave a multi-peak parabola of equally low intensity, having a retention time up to about 20 minutes.

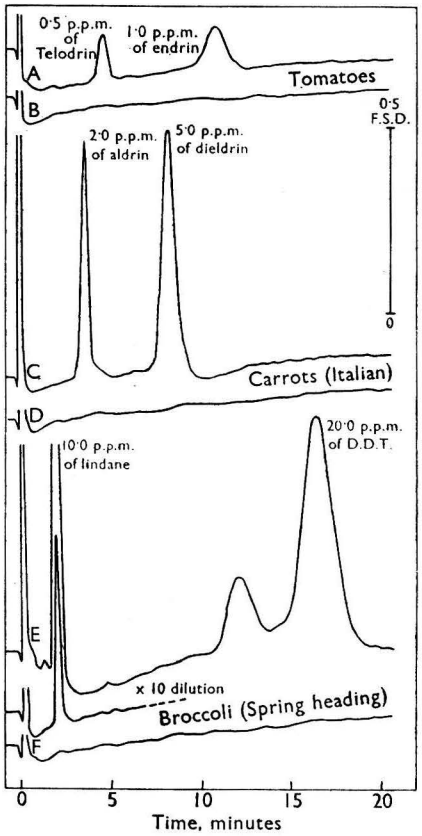
2. Typical "sorting-test" results—

Known amounts of three or four technical insecticides in acetone solution were added at the maceration stage to samples of apple, cabbage and potato, which were then processed as described. The chromatograms produced, which are shown in Fig. 2, indicate the kind of results generally obtained by the use of the standard "sorting-test" procedure. In the test on apple, shown in Fig. 2A, the smaller peak, of 12·3-minutes retention time, is due to the *op'*-DDT present in the technical DDT used. This peak is shown again, more clearly, in Fig. 2C in the test on cabbage. In the treated potato sample (Fig. 2E) the heptachlor

* Telodrin is the Shell Trade Mark name for 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

epoxide added was the biological metabolite, not the one present in the technical heptachlor. The two epoxides have different retention times and are thus readily distinguishable from each other and from the heptachlor itself.





Figs. 2 and 3. Chromatograms of toxicant-treated and control crops obtained by using the standard "sorting-test" conditions

3. Quantitative aspects—

When, in the "sorting test," chromatographic peaks due to insecticide are less than about 70 per cent. of the recorder full-scale deflection, it is possible to make a rapid approximate assessment of the amount of insecticide present. When peaks exceed 70 per cent. of the recorder full-scale deflection, with the result that detector response is no longer reasonably linear, a repeat injection is necessary before making any quantitative assessment. This repeat injection is best carried out with a 5-μl sample after appropriate dilution of the hexane extract. Alternatively, a smaller sample (say down to **1** μl) can be injected, but this leads to reduced precision. Conversely, when peaks are very small, a higher gain setting can be employed, a larger sample—say up to 25 μl—can be injected or a bigger aliquot of the acetone extract can be partitioned into the hexane. Success with any of these procedures depends on the level of crop-background interference which obtains.

For quantitative work, measurement of peak areas was found, as expected, to give more reproducible results than measurement of peak heights. In the preparation of calibration graphs, the use of different volumes (4 to 40 μl) of a single standard insecticide (generally 0·1 μg per ml) solution is preferred to a fixed volume (*e.g.*, 5 μl) of several standard solutions, since preparation of standards in the latter method can be time-consuming when many insecticides are being studied. At the same time, it must be borne in mind that, with the smaller Hamilton micro-syringe pipettes, significant over-injection occurs owing to the

"flashing off" of a portion of the solution contained in the syringe needle. As an example, in this work the over-injection on a 5-μl sample in the 10-μl fixed-needle syringe pipette is $+0{\cdot}4$ μl. The use of a standardised injection procedure makes this increment fairly reproducible, so that allowance for it can be made when the syringe is filled.

An indication of the recoveries obtainable by the standard "sorting-test" procedure was obtained in the following experiment. Known amounts of pairs of insecticides in acetone solution were added at the maceration stage to 50-g samples of control tomato, carrot and broccoli, which were then processed and analysed as described. The areas under the peaks in the chromatograms, which are shown in Fig. 3 (A, C and E), were determined, and the recoveries were calculated from the calibration graph resulting from the direct injection of known amounts of the insecticides on to the column. The results obtained are shown in Table II. For lindane and DDT in broccoli, two chromatograms were run, one under the standard conditions to determine DDT and the other after ten-fold dilution of the hexane extract to determine lindane.

TABLE II

RECOVERIES BY THE STANDARD "SORTING-TEST" PROCEDURE OF PAIRS OF TECHNICAL INSECTICIDES ADDED AT THE MACERATION STAGE TO THREE CONTROL CROPS

Crop treated	Insecticide added	Amount present, p.p.m.	Amount recovered, %
Tomato	Telodrin	0·5	90
	Endrin	1·0	93
Carrot	Aldrin	2·0	78
	Dieldrin	5·0	77
Broccoli	Lindane	10·0	80
	DDT	20·0	83

4. GENERAL OBSERVATIONS—

At the comparatively low temperature of 163° C and with the small loadings used in the "sorting test," column life can be up to several weeks. Detector performance, on the other hand, falls off more rapidly, owing to progressive contamination resulting from a slight "bleed" of stationary phase or crop-decomposition products. This deterioration in sensitivity is due to a drift in the optimum potential for maximum sensitivity to a higher value. When this deterioration becomes serious, high performance can be restored by a combination of abrasive cleaning and solvent washing of the detector.

With careful standardisation of the "sorting-test" conditions, insecticide retention times are generally reproducible to within ± 2 per cent. As might be expected, slight increases in retention times do, however, take place when larger (*e.g.*, 25 μl) aliquots of the hexane extract are used. Reference chromatograms should therefore be run under identical volume-injection conditions.

The time required to carry out one "sorting-test" analysis from receipt of a representative field sample to semi-quantitative assessment of results is about 50 minutes. Of this time about 20 minutes is required for the gas - liquid chromatographic stage, which requires no supervision. In consequence, serial analyses can be completed in about half an hour.

D. EXTENSION OF METHOD TO INCREASE SCOPE

1. TREATMENT OF CROPS SHOWING CHROMATOGRAPHIC INTERFERENCE—

On examining control grain samples by the "sorting test," sharp interference peaks having retention times of 4·2 to 4·3 minutes were observed in the chromatograms. This interference was found present in wheat, oats, barley, maize (slight only) and rice. The rice also gave a large peak at 1·0 minutes; a sample of control onion gave a peak at 0·7 minute. These two last-mentioned peaks are, however, of no serious consequence in that they occur well before the most volatile of the insecticides studied (lindane) appears. The interference peaks appearing in the grain samples at about 4·25 minutes, on the other hand, could be confused with aldrin. Two methods of resolving this problem were examined.

(*a*) *Two-column gas - liquid chromatography*—In the "sorting-test" procedure, gas - liquid chromatography is effected on an essentially non-polar stationary phase, with the result that compounds are eluted in order of volatility. By use of a polar stationary phase, however, the order and times of elution become more dependent on the polarities of the compounds

being chromatographed. Thus, in this instance, a 5-μl aliquot of the control oat sample was chromatographed on a 2-foot column of 100- to 120-mesh kieselguhr supporting 2·5 per cent. by weight of Epikote 1001 maintained at 188° C by refluxing propylene glycol. Chromatograms from both columns were also obtained from the oat sample after the addition at the maceration stage of 0·25 p.p.m. of aldrin. The results obtained, which are shown in chromatograms A, B, C and D in Fig. 4, indicate that, although on the standard non-polar column the oat-interference peak is indistinguishable from aldrin, on the polar column complete resolution is effected.

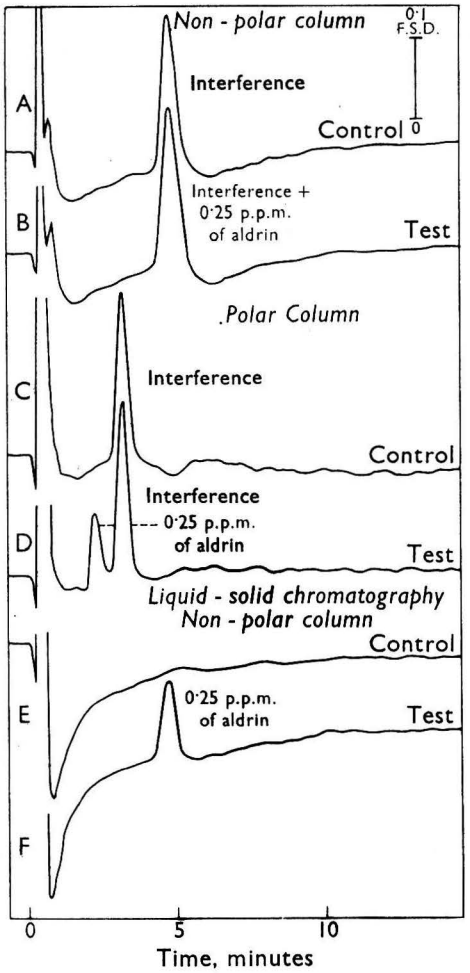


Fig. 4. Chromatograms of control and aldrin-treated oats

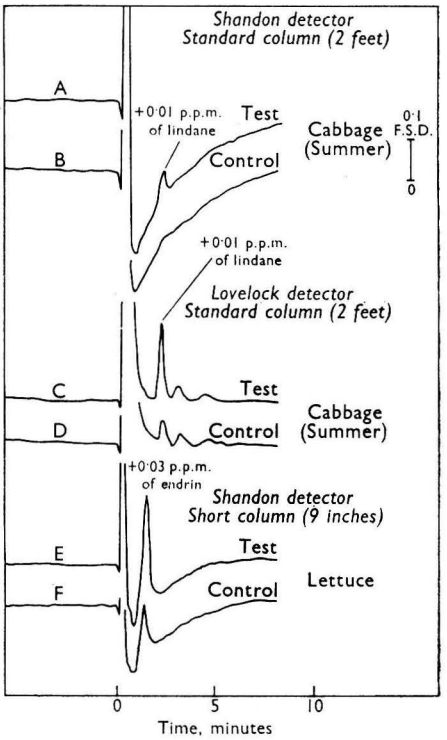


Fig. 5. Chromatogram of crops treated with toxicants at low concentrations

(*b*) *Liquid - solid chromatography*—Differences in compound polarity form the basis of liquid - solid (adsorption - elution) chromatography. Accordingly, 5-ml aliquots of the hexane extracts of the two above-mentioned samples were chromatographed on columns 6 cm long by 1 cm bore containing 5 g of Brockmann grade I/II alumina of about 1 per cent. adsorbed moisture content and eluted with n-hexane until the eluates reached a volume of 25 ml. Gas chromatography of 25-μl aliquots of these eluates on the standard non-polar column at 163° C gave the chromatograms E and F shown in Fig. 4. From these it can be seen that liquid - solid chromatography on alumina has eliminated from the control sample the interference peak at 4·25-minutes retention time and that, in the chromatogram of the treated sample, only the peak due to aldrin remains. Recovery of the insecticide put through this "clean-up" process was found to be quantitative.

2. PROCEDURES FOR MAXIMUM SENSITIVITY—

When small amounts (*e.g.*, <0·1 p.p.m.) of insecticide have to be detected, the level of chromatographic interference due to the crop must be reduced, *e.g.*, by liquid - solid chromatography, and more sample must be used in the analysis, as shown in the following example.

Summer cabbage (50 g) was treated at the maceration stage to contain 0·01 p.p.m. of lindane and processed to give a 100-ml acetone extract. A 20-ml aliquot of this was partitioned into 10 ml of n-hexane, a 5-ml portion of which was chromatographed on a calibrated 5-g column of alumina with hexane - 1 per cent. acetone solution; a 15-ml eluate fraction was taken. Aliquots of 50 μl of this solution were then gas-chromatographed on the standard 2-foot column. In the first instance the Shandon detector was used with a gain-control setting of $\times 100$ and later the Lovelock detector already referred to was employed with a gain-control setting of $\times 20$. The gas chromatograms obtained, which are shown in Fig. 5 (A, B, C and D), indicate that, although some control-crop interference at the retention time of lindane is still evident after liquid - solid chromatography, the presence of 0·01 p.p.m. of lindane in the crop is clearly visible.

For insecticides having longer retention times, increase in sensitivity can be achieved by the use of a shorter column supporting less stationary phase and by the employment of a faster gas-flow rate. A lettuce sample was treated to contain 0·03 p.p.m. of endrin and then processed and "cleaned up" as described above. Gas - liquid chromatography was carried out on a 9-inch column of 100- to 120-mesh kieselguhr supporting 1 per cent. of E301 silicone elastomer and 0·1 per cent. of Epikote 1001. The nitrogen flow rate employed was 21 litres per hour. With the Shandon detector and a gain-control setting of $\times 50$, the chromatograms E and F shown in Fig. 5 were obtained, which indicate that endrin is readily detected at the 0·03 p.p.m. level, despite the presence of some crop interference, and is eluted from the column in 1·5 minutes.

GENERAL DISCUSSION AND CONCLUSIONS

Some of the limitations in the use of bioassay techniques for the screening of foodstuffs for the presence of pesticide residues have recently been discussed by Chilwell and Hartley,[12] who observed that the only serious attention that had been given to the screening aspect of residue analysis was the possibility of identification by dual-system paper chromatography,[13] so far most completely developed for the chlorinated hydrocarbons. Notwithstanding its shortcomings, a bioassay method involving, say, house-flies, fruit-flies or mosquito larvae constitutes at the present time the most potentially useful type of "catch-all" screening procedure. After preliminary screening by such a method, those edible crops exhibiting toxicity must be examined for the identification and determination of the pesticidal residues present. These residues will generally be either organo-phosphorus compounds or chlorinated hydrocarbons. For the latter, gas - liquid chromatographic methods of analysis show much promise and have the advantage over paper-chromatographic procedures of being both rapid and quantitative.

The chief merits of gas - liquid chromatography with electron-capture ionisation detection over other gas - liquid chromatographic methods for determining chlorinated pesticide residues are that it requires no ancillary equipment to effect selectivity, no preliminary concentration stage and, in general, no "clean up" of the extract solution. These factors result in both simplicity and speed. The electron-capture gas - liquid chromatographic "sorting test" described herein could therefore be a useful supplement to the primary bioassay screen and, when only chlorinated pesticides are involved, might even replace it.

From the results obtained it can be seen that the "sorting test" employed in standard form permits the detection of the six chlorinated insecticides lindane, heptachlor, aldrin, Telodrin, dieldrin and endrin in concentrations down to 0·1 to 0·25 p.p.m. in most of the crops examined. For DDT, however, the general limit of detection is rather higher at 1 p.p.m., although for screening purposes this is not serious in view of the comparatively high tolerance limit normally set for this compound. The limit of detection attainable in the standard procedure is obviously dependent on the nature of any chromatographic interference arising from the crop itself. Experience with the method may show that the majority of top fruit, leafy vegetables and root crops produce satisfactorily low chromatographic backgrounds and that only a few types of foodstuffs, *e.g.*, the grains, require special

treatment. If this proves to be so, then most samples could be analysed by the standard "sorting-test" procedure and the two-column gas - liquid chromatographic technique or a simple liquid - solid chromatographic "clean-up" process applied only to the few crops known to be atypical. If, however, interference *is* exhibited in some samples of a wide variety of crops then the "sorting test" should at least serve as a rapid method of screening samples for the *absence* of chlorinated pesticide residues, all crops showing chromatographic peaks being examined by the above-mentioned supplementary techniques. The preliminary screening could then be made still more rapid, at the expense of individual toxicant identification, by reduction of retention times to, say, 5 minutes over-all.

One limitation to the standard "sorting-test" procedure is that chlordane, toxaphene and methoxychlor would not be detected unless present in comparatively high concentration, the two first-mentioned because of their multi-component nature and the last because of its long retention time under the conditions employed. Of these three insecticides, however, the two last-mentioned have high tolerance limits.

In the quantitative work, recoveries from three crops of six insecticides added at the maceration stage in concentrations ranging from 0·5 to 20 p.p.m. average 80 to 90 per cent. and show good reproducibility for any one crop. The use of an appropriate correction factor would therefore seem to be reasonable. Since experiments have shown that the partition stage of the sample-preparation procedure is over 95 per cent. efficient, the losses encountered may well be due to retention of toxicant by the crops. Nevertheless, a rapid crop-preparation process resulting in reasonably high and reproducible recoveries is considered to be preferable for the present purpose to a longer but more exhaustive extraction procedure. When complete maceration of the sample is unnecessary, then the use of some simpler insecticide-removal process, *e.g.*, the solvent stripping of whole fruit, should effect a further saving in time and result in a substantial reduction in the limits of detection, owing to the comparatively small amount of co-extracted crop material present.

As has been shown, the general limits of detection attainable by the standard procedure can be significantly reduced by introducing a simple "clean-up" stage and by altering such variables as partition and injection aliquots and the conditions of gas - liquid chromatography employed. It should be emphasised here that the standard "sorting-test" procedure is necessarily a compromise method designed to give adequate results for the widest possible range of chlorinated insecticides. It follows that in more limited circumstances, when only one or two insecticides need be sought for, operating conditions can be tailored as appropriate to achieve greater sensitivity in the method. As already indicated, for example, reduction in column length and amount of stationary phase, coupled with increase of gas-flow rate, permit an appreciable lowering in the limit of detection of endrin, as well as a marked reduction in the time required for gas chromatography.

The commercial detector used in this work under conditions of electron-capture ionisation detection shows exceptional response to halogenated compounds. This, Lovelock has recently pointed out,[14] is due to their highly "electrophoric" nature. The limits of detection in the "sorting test" are governed by the relative amounts of pesticide and co-extracted crop material present and by their respective "electrophoric" characteristics rather than by any limitation in the sensitivity of the commercial detector. Full advantage can be taken of the even greater sensitivity of the more recent detectors (see Lovelock[8]) only if the ratio of crop interference to pesticide is reduced. The proposed method appears to be comparatively insensitive to the organo-phosphorus insecticides, since they do not in general possess high electron affinity. One exception to this is parathion, in which the presence of the highly "electrophoric" $-NO_2$ group could explain why its limit of detection is no more than fifteen times greater than that for aldrin.

It will be obvious from the foregoing discussion that gas - liquid chromatography with electron-capture ionisation detection is a technique of some potential value for the identification and determination of traces of halogenated compounds. Evidence of compound identity can be made almost certain by use of the two-column gas - liquid chromatographic technique, whereas the analysis of samples containing more heavily interfering extractables than do crops can generally be effected after the application of suitable "clean-up" procedures. As an example, the method has been used with success for determining traces of chlorinated insecticides in "cleaned-up" extracts of animal, avian and insect tissue. The last-mentioned analysis, made possible only by the ability of the method to determine nanogram amounts

of insecticide in very dilute solution, indicates the potentialities that this technique may have in the study of the mechanisms of insect resistance. In the same way have traces of halogenated insecticides, nematicides and herbicides been determined in soil by the method, which readily permits metabolic conversions such as aldrin → dieldrin or heptachlor → heptachlor epoxide to be followed. Finally, it may be noted that preliminary work indicates that the technique could have much value in the determination of traces of chlorinated insecticides in contaminated atmospheres and in samples as varied as wool, wood, hardboard and plastics.

We thank Mr. I. G. Blackwell for carrying out much of the experimental work outlined, Mr. A. Taylor, of the Infestation Control Laboratory, M.A.A.F., Tolworth, Surrey, for a gift of pure heptachlor epoxide (biological metabolite), Dr. R. A. E. Galley, Director of the Woodstock Agricultural Research Centre, for his interest and encouragement and the Directors of "Shell" Research Ltd. for their permission to publish this paper.

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The Determination of Phenothiazine in Commercial Preparations by Ultra-violet Absorption Spectroscopy

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A rapid chromatographic method has been developed for determining phenothiazine in the technical product and in commercial preparations. Separation is achieved on a column of acid aluminium oxide having a Brockmann activity of approximately III, a mixture of diethyl ether and light petroleum being used as eluting solvent. Phenothiazine is determined by means of the characteristic maximum in its ultra-violet absorption spectrum at 254·5 mμ. The method is thought to be specific for phenothiazine.

The need for an accurate method for the specific determination of phenothiazine in commercial preparations has become apparent with the wide-spread interest in the relationship between particle size and purity on the one hand and anthelmintic efficiency on the other. Several methods have been proposed, but most have the disadvantage that they are not specific. Smith[1] determined phenothiazine as ether-soluble material, and Cupples[2] measured the intensity of the red colour produced by oxidising an ethanolic solution with bromine water. Overholser and Yoe[3] utilised the reaction of phenothiazine with a number of inorganic ions to produce red or green compounds. The nitrogen content of phenothiazine is used as a

method of assay in the British Veterinary Codex.[4] Recently, Gunew[5] described a specific method involving chromatographic separation on a mixed adsorbent of silicic acid and a synthetic magnesium silicate. The phenothiazine was determined by direct weighing and was characterised by its melting-point.

Experimental

Gunew's chromatographic method has the disadvantages that the adsorbents recommended are not readily available outside the United States and that the procedure is time-consuming. It has been found that a similar separation can be achieved on a column of acid chromatographic aluminium oxide. The activity of the alumina is approximately Brockmann grade III, and the eluting solvent is a mixture of diethyl ether and light petroleum (boiling range 40° to 60° C). The phenothiazine is determined specifically by the characteristic maximum in its ultra-violet absorption spectrum at 254·5 mμ. As the impurities in commercial phenothiazine are not known, a series of possible impurities was analysed by the proposed procedure; only one, carbazole, showed positive interference. However, from its mode of formation, it seems unlikely that carbazole will constitute more than a trace impurity in phenothiazine.

The preparation of pure phenothiazine was necessary in order to measure the extinction coefficient. Smith and Nelson[6] purified phenothiazine by repeated recrystallisation and obtained a material melting at 184·2° C. However, by sublimation at 130° C and 1 mm pressure, they obtained a product melting at 185·1° C. Baker and Brickman[7] obtained pure phenothiazine by distilling the technical product with super-heated steam; the melting-point of the product was 183° to 185° C. Lazarus and Rogers[8] obtained pale gold or faint green crystals, melting-point 182° C, by sublimation at 260° C and 25 mm pressure. Gunther and Blinn[9] quote the extinction coefficient of pure phenothiazine, melting-point 185·2° to 186·0° C, at 254 mμ as 43,200 ($E^{1\%}_{1cm} = 2170$); the spectrophotometer used was a Beckman model DU, and 95 per cent. ethanol was the solvent. Houston, Kester and DeEds[10] showed that an ethanolic solution of phenothiazine had an absorption maximum at 254 mμ and that two oxidation products, phenothiazine-5-oxide and phenothiazone-3, did not absorb at this wavelength. Brown, Cole and Crowell[11] purified commercial phenothiazine by recrystallisation from a mixture of acetone and iso-octane and then two vacuum sublimations; the product melted at 185·0° to 185·5° C. They showed that the sharp peak at 255 mμ in chloroform disappeared on oxidation.

The absorption peak at 254 to 255 mμ is characteristic of phenothiazine and is not given by its oxidation products. Pure phenothiazine was prepared by repeated vacuum sublimation until there was no further change in the value of $E^{1\%}_{1cm}$.

Diphenylamine is the only impurity identified as being present in technical phenothiazine. Sulphur may be present, but its existence is doubtful, as excess of sulphur can react with phenothiazine to form polymeric products. The existence of polymeric material was reported by Smith,[1] who showed that the ether-insoluble material was isomeric with, or a polymer of, phenothiazine. About 3 to 4 per cent. of technical phenothiazine is insoluble in hot light petroleum (boiling range 100° to 120° C), and this is probably polymeric. The fate of the iodine normally used as catalyst in the reaction between diphenylamine and sulphur is unknown, but about half the iodine is present in the material insoluble in light petroleum. Oxidation products are certainly present and are known to arise from the oxidation of solutions of phenothiazine.[11] Phenothiazone, thionol and diphenylamine sulphoxide are possible oxidation products. If phenothiazine is heated at 250° C, especially in the presence of copper, carbazole is formed. It is unlikely that carbazole could be separated from phenothiazine by sublimation, but, as the melting-point of the pure phenothiazine agreed with that quoted in the literature, the presence of more than traces of carbazole in the technical product is doubtful.

When phenothiazine is subjected to chromatography on alumina, compounds are adsorbed that show ultra-violet absorption characteristics similar to those of phenothiazine. These compounds are not eluted under the conditions described, but it has not been possible to separate them in amounts sufficient for their identification. Phenothiazine was found by the proposed method to contain 2·5 to 5·0 per cent. of these compounds; however, they may be present in much larger proportions by weight if they do not absorb as strongly as does phenothiazine at 254·5 mμ.

In view of the doubts about the nature of the impurities present in commercial phenothiazine, prepared mixtures of recrystallised phenothiazine (having a purity of 97 per cent.) and a number of suspected impurities were analysed by the proposed method; the results are shown in Table I.

TABLE I

RECOVERY OF PHENOTHIAZINE IN PRESENCE OF ADDED IMPURITIES

Impurity	Composition of mixture: Impurity, % w/w	Composition of mixture: Phenothiazine, % w/w	Phenothiazine found, % w/w
Diphenylamine	10·0	87·3	86·7
Carbazole	10·0	87·3	91·1
Carbazole	100·0	0·0	44·0
N-Acetylphenothiazine	10·0	87·3	87·0
Thionol			87·3
Phenothiazone			87·1
Diphenylamine orthosulphoxide			87·0
Sulphur			87·2

PREPARATION OF STANDARD PHENOTHIAZINE—

Recrystallised phenothiazine, prepared in the laboratory from purified diphenylamine and sulphur, was slowly sublimed at 0·05 to 0·10 mm pressure by means of an infra-red lamp, and the sublimate was re-sublimed until there was no significant change in the value of $E^{1\%}_{1cm}$. The residue from the sublimation melted at 250° C, with decomposition. The successive values of $E^{1\%}_{1cm}$ and melting-point are shown in Table II.

TABLE II

CHARACTERISTICS OF SUBLIMED PHENOTHIAZINE

	Value of $E^{1\%}_{1cm}$	Melting-point, °C
Starting material	2290	185·2
First sublimation	2330	185·8
Second sublimation	2344	185·8
Third sublimation	2350	185·8
Fourth sublimation	2352	186·0
Fifth sublimation	2350	186·0

METHOD

REAGENTS—

Diethyl ether—Analytical-reagent grade.

Light petroleum—Analytical-reagent grade, boiling range 40° to 60° C and free from aromatic hydrocarbons.

Light petroleum - diethyl ether mixture—Mix 3 volumes of the light petroleum and 1 volume of the diethyl ether.

Acid aluminium oxide—Brockmann activity grade I. Aluminium oxide Woelm acid (anionotropic) was used.

Ethanol—Heat 4 litres of "absolute ethanol (special for spectroscopy)" under reflux with 10 g of zinc dust and 40 g of potassium hydroxide pellets for 3 hours; the reactants should be of analytical-reagent grade. Immediately distil the ethanol, and reject the first and final 250-ml portions of distillate. The ethanol for use should have minimum transmission in a 1·0-cm cell at 254·5 mμ of not less than 90 per cent.

PREPARATION OF SAMPLE SOLUTION—

Accurately weigh sufficient sample to contain 250 mg of phenothiazine, and dissolve it in approximately 200 ml of diethyl ether (it may be necessary to heat under gentle reflux on a bath of hot water for 5 to 10 minutes). Cool to room temperature, transfer quantitatively to a 250-ml calibrated flask, and dilute to the mark. Allow any insoluble residue to settle, transfer 25·0 ml of the solution to a 100-ml calibrated flask, and dilute to the mark with light petroleum; a 25-ml calibrated flask is preferred for transferring the ether solution. Aqueous

dispersions of phenothiazine may be treated in the same way, but an amount of anhydrous sodium sulphate sufficient to absorb residual water should be added to the ether solution.

Dilute solutions of phenothiazine, especially when exposed to sunlight, tend to be oxidised, and operations from this stage onwards should be carried out as rapidly as possible. It is best to protect the chromatographic column from direct light by means of a suitable shield.

PREPARATION OF ALUMINIUM OXIDE ADSORBENT—

By pipette, place 5·0 ml of distilled water in a dry 250-ml conical flask that can be closed with a ground-glass stopper. Distribute the water over the glass surface, and add 100 g of acid aluminium oxide of Brockmann activity grade I. Shake the flask on a mechanical shaker for 2 hours or until lumps and moist spots can no longer been seen, and set aside for 48 hours at room temperature before use. The criterion of adequacy of the alumina adsorbent is that all the phenothiazine should appear in the first 50 ml of eluate, as described below.

PROCEDURE FOR DETERMINING PHENOTHIAZINE—

Set up a chromatographic tube having the dimensions shown in Fig. 1 in a stand, and place a small wad of cotton-wool in the lower tip of the tube. Add sufficient of the light petroleum - diethyl ether mixture to fill the 10-cm deep section, and pour 8 g of the alumina adsorbent into the centre of the 10-cm section while tapping the tube gently to ensure uniform settling. Allow excess of solvent to drain away, but ensure that the surface of the alumina remains covered by solvent.

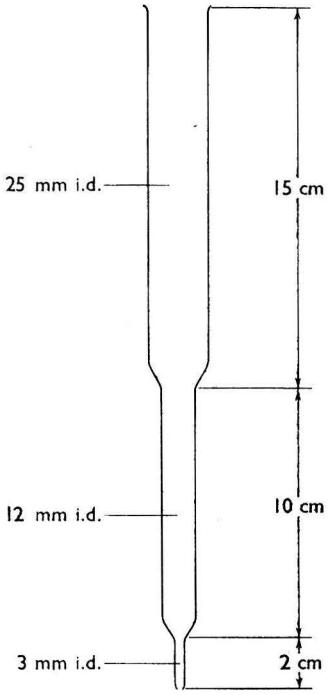

Fig. 1. Chromatographic tube

By pipette, carefully place 10·0 ml of the light peroleum - ether solution of the sample on the column, taking care not to disturb the surface of the alumina, and collect the eluate in a 100-ml calibrated flask. When the surface of the column is nearly exposed, wash the sides of the tube with approximately 5 ml of the light petroleum - ether mixture. Repeat this washing twice more, fill the column with the light petroleum - ether mixture, and collect 50 ml of eluate. Thoroughly wash the tip of the chromatographic tube with the ether

mixture, and collect the washings in the 100-ml calibrated flask. Dilute the contents of the flask to the mark with the solvent mixture, mix thoroughly, place 10·0 ml of this solution, by pipette, in a 100-ml calibrated flask, and dilute to the mark with the spectrographic-grade ethanol.

Measure the optical density of the solution at 254·5 mμ in matched 1-cm silica cells against a solution prepared by diluting 10·0 ml of the light petroleum - ether mixture to 100 ml with spectrographic-grade ethanol. Reverse the cells, again measure the optical density, and use the average of the two readings in the calculation. (For a Unicam SP500 spectrophotometer, a fixed slit width of 0·60 mm is recommended.) Calculate the percentage of phenothiazine in the sample from the value of $E^{1\%}_{1cm}$ for phenothiazine (2350 when a Unicam SP500 instrument is used).

Results

Samples of purified phenothiazine treated by the proposed method gave recoveries from 99·5 to 100·0 per cent. The purity of technical phenothiazine manufactured by two different processes is shown in Table III, in which the term "apparent phenothiazine" refers to results obtained by the proposed method, with omission of the chromatographic stage.

Table III

Results for samples of technical phenothiazine

Sample No.	Apparent phenothiazine content, %	Phenothiazine content, %
Samples manufactured by process A—		
1	90·5	87·1
2	89·8	86·6
3	88·5	86·0
4	88·8	86·5
5	88·4	85·8
6	89·4	86·9
Samples manufactured by process B—		
7	87·4	82·5
8	86·5	83·7
9	88·4	84·3
10	88·0	84·5
11	90·2	85·5
12	88·0	83·7

Conclusion

The method described above has been successfully used for determining phenothiazine in the technical product and in commercial preparations. It appears to be specific for phenothiazine and has been used for the analysis of all commercial forms of phenothiazine so far encountered.

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The Maintenance of Microbiological Assay Organisms as Freeze-dried Cultures

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The possibility of using freeze-dried organisms as inocula for microbiological assays has been investigated. Six assay organisms were freeze-dried and stored at 2° to 4° C for 12 to 16 months.

When unsubcultured freeze-dried cultures were used as inocula and compared with inocula maintained in the standard way, all the organisms investigated gave satisfactory dose-response curves throughout the trial period. Nicotinic acid and thiamine values for dried yeast determined with such inocula also compared favourably with those obtained when standard inocula were used, and the precision and validity of the assays were not affected by use of the freeze-dried organisms.

In assays of thiamine with *Lactobacillus fermenti*, freeze-dried cultures that had been subcultured once before use as assay inocula gave less precise assays; other assays were satisfactory with this subcultured freeze-dried inoculum. However, direct use of freeze-dried organisms has obvious advantages. Maintenance of assay organisms as freeze-dried cultures and direct use of washed suspensions of these cultures as assay inocula are therefore suggested as possible useful modifications of microbiological-assay technique.

Tanguay[1] has pointed out the advantages of maintaining microbiological-assay organisms in a state in which they may be used without subculture. He successfully stored a number of assay organisms for 6 to 12 months at −40° C in M/15 phosphate buffer containing 15 per cent. of glycerol. Deibel, Evans and Niven[2] also used a frozen inoculum for thiamine assay, storing the assay organism, *Lactobacillus viridescens*, in assay basal medium in the freezing compartment of a refrigerator.

Investigation of the possibility of using freeze-dried cultures as assay inocula seemed worth-while, as such a method would have obvious advantages over the above-mentioned methods, *e.g.*, ease of distribution from a standard collection and storage at room or refrigeration temperature. Freeze-dried lactobacilli are fairly stable in character; for example, Sharpe and Wheater[3] demonstrated that no significant change occurred in the physiological and serological characters of forty-one strains of lactobacilli during storage for 6 months as freeze-dried cultures. They also mentioned an observation by Dr. M. F. Gregory that the vitamin-B_{12} requirement of *L. leichmanii* LE2 remained unchanged after the organism had been freeze-dried and stored for 1 month.

This paper reports the results of a number of vitamin assays with freeze-dried inocula that had been stored for up to 16 months.

Methods

Test organisms—

Seven vitamin assays involving use of different organisms were selected for these experiments; the assays were—

(*i*) Nicotinic acid with *L. arabinosus* 17/5.
(*ii*) Thiamine with *L. fermenti* 36.
(*iii*) Thiamine with *L. viridescens* strain S38A.
(*iv*) Folic acid with *Streptococcus faecalis*.
(*v*) Folinic acid with *Pediococcus cerevisiae* 8081.
(*vi*) Pantothenic acid with *L. arabinosus* 17/5.
(*vii*) Riboflavin with *L. helveticus*.

The growth response of the strains of *S. faecalis* and *L. helveticus* was not optimal, but results obtained with use of these organisms are included, as the comparison between assays with freeze-dried and non-freeze-dried cultures is still valid.

FREEZE-DRYING—

Organisms to be freeze-dried were grown for 24 hours in the media listed below.

L. arabinosus 17/5 in glucose - yeast broth at 30° C.
L. fermenti 36 in Difco micro-inoculum broth *plus* 1 μg of thiamine per ml at 37° C.
L. viridescens in Difco APT broth[2] *plus* 1 μg of thiamine per ml at 30° C.
S. faecalis in Difco micro-inoculum broth at 30° C.
P. cerevisiae in Oxoid tomato-juice broth at 30° C.
L. helveticus in glucose - yeast broth at 37° C.

The cultures were then spun in a centrifuge and re-suspended in a medium consisting of 3 parts of horse serum and 1 part of 30 per cent. glucose broth (the "mist-desiccans" of Fry and Greaves). At re-suspension, the concentration of cells was standardised by dry weight to between 230 and 250 μg per ml, and a constant volume of this suspension was freeze-dried by the normal procedure in an Edwardes centrifugal freeze-drier. The freeze-dried cultures were stored at 2° to 4° C.

CONTROLS—

For controls, assay organisms were maintained in the standard way in agar stabs, sub-cultured at fortnightly intervals and stored at 2° to 4° C. The medium used for most of the organisms was the glucose - tryptone agar described previously[4]; for *L. fermenti*, 1 μg of thiamine per ml was added to this medium. For *L. viridescens*, APT agar[2] *plus* 1 μg of thiamine per ml was used, and for *P. cerevisiae* Oxoid tomato-juice agar.

TEST ASSAYS—

Assays involving use of the freeze-dried organisms were carried out at the following intervals after freeze-drying: (*a*) immediately, (*b*) 1 month, (*c*) 3 months, (*d*) 6 months and (*e*) 12 months. Further assays of nicotinic acid and thiamine were carried out as indicated in Tables I, II and III.

Each assay was carried out to compare three types of inoculum, namely—

A. Normal inoculum—An 18- to 24-hour liquid culture, inoculated from stock agar cultures, in the appropriate medium indicated under "Freeze-drying," washed twice and re-suspended in 100 ml of saline (*S. faecalis* re-suspended in 20 ml of saline).

B. Freeze-dried material, subcultured—An 18- to 24-hour culture, inoculated with freeze-dried material, washed twice and re-suspended in 100 ml of saline (*S. faecalis* re-suspended in 20 ml).

C. Freeze-dried material used directly—The contents of one ampoule washed twice and re-suspended in 2 ml of saline.

Standard and test curves were established for the nicotinic acid and thiamine assays on each occasion, the test material for each assay being dried yeast. For the remaining four assays, standard curves only were established.

Results obtained with the three inocula were compared statistically for the nicotinic acid assays and thiamine assays with *L. fermenti*; visual comparison of the curves was used in the remaining assays.

Assay methods were in general those described in the Difco Manual,[5] and Difco dehydrated media were used throughout. Exceptions to this were the folinic acid and the two thiamine assays, which were carried out by adapted methods involving use of smaller volumes.[4,6]

RESULTS

NICOTINIC ACID ASSAY—

Preliminary plots indicated that log(response) bore a linear relationship to log(dose), except that curvature sometimes occurred at one extreme of the range of responses; in such instances, the appropriate extreme doses were omitted from the analyses.

Analysis of the slopes of the lines showed that these varied somewhat from one experiment to another, but that, except in experiment No. 4 (Table I), no significant variation occurred within experiments, *i.e.*, on the grounds of slope, no one of the three inocula could be preferred. (It should be noted that, in most experiments, the slopes of dose-response curves obtained with freeze-dried inocula were slightly less than those obtained with standard inocula.)

Potencies were calculated in the usual way and are shown, with the 95 per cent. confidence limits, in Table I. With the exception of experiment No. 2, the three inocula gave potencies not differing significantly from each other. In experiment No. 2, which gave a low potency with inoculum B, the growth in both test and standard was much less than normal. No explanation was found for this phenomenon, and it was observed only once. The growth response was so markedly decreased that such an experiment would automatically be repeated; a normal response was obtained in the repeat assay on this occasion.

TABLE I

NICOTINIC ACID VALUES FOR DRIED YEAST OBTAINED WITH NORMAL AND FREEZE-DRIED CULTURES OF *L. arabinosus* 17/5

The inocula used were those described on p. 715. Figures in brackets are 95 per cent. confidence limits

Experiment No.	Time after freeze-drying	Nicotinic acid found with— inoculum A, μg per g	inoculum B, μg per g	inoculum C, μg per g
1	0 weeks	514 (498 to 531)	505 (488 to 523)	495 (483 to 506)
2	4 weeks	463 (443 to 485)	349 (316 to 385)	479 (462 to 495)
3	11 weeks	501* (478 to 526)	519 (500 to 538)	577* (524 to 634)
4	11½ weeks	497 (465 to 531)	495 (476 to 514)	503 (482 to 526)
5	12 weeks	491* (476 to 506)	484 (468 to 502)	502 (478 to 528)
6	27 weeks	509 (497 to 521)	512 (502 to 523)	505 (487 to 524)
7	12 months	575 (495 to 668)	556* (517 to 599)	587 (558 to 616)
8	16 months	484* (435 to 538)	484 (459 to 511)	472 (391 to 569)

* Assay of doubtful validity.

Some of the assays were of doubtful validity, three occurring with inoculum A and one each with inocula B and C. Comparison of residual variations also showed no significant difference between inocula.

Freeze-dried inocula, subcultured or unsubcultured, of *L. arabinosus* gave, therefore, results similar to those obtained by the standard method for nicotinic acid assay.

TABLE II

THIAMINE VALUES FOR DRIED YEAST OBTAINED WITH NORMAL AND FREEZE-DRIED CULTURES OF *L. fermenti* 36

The inocula used were those described on p. 715. Figures in brackets are 95 per cent. confidence limits

Experiment No.	Time after freeze-drying	Thiamine found with— inoculum A, μg per g	inoculum B, μg per g	inoculum C, μg per g
1	0 weeks	73 (66 to 82)	70 (62 to 77)	73 (59 to 90)
2	4 weeks	95 (85 to 107)	76 (56 to 104)	76 (68 to 86)
3	7 weeks	72 (58 to 90)	48 (36 to 65)	79 (64 to 97)
4	8 weeks	57 (49 to 68)	50 (38 to 66)	52 (47 to 58)
5	9 weeks	64 (57 to 72)	66 (56 to 77)	55 (37 to 81)
6	12 weeks	49 (37 to 63)	65 (47 to 91)	47 (32 to 69)
7	24 weeks	—*	—*	—*
8	25 weeks	—*	56 (48 to 65)	—*
9	12 months	76 (66 to 88)	73 (59 to 90)	77 (63 to 93)
10	16 months	—*	57 (51 to 63)	—*
11	16 months	—*	57 (50 to 64)	—*

* Invalid assay.

THIAMINE ASSAY WITH *L. fermenti*—

Statistical comparison was made of the slopes of lines obtained by plotting log(response) against log(dose) for the three inocula. As with the nicotinic acid assays, there was some variation from experiment to experiment, but no significant difference in this respect between inocula in any one experiment, although the freeze-dried inocula on the whole gave better growth than did the standard inoculum.

The potencies obtained with the three inocula, together with the 95 per cent. confidence limits, are shown in Table II and did not differ significantly from each other. Some of the assays were invalid, but this occurred no more frequently with inoculum C than with A and less frequently with B. The residual variance, however, was found to be higher in most assays (nine out of eleven) with inoculum B than with A or C.

In so far as any difference was found between the inocula, therefore, the subcultured freeze-dried inoculum gave results rather less satisfactory than those found with the standard and the unsubcultured freeze-dried inocula of *L. fermenti*.

THIAMINE ASSAY WITH *L. viridescens*—

In the two series of assays described above, graphical estimates of potency were also made, and these estimates agreed well with the statistically calculated figures. In view of this and of the time-consuming nature of a full statistical analysis, only graphical estimates of potency were used in assessing this third series of experiments.

Visual comparison of log(dose) - log(response) curves obtained with the three inocula revealed in most experiments a linear response, with no difference in slope between the three inocula in any experiment. In experiments Nos. 10 and 11 (see Table III) the log(dose) - log(response) graph for inoculum A was slightly curved, a type of variation in response already noted in this assay,[4] and could therefore less readily be compared with the corresponding graphs for inocula B and C. No marked difference between the dose-response curves for the three inocula was, however, apparent, except that in most experiments inoculum B gave curves of slightly less slope than did inocula A and C.

TABLE III

THIAMINE VALUES FOR DRIED YEAST OBTAINED WITH NORMAL AND FREEZE-DRIED CULTURES OF *L. viridescens*

The inocula used were those described on p. 715

Experiment No.	Time after freeze-drying	Thiamine found with— inoculum A, μg per g	inoculum B, μg per g	inoculum C, μg per g
1	0 weeks	56	52	57
2	4 weeks	80	80	65
3	7 weeks	74	71	—*
4	8 weeks	56	55	53
5	9 weeks	59	63	66
6	24 weeks	59	55	61
7	25 weeks	54	58	53
8	12 months	47	47	52
9	12½ months	—*	—*	45
10	16 months	61	67	—*
11	16 months	61	65	58

* Invalid assay.

Potency estimates obtained with the three inocula were generally in excellent agreement with each other, showing less variation than the values obtained by the *L. fermenti* method. No one inoculum showed a particular tendency to give invalid assays. Replicate results were excellent, showing less variation than those obtained by the *L. fermenti* method.

FOLIC ACID ASSAY—

Dose-response curves for *S. faecalis*, although not optimal with the strain used, were almost identical for the three inocula in all experiments. Replicate readings showed good agreement with all inocula.

FOLINIC ACID ASSAY—

Visual comparison showed that linear dose-response curves were obtained with the three inocula; the two freeze-dried inocula, as in the nicotinic acid assay, showed a tendency

to give dose-response curves of slightly less slope than did inoculum A. With all inocula, replicate readings showed good agreement.

PANTOTHENIC ACID ASSAY—

In this assay, log(dose) bore a linear relationship to response. Visual comparison again showed no detectable difference in slope, with two exceptions, in one of which inoculum B and in the other inoculum C showed slight variation from inoculum A. Graphs were still linear, however, and the difference in slope was insufficient to affect the precision of the assay to any extent. Again, the freeze-dried inocula gave dose-response curves of slightly less slope than did the standard inoculum. Agreement between replicate results was excellent with all three inocula.

RIBOFLAVIN ASSAY—

The strain of *L. helveticus* used did not give optimal dose-response curves in this series of assays, and no linear relationship was apparent. However, visual comparison of dose-response curves showed good agreement between the three inocula. In some assays, the freeze-dried inocula gave slightly lower responses, and once or twice slightly greater responses than were obtained with the standard inoculum. Replicate readings again showed good agreement with all three inocula.

DISCUSSION OF THE ASSAYS

The normal methods for maintaining assay organisms and producing cultures for use as assay inocula are laborious and time-consuming; further, they fail to minimise the possibility of variation occurring in such organisms. Maintenance of assay organisms as freeze-dried cultures and direct use of these cultures as assay inocula therefore have considerable advantages over the methods currently employed.

This survey of a collection of six assay organisms maintained for 12 to 16 months in a freeze-dried state has shown that, in these assays, the dose-response curves obtained with inocula prepared from freeze-dried cultures subcultured once before use or merely washed and re-suspended in saline did not differ significantly in slope from those obtained with a standard inoculum. In many experiments, a lesser slope of the standard curves obtained with freeze-dried inocula was observed, *e.g.*, in the nicotinic acid assays, in thiamine assays with *L. viridescens* and in folinic acid and pantothenic acid assays. It was shown, however, for nicotinic acid and thiamine assays, that the final results were not affected, as the test curve showed a corresponding slight depression. The cause of the depressed growth curve has not been ascertained; it might be due to a decreased rate of growth after freeze-drying or possibly to altered growth-factor requirements, and further observations on this point would be of interest. However, as it is not a progressive change—similar results are obtained both immediately and at 12 and 16 months after freeze-drying—this observation would not appear to be important from the point of view of maintaining assay organisms, since the observed depression of the dose-response curve was not sufficient significantly to alter the precision of the assays (as shown by the results of the assays for nicotinic acid and thiamine).

The use of unsubcultured freeze-dried organisms as inoculum did not affect the precision or validity of the assays. Subcultured freeze-dried inoculum of *L. fermenti* used in thiamine assays gave results of lower precision than those found with standard or unsubcultured freeze-dried inoculum. However, the use of unsubcultured freeze-dried organisms is preferable, in any case, on grounds of convenience. This material can therefore be tentatively recommended as inoculum for microbiological assays. It is hoped to carry out further assays in this series to determine for how long organisms may satisfactorily be maintained in a freeze-dried state for use as assay inocula.

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Collaborative Work on the Determination of Alkylbenzenesulphonates in Sewage, Sewage Effluent, River Waters and Surface Waters

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The results of collaborative work by three laboratories on the determination of alkylbenzenesulphonates in sewage, sewage effluent, river water and surface water are presented. The methods used were those described by Longwell and Maniece (both with and without preliminary hydrolysis), Webster and Halliday and Sallee and his co-workers (as modified by Ogden, Webster and Halliday).

As a result of preliminary work directed towards a reduction of blank values, slight modifications to Longwell and Maniece's and Webster and Halliday's methods were introduced; these modifications did not affect the basic principles of either method. Results have been examined statistically, and conclusions drawn from this examination are given. Experience gained during this work has led to the simplification and improvement of both colorimetric methods; the improved methods are outlined.

IN 1958, the Standing Technical Committee on Synthetic Detergents initiated a large-scale investigation into the biochemical break-down of alkylbenzenesulphonates during sewage treatment as practised in a well managed sewage works (the Luton Experiment). For this purpose, it was necessary to decide on a method for determining alkylbenzenesulphonates in sewage, sewage effluent and river waters, and the procedure described by Longwell and Maniece[1] was officially adopted.

During the investigation, it was stated that, in some circumstances, this method gave high results as compared with those obtained when Webster and Halliday's procedure[2] or the infra-red method of Sallee and his co-workers[3] (as modified by Ogden, Webster and Halliday[4]) was used. In consequence, a programme of collaborative work on these three methods was drawn up to be carried out in our three laboratories. As Longwell and Maniece's method was developed to determine anionic detergents as a class, and the object of this work was to determine alkylbenzenesulphonates only, their method was applied both with and without preliminary hydrolysis in order to correct for any alkyl sulphates that might be present.

The work was divided into the three stages listed below.

Part 1. On synthetic sewage and sewage effluent free from alkylbenzenesulphonates.

Part 2. On Luton settled sewage and final effluent.

Part 3. On river Lea water and surface water from Birmingham mains.

TABLE I

SUMMARY OF RESULTS OF COLLABORATIVE WORK

The figures in parenthesis are mean values

Description of sample	Alkylbenzene-sulphonate added, p.p.m.	Longwell and Maniece method: No. of determinations	Longwell and Maniece method: Alkylbenzene-sulphonate found, p.p.m.	Longwell and Maniece method (with hydrolysis): No. of determinations	Longwell and Maniece method (with hydrolysis): Alkylbenzene-sulphonate found, p.p.m.
Part 1 of work—					
Synthetic settled sewage	Nil	5	0·15 to 0·4 (0·34)	6	0·0 to 0·2 (0·10)
	3·14	7	3·0 to 3·8 (3·22)	8	2·5 to 3·0 (2·90)
	9·03	11	8·5 to 10·0 (8·89)	11	7·7 to 9·7 (8·54)
Effluent from synthetic sewage..	Nil	5	0·0 to 0·18 (0·07)	6	0·0 to 0·20 (0·06)
	0·79	13	0·5 to 1·0 (0·81)	10	0·61 to 0·81 (0·74)
	2·7	8	2·5 to 2·8 (2·60)	8	1·9 to 2·6 (2·33)
Part 2 of work—					
Luton settled sewage ..	Nil	7	3·5 to 3·9 (3·81)	6	3·0 to 3·3 (3·17)
	1·05	7	4·5 to 4·9 (4·71)	6	3·9 to 4·15 (4·03)
Luton final effluent ..	Nil	8	1·5 to 1·7 (1·59)	8	1·4 to 1·6 (1·51)
	0·60	8	1·9 to 2·28 (2·13)	8	1·94 to 2·4 (2·13)
Part 3 of work—					
River Lea water ..	Nil	6	0·58 to 0·67 (0·62)	6	0·57 to 0·64 (0·60)
	0·26	6	0·83 to 0·85 (0·84)	6	0·75 to 0·84 (0·81)
Surface water	Nil	6	0·0 to 0·02 (0·01)	6	0·0 to 0·03 (0·01)
	0·16	6	0·15 to 0·18 (0·17)	6	0·13 to 0·17 (0·15)

DESCRIPTION OF SAMPLES

Fourteen samples were examined in all three laboratories; those examined under Part 1 were—

Synthetic settled sewage without alkylbenzenesulphonates.
Synthetic settled sewage with a low concentration of added alkylbenzenesulphonates.
Synthetic settled sewage with a high concentration of added alkylbenzenesulphonates.
Effluent from treated synthetic sewage without alkylbenzensulphonates.
Effluent from treated synthetic sewage with a low concentration of added alkylbenzenesulphonates.
Effluent from treated synthetic sewage with a high concentration of added alkylbenzenesulphonates.

Those examined under Part 2 were—

Luton settled sewage without added alkylbenzenesulphonates.
Luton settled sewage with added alkylbenzenesulphonates.
Luton final effluent without added alkylbenzenesulphonates.
Luton final effluent with added alkylbenzenesulphonates.

Those examined under Part 3 were—

River Lea water without added alkylbenzenesulphonates.
River Lea water with added alkylbenzenesulphonates.
Surface water without added alkylbenzenesulphonates.
Surface water with added alkylbenzenesulphonates.

Samples were prepared by the Water Pollution Research Laboratory with use of a standard solution of tetrapropylenebenzenesulphonate (supplied by Thomas Hedley & Co. Ltd.) for all additions, and each sample was sterilised by the addition of 10 mg of mercuric chloride per litre to prevent loss of alkylbenzenesulphonate by biochemical break-down before analysis. The analysts had no knowledge of the concentrations of alkylbenzenesulphonate present in the samples.

TABLE I (*cont.*)

SUMMARY OF RESULTS OF COLLABORATIVE WORK

The figures in parenthesis are mean values

Description of sample	Alkylbenzene-sulphonate added, p.p.m.	Webster and Halliday method: No. of determinations	Webster and Halliday method: Alkylbenzene-sulphonate found, p.p.m.	Infra-red method: No. of determinations	Infra-red method: Alkylbenzene-sulphonate found, p.p.m.
Part 1 of work—					
Synthetic settled sewage	Nil	6	0·1 to 0·33 (0·22)	2	0·0 (0·0)
	3·14	9	2·6 to 3·0 (2·86)	4	2·9 to 3·0 (2·95)
	9·03	7	7·6 to 9·6 (8·50)	—	—
Effluent from synthetic sewage..	Nil	5	0·0 to 0·04 (0·02)	3	0·0 to 0·07 (0·04)
	0·79	7	0·4 to 0·79 (0·68)	6	0·62 to 0·7 (0·68)
	2·7	7	2·2 to 2·6 (2·41)	—	—
Part 2 of work—					
Luton settled sewage ..	Nil	6	2·6 to 3·2 (2·92)	5	2·8 to 3·0 (2·92)
	1.05	6	3·4 to 4·0 (3·73)	6	3·3 to 3·8 (3·57)
Luton final effluent ..	Nil	8	1·17 to 1·4 (1·26)	5	1·16 to 1·8 (1·31)
	0·60	9	1·60 to 2·04 (1·82)	4	1·60 to 2·6 (1·90)
Part 3 of work—					
River Lea water ..	Nil	6	0·38 to 0·45 (0·40)	4	0·36 to 0·39 (0·38)
	0·26	6	0·58 to 0·65 (0·62)	4	0·56 to 0·62 (0·60)
Surface water	Nil	6	0·0 (0·0)	3	0·0 to 0·03 (0·02)
	0·1	6	0·13 to 0·16 (0·14)	4	0·13 to 0·19 (0·16)

MODIFICATIONS TO THE METHODS OF ANALYSIS AS DESCRIBED

As the concentrations of alkylbenzenesulphonate in some of the samples were low, it was considered necessary to reduce the blank values of both colorimetric methods to a minimum, and to this end minor modifications were introduced. It is intended to publish full details of the revised colorimetric methods in the near future.

LONGWELL AND MANIECE METHOD—

(*a*) To ensure that the chloroform used was free from detergent, it was distilled from lime, washed with de-mineralised water, dried with anhydrous sodium sulphate and filtered through cotton-wool. All water used was prepared by passing distilled water through a column of Bio-deminrolit to ensure that it was free from detergent.

(*b*) Three extractions, each with a 15-ml portion of chloroform, were used in place of one with 15 ml and two with 10 ml, with intermediate rinsing. In the first extraction, a buffer of pH 10·5 was used (as in Webster and Halliday's method) instead of one of pH 10·0.

(*c*) One solution of methylene blue (0·2 g per litre) was used, and this was washed daily with three 10-ml portions of chloroform per 100 ml of dye solution.

(*d*) All spectrophotometric readings were made against chloroform.

Subsequently, further modifications were found desirable. These include (*i*) replacement of the alkaline phosphate buffer by an alkaline borate solution of pH 11·2, which leads, at extraction, to a pH essentially independent of the type and volume of sample examined, (*ii*) pre-extraction of the methylene blue solution, which removes interference caused by impurities normally present in that compound and (*iii*) rinsing the cuvette with the solution to be tested before a reading of optical density is made, which avoids errors caused by adsorption of alkylbenzenesulphonate on the glass.

WEBSTER AND HALLIDAY METHOD—

(*a*) The chloroform was subjected to the same treatment as described under (*a*) above.

(*b*) After acid hydrolysis, the solution was neutralised to phenolphthalein with a 10 per cent. solution of sodium hydroxide; it was then titrated with 0·1 N sulphuric acid until just decolorised. When the buffer of pH 7·5 is added, the solution should remain decolorised.

(*c*) Optical densities were read against chloroform.

The method was later simplified, thereby decreasing the time of analysis by half and eliminating emulsion troubles that sometimes occurred. The essential changes are listed below.

Methylene blue solution—After preparation, this is extracted with chloroform and then with light petroleum to remove traces of chloroform. Prepared in this way, the solution is stable for several weeks, and the blank values are less than those for other colorimetric methods

Hydrolysis—This is no longer necessary.

Chloroform - amine extraction—This is no longer required.

Light petroleum - amine extraction—Two extractions from strongly acid solution (each with a 50-ml portion of extractant) replace the previous four extractions (each with 25 ml of extractant) from neutral solution. The 1-methylheptylamine can be replaced by the cheaper n-heptylamine, which is available in this country.

Adsorbed alkylbenzenesulphonate—This is removed from glassware by rinsing with ethanol.

Removal of amine from light petroleum extract—The pH is adjusted to 10·5, and the solution is boiled for 15 minutes. No further adjustment of pH is then needed.

Extraction of methylene blue complex—Two extractions, one with 25 ml of extractant and the other with 20 ml, replace the three extractions previously used.

INFRA-RED METHOD—

This method[3] was as modified by Ogden, Webster and Halliday,[4] except that a sample containing approximately 4 mg of alkylbenzenesulphonate was passed through the column and the optical density was measured at 9·9 μ only.

RESULTS

The results are summarised in Table I and are based on calibration curves relating optical density to concentration of alkylbenzenesulphonate. These curves were plotted from the results obtained when suitable dilutions of a standard solution of sodium tetrapropylenebenzenesulphonate were treated in the same way as the samples.

CONCLUSIONS

Statistical examination of these results leads to the broad conclusions listed below.

(1) There is no significant difference in precision between the three methods.

(2) The standard deviation tends to increase with increasing concentration of detergent. For concentrations in the range 2 to 4 p.p.m., the standard deviation (expressed as alkylbenzenesulphonate) for each of the three methods is 0·18 p.p.m.; for the range 0·4 to 0·8 p.p.m., it is 0·02 p.p.m.

(3) For detergent-free synthetic sewage and detergent-free effluent, both Longwell and Maniece's method[1] (with hydrolysis) and Webster and Halliday's method[2] show a small apparent alkylbenzenesulphonate content of the same magnitude.

(4) Longwell and Maniece's method (with hydrolysis) sometimes, but not always, gives results significantly lower than those found by the same method without hydrolysis.

(5) There is no significant difference between the Longwell and Maniece method (with hydrolysis) and the Webster and Halliday method in the determination of added alkylbenzenesulphonate.

(6) Except with surface water, for which recovery was 100 per cent., each method recovered about 90 per cent. of the alkylbenzenesulphonate added to the sample.

(7) For Luton settled sewage, Luton final effluent and river Lea water, results by the Longwell and Maniece method, with and without hydrolysis, are significantly higher than those by the Webster and Halliday or the infra-red method.

With Luton settled sewage, results by the Longwell and Maniece method with hydrolysis are intermediate between those obtained by the same method without hydrolysis and by the Webster and Halliday method, which might be partly explained by the presence of alkyl sulphates in the sewage. Nevertheless, there remains for Luton settled sewage, Luton effluent and river Lea water a significant difference in alkylbenzenesulphonate content found by the Longwell and Maniece method, with or without hydrolysis, as compared with those found by the other methods. The reason for this difference has not yet been satisfactorily explained, but it is likely to be due to the presence in these liquors of partly degraded alkylbenzenesulphonate products that respond to the Longwell and Maniece method only.

This paper is published at the request of the Standing Technical Committee on Synthetic Detergents.

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The Determination of Thiamine (Vitamin B_1) in Cereal Products: Further Refinements

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A method for determining thiamine is described; it involves base exchange on sand and is capable of high precision. Selected sands were found to be much more rapid, versatile, selective and reliable than other base-exchange materials used for purifying extracts for thiamine determination. The method, which has been extensively applied to wheat and oat products, has revealed the nature of systematic errors that can affect this determination. The errors due to light absorption and quenching, and to fluorescence not derived from thiamine ("blank"), have been eliminated, so that accurate determinations can be made on many materials for which these disturbances are so severe that the use of unpurified extracts leads to gross errors. For white flour, a comparison between results by the proposed method and those given by direct determination leads to the important conclusion that, in the direct method, the blank should not be deducted if accurate results are to be obtained, since the blank almost exactly compensates opposing errors due to light absorption and quenching. The method can be used with the method of digestion also described, for determining combined as well as free thiamine.

The use of base exchange on sand for the determination of thiamine, briefly described previously,[1] has been of value in solving problems underlying discrepancies that have been found between workers taking part in various collaborative tests. It is now possible to report further improvements in the method that give it a high degree of precision. The method was at first applied only to acid extracts of cereals, but has now been extended to extracts from cooked products digested in order to release combined thiamine.

Sands do not show complete recovery of adsorbed thiamine with cold elution, but material much better than synthetic zeolite in this respect can be selected. The sand originally recommended and used for several years (Boam No. 4) gave recovery of about 98·5 per cent.

in the cold, and this method was used because of its convenience until all the sand was mined away. Investigation at the pits showed that strata a few inches apart had wide differences in properties, from complete and irrecoverable adsorption to a low base-exchange capacity. It was found possible to select sands giving recoveries of 96 to 97 per cent. in the cold; by incorporating a supplementary hot elution, 98 to 100 per cent. of the thiamine applied to the column could be recovered.

For the determination of free thiamine, extracts prepared with 0·24 N hydrochloric acid are used, and this is usually most convenient for determining thiamine in wheat and oat products, where most of the thiamine is free. For determination of combined thiamine, digestion with an enzyme at the optimum concentration of hydrogen ions is essential. When sands are used, it has been considered desirable to keep the concentration of salts used as buffers as low as possible. The proposed method has given reproducible results and is convenient in practice.

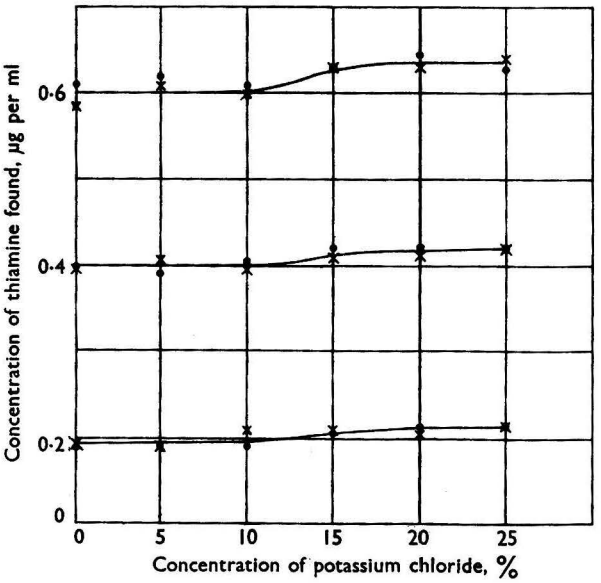

Fig. 1. Effect of potassium chloride concentration on apparent-thiamine concentration

The method described for controlling volume has been in use for about 10 years; it has great advantages in speed and accuracy, since it avoids repeated centrifugation, quantitative transference of digests from one tube to another and dilution of extracts by repeated extraction of residual solids. It depends on the fact that carbohydrate material mixed with water does not change in volume during solution or digestion of the solid material. This can readily be shown by placing flour in a bulb having a graduated neck, *e.g.*, a Hirschsohn phenol-determination flask (British Standard 676:1953), and carrying out any of the boiling or digestion procedure described with the liquid level resting on one of the graduations. This level will remain the same at the same temperature of reading. The increase in volume of liquid on digestion can be measured by evaporating to dryness an aliquot of the extract and calculating the total solid that has passed into solution, allowing for approximately one molecule of water of hydrolysis for each molecule of monosaccharose formed. Careful checks with a number of flours, including such methods as the addition of a definite amount of acid to the extract and subsequent titration to ascertain the resulting concentration, as well as much work with thiamine, have indicated that multiplication of the weight of solid passing into solution by the factor 0·65 gives accurate results with wheat flour. The method described avoids the use of buffers containing strong bases, which might compete with thiamine in the base-exchange process; if such are used, a corresponding correction to the weight of solid left after evaporation must be made.

A major source of error in base-exchange methods of purifying extracts for the determination of thiamine lies in the effect of the potassium chloride solution used for elution on the partition coefficients involved at the subsequent extraction stage. The salt acts in three ways: (*a*) it increases the proportion of thiochrome passing to the isobutyl alcohol phase, (*b*) it withdraws water from the isobutyl alcohol phase and (*c*) it causes methanol to pass from the aqueous phase to the isobutyl alcohol phase. As shown by Jansen,[2] methanol increases the permissible concentration of ferricyanide; in the absence of the alcohol the necessarily low concentration may give low results, even with purified extracts. The relative amount recommended in the proposed method is half that used in the "direct" method,[3] in which unpurified acid extracts are used. In order to ascertain the extent of the salt effect, solutions containing various concentrations of potassium chloride, but all with the same concentration of thiamine, were examined by the technique of oxidation and extraction used in the "direct" method[3]; the results are shown in Fig. 1. It can be seen that the position of most rapid change in the apparent-thiamine concentration corresponds to the level of concentration of potassium chloride found in eluates from base-exchange materials. It was found to be impossible to control this, other than by repeated extraction with isobutyl alcohol, but the use of four extractions gives high precision.

METHOD

APPARATUS—

Test-tubes—200 mm × 35 mm, 175 mm × 30 mm and 150 mm × 25 mm.

Stoppered flasks—Conical or otherwise, of capacity 250 ml.

Stirring rods—Formed into a ring at the lower end, the ring being at a right angle to the stem of the rod.

Base-exchange column—As previously described,[1] this has internal dimensions of 230 mm × 15 mm and contains 40 c.c. of sand supported on a small plug of glass-wool above a terminal 2-mm capillary. An upper reservoir of capacity at least 30 ml is necessary.

High-speed pipettes—The use of pipettes of the types described previously[4] is of the greatest advantage in the elution and oxidation stages.

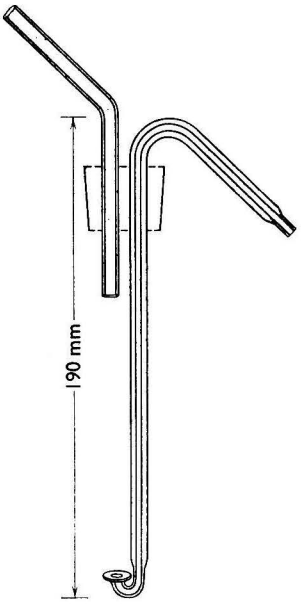

Fig. 2. "Blow-off" tube

Bubblers—These are made from glass tubing of 5 mm outside diameter bent, 20 cm from the bottom, to an angle of about 80° and drawn out at the bottom into a stout capillary

of 1 mm bore. The tip of the capillary is ground to an angle of 45°, sharp edges being rounded off, in order to prevent the flow of air from being checked at any time by contact between the tip and the bottom of the test-tube.

"Blow-off" tube—This is made in the form shown in Fig. 2. The small ground plate at the top of the upturned capillary greatly assists good separation of the two phases, and a full bore of 2 mm throughout the length of the capillary, including bends and jet, is important for convenient operation. A small rubber bellows (as used for sprays), preferably without a reservoir, is helpful for "blowing off" the isobutyl alcohol.

REAGENTS—

All reagents should be of recognised analytical grade, and all water distilled from glass apparatus.

Sulphuric acid, 0·025 N.

Hydrochloric acid, 2 per cent. v/v (approximately 0·24 N) and 10 per cent. v/v.

Potassium chloride in 2 per cent. acid—At room temperature, saturate 2 per cent. hydrochloric acid with potassium chloride.

Potassium chloride in 10 per cent. acid—At room temperature, saturate 10 per cent. hydrochloric acid with potassium chloride.

Acid potassium chloride solution (2 + 1)—Mix 2 volumes of potassium chloride in 2 per cent. acid with 1 volume of 2 per cent. hydrochloric acid.

Standard thiamine solutions—Prepare as required (usually to contain 0·05 to 0·4 μg of thiamine per ml) in acid potassium chloride solution (2 + 1). It is most important that these solutions should be prepared with the precautions previously described.[3]

Barium acetate solution, 3·5 g per litre.

Acetic acid—Dilute 20 g of glacial acetic acid to 1 litre.

Enzyme—Yeast phosphatase[5] or takadiastase.

Sodium hydroxide solution, 40 per cent. w/v.

Potassium ferricyanide solution, 5 per cent. w/v.

Methanol—Re-distil in the laboratory before use.

Isobutyl alcohol—Shake the alcohol as received with about one eighth of its volume of 10 per cent. sodium hydroxide solution and some solid potassium ferricyanide for at least 1 hour, transfer to a separating funnel, remove the aqueous layer, and wash the alcohol with four or five portions of distilled water. Distil the alcohol in steam (from boiling distilled water), set aside overnight, and use water-saturated. The isobutyl alcohol can be recovered after use by separating it from the aqueous layers, washing with three portions of water, steam-distilling and drying (first with sodium chloride and then with anhydrous sodium sulphate). It is then dry distilled, with rejection of the fraction distilling below 100° C and a small final fraction; finally, it is steam-distilled as before. (The drying and dry distillation can be omitted when only acid extracts have been oxidised, and rejected fractions can be recovered by further treatment.)

Blank determinations carried out with the reagents listed above should show only an unmeasurably small fluorescence.

PERPARATION OF EXTRACT

Acid extraction—When nearly all the thiamine is present in the free state, as in uncooked wheat products, extraction with 2 per cent. hydrochloric acid as previously described[3] can be used with advantage.

Digestion—For determining combined as well as free thiamine digestion is essential. Transfer 10 g of the ground product to a test-tube (200 mm × 35 mm), add 50 ml of 0·025 N sulphuric acid, and mix thoroughly by using a glass rod formed into a ring at its lower end; this rod remains in the test-tube until digestion is complete. Weigh the tube and its contents, place in a bath of boiling water for 15 minutes, with frequent stirring, cool, and weigh again. Add sufficient acetic acid (20 ml for samples of wheat flour) to bring the pH of the mixture to 3·7 if yeast phosphatase is used or sufficient barium acetate solution (30 ml for wheat flour) to bring the pH to 4·5 if takadiastase is used. In order to avoid loss of material from tubes used for determinations, check the pH value at this stage by means of an additional test-tube treated as the first; subsequently, discard this tube. (For a number of determinations on similar material, only one such additional tube is necessary.) Add, with stirring, 10 ml of enzyme suspension (or 0·2 g of solid takadiastase) and then, without stirring, sufficient water

to bring the total volume of liquid added to 100 ml (*e.g.*, 20 ml with flour and yeast phosphatase); this water serves to wash down and cover all previously added material. Weigh the tube and its contents, and cover with a small beaker. If yeast phosphatase has been used, set aside in a warm room for 30 minutes or in an incubator at 37° C overnight; for takadiastase, place in the incubator overnight. (It has not been found necessary to protect these digests with toluene or chloroform.) After digestion, again weigh the tube, thoroughly stir its contents, and check the pH. Transfer most of the liquid to a 100-ml centrifuge tube, and spin in a centrifuge until it is as clear as possible (yeast phosphatase alone does not give a completely clear solution). Transfer 10 ml of the clear solution to a weighed flat-bottomed dish, and dry by heating on the water bath and then for 10 minutes in an oven at 105° C. Allow to cool in a desiccator, weigh, set aside overnight in the open laboratory, and weigh again. The solid material absorbs about 5 per cent. of moisture; use this moist weight, n, for calculating the gain in volume, p, from the formula $p = \frac{0{\cdot}65\ n\ (100 - m)}{10 - 0{\cdot}65\ n}$, where m is the total loss in weight, in grams, during boiling and digestion. The final volume is then equal to $(100 - m + p)$. Differences in volume between members of a series treated together, or even on different days if the enzyme is the same, usually do not exceed 0·5 ml between extremes.

BASE-EXCHANGE PURIFICATION—

Wash the sand by transferring it several times from one beaker to another with portions of 2 per cent. hydrochloric acid, and reject the fine suspension with the liquid. Then wash it into the column with a jet of 2 per cent. hydrochloric acid from a wash-bottle, and gently tap the column until the straight part (40 ml) is full. Transfer 10 ml of the acid extract or digest to the reservoir above the sand, and allow to drain through at a rate not exceeding 3 ml per minute. Rinse the reservoir with two or three portions of 2 per cent. acid, and then allow 25 ml of this acid to pass through the column for acid extracts or 200 ml for digests. With digests, material giving "blank" fluorescence is lightly adsorbed on the column but is removed by adequate washing. However, the use of too great a volume of acid for washing tends to increase slightly the trace of thiamine irreversibly adsorbed by the sand, as shown by recovery experiments. A 200-ml calibrated flask filled with acid and inverted in the reservoir facilitates the application of the larger volume, or a stoppered tap funnel having a sufficiently wide bore can be used. Use a jet of the acid to wash any displaced sand to an even surface.

ELUTION—

Place in a boiling-water bath close to the columns three 200-mm × 35-mm test-tubes, one containing 2 per cent. hydrochloric acid, the second potassium chloride in 2 per cent. acid and the third potassium chloride in 10 per cent. acid; cold potassium chloride in 2 per cent. acid should also be available. Place on each column in turn 1 ml of the cold potassium chloride in 2 per cent. acid, allow to drain through, and add a second 1-ml portion. Then add 5 ml of the hot potassium chloride in 2 per cent. acid and then three successive 2-ml portions of hot 2 per cent. acid, allowing each portion to drain before adding the next. (Liquid leaving the column is rejected up to this point.) Place a 175-mm × 30-mm test-tube (collecting tube I) under the column, and elute with three successive 5-ml portions of hot potassium chloride in 10 per cent. acid. Then place a second tube (collecting tube II) similar to the first under the column, and elute with 5 ml of hot potassium chloride in 10 per cent. acid and then with two 5-ml portions of hot 2 per cent. acid. (This sequence of operations is used because it has been found that hot 10 per cent. acid coming in contact with thiamine held on the column drives appreciable amounts into the irreversibly adsorbed state, so that it is desirable first to remove most of the thiamine by milder elution.)

From the washing of the sand until completion of elution there should be no delay. The eluates, if covered, may stand for up to 2 days before oxidation.

OXIDATION—

Prepare standards to cover the range of unknowns by placing 15 ml of each standard solution in a test-tube similar to the collecting tubes, and provide for each of these a tube containing 15 ml of acid potassium chloride solution (2 + 1) to represent collecting tube II. Add 7·5 ml of methanol to the contents of each tube (I and II). Arrange the pairs of tubes

in racks, with the standards distributed among the unknowns and with tube II placed behind tube I. Behind this, place two 150-mm × 25-mm test-tubes, described below as tubes III and IV, each containing 25 ml of isobutyl alcohol.

Place in tube I a bubbler conveying a stream of air, and add 6 ml of 40 per cent. sodium hydroxide solution and, 3 seconds later, 1 ml of 5 per cent. potassium ferricyanide solution. After 30 seconds, add 25 ml of isobutyl alcohol, and allow the air to bubble through for a further minute. Lift the bubbler from the liquid, allow it to drain for a moment, and transfer it to tube II. Repeat the sequence of operations, and transfer the bubbler to the isobutyl alcohol in tube III for washing before placing it in the next tube I. (It is convenient to have four bubblers and to oxidise the solutions in pairs, when the sequence of operations follows in correct timing without haste or waiting.) These operations must be carried out in dim indirect light. When the contents of all the tubes have been oxidised, allow the entire set to stand in darkness until the two phases have separated; this usually takes 15 minutes or less.

With a "blow-off" tube, transfer the isobutyl alcohol to a stoppered 250-ml conical flask, allow the "blow-off" tube to drain (a small puff of air at the jet end assists in this), and transfer it to tube II. Blow the isobutyl alcohol from this tube into tube I, and allow air to bubble through it for about a minute while the "blow-off" tube is being drained and then washed in the isobutyl alcohol in tube III; then pour the isobutyl alcohol from tube III into tube II. Lift the bubbler from the liquid in tube I, allow it to drain, and transfer it to tube II for 1 minute, then to tube IV for washing, then to the next tube I and so on. Again set the tubes and flasks aside in the dark for 15 minutes, and then repeat the process, the isobutyl alcohol from tube I being transferred to the stoppered flask, that from tube II to tube I and that from tube IV to tube II. (The final washing of the "blow-off" tube and bubbler is perforce omitted at this stage, but the solutions are now so dilute that, with careful draining, the traces of fluorescent material left on the tube and bubbler do not detectably affect the result.) After two more transfers of isobutyl alcohol, all four portions of it will be in the stoppered flask; shake this well, transfer its contents to a 175-mm × 30-mm test-tube, and allow to settle overnight in complete darkness. Measure the fluorescence as previously described[3] with a Spekker or other fluorimeter. Return the isobutyl alcohol from the fluorimeter cuvette to the tube, and allow the cuvette to drain on a pile of filter-papers. Before it is refilled, the cuvette can, if desired, be rinsed with a few drops of isobutyl alcohol from the next tube, but this precaution has not been found necessary. The solutions should preferably be graded more or less in order of concentration.

To take full advantage of the precision of the method, measure the fluorescence again on two or more occasions, the solutions being set aside in darkness for at least 6 hours after each set of readings. This has been found to reduce the effect of small changes that occasionally affect the solutions relative to one another and that may arise from light scattering by invisible crystals of sodium carbonate or by material derived from protein and not removed by the base-exchange process. These changes may develop at any time, especially when the solution is disturbed (see previous paper by Ridyard[6]). Each set of readings from the standards is plotted on a curve, from which the concentrations of the unknowns can be read.

Discussion of the method

The results for 116 samples, for each of which fluorescence readings had been made on three consecutive days, were submitted to an analysis of variance in order to estimate the standard error; this was found to be $\pm 0{\cdot}005$ μg of thiamine per ml, based on 230 degrees of freedom. The means of the 116 readings for each day were statistically indistinguishable at 0·212, 0·212 and 0·211 μg per ml. It would be unwise to assume that the distribution for single determinations is Gaussian, but the Camp - Meidell inequality[7] indicates that, for individual readings, the error should be less than 3S (*i.e.*, 0·015 μg per ml) in at least 95 per cent. of the determinations. When several replicates (N) are available, the standard error of the mean will be correspondingly reduced to $\pm \frac{0{\cdot}005}{\sqrt{N}}$ and the error distribution more normal. For three replicates, therefore, the standard error should be $\pm \frac{0{\cdot}005}{\sqrt{3}}$, *i.e.*, $\pm 0{\cdot}003$ μg per ml, with 95 per cent. Gaussian confidence limits of ± 2S, that is, $\pm 0{\cdot}006$ μg per ml.

Thus, the practice of taking readings on three days, or two on one and another on a later day, which commonly fits well into laboratory routine, reduces the error by more than 50 per cent. The error is increased by a factor of 3/2 when correcting for the change in volume from 10 to 15 ml in the base-exchange process, but it is obvious that, if an error of $\pm 0{\cdot}02$ μg per ml is unimportant, only a single reading need be made. A few tentative experiments also suggest that this error is not likely to be greatly exceeded if the isobutyl alcohol extracts are set aside in darkness for only 1 hour and then read in a cloudy condition; alternatively, 25 ml of each extract may be added to 1 ml of ethanol in a separate tube and well mixed, the visibly clear solution then being examined with the fluorimeter. This might be of value if a result was urgently required, but should be checked by subsequent readings until such treatment has been tested more fully.

TABLE I

THIAMINE FOUND IN ACID EXTRACTS OF FLOURS

Each extract contained 10 g of 70 per cent. flour per 50 ml

Thiamine added, μg per ml	Thiamine found in extract of— flour No. B6254, μg per ml	flour No. B6283, μg per ml
Nil	0·128	0·138
Nil	0·135	0·141
0·40	0·522	0·545
0·60	0·708	0·725
0·60	0·707	0·729

TABLE II

THIAMINE FOUND IN YEAST PHOSPHATASE DIGESTS OF FLOUR AND BREAD

Thiamine added, μg per ml	Thiamine found in digest containing, per 100 ml— 5 g of flour No. B6283, μg per ml	10 g of flour No. B6283, μg per ml	10 g of bread No. B6317, μg per ml
Nil	0·051	0·102	0·081
Nil	0·054	0·102	0·078
0·0625	0·113	0·158	0·134
0·1250	0·171	0·220	0·200
0·1250	0·171	0·228	0·198

TABLE III

COMPARISON OF RESULTS BY "DIRECT" AND PROPOSED METHODS FOR ACID EXTRACTS OF FLOURS

Sample No.	Description of sample	Sub-sample	Blank value by direct method, μg per ml	Thiamine found by— "direct" method (blank not deducted) Extract No. 1, μg per ml	"direct" method (blank not deducted) Extract No. 2, μg per ml	proposed method Extract No. 1, μg per ml	proposed method Extract No. 2, μg per ml
B6616	Bakers-grade flour (10 g per 42·5 ml)	A	0·06	0·695	0·710	0·713	—
		B	0·08	0·715	0·710	0·707	—
B6558	81 per cent. extraction flour (10 g per 42·5 ml)	B	0·07	0·589	0·574	0·590	—
		G	—	0·580	0·582	0·582	—
B6579	70 per cent. flour, fortified (10 g per 42·5 ml)	B	0·06	0·549	0·568	0·557	—
		G	—	0·568	0·560	0·585	—
B6575	Wholemeal (5 g per 42·5 ml)	B	0·09	0·464	0·465	0·491	—
		G	—	0·472	0·464	0·479	—
B6669	70 per cent. extraction flour (10 g per 50 ml)	—	—	0·151	0·156	0·146	0·140
	As above *plus* 0·05 μg of thiamine per ml	—	—	0·198	—	0·186	—
	As above *plus* 0·10 μg of thimaine per ml	—	—	0·235	0·232	0·234	0·236

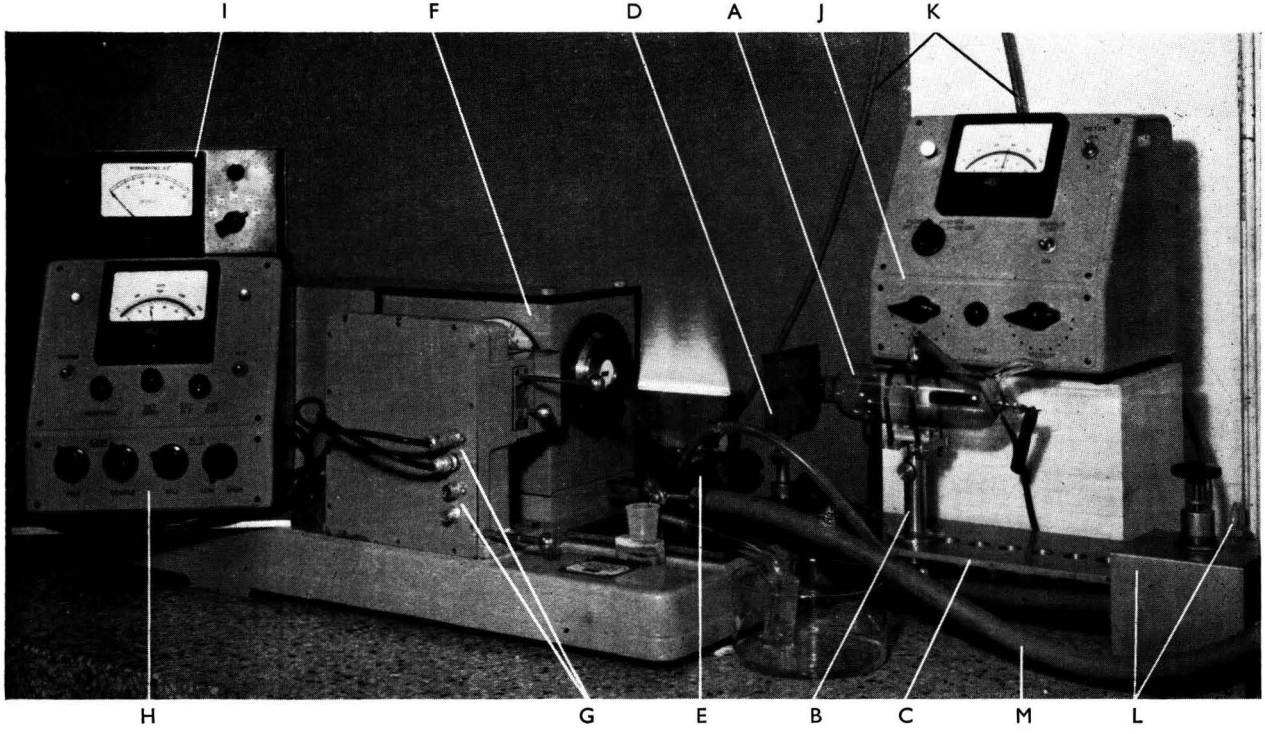

Fig. 1(*a*). Atomic-absorption spectrophotometric apparatus (for key to lettering, see p. 731)

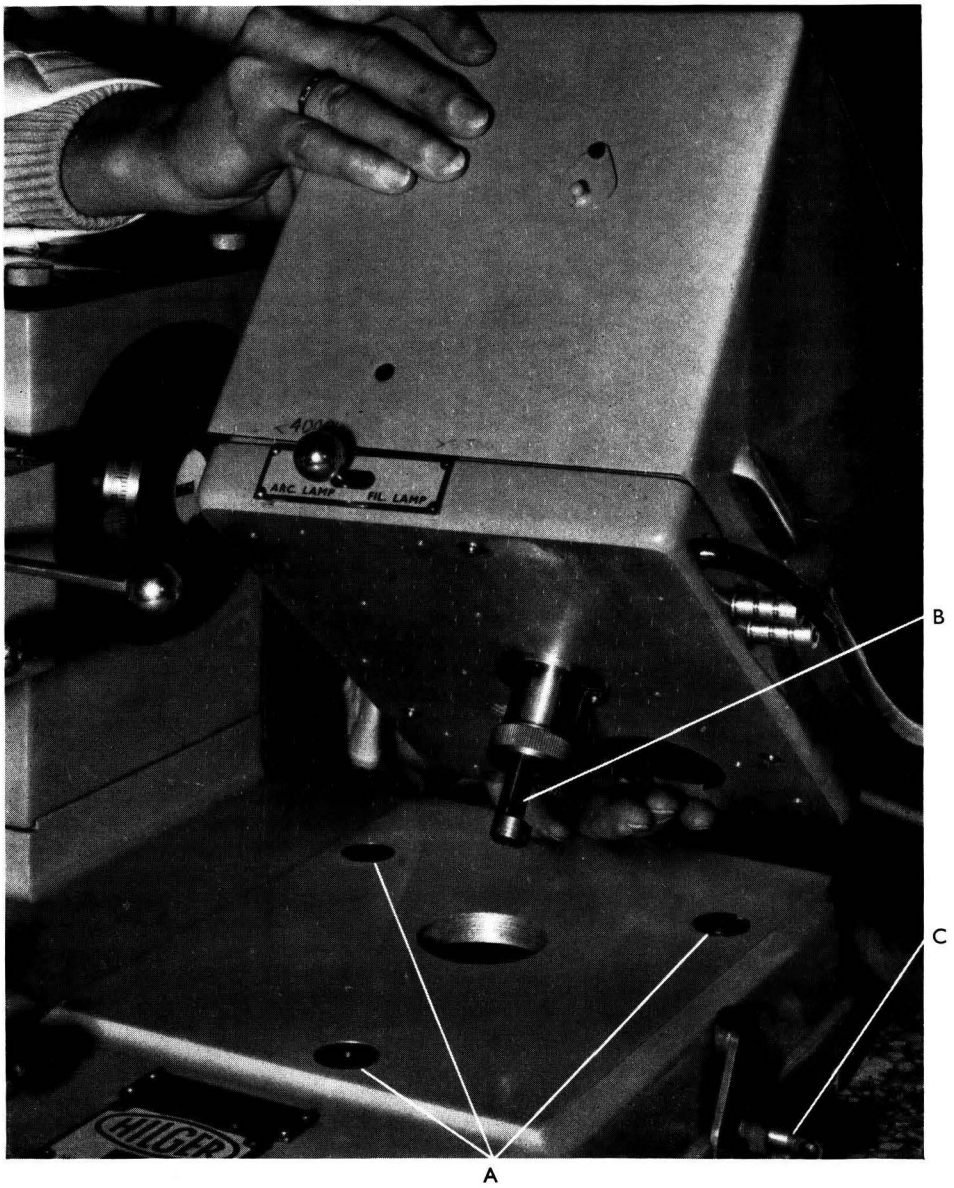

Fig. 1(*b*). Point, plane and groove mounting by means of which rapid conversion to conventional absorptiometric apparatus can be effected (for key to lettering, see p. 731)

To face page 731]

The high precision attainable by the proposed method is shown by the results in Tables I and II.

Acid extracts of several flours have been examined both by the "direct" method[3] and by the purification and oxidation procedure described above. The results show remarkable agreement, as with earlier studies,[1] and indicate that the "direct" method is simple and reliable for determining free thiamine in flour, but that the blank value[3] should *not be deducted*, as it almost exactly compensates for the loss in fluorescence caused by interferences in the optical measurements.[6] This is naturally only true if the reagent blank is negligible. The compensation of the two errors becomes less exact as the proportion of bran increases, is unreliable with oats and may need checking if an instrument other than a Spekker fluorimeter is used, since instrument design affects the magnitude of the light absorption and quenching interferences. Typical results are shown in Table III.

Certain peculiarities in the behaviour of the enzymes used for digestion have been observed and are being further investigated. Proteolytic enzymes liberate from wheat gluten[8] a substance that behaves similarly to thiamine in analysis, but is less strongly held on base-exchange columns. A limited amount of the sand used in this work is available to interested workers.

I thank Dr. J. B. Hutchinson for the statistical analysis and Mr. K. H. Willis for the thiamine determinations.

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The Determination of Molybdenum by Atomic-absorption Spectrophotometry

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An examination of the factors affecting the determination of molybdenum in aqueous solutions by atomic-absorption flame methods, and the apparatus used, are described. Sensitivity in analysis increased from zero under highly oxidising flame conditions to a limit of detection of about 0·5 p.p.m. in solution (when the molybdenum line at 3132·6 A was used) under reducing flame conditions. It was found that, in a reducing flame, calcium, strontium, manganese, iron and sulphate ions in the solutions caused different degrees of depression in the molybdenum absorption and that all of these interferences were completely suppressed by the addition of an excess of aluminium chloride. Nitric acid also caused slight depression of the molybdenum absorption.

Results of recovery experiments, of tests of precision and of comparison of atomic absorption with colorimetric methods are given.

Although the determination of molybdenum is apparently possible by flame-emission methods, the literature on the subject is scant. For instance, Mavrodineanu's exhaustive bibliography[1] on analytical flame spectroscopy, which contains 766 references and covers the period 1956 to March, 1959, has no entry on the determination of molybdenum. Burriel-Martí and Ramírez-Mũnoz[2] listed a detection limit of 3 p.p.m. when a diffuse molybdenum-emission band at about 6000 A was used, but no mention was made of the use of this band in analytical practice.

Thiocyanate[3,4] and dithiol[3] have generally been used for the colorimetric determination of molybdenum down to trace concentrations in a variety of materials.

Since the recognition, by Walsh and his co-workers,[5,6] of the advantages to be gained from the application of atomic absorption to chemical analysis and their provision of the means of achieving this application, thirty or more elements have been determined by the method. Molybdenum, however, was not among these elements; on the contrary, it has been cited[7] as an example of an element not conducive to determination by atomic-absorption flame methods.

This paper reports the results of detailed investigation into the use of atomic-absorption apparatus for determining molybdenum; it follows preliminary investigations, the results of which have already been published.[8]

Description of apparatus

Fig. 1(*a*) shows the atomic-absorption apparatus used, and Fig. 1(*b*) the manner in which the atomic-absorption optical array can be rapidly interchanged with a modified Hilger lamp-housing for conventional absorptiometric analysis. The atomic-absorption optical array was designed by me and constructed at the Division of Plant Industry. The point, groove and plane mounting—A in Fig. 1(*b*)—and associated spring-loaded, grooved rod—B in Fig. 1(*b*)—and lever and cam fixing mechanism—C in Fig. 1(*b*)—replacing the original mounting system of a Uvispek monochromator were designed and constructed at the Division of Chemical Physics, C.S.I.R.O. A mechanical chopper interposed in the beam between the Hilger lamp-housing and the entrance slit of the monochromator is necessary if measurement in conventional absorptiometric analysis is to be effected with use of a.c. amplification. The components visible in Fig. 1(*a*) are listed below.

A—Molybdenum hollow-cathode discharge tube having a silica window, tungsten anode and a cathode of graphite lined with molybdenum metal. The cathode was made from a $1\frac{1}{2}$-inch length of $\frac{3}{8}$-inch diameter spectroscopic graphite rod by drilling it longitudinally with a $\frac{5}{16}$-inch diameter drill to a depth of $1\frac{1}{4}$ inches, pushing a press-fitting cylindrical pellet, $\frac{3}{16}$ inch in length, made from powdered molybdenum metal to the bottom of the hole and lining the hole with thin molybdenum sheet formed into a slightly over-sized cylinder so that it could be sprung in. The cathode was mounted in the tube on a piece of $\frac{1}{16}$-inch diameter tungsten wire by pressing the wire into a hole of appropriate diameter drilled longitudinally in the solid end of the cathode. The aim in so constructing the tube was to avoid the introduction of any electrically conducting component more volatile than the molybdenum inside the graphite cathode, on the assumption that this would give maximum stability in emission of molybdenum from the tube by confining most of the discharge to the inside of the cathode. Although emission of molybdenum from this tube was steady, no tube having a cathode composed entirely of molybdenum was available for testing the validity of the assumption. The tube was evacuated and filled in the usual manner[9] with argon under a pressure of 3·5 cm of silicone oil.

B—Mounting for hollow-cathode discharge tube; this permits vertical adjustment of the lamp by means of a telescoped rod and horizontal and rotational adjustment by means of the flange and screw clamp at its base and the over-size hole in the base plate.

C—Base plate made of $\frac{1}{4}$-inch stainless-steel sheet, which is point, plane and groove mounted on the monochromator and supported by an adjustable leg (hidden) at the other extremity. The base plate has two grooved bars attached to it, one on each side of the burner, for mounting optical accessories and a series of holes $\frac{3}{4}$ inch in diameter drilled along the optical axis to permit adjustment of the position of the hollow-cathode discharge tube.

D—Adjustable diaphragm, which, in conjunction with the entrance slit of the monochromator, confines the beam from the hollow-cathode tube to the flame. The opening in the diaphragm was adjusted to a width of 1·5 mm and a height of 4 mm, and the monochromator entrance slit was masked to 4 mm in length for all the work described in this paper.

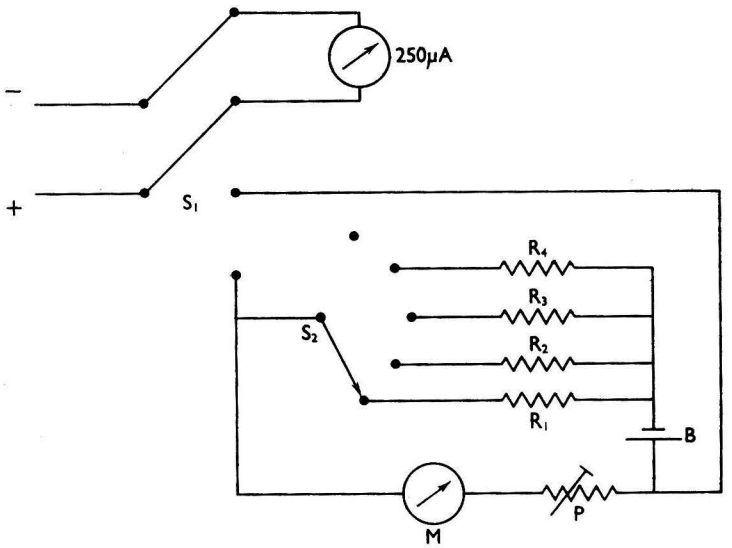
E—E.E.L. (Evans Electroselenium Ltd.) atomiser and spray-chamber assembly on which is mounted a laboratory-constructed burner having an opening 10 cm long and about 0·3 mm wide. The height from the base plate to the slit necessitated the mounting of the spray chamber on its side and its attachment to the burner by means of a right-angled stainless-steel tube $\frac{3}{4}$ inch in diameter. The mounting permits vertical, horizontal and rotary adjustment of the burner.

F—Uvispek quartz monochromator, in the photocell housing of which are mounted interchangeable RCA 1P22 and 1P28 photomultiplier tubes.

G—High-tension and output sockets for each photomultiplier.

H—WMA indicator comprising high-tension supply for photomultiplier, a.c. amplifier and meter (manufactured by Techtron Appliances Pty. Ltd., Melbourne, to the design of Box and Walsh[10]).

I—Scale-expansion unit (circuit shown in Fig. 2), effecting 5-fold expansion of the scale on the WMA indicator. Zero to full-scale deflection (50 divisions) on the expanded scale is equal to 80 to 100 per cent. transmission on the original scale, *i.e.*, each division of absorption on the expanded scale is equal to 0·4 per cent. absorption. The suggestion that this principle could be applied to give increased sensitivity in atomic-absorption analysis was made by Dr. J. B. Willis in a personal communication.



S_1 = Double-pole double-throw switch
S_2 = Rotary switch
M = Microammeter (full-scale deflection, 50 μA)
P = 5000-ohm potentiometer
B = 1·5-volt battery
R_1 = 7500-ohm resistor
R_2 = 10,000-ohm resistor
R_3 = 15,000-ohm resistor
R_4 = 30,000-ohm resistor

Fig. 2. Circuit of scale-expansion unit shown at I on Fig. 1(*a*)

J—Power pack for supplying hollow-cathode discharge tubes (manufactured by Techtron Appliances Pty. Ltd., Melbourne, to the design of Box and Walsh[10]). It was used at a modulation of 50 cycles per second throughout the work described in this paper.

K—Leads from a.c. electronic voltage-stabiliser (obtained from Stabilac Ltd., Sydney) supplying power pack and WMA indicator at 230 volts ± 0·25 per cent. for ±10 per cent. mains variation.

L—Diaphragm valve and water manometer for controlling supply of acetylene.

M—Air supply controlled by two 3-inch diameter diaphragm valves.

For the work described here, the burner was adjusted so that its opening was parallel to and about 3 mm vertically below the base of the beam formed by the adjustable diaphragm and masked monochomator slit. The molybdenum hollow-cathode discharge tube was operated at 30 mA, and the supplies of air and acetylene to the burner were set at various pressures depending on the sensitivity required for a particular experimental run, but kept constant throughout a given run. Neither a strongly reducing highly luminous flame nor

a strongly oxidising non-luminous flame was used, but the gas pressures were set to give different degrees of slight luminosity at the base of the flame. Both entrance and exit slits were set at 0·2 mm, and all work was carried out with the 1P28 photomultiplier tube.

The apparatus was operated in the manner described previously,[11] except that the expanded scale was used rather than the original scale on the WMA indicator.

Experimental

Analytical sensitivity—

After the initial finding[8] that determination of molybdenum by atomic absorption was possible, a photographic examination of the molybdenum spectrum for absorbing lines was carried out in a manner similar to that described by Allan.[12] Visual examination of the plate indicated (*a*) that most of the energy emitted from the molybdenum atom was concentrated in nine lines, all of wavelength longer than 3100 A, ending in the ground state, (*b*) that most of these lines showed appreciable absorption and (*c*) that no lines of the molybdenum spectrum other than these nine would be useful in atomic-absorption analysis. Measurement of the relative strengths in absorption of the nine strong lines was carried out on a solution containing 50 p.p.m. of molybdenum (as ammonium molybdate) with the apparatus shown in Fig. 1(*a*). The wavelengths of these nine lines, the lower and upper terms of their multiplets and their relative strengths in absorption are shown in Table I.

Table I

Relative strengths in absorption of molybdenum resonance lines

Wavelength, A	Multiplet	Absorption, divisions on expanded scale*
3798·26	a^7S to z^7P^0	27
3864·12		21
3902·97		15
3112·13	a^7S to z^7D^0	2
3158·16		12
3208·84		5
3132·59	a^7S to y^7P^0	45
3170·33		31
3193·97		26

* See description of scale-expansion unit, p. 732.

It has already been stated[8] that, unlike most other elements, the sensitivity in absorption of molybdenum varies widely according to the reducing potential of the flame; no molybdenum absorption, even at extremely high concentrations of molybdenum in the solutions sprayed into the flame, was evident if a large excess of air over acetylene was supplied to the burner. Table II shows the manner in which the sensitivity in absorption of the molybdenum line at 3132 A varied as the acetylene pressure was varied at constant air pressure.

Table II

Effect of variation in oxidation - reduction conditions of flame on molybdenum absorption

Atomic-absorption measurements for molybdenum were made at 3132·6 A.
Air pressure was kept constant at 18 lb per sq. inch

Acetylene pressure, cm of water	Molybdenum absorption, divisions on expanded scale*	Type of flame
28	50	Moderately reducing; incandescence visible along whole of base and diffuse edge on inner cone
27	44	
26	40	
25	33	
24	21	
23	17	Gradation between these conditions
22	12	
21	5	
20	3	
19	1	Highly oxidising; no incandescence and sharp edge on inner cone
18	0	

* See description of scale-expansion unit, p. 732.

In analytical practice, it was found that this wide variation in sensitivity with oxidation - reduction conditions in the flame caused little inconvenience, provided that the air and acetylene pressures were closely controlled throughout an analytical run, that the burner was operated for a warming-up period of about 15 minutes before the beginning of a run and that water was sprayed from the atomiser into the flame for about 3 minutes immediately before starting a run. It appeared that, at fixed air and acetylene pressures, the oxidation - reduction conditions in the flame varied according to the temperature of the burner. It was necessary, therefore, to equilibrate the burner with its surroundings under analytical conditions in order to obtain precise results.

A gain in sensitivity of up to about 2-fold over the maximum shown in Table II was found to be attainable by imposing highly reducing flame conditions, but the high concentration of particles of incandescent carbon associated with such a flame caused some loss of precision. Also, the spraying of pure water into a highly luminous flame resulted in a positive transmission reading, which was apparently caused by a diminution in the concentration of particles of carbon in the flame, thereby permitting the passage of more light from the hollow-cathode tube.

The optimum height of passage of the beam from the hollow-cathode tube through a moderately reducing flame was found to be 3 to 7 mm above the top of the burner. Passage at heights greater or lesser than this resulted in markedly poorer sensitivity.

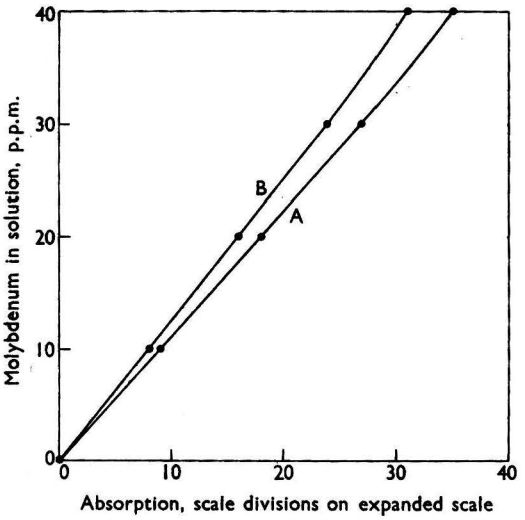


Fig. 3. Calibration curves relating absorption of the molybdenum line at 3132·6 A to concentration of molybdenum (as ammonium molybdate) in solution: curve A, water; curve B, mixture of 0·82 N nitric acid and 0·06 N hydrochloric acid

EFFECT OF AN OXIDISING AGENT—

Fig. 3 shows calibration curves based on solutions containing molybdenum (as ammonium molybdate) at various concentrations in water and in a mixture of 0·82 N nitric acid and 0·06 N hydrochloric acid. The depression of molybdenum absorption indicated by curve B may have been the result of a viscosity or surface-tension effect in the production of the sample spray, but a more feasible explanation of it is the diminution in the number of molybdenum atoms available for absorption by the imposition of more oxidising conditions on the flame by the nitric acid. If, then, oxidising agents are used in the extraction or solution of samples for analysis, the amounts should be kept as small as possible and the standard solutions should be so prepared that they contain approximately the same concentration of the oxidising agent as do the sample solutions.

INTERFERENCES—

In the determination by atomic absorption of some elements, notably calcium,[13] it has been found that the unsuspected presence of certain extraneous ions in the sample solutions causes erroneous results, owing to the formation of refractory compounds only partly dissociated in the flame. In order to ascertain whether or not interferences of this type would influence the determination of molybdenum by atomic-absorption methods, the effects of a number of ions were tested. Table III lists the ions tested, the compounds used, their effects on molybdenum absorption relative to that of the same concentration of molybdenum in the absence of extraneous ions and the blank readings on solutions of the ions tested.

The results in Table III indicated (*a*) that most of the ions tested had little effect, or no more than could be ascribed to experimental error, (*b*) that serious interference with molybdenum absorption occurred in the presence of manganese, calcium or strontium and (*c*) that slight interference could result from the presence of sulphate, magnesium or ferric ions. Also, there was an indication that the effect of a given interfering ion was influenced by the natures of ions of opposite charge in the solutions.

TABLE III

INFLUENCE OF EXTRANEOUS IONS ON MOLYBDENUM ABSORPTION

Atomic-absorption measurements for molybdenum were made at 3132·6 A. The concentration at which each element in column 1 was tested for interference was 500 p.p.m. The ion involved in each experiment is evident from the compounds listed in column 2

		Absorption, on expanded scale,* of—		
Element tested	Compound used	solution containing 10 p.p.m. of molybdenum, divisions	solution containing 40 p.p.m. of molybdenum, divisions	blank solution, divisions
—	—	8·0	29·5	
Zn	$ZnCl_2$	8·5	29·0	
K	KCl	8·0	28·0	0·0
Al	$AlCl_3$	8·0	29·5	
S	H_2SO_4	7·0	26·0	
Cl	HCl	7·5	28·0	
Si	Na_2SiO_3	8·5	29·5	0·5
P	H_3PO_4	8·0	31·0	
Mn	$MnCl_2$	3·5	14·5	
Mn	$MnSO_4$	3·0	6·0	
Mn	$KMnO_4$	6·5	23·0	
Cr	K_2CrO_4	7·5	29·5	
Ni	$NiCl_2$	8·5	30·0	0·0
Ca	$CaCl_2$	1·0	11·0	
Sr	$SrCl_2$	0·5	6·5	
Mg	$MgCl_2$	5·0	19·5	
Na	NaCl	8·5	29·5	
Fe	$FeCl_3$	6·0	24·5	

* See description of scale-expansion unit, p. 732.

An attempt was made (see Table III) to determine whether or not the interference from manganese with molybdenum absorption varied according to the nature of the manganese ion used. However, a subsequent test carried out on a mixed solution of potassium and manganous chlorides indicated that the lesser interference shown in Table III for potassium permanganate compared with either manganous chloride or manganous sulphate was caused by suppression of interference from manganese by potassium rather than by the presence of manganese as permanganate.

In view of the apparent suppression by potassium of the interference from manganese with molybdenum absorption, experiments were carried out to discern whether or not (*a*) sodium had any effect on interference from manganese and (*b*) sodium or potassium affected interference from strontium or calcium with molybdenum absorption. It was found that both alkali-metal ions would suppress interference from manganese, that potassium would partly suppress interference from calcium and that both sodium and potassium in the presence

of strontium caused greater depression of molybdenum absorption than did the same concentration of strontium alone. Neither sodium nor potassium could therefore be relied on as a universal suppressor of interferences from extraneous ions with molybdenum absorption.

Since an anion is likely to be more effective than a cation in suppressing interference from extraneous ions and since earlier work[8] had indicated that one of the anions in superphosphate must at least partly suppress interference from calcium, the effects of anions on suppression of the interference was next tested. The ions chosen for examination were phosphate and aluminium. Although aluminium was present as a cation in the solutions tested, it would probably have been converted to aluminate in molten particles in the flame and therefore effectively have acted as an anion. Neither nitrate nor sulphate was tested, as the oxidising effect of nitrate precluded its use, and the results in Table II indicated that sulphate would not be effective against interference from manganese. The relative effectiveness of phosphate and aluminium in suppression of the combined interference from calcium, strontium and manganese with molybdenum absorption is indicated in Table IV.

TABLE IV

EFFECTS OF ALUMINIUM AND PHOSPHATE ON COMBINED INTERFERENCE FROM CALCIUM, STRONTIUM AND MANGANESE

Atomic-absorption measurements for molybdenum were made at 3132·6 A

Calcium present, p.p.m.	Strontium present, p.p.m.	Manganese present, p.p.m.	Aluminium present, p.p.m.	Phosphate present (as P), p.p.m.	Absorption, on expanded scale,* of solution containing— 10 p.p.m. of molybdenum, divisions	40 p.p.m. of molybdenum, divisions
0·0	0·0	0·0	0·0	0·0	8·5	32·0
500	500	500	0·0	0·0	2·0	8·0
				250	2·0	11·5
				500	4·0	14·0
				1000	6·5	23·0
				2500	8·0	30·0
500	500	500	250	0·0	8·5	26·5
			500		8·5	31·0
			1000		9·0	31·5
			2500		10·0	31·0
0·0	0·0	0·0	2000	0·0	9·0	33·0
				1000	9·5	35·0

* See description of scale-expansion unit, p. 732.

It was obvious from the results in Table IV that aluminium was much more effective in suppression of interference than was phosphate. Since comparison of the results in Table IV with those in Table II indicated that the interferences from calcium, strontium and manganese with molybdenum absorption were not additive, the effect of aluminium on the separate interferences from these ions and on the interferences from ferric and magnesium ions was tested. For all these ions, it was found that 1000 p.p.m. of aluminium completely suppressed the effect of 500 p.p.m. of the interfering element on the absorption of 10 and 40 p.p.m. of molybdenum in solution. Also, in another experiment, it was found that aluminium suppressed interference from manganese regardless of whether the manganese was present as sulphate or chloride.

RECOVERY EXPERIMENTS—

As the addition of aluminium to the solutions for analysis appeared to be a satisfactory means of suppressing all major interferences encountered, the efficiency of this element in analytical practice was tested by carrying out recovery experiments for molybdenum added to solutions of "molybdenised" superphosphate and stainless steel.

A 9·5-g sample of "molybdenised" superphosphate was extracted by boiling for a few minutes with a mixture of 15 ml of concentrated nitric acid and 1 ml of concentrated hydrochloric acid. This solution was allowed to cool, diluted with cold water and filtered, and the beaker, residue and filter were washed repeatedly with cold water to give a final volume of filtrate and washings of 200 ml. Two 2·93-g samples of stainless steel were dissolved by warming with a mixture of 15 ml of concentrated nitric acid and 30 ml of concentrated

hydrochloric acid; the solutions were diluted with cold water and filtered; and the beakers and filters were washed with cold water to give final volumes of 200 ml.

Two 2-ml aliquots were taken from each of these solutions. One of the aliquots of the superphosphate solution was mixed with 6 ml of a solution containing 2667 p.p.m. of aluminium (as aluminium chloride) and 24 p.p.m. of molybdenum, and the other was mixed with 6 ml of a solution containing 2667 p.p.m. of aluminium (as chloride) only. To one aliquot of each of the solutions of stainless steel were added 10 ml of a solution containing 2400 p.p.m. of aluminium (as chloride) and 24 p.p.m. of molybdenum, and to the other were added 10 ml of a solution containing 2400 p.p.m. of aluminium (as chloride) alone. All of these solutions were analysed, with the atomic-absorption apparatus described above, against standards containing 2000 p.p.m. of aluminium (as chloride), 4 ml of concentrated nitric acid per 100 ml and up to 25 p.p.m. of molybdenum at 5 p.p.m. intervals of concentration. Calculations of recoveries of added molybdenum yielded the results shown in Table V.

TABLE V

RECOVERY OF MOLYBDENUM ADDED TO SOLUTIONS OF SUPERPHOSPHATE AND STAINLESS STEEL

Atomic-absorption measurements for molybdenum were made at 3132·6 A. Both sample and standard solutions contained 2000 p.p.m. of aluminium and about 4 ml of concentrated nitric acid per 100 ml

Sample	Molybdenum present in sample solution, μg	Molybdenum added, μg	Total molybdenum found, μg	Recovery of added molybdenum— μg	Recovery of added molybdenum— %
Superphosphate ..	46·4	144	205	158	110
Stainless steel A ..	75·6	240	311	235	98
Stainless steel B ..	69·6	240	302	232	97

The experiment on the solution of the sample of superphosphate was repeated and gave a recovery of 112 per cent. of the added molybdenum, thereby indicating that the high result in Table V was not due to experimental error. Since phosphate was considered to be the ion most likely to cause this enhancement and since there was evidence of enhancement by phosphate of molybdenum absorption (from the results in Tables III and IV), duplicate recovery experiments were carried out on the solution from the sample of superphosphate in a manner identical with that already described, except that the standards used each contained 1000 p.p.m. of phosphorus (as orthophosphoric acid) in addition to the aluminium and nitric acid. These experiments gave recoveries of 98 and 99 per cent. of the added molybdenum.

It appears, then, that the addition of aluminium chloride to both sample and standard solutions will suppress all major interferences with molybdenum absorption and for any sample solution will lead to a result not in error by more than about 10 per cent. If a more accurate result is required, it is necessary to take into account the major-element composition of each sample solution and to prepare the standards accordingly, aluminium chloride still being added to both sample and standard solutions to compensate for minor differences in composition. This causes little inconvenience in the routine analysis of samples having approximately uniform compositions.

COMPARISON WITH OTHER METHODS—

The results of analysing the solutions of the samples of stainless steel and superphosphate used in the recovery experiments by both colorimetric and atomic-absorption methods are shown in Table VI. The atomic-absorption analyses of the stainless-steel samples were carried out with 2000 p.p.m. of aluminium (as chloride) added to both standard and sample solutions. Those of the sample of superphosphate were carried out with 2000 p.p.m. of aluminium and 1000 p.p.m. of phosphorus (as orthophosphoric acid) added to the standard solutions and 2000 p.p.m. of aluminium added to the sample solutions.

Comparison between the results by atomic absorption and colorimetry in Table VI indicates good agreement for the sample of superphosphate, but a consistent difference, which appears to be slightly in excess of random error, for the samples of stainless steel. If this difference is significant, it can probably be ascribed to incomplete removal of interference (with the colorimetric method) from the high concentration of chromium in stainless steel.

TABLE VI

COMPARISON OF RESULTS FOR MOLYBDENUM BY VARIOUS METHODS

Atomic-absorption measurements for molybdenum were made at 3132·6 A

Sample	Concentration of molybdenum found by— colorimetric method, %	atomic-absorption method, %
Superphosphate	0·046*	0·045
	0·043*	0·048
	0·045*	0·048
	0·044†	0·046
	0·044†	0·046
	0·046†	—
Stainless steel A	0·28†	0·25
	—	0·25
	—	0·26
Stainless steel B	0·27†	0·24
	—	0·24
	—	0·24

* Dithiol method described by Sandell.[3]
† Thiocyanate method described by Grigg.[4]

REPRODUCIBILITY—

The absorptions of five portions of a solution containing 10 p.p.m. of molybdenum and five of a solution containing 40 p.p.m. of molybdenum (each as ammonium molybdate) were read in rotation until twenty readings for each solution had been made; the apparatus described above and the molybdenum line at 3132·6 A were used. Calculation of standard deviations of single determinations from these readings gave results of 7·30 $\pm$ 0·091 divisions on the expanded scale for 10 p.p.m. of molybdenum and 28·68 $\pm$ 0·075 divisions for 40 p.p.m. of molybdenum.

Short-term fluctuations of about $\pm$0·5 division on the expanded scale occurred regardless of whether or not the flame was in operation. This and the fact that the standard deviation was approximately independent of the magnitude of the absorption reading indicated that fluctuation in light output from the hollow-cathode discharge tube rather than fluctuation in the flame was the main source of error. The short-term fluctuations were such that a mean position of the pointer could be accurately assessed when setting to zero or taking a reading.

EFFECT OF HIGH CONCENTRATIONS OF SALTS—

With a highly oxidising flame in which molybdenum could not absorb, it was found that some apparent absorption of molybdenum resonance radiation was produced when concentrated solutions of salts were sprayed into the flame. It seemed likely that this was caused by interruption of the hollow-cathode beam by particles of salts in the flame, and measurements made on highly concentrated solutions of salts with both absorbing molybdenum lines and an oxidising flame and with non-absorbing lines confirmed this view. For a given solution, the degree of interruption was found to be independent of the absorbing power of the line used, but solutions of different salts gave different readings at similar concentrations. Since aluminium and calcium salts were found to give higher readings than did alkali-metal salts, it appeared that the more refractory the compounds formed in the flame, the greater was the effect.

Unless a correction for this extraneous absorption can be made, it will impose a sensitivity limit beyond which an element cannot be accurately determined by atomic-absorption methods. For the determination of molybdenum with the apparatus described above, the effect can be neglected, provided that the concentration of salt in the solution analysed does not exceed 1 per cent. A solution containing 5·5 per cent. of calcium chloride gave a reading of 2·5 divisions on the expanded scale when the molybdenum line at 3158 A and an oxidising flame were used. If we suppose the lower limit of accurate determination to be 0·5 p.p.m. of molybdenum in solution, then the lower limit with respect to the soluble solids from which the sample solution is prepared will be 50 p.p.m. of molybdenum unless the standard solutions are prepared in a manner such that they simulate the sample solutions in general composition.

Conclusions

Before the possibility of determining molybdenum by atomic absorption was examined, it was apparent that the element should be highly volatile in the flame in the form of its trioxide, which sublimes at 795° C.[14] However, consideration of the low volatility of the metal (melting-point, 2620° C; boiling-point 4800° C[14]) introduced some uncertainty as to whether or not any free atoms of molybdenum produced by dissociation of the oxide vapour in the flame would condense into particles of metal and so prevent absorption of molybdenum resonance radiation. The fact that the introduction of molybdates results in strong absorption of molybdenum resonance radiation in a reducing flame, but not in an oxidising flame, indicates first that molybdates dissociate at low temperature to give free molecules of molybdenum trioxide in a flame and secondly that free atoms of molybdenum produced by dissociation of the oxide vapour near the base of a reducing flame have a finite lifetime before condensation to particles of metal or reversion to oxide in the higher regions of the flame.

Molybdenum is the first element existing predominantly as an anion to be determined by atomic-absorption methods. The fact that the most serious interferences with its determination arise from the presence of certain cations indicates that, as in the determination of other elements, all serious interferences result from the formation of refractory compounds not easily dissociated in the flame.

It is likely that, with burners of conventional design, greater sensitivity will always be attainable in the determination of molybdenum by atomic-absorption flame methods than by flame-emission methods, as the former require only free ground-state atoms for operation, whereas the latter require excited atoms. In producing the hottest possible flame from a burner in an attempt to attain the maximum possible concentration of excited atoms at the expense of ground-state atoms in flame-emission analysis, it is inevitable that a highly oxidising flame will result; this will act against sensitivity in both emission and absorption by favouring the oxide state rather than the free atomic state of molybdenum. In a reducing flame, on the other hand, it is possible to produce an abundance of molybdenum atoms from a sample; this will give high sensitivity in absorption, but the temperature of such a flame is not high enough to excite sufficient of these atoms to give high sensitivity in emission.

The results of this investigation suggest two simple means by which the sensitivity of atomic-absorption apparatus may be varied to deal with samples differing widely in molybdenum content. These means are (*i*) the choice of a line (see Table I) appropriate in sensitivity to the range of concentrations covered by a given batch, as suggested by Willis[15] for determining sodium, or (*ii*) the choice of flame conditions appropriate to the range of concentrations. The use of one of these methods obviates the necessity for diluting sample solutions or replacing the burner by one of different length in order to vary the sensitivity.

The work described in this paper was undertaken, in part, to examine the possibility of directly analysing solutions from plants and soils for molybdenum. Although it has been shown that all problems arising from the interference of extraneous ions can be overcome, the sensitivity of the apparatus is as yet inadequate for this purpose. The introduction of refinements in the design of atomic-absorption instruments may make direct analysis of solutions from plants and soils possible in the future; in the meantime, however, such samples could only be analysed after preliminary concentration involving use of ion-exchange resins, extraction of molybdenum complexes into organic solvents or co-precipitation. As has been shown, atomic-absorption apparatus at its present stage of development is adequately sensitive for most industrial applications, in which samples containing relatively larger concentrations of molybdenum (50 p.p.m. or more) are encountered. The speed and convenience in use of the atomic-absorption method gives it advantage over colorimetric methods in the determination of molybdenum in such samples.

I thank Mr. P. C. Tracey for making components of the instrument shown in Fig. 1(*a*), Mrs. F. J. Taylor for technical assistance, Mr. C. H. Williams for useful discussion during the work and Mr. S. W. Thorne for designing and constructing the scale-expansion system shown in Fig. 2.

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Volumetric Determination of Sulphate

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A modified version of Sporek's volumetric method for determining sulphate is described. Sulphate is precipitated as lead sulphate from a boiling aqueous ethanolic solution by lead nitrate solution. After standing for 3 hours, the lead sulphate is separated, washed and dissolved in an excess of disodium ethylenediaminetetra-acetate solution, and the excess is determined by titration with zinc chloride solution; Eriochrome black T is used as indicator.

THE work described in this paper was undertaken in order to resolve the erratic results found when Sporek's volumetric method[1] was used for determining sulphate in materials obtained in the extraction of uranium from its ores. In this method, sulphate is precipitated as lead sulphate from a 50 per cent. v/v solution of isopropyl alcohol containing 10 per cent. v/v of nitric acid. The precipitate, after being set aside for 15 minutes, is separated from the supernatant liquid by decantation, washed and dissolved in an excess of disodium ethylenediaminetetra-acetate (EDTA) solution. The excess of EDTA is then titrated with 0·05 M zinc chloride, Eriochrome black T being used as indicator. As the volumetric finish of this procedure was satisfactory, the conditions under which the lead sulphate was precipitated, separated and washed were investigated, and the effects of elements that might be encountered in the samples were studied.

EXPERIMENTAL

Preliminary tests showed that the method used for separating the precipitated lead sulphate from the supernatant liquor (decantation through a fine-porosity frit) was slow. The use of two thicknesses of Whatman No. 3 filter-paper was found to be much more rapid, and the papers were just as retentive as the frit. In the experiments described below, the supernatant liquor was filtered, with suction, through two 5·5-cm filter-papers supported on a Buchner funnel, and, as a small amount of precipitate was always decanted with the liquor, the papers were removed from the funnel and placed in the original beaker containing the lead sulphate.

Sporek carried out precipitation in a Buchner flask, but we considered this to be unnecessary, as pouring was inconvenient, owing to the wide lip; precipitation in beakers was easier and more convenient.

EFFECTS OF CONCENTRATIONS OF REAGENTS, TEMPERATURE AND TIME OF STANDING—

A sodium sulphate solution of known concentration was used in experiments to determine the most suitable concentrations of nitric acid and isopropyl alcohol and the effect of time of

standing after precipitation of the lead sulphate. Precipitations were carried out at room temperature, and the samples were set aside at room temperature for periods up to 18 hours. A 10-ml portion of M lead nitrate was used to precipitate sulphate, and the final volume of solution in all experiments was 100 ml.

These experiments showed that a concentration of 20 per cent. v/v of nitric acid could not be used with any concentration of isopropyl alcohol from 10 to 50 per cent. v/v, as oxidation of the alcohol occurred readily and was accompanied by violent reaction. With 10 per cent. v/v of nitric acid, oxidation occurred in the presence of 25 and 50 per cent. v/v of isopropyl alcohol after the solutions had been set aside for 1 to 2 hours. With 10 per cent. v/v each of nitric acid and isopropyl alcohol, the precipitate formed was to a large extent insoluble in an ammoniacal solution of EDTA, even after boiling.

Experiments in which 5 per cent. v/v of nitric acid and from 10 to 25 per cent. v/v of the alcohol were used gave results nearer to the amounts of sulphate present; although these results were not consistent, they indicated that the best concentrations of acid and isopropyl alcohol were 5 and 10 to 25 per cent. v/v, respectively.

The temperature at which the samples were set aside after precipitation was then decreased from room temperature to 3° C. Precipitation from samples containing 5 per cent. v/v of nitric acid and 10 or 25 per cent. v/v of isopropyl alcohol was carried out at room temperature, and the solutions were set aside for up to 2 hours at 3° C. The results showed an increase in precision, but accuracy was poor; recovery of sulphate from samples containing 25 per cent. v/v of isopropyl alcohol was better than that from those containing 10 per cent.

PRECIPITATION FROM BOILING SOLUTION—

Precipitation from boiling solution is used in the gravimetric determination of sulphate as barium sulphate, and the precipitation of lead sulphate from boiling aqueous alcoholic solutions was studied in order to ascertain whether or not better precipitation and a coarser precipitate could be obtained. Tests were carried out as described above on solutions containing 25 per cent. v/v of isopropyl alcohol, but the lead nitrate solution was added after the sample solution had been heated to boiling-point; the samples were then set aside for up to 2 hours at room temperature. The precipitates formed were fairly coarse and readily separable from the supernatant liquor by decantation. The results of these experiments showed greatly improved accuracy and precision.

The effect of the temperature at which precipitation took place was also investigated, sulphate being precipitated at from 30° to 88° C (the boiling-point of the solution) and also after the solution had been boiled for 1 and 3 minutes. The results showed that the best accuracy and precision were attained when precipitation occurred as the solution was brought just to boiling-point. There was a small decrease in recovery when the solution was boiled for any length of time before precipitation.

INCREASE IN TIME OF STANDING AFTER PRECIPITATION—

The time for which the samples were set aside after precipitation was increased to 3 hours; precipitations were carried out both at room temperature and at the boiling-point of the solution. The results for a solution found by the barium sulphate method to contain 71·3 g of sodium sulphate per litre were—

Sodium sulphate found (precipitation at boiling-point), g per litre	71·0	71·6	71·8	71·8	71·3	71·0
Sodium sulphate found (precipitation at room temperature), g per litre	74·1	74·7	74·4	74·4	74·1	73·8

These results show good accuracy and precision when precipitation was carried out at the boiling-point of the solution; precision was also good when precipitation was made at room temperature, but results were high by approximately 3 g per litre. Addition of precipitant at the boiling-point of the solution is evidently necessary for complete precipitation of lead sulphate.

USE OF ETHANOL—

A solution containing 25 per cent. v/v of isopropyl alcohol is fairly viscous; consequently, the rate at which the lead sulphate settled during washing with such a solution was rather

slow. Further, washing with a viscous solution is not ideal, so the use of ethanol was tried.

Sulphate was precipitated from boiling aqueous solutions containing 25 per cent. v/v of ethanol and 5 per cent. v/v of nitric acid by 10-ml portions of M lead nitrate. The samples were set aside at room temperature for up to 3 hours, and the precipitates were washed with a 25 per cent. v/v solution of ethanol in water. The results are shown in Table I and

TABLE I

RECOVERY OF SULPHATE FROM ETHANOLIC SOLUTIONS

Sulphate present, g per litre	Time of standing, hours	Sulphate recovered, g per litre
67·0	1	69·6, 67·5
	2	66·8, 66·1
	3	66·8, 67·4
81·3	1	73·9, 78·1
	2	79·5, 78·8
	3	80·9, 81·7

indicate that the same orders of accuracy and precision were attained after the solutions had been set aside for 3 hours as were obtained when isopropyl alcohol was used. Recoveries were low when the solutions were set aside for 1 or 2 hours.

CONCENTRATION OF NITRIC ACID—

Since the samples could contain different amounts of nitric acid, the range of concentrations of this acid that could be tolerated was investigated, concentrations from 1 to 20 per cent. v/v being tried. Precipitation was carried out as described above, and the samples were set aside at room temperature for 3 hours. The results of these experiments (see Table II)

TABLE II

EFFECT OF NITRIC ACID ON RECOVERY OF 0·169 g OF SULPHATE

Concentration of nitric acid, % v/v	Sulphate recovered, g	Error, g
1	0·169	0·00
2	0·170	+0·001
3	0·170	+0·001
4	0·167	−0·002
5	0·168	−0·001
6	0·166	−0·003
7	0·163	−0·006
8	0·152	−0·017
9	0·140	−0·029
10	0·132	−0·037
15	0·104	−0·065

showed that the maximum allowable concentration of nitric acid was 6 per cent. v/v, above which level recovery of sulphate rapidly decreased.

INTERFERENCE—

The effects of thirteen elements on the determination of sulphate are shown in Table III. Phosphate, molybdate and selenate cause considerable interference, leading to high results; arsenic and antimony do not interfere.

METHOD

REAGENTS—

Ethanol, 95 per cent.

Wash solution—Aqueous solution containing 25 per cent. v/v of ethanol.

Lead nitrate, M—Dissolve 331 g of lead nitrate in water, and dilute to 1 litre.

Indicator mixture—Grind 0·1 g of Eriochrome black T with 100 g of ammonium chloride.

EDTA solution, 0·1 M—Accurately weigh 37·255 g of disodium ethylenediaminetetra-acetate dihydrate, dissolve in water, and dilute to 1 litre.

Zinc chloride, 0·1 M—Accurately weigh 6·538 g of pure zinc, dissolve in diluted hydrochloric acid (1 + 1), and dilute to about 800 ml with water. Almost neutralise by adding ammonia solution, cool, and dilute to 1 litre. Standardise against the 0·1 M EDTA solution; use the indicator mixture at a pH of about 10.

TABLE III

EFFECTS OF VARIOUS ELEMENTS ON DETERMINATION OF SULPHATE

Element	Amount of element added, g	Sulphate present, g	Sulphate recovered, g
Aluminium added as $Al(NO_3)_2$	0·25	0·169	0·168
Calcium added as $CaCO_3$			0·169
Chromium added as K_2CrO_4			0·167
Copper added as $Cu(NO_3)_2$			0·171
Iron added as $Fe(NO_3)_3$			0·168
Magnesium added as $Mg(NO_3)_2$			0·171
Manganese added as $KMnO_4$			0·166
Uranium added as $UO_2(NO_3)_2$			0·168
Vanadium added as NH_4VO_3			0·165
Zinc added as $Zn(NO_3)_2$			0·165
Phosphorus added as Na_2HPO_4	0·25	0·169	0·201
	0·05	0·107	0·108
	0·025	0·107	0·110
	0·005	0·107	0·108
Selenium added as H_2SeO_3	0·25	0·169	0·211
	0·05	0·107	0·125
	0·025	0·107	0·117
	0·005	0·107	0·106
Molybdenum added as $(NH_4)Mo_7O_{24}$	0·25	0·169	0·235
	0·05	0·107	0·141
	0·025	0·107	0·116
	0·005	0·085	0·109

PROCEDURE—

For solid samples, weigh a suitable portion into a 250-ml conical flask, and add 10 to 15 ml of concentrated nitric acid and 5 ml of hydrobromic acid. Boil gently until evolution of bromine ceases, add a further 5 ml of hydrobromic acid, and boil gently until the volume has been decreased to about 5 ml. Dilute with water, and filter under suction. Treat the filtrate as described below for liquid samples.

Transfer a portion of solution containing 0·02 to 0·2 g of sulphate to a 400-ml beaker, and neutralise, if necessary, with ammonia solution or concentrated nitric acid. Add 3 to 5 ml of nitric acid and sufficient water to give a volume of 65 ml. (If the sample solution contains carbonate, cover, and boil gently for 5 minutes.) Add 25 ml of 95 per cent. ethanol, heat just to boiling-point, and add 10 ml of M lead nitrate. Rinse the sides of the beaker with the wash solution, set aside in a cool place for 3 hours, and decant the supernatant liquor through two thicknesses of Whatman No. 3 filter-paper supported on a 5·5-cm Bucher funnel under suction. Mix the precipitate with 15 to 20 ml of the wash solution, allow to settle, and decant the supernatant liquor; repeat the washing and decantation twice. Wash the filter-paper and funnel with the wash solution, transfer the papers to the beaker containing the precipitate, rinse the funnel with water, and add the rinsings to the contents of the beaker. Add 0·1 M EDTA solution until about 10 ml more than the amount needed to dissolve the precipitate are present, then add 10 ml of ammonia solution, and swirl to dissolve the precipitate. Titrate the excess of EDTA solution with the 0·1 M zinc chloride; use the Eriochrome black T mixture as indicator, and take the end-point as being when the colour changes from blue to violet.

1 ml of 0·1 M EDTA solution $\equiv$ 0·0096 g of SO_4^{2-}.

RESULTS AND DISCUSSION OF THE METHOD

Results obtained by the proposed method are shown in Table IV and are in good agreement with those obtained by the gravimetric (barium sulphate) and furnace methods.

TABLE IV

TYPICAL RESULTS FOUND BY PROPOSED PROCEDURE

	Result found by—	
	proposed method	alternative method
Sulphate content of sodium uranate, %	0·43	0·47*
	0·27	0·27*
Sulphur content of uranium ore, %	0·46	0·48†
Sulphate content of ore leach solution, as Na_2SO_4, g per litre	73·4	73·3†
	82·0	81·3†

* Result found by furnace method.
† Result found by gravimetric (barium sulphate) method.

Aqueous solutions of both ethanol and isopropyl alcohol are satisfactory. Ethanol has certain advantages that merit its use and has therefore been adopted in the final procedure. The time taken for a determination is considerably shorter than that required for the gravimetric barium sulphate method and is well suited to routine analysis.

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Further Studies on the Zone-Strip Technique

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An equation has been derived correlating the length and content of a loaded zone on a paper chromatogram with the distance travelled by the developing solvent. Use of this equation leads to increased accuracy and avoids the need for plotting calibration graphs.

SHIMI and co-workers[1] have described micro-quantitative analysis by the zone-strip technique. However, during repeated applications of the technique, some difficulty has been encountered. The rates at which the developing solvent travelled through various strips in the chromatographic tank could not be controlled to permit the simultaneous attainment of 40 cm down from the starting line. In practice, the distances travelled by the solvent front differed considerably, thereby affecting the lengths of zones and so producing misleading results. Moreover, in order to obtain satisfactory resolution of the components of biological fluids and miscellaneous mixtures, it may be necessary to investigate several developing solvents. In such instances, a calibration curve for each solvent would be plotted for each compound to be determined, and the establishment of numerous calibration curves is rather tedious and most inconvenient. This paper describes work carried out to overcome these difficulties and so to facilitate manipulation of the technique.

EXPERIMENTAL

The procedure used was that described previously,[1] with some minor modifications. Fifty paper strips (0·5 cm wide) were loaded with equal amounts of the compound to be assayed. A set of five strips was suspended from one half of a Petri dish (10 cm in diameter) supported by a stand resting on the floor of the chromatographic tank (see Fig. 1). The glass plate covering the tank contained several holes, 1 cm in diameter, through each of which passed the jet of a 25-ml pipette filled to the mark with the developing solvent. The upper end of each pipette was fitted with a rubber bulb, and the pipettes were held in the glass plate

by means of rubber stoppers. The dishes were so arranged that each was below the jet of a pipette, and solvent was transferred to the dishes by pressing the rubber bulbs; this was carried out in an order such that the dishes were successively filled with solvent at 1-hour intervals. The strips were allowed to remain in the tank for 1 hour after the last dish had been filled, *i.e.*, the time elapsing between the beginning of development of the first set of five strips and the removal of all the strips from the tank was 10 hours. The distances travelled by the solvent were immediately measured, the strips were allowed to dry, and, finally, the loaded zones were identified as before.[1] Several developing solvents were tested by this technique, and the results are shown in Table I (see p. 747).

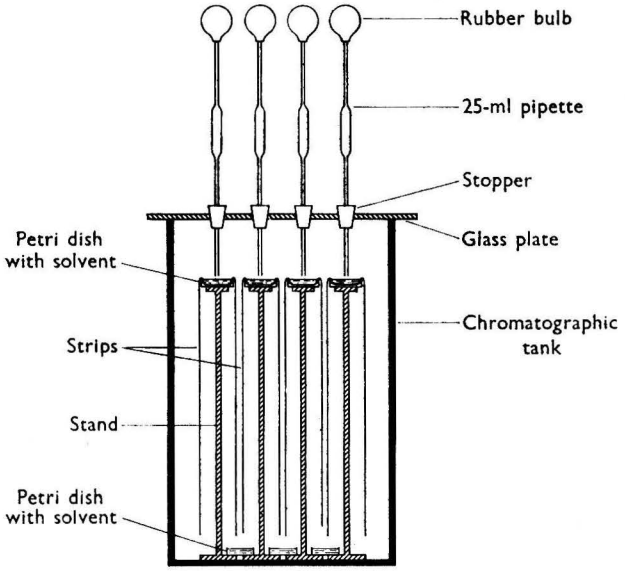

Fig. 1. Apparatus used for developing strips

The technique was then applied to the determination of various concentrations of different compounds and to some solutions of unknown concentration. For the latter solutions, "guide" strips were included among the strips treated with the sample solution; these "guide" strips were exactly the same as those containing the sample, but were loaded with known amounts of an authentic specimen of the compound being assayed (the significance of using the "guide" strips is mentioned below). The sample solutions were also analysed by standard chemical and biological methods for comparative purposes.

Results and discussion of the method

For any particular concentration of any of the compounds tested, the graph correlating length of loaded zone and logarithm of distance travelled by the solvent through the strip was linear. Typical graphs are shown in Figs. 2, 3, 4, 5 and 6, and the results from which these were plotted led to the derivation of the equation—

$$l = k \log d$$

in which l is the length of loaded zone in centimetres, d is the distance travelled by the solvent in centimetres and k is a constant. Similarly, an analogous equation could be derived from the relationship previously found[1] between the length and the logarithm of the content of a loaded zone, *viz.*—

$$l = k_1 \log c$$

where c is the content of the zone in micrograms and k_1 is another constant.

From these equations, it was found that the relationship between the length of a zone,

its content and the distance travelled by the developing solvent could be expressed by the equation—

$$l = K \cdot \log c \cdot \log d \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

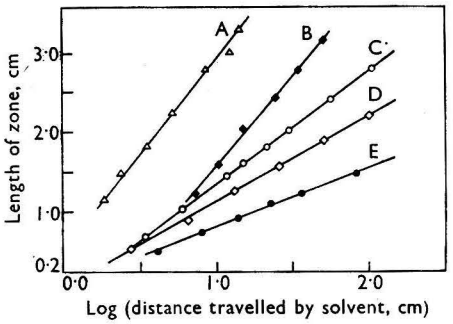

Fig. 2. Curve A, citric acid; curve B, ascorbic acid; curve C, succinic acid; curve D, glutamic acid; curve E, glycine. Developing solvent n-butyl alcohol - acetic acid - water (4 : 1 : 5)

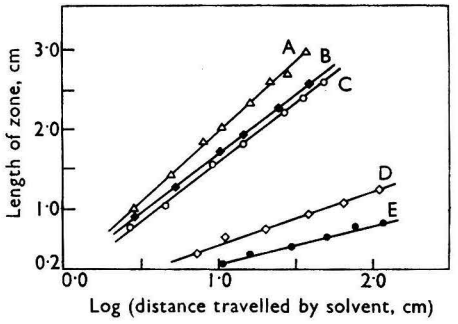

Fig. 3. Curve A, oxytetracycline; curve B, tetracycline; curve C, chlortetracycline; curve D, glucose; curve E, sucrose. Developing solvent as for Fig. 2

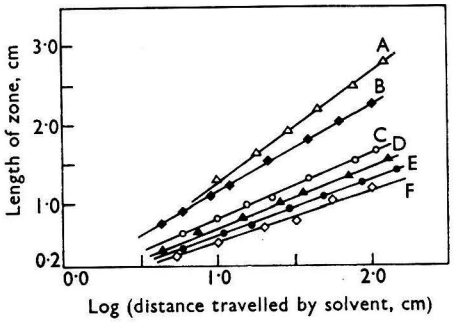

Fig. 4. Curve A, glucose; curve B, glycine; curve C, glutamic acid; curve D, sucrose; curve E, succinic acid; curve F, citric acid. Developing solvent n-butyl alcohol - 29 per cent. ammonia solution (9 : 4)

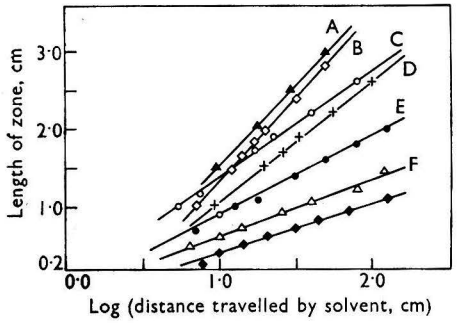

Fig. 5. Curve A, glutamic acid; curve B, glycine; curve C, ascorbic acid; curve D, citric acid; curve E, succinic acid; curve F, sucrose; curve G, glucose. Developing solvent ethanol - 29 per cent. ammonia solution - water (80 : 4 : 16)

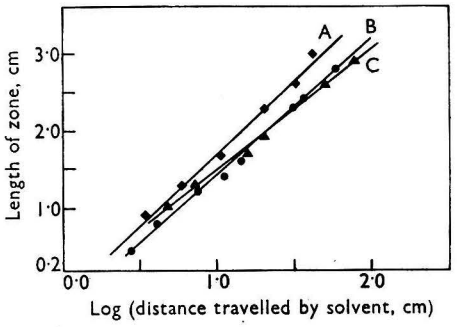

Fig. 6. Curve A, glycine; curve B, alanine; curve C, glutamic acid. Developing solvent acetone - 0·5 per cent. aqueous urea solution (60 : 40)

Figs. 2 to 6. Graphs showing relationships between distances travelled by developing solvents and lengths of zones containing various compounds

where K is a third constant. This equation differs from that suggested by Fowler[2] in that the latter is only applicable to regular spots, which occur infrequently on developed chromatograms. Fowler's equation does not take account of the distance travelled by the developing solvent, which we have found to be an important factor controlling the accuracy of results.

The value of K for each of the compounds tested was calculated and found to be constant under specified conditions; when different developing solvents were used in assaying any compound, several values of K were obtained, and these are shown in Table I.

TABLE I

VALUES OF K FOR VARIOUS COMPOUNDS WITH DIFFERENT SOLVENTS

Compound	Developing solvent*	Value of K	Compound	Developing solvent*	Value of K
Citric acid	A	1·58 ± 0·0361	Glucose	A	0·31 ± 0·0225
	B	0·26 ± 0·0273		B	0·70 ± 0·0108
	C	0·65 ± 0·0252		C	0·24 ± 0·0224
Succinic acid	A	0·70 ± 0·0434	Sucrose	A	0·19 ± 0·0285
	B	0·33 ± 0·0417		B	0·39 ± 0·0245
	C	0·50 ± 0·0		C	0·33 ± 0·0261
Ascorbic acid	A	0·73 ± 0·0115	Malic acid	A	1·0 ± 0·0442
	B	0·35 ± 0·0365	DL-Alanine	D	1·10 ± 0·0521
	C	0·83 ± 0·0	Oxytetracycline	A	2·20 ± 0·0357
Glutamic acid	A	0·60 ± 0·0217	Chlortetracycline		1·66 ± 0·0284
	B	0·44 ± 0·0285	Tetracycline		1·80 ± 0·0187
	C	1·00 ± 0·0196	Penicillin G	E	0·87 ± 0·0388
	D	1·26 ± 0·0324	Pencillin K		0·76 ± 0·0182
Glycine	A	0·24 ± 0·0220	Pencillin V		0·56 ± 0·0202
	B	0·68 ± 0·0317			
	C	0·84 ± 0·0102			
	D	1·35 ± 0·0348			

* The developing solvents used were—

A. Mixture of n-butyl alcohol, acetic acid and water (4:1:5).
B. Mixture of n-butyl alcohol and 29 per cent. ammonia solution (9:4).
C. Mixture of ethanol, 29 per cent. ammonia solution and water (80:4:16).
D. Mixture of acetone and 0·5 per cent. aqueous urea solution (60:40).
E. Aqueous ether.

The technique yields fairly accurate results when pre-determined values of K are used. The "guide" strips are used to derive the value of K for any compound to be assayed when this value is unknown. When K, d and l have been found, the zone content can be calculated from equation (1), thereby avoiding the need for plotting calibration graphs.

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Recommended Methods of Assay of Crude Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS OF ASSAY OF CRUDE DRUGS

The Colorimetric Determination of Rotenone

THE first Report on the work of the Panel* on lonchocarpus and derris of the Joint Committee set up by the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry to study recommended methods for the assay of crude drugs was published in 1959.[1] In this Report on the determination of rotenone in rotenone-bearing plants, with special reference to lonchocarpus, the Panel described a gravimetric method consisting essentially in weighing a rotenone - carbon tetrachloride complex crystallised from an extract of derris or lonchocarpus roots. The application of the method is limited by the size of the sample, since, to obtain worth-while results, it is necessary to have about 5 g of extractive or about 30 g of finely ground root for each determination. Another limitation of this gravimetric method is that, unless careful purification procedures are incorporated in the assay, it is not always possible to obtain crystalline rotenone from formulated products, and, further, materials present in these products may well interfere with the crystallisation of the complex. Thus it is clear that, for purposes other than trading in derris and lonchocarpus roots and extractives, a method is needed that will determine small amounts of rotenone in formulated products.

No generally accepted method for determining rotenone, other than the gravimetric methods reviewed in the earlier Report, has been described in the literature. However, several published papers describe colorimetric methods based on the reactions of the various constituents present in derris and lonchocarpus. The earlier publication of the Panel described six of the known rotenoids present in these materials; one method of analysis, proposed by Goodhue[2] and based on an earlier method recommended by Gross and Smith,[3] measures certain of these rotenoids and not others. Goodhue found that, if a weak solution of rotenone was treated with an alkaline solution of sodium nitrite and then acidified with sulphuric acid, a rose-pink colour developed and appeared to be proportional to the amount of rotenone present in the solution. When he examined various extracts in this way, Goodhue observed that the colour response was greater than could be accounted for by the amount of rotenone indicated by the gravimetric methods; the technique was therefore limited by the fact that it could only indicate a "rotenoid," and not a rotenone, content. It is probable that rotenone, elliptone and deguelin give the colour developed in Goodhue's method, and it is equally probable that toxicarol, sumatrol and malaccol do not. Even with this limitation, the method is of value for control purposes, since, if the "Goodhue value" of any particular rotenone-containing extract is known, then it is possible to estimate the amount of rotenone in a formulated product. For example, if an extract containing 30 per cent. of rotenone, as determined by the crystallisation procedure, is analysed by Goodhue's method, it will indicate a total-rotenoids content of about 57 per cent. If analysis by Goodhue's method of a product fashioned from such an extract indicates a "Goodhue value" of 0·57 per cent., it is reasonable to assume that the rotenone content of the product is about 0·3 per cent. Therefore Goodhue's method is of value for analysing products, complex or simple, that contain rotenone if the analyst has foreknowledge of the rotenone content and of the "Goodhue value" of the extract used.

In 1936, Meijer[4] described a method of analysis that depended upon the development of a reddish purple colour when solutions containing extracts of derris or lonchocarpus were heated with a solution of sodium nitrite in concentrated sulphuric acid. It appeared probable that all the constituents of such extracts contributed to the colour developed, and thus by Meijer's method it seemed possible to analyse any material for its derris or lonchocarpus extractive content. The results obtained by Meijer's original method were somewhat variable, but work by Cahn, Phipers and Boam[5] showed that, with certain modifications, the method

* The Constitution of the Panel presenting this second Report was: Dr. R. F. Phipers (Chairman), Mr. R. Buckley, Mr. J. A. Dawson, Mr. W. E. Drinkwater, Mr. R. V. Foster, Mr. S. C. Jolly, Mr. R. A. Rabnott and Mr. F. H. Tresadern, with Miss A. M. Parry as Secretary.

could be made reliable. In 1947, Meijer[6] published a further communication in which he described a method similar to that developed by Cahn, Phipers and Boam, but, in comparative tests, the results were found to be more consistent by the latter method.

During the second World War there was great interest in derris as a source of rotenone, the active ingredient of the anti-louse powder supplied to the British military forces, and this large consumption of derris naturally necessitated many analyses. These analyses, which controlled the purchase of the raw materials and the preparation of the anti-louse powder, were based on the gravimetric method current at that time, but the manufacturers took the opportunity to carry out parallel colorimetric determinations by both the Goodhue method and the modified Meijer method. As a result of about 2000 analyses, it became possible to establish an arbitrary relationship between the two colorimetric methods and the rotenone content of the material as determined by the gravimetric method. It appeared that the rotenone content of an extract, expressed as a percentage, *plus* 27 approximately equalled the "Goodhue value," *i.e.*, the Goodhue-positive rotenoids, also expressed as a percentage. This meant that if a formulated product were suitably extracted with a solvent, such as acetone, and the extract was diluted to the appropriate concentration, then application of Goodhue's method would give the percentage content of the Goodhue-positive rotenoids, and a determination by the modified Meijer method would indicate the total extractive content. Clearly, from these two values the percentage of "Goodhue materials" in the extract could be calculated. If 27 was then deducted from this value an approximate percentage of rotenone in the extract could be obtained. From this figure and that for the apparent extract content, found by Meijer's method, the probable rotenone content of the product could be calculated.

With use of derris roots of known rotenone content, many products were analysed by this combined colorimetric technique, and it was found possible to deduce a figure sufficiently accurate for the purposes of manufacturing control. This method was also capable of detecting any decrease in the rotenone content that might take place in products subject to bad conditions of storage, such as high humidity or excessive exposure to light. Owing to war-time security, the combined application of these two colorimetric methods was not published, and their use was restricted to the commercial organisation concerned. However, when the Panel required a method for determining rotenone in formulated products, this information was provided for its consideration. It is unlikely that the proportion of material present in derris or lonchocarpus extract responding to Goodhue's method, but which is not rotenone, will be constant. However, as has been mentioned earlier, many tests showed that the use of a constant figure would permit a reasonable degree of accuracy in the assessment of the rotenone content. As the original figure used by Cahn, Phipers and Boam might not be correct if the rotenone content is determined by the revised gravimetric method recommended by the Panel, which tends to give a higher result for the proportion of rotenone in any particular lonchocarpus root or extract than did the earlier methods, it was necessary to establish a revised figure for calculating the rotenone content from the "Goodhue value."

Experimental

First, the Panel investigated Meijer's method as modified by Cahn, Phipers and Boam, and the preliminary results obtained gave rise to some doubts as to the optimum time of heating for the maximum development of colour. Two different samples of lonchocarpus root were then examined, heating times of 3 or 5 minutes being employed. The results (see Table I) show that this colorimetric method does give fairly concordant results.

Each of the roots contained 5·6 per cent. of rotenone when assayed by the gravimetric method recommended by the Panel. A large number of replicates demonstrated that root A contained 18·1 per cent. of extractives and root B 14·7 per cent.

Goodhue's colorimetric method was then applied to these two roots, and the total Goodhue-positive rotenoids content was determined. The correction factor found necessary to convert the Goodhue-positive rotenoid content to the known rotenoid content is shown in Table II.

It can be seen that the mean figure differs from the figure of 27 to 28 mentioned earlier by Cahn, Phipers and Boam, and the Panel considered that a figure of 23 would probably be suitable.

The Panel then turned its attention to a sample of genuine *Derris elliptica*, which, by

the Panel's gravimetric method, was shown to contain 10·8 per cent. of rotenone. The results of collaborative trials by the colorimetric methods are shown in Table III.

TABLE I

APPARENT EXTRACTIVE CONTENT AS DETERMINED BY THE MODIFIED MEIJER METHOD

	Extractive content of—			
	lonchocarpus root A, %		lonchocarpus root B, %	
Heating time	3 minutes	5 minutes	3 minutes	5 minutes
Laboratory				
A	—	—	15·4	15·1
	—	—	14·6	14·9
B	17·3	17·4	14·0	14·0
	17·3	17·3	14·0	13·9
C	18·7	18·4	16·3	15·5
	18·5	19·5	16·4	15·4
D	18·3	17·9	14·3	13·9
	18·4	17·6	14·4	13·6
E	17·1	18·0	15·4	16·3
	—	—	16·0	16·2
F	18·9	18·8	14·6	14·6
	19·3	18·6	14·2	14·3
G	18·1	18·1	14·8	14·5

TABLE II

DETERMINATION OF THE CORRECTION FACTOR FOR THE CONVERSION OF GOODHUE-POSITIVE ROTENOIDS TO ROTENONE

	Lonchocarpus root A		Lonchocarpus root B	
Laboratory	Goodhue-positive rotenoids, %	Correction factor (K)	Goodhue-positive rotenoids, %	Correction factor (K)
A	—	—	9·37	25
B	9·41	21	8·47	20
C	9·99	24	9·21	24
D	10·03	24	8·89	22
E	9·81	23	8·67	22
F	10·11	24	9·37	25
	Mean	23		Mean 23

TABLE III

DETERMINATION OF ROTENONE IN A SAMPLE OF *Derris elliptica* ROOT

		Rotenone content	
Laboratory	Extractive content, %	with K = 23, %	with K = 28, %
A	26·2	11·3	10·0
B	25·0	12·1	10·8
C	26·9	12·1	10·8
D	27·3	12·5	11·2
E	25·5	11·7	10·5
G	24·9	12·5	11·2
Mean	25·96	12·0	10·75

It can be seen from a comparison of these results with the figure obtained by the gravimetric method that, in this instance, a correction factor of 23 was not suitable, but that a factor of 28 gave a rotenone figure reasonably close to that determined by the gravimetric method. It is of interest to note that this figure of 28 is the same as that obtained by Cahn, Phipers and Boam in their original experiments on derris, but not lonchocarpus, roots.

The Panel is of the opinion, therefore, that a factor of 23 should be used in the analysis of products derived from lonchocarpus and a factor of 28 when the source is derris. For materials of unknown botanical origin, it is suggested that the use of a factor of 25 would be satisfactory.

While the work of the Panel was in progress, Bryan and Lincoln[7] suggested various alterations in Goodhue's original method. Collaborative trials were carried out to compare the method suggested by Bryan and Lincoln with that being used by the Panel, but the latter was preferred, since it gave more consistent results.

Further investigations into the details of the modified Meijer method showed that more concordant results were obtained if recrystallised rotenone, instead of the resinous extractive, was used as the component for preparing the standard solution. However, Cahn, Phipers and Boam had observed that, when recrystallised rotenone was used for the preparation of the standard solution and its optical density was compared with that of a standard solution of the same concentration prepared from resinous extractive, the latter was 90 per cent. of the former. It thus appeared that not all the extractives were "rotenoids" and, if recrystallised rotenone is used as the standard for the determination of the amount of resinous extractive, then a factor of $\frac{100}{90}$, *i.e.*, 1·1, must therefore be introduced in calculating the amount of resinous extractive.

In experiments carried out on the conditions for optimum development of the colour in Meijer's method, it was found that the temperature originally used, *i.e.*, 70° C for 3 minutes, was too high, since the colour began to fade at temperatures above 50° C; it also appeared to be preferable to add the acetone solution of the sample under test to the acid reagent, rather than the reverse.

As a result of these collaborative trials the methods described in Appendix I are considered to be useful for the determination of rotenone when the gravimetric method cannot be applied. The empirical nature of the methods does not permit them to be regarded as standard procedures.

Results

The methods were tested by the analysis of certain commercial preparations. Three products were examined: a dusting powder containing finely ground lonchocarpus root, a wettable powder containing finely ground lonchocarpus root together with dried soap, and an experimental emulsifiable fluid, which was a solution of lonchocarpus extract and emulsifying agents in a blend of solvents. The results obtained on these three samples are shown in Tables IV, V and VI.

Table IV

Rotenone content of a dusting powder

Laboratory	Extractive content, %	Mean, %	Rotenone content, %	Mean, %
A	2·68		1·2	
	2·70	2·69	1·2	1·2
B	2·59		1·07	
	2·75	2·67	1·08	1·05
	2·74		1·01	
C	2·81		1·09	
	2·83	2·81	1·19	1·14
	2·78		1·14	
D	2·89		1·1	
	2·89	2·86	1·12	1·12
	2·81		1·14	
E	2·92		1·20	
	2·91	2·93	1·11	1·13
	2·96		1·09	
F	2·89		1·09	
	2·95	2·91	1·02	1·08
	2·88		1·13	
G	2·74	2·74	1·08	1·08
Mean	2·82		Mean 1·11	

TABLE V

ROTENONE CONTENT OF A WARBLE-FLY POWDER

Laboratory	Extractive content, %	Mean, %	Rotenone content, %	Mean, %
A	5·84		2·45	
	5·75	5·8	2·55	2·5
B	6·51		2·51	
	6·42	6·51	2·33	2·34
	6·61		2·18	
C	5·66		2·24	
	5·65	5·69	2·25	2·26
	5·75		2·30	
D	5·9		2·4	
	5·79	5·8	2·2	2·3
	5·7		2·3	
E	5·81		2·51	
	5·80	5·81	2·44	2·45
	5·81		2·40	
F	5·80		2·57	
	5·88	5·85	2·55	2·52
	5·87		2·45	
G	5·76		1·83	
	5·80	5·78	2·20	2·04
	—		2·08	
Mean	5·90		Mean 2·34	

TABLE VI

ROTENONE CONTENT OF AN EMULSIFIABLE FLUID

Laboratory	Extractive content, %	Mean, %	Rotenone content, %	Mean, %
A	2·73		0·83	
	2·70	2·71	0·85	0·84
B	1·63		0·90	
	1·59	1·61	0·89	0·92
	1·60		0·97	
C	2·68		0·99	
	2·67	2·67	0·98	0·97
	2·65		0·95	
D	2·6		0·8	
	2·77	2·69	0·93	0·86
	2·69		0·86	
E	2·84		0·76	
	2·83	2·86	0·78	0·77
	2·90		0·77	
F	2·72		0·94	
	2·70	2·77	0·92	0·93
	2·89		0·92	
G	3·36		0·77	
	3·37	3·35	1·00	0·97
	3·31		0·94	
Mean	2·66		Mean 0·89	

The results in Table VI show that one collaborator (E) obtained results for the rotenone content significantly lower than other workers; another collaborator (B), whose figures for the extractive content appeared anomalous, analysed this fluid again and obtained results that indicated a rotenone content of 0·55 per cent. This worker observed that the material had altered in its physical condition from the time he had first received it and that a crystalline deposit had appeared. Examination of this experimental fluid at different periods of time gave the results shown in Table VII.

TABLE VII

DETERMINATION OF ROTENONE IN AN EMULSIFIABLE FLUID AFTER DIFFERENT INTERVALS OF TIME

Date of examination	Extractive content, %	Rotenone content, %
24.4.61	2·55	0·91
4.5.61	2·43	0·67
8.5.61	2·47	0·55

These results show that the application of the proposed colorimetric method gives a clear indication of the instability of the product and also a measure of the degree of deterioration. The figure quoted for rotenone cannot be correct, because that would be to assume that only the rotenone is degraded, whereas it is most probable that all the Goodhue-positive rotenoids are subject to deterioration. Little is known of the deterioration of such rotenoids, but the point is academic, since the set of results indicates clearly the degree of instability. It is known that simple solutions of rotenoids are reasonably stable, and it is conjectured that the instability of this sample arises from the introduction of unsuitable emulsifying agents.

Appendix I

METHOD FOR THE DETERMINATION OF ROTENONE IN FORMULATED PRODUCTS

INTRODUCTION—

Previous experience had indicated that a combination of two colorimetric methods permitted calculation of an approximate value for the rotenone content of derris or lonchocarpus extractives. Investigations by the Panel confirmed that this arbitrary application of the two methods gave results reasonably close to the rotenone content as known from the gravimetric method already recommended by the Panel. Despite the arbitrary nature of the calculation employed, the results obtained by the Panel demonstrate the practical value and application of the method. However, it must be stressed that the methods cannot be regarded as standard procedures.

PRINCIPLE OF METHOD—

Two colorimetric methods are employed, one of which gives the so-called derris or lonchocarpus extractive content of the sample to be assayed and the other measures rotenone and other closely-related compounds (Goodhue-positive rotenoids). By the use of a correction factor, established experimentally, which relates the two methods, the rotenone content can be calculated.

APPLICABILITY—

For determining rotenone in samples of ground lonchocarpus or derris roots or their extractives that are not available in sufficient amount for the gravimetric method.[1] Also for determining rotenone in formulated products in which the adjuvants will interfere with the crystallisation procedure of the gravimetric method. With suitable techniques a few milligrams of rotenone can be determined.

DETERMINATION OF EXTRACTIVE CONTENT

REAGENTS—

All reagents except rotenone must be of analytical grade.

Acetone.

Sulphuric acid - nitrous acid solution—A 0·01 per cent. w/v solution of sodium nitrite in sulphuric acid, sp.gr. 1·84. This solution must be stored in the dark.

Stock standard rotenone solution—A 0·5 per cent. w/v solution of pure rotenone (melting-point 162° to 164° C after two consecutive recrystallisations from ethanol) in acetone. This solution must be stored in the dark; under these conditions it is stable for 14 days.

Working standard rotenone solution—A 0·01 per cent. w/v solution in acetone, prepared by suitable dilution of the stock solution. This dilute solution should be prepared immediately before use.

PROCEDURE—

Reagent blank—Carry out a blank determination by the entire procedure with the same amount of reagent as is used in the test and 1 ml of acetone instead of the sample solution.

Standard colour—Treat 1 ml of the working standard rotenone solution in a similar manner to the test solution.

Treatment of sample—If the sample is a liquid preparation, dilute it with acetone to give a solution containing about 0·01 per cent. of extractive. If the product is a powder, it should be extracted, when possible, with acetone; if this is impracticable, other solvents may be used, but they should then be removed and the residue dissolved in acetone to give a solution containing about 0·01 per cent. of extractive. The optical densities of the colours of the standard and test solutions should be as close as possible, and in any event should not differ by more than 20 per cent.

Transfer 1·0 ml of this solution to a 25-ml conical flask (see Note 1) containing 10 ml of sulphuric acid - nitrous acid solution at 20° C, swirling the flask during the addition to ensure uniform mixing of the contents. Immediately place the flask in a water bath, maintained at 40° C, for 15 minutes, during which time a reddish purple colour develops. Transfer the flask to a water bath at 20° C for 5 minutes.

Within 30 minutes of the initial mixing of the acid and acetone solutions, measure the optical densities of the colours of the test and standard solutions in turn against the blank in a 1-cm cell; use a spectrophotometer at 540 mμ or a photo-electric absorptiometer with a yellow filter (Ilford 606 is suitable).

The percentage of extractive in the test solution can be calculated from the expression—

$$\frac{\text{Optical density of test solution}}{\text{Optical density of standard colour}} \times 0{\cdot}01 \times 1{\cdot}1.$$

From a knowledge of the dilution rates used in the preliminary treatment of the sample, calculate the percentage of extractive in the sample.

DETERMINATION OF GOODHUE-POSITIVE ROTENOIDS

REAGENTS—

All reagents except rotenone must be of analytical grade.

Acetone.

Working standard rotenone solution—As for "Determination of Extractive Content" (see above).

Sulphuric acid, diluted—Dilute 1 volume of sulphuric acid, sp.gr. 1·84, with 3 volumes of distilled water.

Sodium nitrite solution—Dissolve 1 g of sodium nitrite in 10 ml of distilled water, and dilute to 1 litre with ethanol (95 per cent. v/v). This solution must be stored in the dark.

Potassium hydroxide solution—Dissolve 40 g of potassium hydroxide pellets in 100 ml of distilled water. This solution should not be used if discoloured by ageing or if it contains appreciable amounts of carbonate.

Alkaline sodium nitrite solution—Mix 1 volume of potassium hydroxide solution with 7 volumes of sodium nitrite solution (see Note 2). Prepare this solution freshly as required.

PROCEDURE—

Reagent blank—Carry out a blank determination by the entire procedure with the same amount of reagents as are used in the test and 2 ml of acetone instead of the sample solution.

Standard colour—Treat 1 ml of the working standard rotenone solution in a similar manner to the test solution.

Treatment of sample—If the sample is a liquid preparation, dilute it with acetone to give a solution containing about 0·02 per cent. of extractive. If the product is a powder, it should be extracted, when possible, with acetone; if this is impracticable, other solvents may be used, but they should then be removed and the residue dissolved in acetone to give a solution containing about 0·02 per cent. of extractive. The optical densities of the colours of the

standard and test solutions should be as close as possible, and in any event should not differ by more than 20 per cent.

Transfer 1·0 ml of this solution to a 25-ml conical flask (see Note 1), and add 1 ml of acetone and 2 ml of alkaline sodium nitrite solution. Place the flask in a water bath, maintained at 20° C, for 7 minutes. Add 5 ml of diluted sulphuric acid, insert the stopper in the flask, mix the contents by swirling, and immediately return the flask to the water bath for 15 minutes, during which time a rose-pink colour develops. This colour is stable for about 1 hour.

Within 30 minutes of developing the colour, measure the optical densities of the colours of the test and standard solutions in turn against the blank in a 1-cm cell; use a spectrophotometer at 540 mμ or a photo-electric absorptiometer fitted with a yellow filter (Ilford 606 is suitable).

The percentage of Goodhue-positive rotenoids in the test solution can be calculated from the expression—

$$\frac{\text{Optical density of test solution}}{\text{Optical density of standard colour}} \times 0{\cdot}01.$$

From a knowledge of the dilution rates used in the preliminary treatment of the sample, calculate the percentage of Goodhue-positive rotenoids in the sample.

Calculation of rotenone content

$$\text{The percentage of rotenone in the sample} = r - \frac{KD}{100},$$

where r = percentage of Goodhue-positive rotenoids,
D = percentage of extractive in the sample and
K = 23 for products derived from lonchocarpus,
28 for products derived from derris or
25 for products of unknown origin.

Notes—

1. The flasks used in the test must be scrupulously clean before use, as both tests are very delicate and the slightest trace of organic matter will be detrimental to the development of the colours.

2. The alkaline sodium nitrite solution may be mixed in a measuring cylinder, *e.g.*, 3 ml of potassium hydroxide and 21 ml of ethanolic sodium nitrite in a 25-ml cylinder.

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Notes

COLORIMETRIC DETERMINATION OF 'KELTHANE' RESIDUES

THE method described by Rosenthal, Frisone and Gunther[1] for determining Kelthane (1,1-bis-*p*-chlorophenyl-2,2,2-trichloroethanol) is based on its decomposition to chloroform when heated with a 50 per cent. aqueous solution of sodium hydroxide; specially constructed apparatus is needed, and the procedure involves a complex sequence of operations. It has been found that Kelthane is decomposed by boiling pyridine containing ammonia and that good recoveries of chloroform can be obtained from microgram amounts of Kelthane by heating under reflux with pyridine and ammonia solution, distilling and applying the Fujiwara colour reaction to the distillate. This simplified procedure provides the basis for determining Kelthane in residues from solvent extracts of plant materials and readily lends itself to the simultaneous treatment of a number of samples. A further advantage is that the residual dichlorobenzophenone in the distillation tube can be independently determined to check the possibility of some decomposition having occurred within the plant. Of the possible methods for determining the dichlorobenzophenone, a modification of that described by Schechter, Soloway, Hayes and Haller[2] for determining DDT gave satisfactory results. This method described in this Note is derived from those used by Harris[3] and Skerrett and Baker[4] for chlorobenzilate, which are based on that for DDT.[2] In these methods, the nitrated product in benzene is mixed with a solution of sodium methoxide[2,3] or potassium hydroxide[4] in methanol. A simplified procedure used by Downing and Norton[5] for determining the nitrated DDT consists in extracting the nitrated product directly into benzene, which overcomes the need for preliminary extraction into ether. This procedure was also found to be satisfactory for determining dichlorobenzophenone and was adopted. Examination by the combined analytical methods of tomato foliage after treatment with Kelthane failed to demonstrate that any substantial break-down of the type just mentioned occurred.

The scope of the methods can be seen from the results summarised below. Known amounts from 7 to 39 μg of chloroform in 5 ml of pyridine yielded, after distillation, a mean recovery of 96·5 per cent. (coefficient of variation, 1·3 per cent.) as compared with similar amounts of chloroform determined directly. In a calibration experiment with known amounts from 12·4 to 124 μg of pure Kelthane, recoveries were 81·4 per cent. by the chloroform-distillation method and 90·3 per cent. by the dichlorobenzophenone-nitration method in the same test (coefficients of variation, 5·6 and 6·9 per cent., respectively).

Results of determinations of Kelthane and dichlorobenzophenone in a series of prepared mixtures are shown in Table I. The amount of dichlorobenzophenone found (initially present in the sample) is the calculated difference between the total dichlorobenzophenone determined by the nitration procedure and that equivalent to the Kelthane found by the chloroform-distillation method. Negative results may arise through experimental error when the amount of dichlorobenzophenone in the sample is small or zero, and careful standardisation of reagents and conditions is particularly necessary for consistent results.

TABLE I

AMOUNTS OF KELTHANE AND DICHLOROBENZOPHENONE FOUND IN MIXTURES

Mixture No.	Kelthane present, μg	Dichlorobenzophenone present, μg	Kelthane found, μg	Dichlorobenzophenone found by difference,* μg
1	0·0	85	0·0	80
2	25	68	25	63
3	50	51	50	50
4	74	34	72	27
5	99	17	101	15
6	124	0·0	122	−2

* See text.

METHODS

PROCEDURE FOR DETERMINING KELTHANE BY CHLOROFORM DISTILLATION—

Shake the plant material with light petroleum (boiling range 40° to 60° C), concentrate the extract in a Kuderna - Danish evaporator,[6] and remove residual solvent in the tube by means of a current of air. Place in the tube 5 ml of pyridine containing 1 per cent. v/v of ammonia

solution, sp.gr. 0·880, and connect the tube to a small vertical Liebig condenser; use all-glass joints. Heat under reflux for 10 minutes over a small flame so adjusted that a ring of condensing pyridine remains steady midway up the tube. Then increase the rate of heating, and slowly distil the pyridine into a 5-ml graduated test-tube fitted with a standard ground-glass joint. Collect 3 to 4 ml of distillate, adjust its volume to 5 ml with fresh pyridine, and add 0·5 ml of a 20 per cent. aqueous solution of sodium hydroxide. Insert a stopper, shake the tube vigorously, and immerse it for 2·5 minutes in a boiling-water bath. Cool the tube to room temperature by immersion in cold water, measure the optical density of its contents at 530 mμ within 15 to 30 minutes, and determine the amount of Kelthane present by reference to the optical densities obtained with known amounts of this compound.

PROCEDURE FOR DETERMINING DICHLOROBENZOPHENONE BY NITRATION—

Remove the pyridine remaining after the distillation stage in the procedure described above by volatilisation at 50° to 60° C; assist volatilisation with a jet of air. Add to the residue 4 ml of nitrating acid (a mixture of equal volumes of concentrated sulphuric and fuming nitric acids), and place the tube in a boiling-water bath for 1 hour. Cool the tube in running water, dilute its contents to 25 ml, with mixing and continued cooling, and add a measured volume of benzene. Insert a stopper, and shake the tube vigorously for 30 seconds; allow the layers to separate, shake again for 30 seconds, and, after separation, shake a third time for 30 seconds. After the final separation of the layers, withdraw the aqueous layer by aspiration, add 5 ml of a 5 per cent. solution of sodium hydroxide to the benzene layer, shake the mixture vigorously for 30 seconds, allow to separate, and remove the aqueous layer as before. Repeat the extraction with a further 5 ml of the 5 per cent. solution of sodium hydroxide. Finally, add 1 to 2 g of anhydrous sodium sulphate to the benzene, shake thoroughly, and set aside for 5 minutes. Remove a measured volume of the dry benzene solution, and treat it with an equal volume of a 7·5 per cent. w/v solution of sodium methoxide in methanol. (Use of this concentration of sodium methoxide and a 1-to-1 ratio of volumes gave greater sensitivity than was attained with the 10 per cent. solution and the 1-to-2 ratio of volumes proposed for DDT.[2]) Measure the optical density of the coloured solution at 538 mμ after not less than 30 minutes, and determine the amount of dichlorobenzophenone present by reference to the optical densities obtained with known amounts of this compound.

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NITROSOPHENOL COMPLEXES IN THE DETECTION AND DETERMINATION OF NITRITES

THE use of resorcinol for detecting and determining nitrites has been discussed.[1] The method differs from the Griess method[2] and Bark and Caterall's related procedure[3] in that the nitrite is used to nitrosate a phenol, which then forms a coloured complex with iron or cobalt under the correct conditions of acidity. This Note discusses the sensitivities of various phenols as reagents for determining nitrite and the advantages and limitations of their use.

The compounds studied were *m*-methoxyphenol (resorcinol monomethyl ether) and resorcinol (mentioned in the previous Note[1]), dimedone, sodium 2-naphthol-1-sulphonate, 4-chloro- and 2-methylresorcinol and sodium 1-naphthol-4-sulphonate. The colours of the complexes formed with dimedone were very slow to develop; sodium 2-naphthol-1-sulphonate appeared to be a satisfactory reagent, but development of colour was again slow. 4-Chloro- and 2-methylresorcinol (both of which, when nitrosated, I have used successfully as reagents for iron and cobalt) were

disappointing in that they did not react to form a green solution with ferrous iron. Sodium 1-naphthol-4-sulphonate (Neville Winther acid) appeared to react only when the concentration of nitrite was high.

Experimental

A solution of sodium nitrite containing 0·1 g of NO_2^- per litre was prepared in water; for calibration purposes, 0·5-, 1-, 1·5-, 2-, 3-, 4-, 5- and 6-ml portions of this solution were diluted to approximately 49 ml in separate 50-ml calibrated flasks. The selected phenol was added to the contents of each flask and dissolved, 0·5 ml of glacial acetic acid was added, the solutions were mixed, and 0·1 g of ammonium ferrous sulphate or cobalt sulphate was added; each solution was then diluted to the mark and thoroughly mixed. (A 0·1-g portion of the phenol was used, or a suitable amount of a solution of the phenol, usually in the glacial acetic acid; no further addition of acid was necessary in the latter instance.) Under these conditions, the pH was between 3·1 and 3·2, and small variations in this figure had no significant effect on optical density.

The complexes with ferrous iron of all the phenols studied were soluble. The complex of resorcinol monomethyl ether with cobalt was insoluble, but could be dissolved by shaking the mixture with isopentyl alcohol, allowing the layers to separate and washing and filtering the non-aqueous layer, which was then used for optical-density measurements. Except for the complex of naphthol sodium sulphonate with ferrous iron, the iron and cobalt complexes of the other phenols studied could be partitioned into isopentyl alcohol; this procedure provided an opportunity for increasing the sensitivity of the reaction by extracting the colour from 50 ml of aqueous solution into, for example, 25 ml of non-aqueous solvent.

Results

Comparative results for the phenols examined are shown in Table I; measurements were made in 1-cm glass cells with a Unicam spectrophotometer.

Table I

Development of colour with various phenols

Phenol	Ferrous complex: Wavelength of maximum optical density, A	Ferrous complex: Time for development of maximum colour at 20° C, minutes	Ferrous complex: Optical density of solution containing, per 50 ml— 2 ml of nitrite solution	Ferrous complex: Optical density of solution containing, per 50 ml— 4 ml of nitrite solution	Wavelength of maximum optical density of cobalt complex, A
Resorcinol monomethyl ether ..	7050	60	0·225	0·500	3525
Dimedone	5950	360	0·062	0·225	3425
Sodium 2-naphthol-1-sulphonate	7000	240	0·275	0·620	3825
Resorcinol	7000	20	0·112	0·290	3962

When the temperature of the experiments with dimedone and sodium 2-naphthol-1-sulphonate was increased to 50° C, only 60 minutes were needed for development of colour, but, for the latter reagent, maximum optical density was decreased. Nitrates, either alone or mixed with nitrites, had no effect.

The use of this type of reagent in qualitative analysis has been discussed[1]; other uses are in the examination of water for nitrite content, in the food industry and, possibly, in the detection of nitrates after reduction to nitrites. The method can also be applied to the determination of nitrite-inhibited anti-freeze additives to engine coolants. When the formulation of the anti-freeze is known, the proportion of it in the coolant can be rapidly ascertained by reference to a calibration graph for nitrite.

I thank Dr. J. S. Wignall of this department for much encouragement and help in the preparation of this Note.

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Book Reviews

STANDARD METHODS OF CLINICAL CHEMISTRY. Volume 3. By the American Association of Clinical Chemists. Editor-in-Chief: DAVID SELIGSON. Pp. x + 230. New York and London: Academic Press Inc. 1961. Price $6.50; 52s.

The third volume of this excellent series includes some toxicological methods, some techniques for measuring enzyme activity in body fluids and a biological method, as well as descriptions of several typical clinical chemical analyses.

The methods described for determining alcohol in blood, arsenic in tissues and body fluids and barbiturates and sulphonamides are in general similar to those accepted in this country. It is, however, surprising that the Brody method for determining salicylate has been described rather than Trinder's method, which is not only simple and rapid, but avoids extraction by a toxic solvent.

The determinations of enzyme activity are usually those ordinarily employed, but it is surprising that the description of choline-esterase determination—which depends on the measurement of changes in pH—does not indicate the degree of accuracy desirable nor the kind of pH meter needed. The clinical determination of catechol amines is confined to Lund's determination of noradrenalin and adrenalin by the trihydroxyindole fluorimetric method; no mention is made of the determination of dopamine or of vanillylmandelic acid. The biological technique depending upon the reaction of strips of rabbit aorta is also described in full detail.

The ascorbic acid method is based upon the original Roe and Koether technique, which depends on the coupling of dehydroascorbic acid with 2,4-dinitrophenylhydrazine, a method that has been somewhat neglected in this country. There is a very elegant automatic coulometric titration for chloride, depending upon the precipitation of chloride by silver ions liberated from a silver electrode: the end-point occurs when the sudden increase in silver ions causes a rapid increase in the current passed between two reference electrodes; this activates a relay, cutting off the current and recording the time taken. Although there is a reference to the original paper and to a commercial source of the equipment, it is a pity that the apparatus is not described in detail. The description of the chromatography of haemoglobins is excellent and includes most of the techniques usually employed, but the techniques described for determining creatinine and fibrinogen are out of date; the investigation of gastric acidity makes no use of the modern augmented histamine test-meal. The description of the determination of hydrocortisone in plasma involves the Porter - Silber reaction carried out on a crude extract, with little or no preliminary purification. This method is not acceptable to many workers, since plasma contains phenylhydrazine-reacting chromogens other than cortisol. The method recommended for lactic acid is good and based upon the standard Barker and Summerson procedure.

This volume does not maintain the high standards set by the previous two. It is a pity that a more general treatment is not always given, for details of calculation are often presented that refer to one particular kind of photometer. Nevertheless, there is sufficient in this volume for it to be indispensable to all clinical chemical laboratories. C. H. GRAY

PROTIDES OF THE BIOLOGICAL FLUIDS. Proceedings of the Eighth Colloquium, Bruges, 1960. Edited by H. PEETERS. Pp. x + 356. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1961. Price 86s.

It is not easy to review adequately a volume of this kind, containing within the compass of about 350 pages no less than eighty papers given at a colloquium in Bruges in the early summer of 1960. A quarter of the papers are written in French, another quarter in German and the remainder in English. Although a considerable proportion of the papers deals with techniques, most of the remainder appear to reflect a somewhat static biochemistry of the proteins of the body fluids. The section on labelled compounds mainly consists of seven papers within the compass of only 30 pages, although a few papers in the rest of the volume discuss the kinetics of protein synthesis and break-down, *e.g.*, that by Gordon and Humphrey on intra-cellular albumin in the liver and that by Cohen and Freeman on the metabolism of human gamma-globulin. The dynamic aspects of protein metabolism received mention in the concluding round-table discussion. It can be confidently predicted that the dynamic aspects of protein metabolism will receive greater attention in future colloquia.

The volume begins with an excellent survey of chromatographic methods in protein chemistry by Huisman. Most of the following 100 pages are concerned with methods and techniques; in this section papers on gel composition and serological reactions, on the properties of paper etc. for electrophoresis and chromatography and a mathematical treatment of the problems of diffusion and precipitation in gels are particularly important. In the following section, of nearly 80 pages, immuno-electrophoresis receives fairly comprehensive attention, and the section includes specially interesting papers in which this technique is combined with autoradiography and used in investigating the binding properties of the serum proteins for substances such as insulin and the thyroid hormones. This section also includes contributions by Heremans on the antigenetic relationships of the gamma-globulins and by workers in Cambridge and Warsaw on the antigenetic specificity of fractions obtained by the action of papain on these proteins. The remainder of the book includes a miscellany of topics, including glycoproteins and lipoproteins, with a 60-page section on the pathology of the body fluids, in which the dysproteinaemias (especially in myeloma and related conditions) are discussed. Fahey and Askonas have summarised their elegant work in which ^{14}C-lysine was used to study myeloma protein synthesis and its relation to the plasma cells, and Freeman and Cohen have discussed plasma protein metabolism in the nephrotic syndrome.

It is unlikely that this book will be a general practical laboratory aid or standard reference. It is directed mainly to those specialised workers in the various fields faced with common problems of protein chemistry and biochemistry; certain topics are of special interest to the clinical chemist and chemical pathologist. The volume is magnificently produced and illustrated.

C. H. GRAY

POLYNUCLEOTIDES: NATURAL AND SYNTHETIC NUCLEIC ACIDS. By ROBERT F. STEINER and ROLAND F. BEERS, jun. Pp. viii + 404. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1961. Price 85s.

This book is stated in the preface to be intended for the active worker in the nucleic acid field or in related fields and to provide a systematic account of the properties of natural and synthetic polynucleotides, including a discussion of their structures, syntheses, physical properties and biological functions. There is no doubt, however, that the main emphasis of the book is on the physical properties of polynucleotides, and in this respect it resembles the recent book by Jordan. The discussion of biological function is virtually confined to the last chapter, and this topic is covered so briefly and in such an elementary way that the active worker in the polynucleotide field will find himself reading material with which he should already be well acquainted. On the other hand, the physico-chemical aspects of polynucleotides are expertly dealt with at some length and in considerable detail, and it is for this that the book will be of most value to most readers. Even so, it is unfortunate that the period covered terminates at about the middle of 1960, so that much of the exciting recent work of Doty, Marmur and their colleagues is not fully described.

The treatment of the subject tends to be uneven. For example, the enzyme polynucleotide phosphorylase is given the best part of two chapters, whereas DNA polymerase, referred to by the authors as polynucleotide pyrophosphorylase, is dismissed in three pages, and the mechanisms for the biosynthesis of RNA by enzyme systems other than polynucleotide phosphorylase are given very scanty treatment indeed.

A minor defect is that authors cited in the references are given their first initial only, so that some well known names are not immediately recognisable.

In spite of these criticisms, there is no doubt that this book will be of great value to those working in the polynucleotide field and especially to those interested in the physico-chemical aspects of the subject.

J. N. DAVIDSON

SPECTROCHEMICAL ANALYSIS. By L. H. AHRENS, M.A., D.Sc., F.R.I.C., and S. R. TAYLOR, M.A., M.Sc., Ph.D. Second Edition. Pp. xxiv + 454. Oxford, London, New York and Paris: Pergamon Press; Reading, Mass., and London: Addison-Wesley Publishing Company Inc. 1961. Price 105s.

The scope of this book is adequately defined by its sub-title, "A treatise on the d.c. arc analysis of geological and related materials." The authors have specifically limited themselves to the analysis of non-metallic samples, such as minerals, rocks, meteorites, soil, refractories, slag and ash, and have surveyed in detail the extensive literature dealing with analysis of such materials

by means of the d.c. arc. This second edition has been brought up to date by including developments since the appearance of the first (in 1950), such as the use of spectrochemical techniques for determining elements at comparatively high concentrations, the development of general sensitive methods for the simultaneous determination of many elements and the use of enrichment techniques.

The first part of the book, comprising some 180 pages, deals with general principles, including such matters as origin of spectra, features of the arc discharge, sample preparation, selective volatilisation, internal standards, matrix effects and photographic measurement. Many of these are discussed well and informatively, and in this respect particular mention may be made of those parts dealing with factors influencing the origin of radiation in the discharge, matrix effects, the choice of an internal standard and photographic aspects. However, because the mechanism of the arc discharge is complex and as yet not fully understood—as the authors have rightly pointed out—no clear correlation can be expected between theory and experimental results. In spite of this fundamental difficulty, the authors have high-lighted many salient points from the confused tangle of the literature, in which each investigator seems to have developed his own individual techniques in the absence of adequate theoretical background. Although instrumental aspects have been specifically excluded, some discussion on the effects of optical arrangements on distribution of line intensities would perhaps not have been out of place. Further, those without prior knowledge could be misled by the brief section on statistical interpretation of results.

The second part of the book (120 pages) outlines and reviews methods for specific elements or groups of elements, incorporating most techniques described in the literature, together with comments based on the authors' extensive experience. It would have been advantageous if these comments could have been given greater prominence and firmer guidance provided by linking individual methods with the general principles established in the first part, but this is hardly possible because of the unsystematic nature of the literature, and the authors have done well to reduce it to manageable proportions. In any event, they set out to write a treatise and not a monograph.

The bibliography, some 50 pages, is up to date and of wide coverage. An appendix (70 pages) is devoted to wavelength tables, listing sensitive lines of the elements and possible interfering lines. It also is comprehensive, but it could perhaps be supplemented by references to authoritative lists of lines for particular elements. A case in point is Shenstone's list for the first spectrum of copper (*Phil. Trans. A*, 1948, **241**, 297), which would be useful for anyone employing copper electrodes.

The book is primarily directed to geochemists, who will doubtless welcome this second edition. Other analysts, as well as metallurgists, should find several items of interest and stimulation.

M. MILBOURN

EXPERIMENTS FOR INSTRUMENTAL METHODS. By CHARLES N. REILLEY and DONALD T. SAWYER. Pp. xii + 412. New York, Toronto and London: McGraw-Hill Book Company Inc. 1961. Price 46s.

The wide-spread acceptance of new physical methods of analysis has been accompanied by the publication of specialised monographs detailing the fundamental theory and applications of each method, but, so far, few practical books describing the manipulation and maintenance of analytical instruments and suitable for the laboratory training of future analysts have appeared. This laboratory manual, which is presumably based on practical courses conducted at the Universities of North Carolina and California, has been written to meet this need. The authors, however, do not indicate the technical level of the student for whom the course has been designed. Moreover, there is always a divergence of opinion as to what the scope and emphasis of a book of this kind should be.

The manual has been planned on a broad basis to cover experimental procedures for most of the instrumental techniques used routinely in a modern analytical laboratory. The text has been divided into five parts, covering electrometric methods (potentiometric, conductimetric, polarographic, amperometric and electrolytic), optical methods (emission and ultra-violet and infra-red absorption spectroscopy), separation methods (column, paper and gas chromatography and ion exchange), radiochemical methods and instrumentation (elementary electronics and the servicing of instruments). A useful appendix, containing tables of selected physical data and colour-coding schemes for electrical components, has been added. X-Ray and nuclear magnetic resonance techniques are mentioned, but their laboratory use is not described.

Each chapter is introduced by a short summary of the relevant theory and includes an adequate

up-to-date bibliography to encourage wider reading. The directions for each experiment are preceded by a more detailed account of the basic theory and are followed by a set of questions designed to test the student's understanding of the experiment. Most of the experiments are described in sufficient detail for a degree student or a skilled laboratory technician to go ahead on his own, but it is immediately evident that the course should be conducted by a supervisor who knows the answers and is able to demonstrate specialised pieces of equipment, such as the press for making potassium bromide discs (see page 199). The experiments have, in general, been well selected to demonstrate the basic principles and analytical applications of each instrumental method.

The book has, from the British point of view, the serious defect that many experiments, including those on polarography and spectroscopy, are discussed in relation to American commercial instruments. This might confuse a student, but a supervisor should have no difficulty in amending the instructions to suit equivalent British instruments.

The manual, which has been printed by off-set reproduction of veritype and has a paper cover, is by present-day prices for American books surprisingly cheap. It can be warmly recommended as an introductory text for chemists interested in the handling of analytical instruments and as a laboratory-training manual for all students of analytical chemistry.

J. E. PAGE

LES MÉTHODES DE LA CHIMIE ANALYTIQUE: ANALYSE QUANTITATIVE MINÉRALE. By G. CHARLOT. Fourth Edition. Pp. viii + 1024. Paris: Masson et Cie. 1961. Price (paper) 100 NF; (cloth boards) 110 NF.

As this book has not been reviewed in *The Analyst* before, it is justifiable to give it a more lengthy notice than is usually accorded to a fourth edition. It is a very individual work, packed with information concisely presented, and can be said to summarise its author's method of teaching and his views. This does not make the book easy to read; the English analyst, especially if he is middle-aged, learnt his analytical chemistry in a different way, but he will find the effort worth making.

The first 550 pages present the theoretical foundation in four parts; chemical reactions in solution, electrochemical reactions, methods of measurement and non-aqueous solvents. There is a brief note (20 pages) on various techniques, then comes the second part (410 pages) on the determination of the principal elements.

It is most difficult to summarise this book, itself a concentrated essence, so to speak, of modern analytical chemistry. In it, acids are Brönsted acids, and oxidations and reduction are reactions involving exchange of electrons, as are electrochemical reactions of every kind; the treatment is formal and rigorous. Numerous graphs are given, but many are not easy to understand unless one is familiar with the author's other works. Part III, on methods of measurement, begins with a discussion on precision and a brief statistical section, followed by spectrophotometry, gravimetry and thermogravimetry and radiometric determinations; volumetric analysis is not reached until the fifth chapter. There are 80 pages on amperometry, potentiometry and conductivity and 24 on coulometry, and the theoretical portion ends with a thorough discussion of reactions in non-aqueous solvents.

Each sub-section is followed by a selective bibliography, papers and text-books as recent as 1960 being cited. The quantity of information included is vast. From time to time the reader is astounded by the cheerful disrespect with which his cherished beliefs are dismissed. "Precipitation is the oldest method of separation, and is still often used. It has numerous inconveniences, the operations are lengthy, the separations are imperfect because of adsorption and co-precipitation. The old classical methods using hydrogen sulphide, ammonia, sodium hydroxide, etc., are particularly imperfect and only of interest in a few exceptional cases. In modern methods precipitation is avoided as far as possible." This is more or less true, but at the same time it overstates the case, as these old methods are still very useful (to those who have learnt by experience how to use them), particularly as preliminary steps in dealing with unusual and complex samples.

The second part of the book deals with the determination of sixty-six elements. It gives selected methods of separation and determination of major amounts and traces, each paragraph being followed by a few references. Brief practical instructions are given for the more important procedures, enough for a practised analyst, certainly not enough for a tyro; when the author's personal views do not accord with the usual practice, the latter is neglected. For example, under "iron," separation by precipitation as hydroxide is not mentioned: we find instead separation

by solvent extraction as ferric chloride, isobutyl methyl ketone being preferred, with a long list of other ions wholly or partly extracted. Cupferron, oxine and 1,10-phenanthroline are also mentioned. The volumetric determination of iron after its reduction to the ferrous state follows and is well treated. Brief accounts of the titration of Fe^{3+} by titanous chloride and by EDTA follow. Under "Gravimetric determination," only precipitation of ferric hydroxide and its conversion to Fe_2O_3 is described. Under "Colorimetry," thiocyanate and 1,10-phenanthroline are discussed, with lists of interferences, extinction coefficients and—under thiocyanate—the effect of adding acetone and dioxan to lower the dielectric constant of the medium. 2,2'-Bipyridyl, sulphosalicylic acid and cupferron have a line each and a few literature references. There are a note on precision spectrophotometry and references to a few books on the analysis of ferrous metals. The A.S.T.M. and A.F.N.O.R. standard methods are listed, but no corresponding British Standards are included.

This is a representative sample of the treatment accorded to an important element. A rare element, such as indium, is given only 4 pages, beginning with its separation by extraction as iodide with cyclohexanone; the only reference to flame photometry is an obscure Russian publication, and polarography is not mentioned. In fact, the author tends to neglect the polarograph in the practical part of the book. One cannot expect any author to discuss all the elements equally well, and most readers with specialised interests are likely to find omissions. For example, under potassium, there is no mention of the best gravimetric method, the weighing of potassium platinic chloride. (There is an irrational prejudice against this method on the Continent, where it is seldom used.) We are often told that the day is past when a single author can cover a subject, there must instead be an editor controlling a squad of collaborators; the result too often shows that one or more of the collaborators would have been more usefully employed in some less exacting occupation. But there is probably not another volume that presents such a mass of information on inorganic analysis at the present time, and such a useful list of references.

Finally, those who have suffered from the inadequacy of the index in French books may be glad to know that the index is good. H. N. WILSON

MONOGRAPHS ON THE RADIOCHEMISTRY OF THE ELEMENTS. Washington, D.C.: U.S. Department of Commerce, Office of Technical Services. 1960–61. Nuclear Science Series: NAS—NS–3001 to 3011, 3013 to 3018 and 3020 to 3029.

These monographs are part of an as yet incomplete series of publications on the radiochemistry of the elements, prepared under the auspices of the U.S. National Academy of Sciences—National Research Council Subcommittee on Radiochemistry. The stated object is to present "up-to-date compilations of radiochemical information and procedures. . . . Each monograph collects in one volume the pertinent information required for radiochemical work with an individual element or a group of closely related elements."

The elements or groups dealt with so far are cadmium (monograph No. NAS—NS–3001), arsenic (3002), francium (3003), thorium (3004), fluorine, chlorine, bromine and iodine (3005), americium and curium (3006), chromium (3007), rhodium (3008), molybdenum (3009), barium, calcium and strontium (3010), zirconium and hafnium (3011), beryllium (3013), indium (3014), zinc (3015), protactinium (3016), iron (3017), manganese (3018), rare earths, scandium, yttrium and actinium (3020), technetium (3021), vanadium (3022), tin (3023), magnesium (3024), rare gases (3025), mercury (3026), copper (3027), rhenium (3028) and ruthenium (3029).

Although not completely uniform in presentation, the monographs typically provide information under the following headings: references to reviews of the inorganic, analytical and radiochemistry of the element; table of isotopes; review of those aspects of the chemistry of the element of interest to radiochemists; solution of samples; counting techniques; radiochemical separation procedures. As would be expected, the real meat is in the sections on the chemistry of the element and on radiochemical procedures, which together comprise the major part of each monograph.

Although some criticism can be made (it seems curious that, often, the energy of the radiations is not included in the table of isotopes), these monographs clearly offer a valuable source material for radiochemists. Indeed, because of the analytical information provided, particularly on solvent extraction and ion exchange, some of the monographs might profitably be read by analysts not concerned with radiochemistry.

The monographs are paper-backed. With four exceptions (notably, the monograph on the rare earths, 282 pages, price 3 dollars), the monographs comprise some 20 to 70 pages and cost 50 or 75 cents. H. J. CLULEY

Publications Received

COLORIMETRIC METHODS OF ANALYSIS: INCLUDING PHOTOMETRIC METHODS. Volume IIIA. By FOSTER DEE SNELL, Ph.D., and CORNELIA T. SNELL, Ph.D. Assisted by CHESTER ARTHUR SNELL, Ph.D. Pp. x + 576. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1961. Price 96s.

ABSORPTION SPECTRA IN THE ULTRAVIOLET AND VISIBLE REGION. (A Theoretical and Technical Introduction.) Second Edition. Pp. 80. Edited by Dr. L. LANG, in collaboration with Dr. J. SZOKE, Dr. G. VARSANYI and M. VIZESY. *Also* Volume I, Second Edition, Loose-leaf, pp. 414, and Volume II, First Edition, Loose-leaf, pp. 408. Budapest: Publishing House of the Hungarian Academy of Sciences. 1961. Price (Text Volume and Volume I together) $18.00; (Volume II) $16.00.

Volumes I and II contain spectra and spectroscopic data.

CARBON-14 COMPOUNDS. By JOHN R. CATCH. Pp. viii + 128. London: Butterworths Publications Ltd. 1961. Price 30s.

ORGANIC PEROXIDES. By ALWYN G. DAVIES, Ph.D., D.Sc. Pp. x + 215. London: Butterworths Publications Ltd. 1961. Price 50s.

SEPARATION OF HEAVY METALS. By ANIL K. DE, D.Phil. Pp. 308. Oxford, London, New York and Paris: Pergamon Press. 1961. Price 60s.; $9.00.

XV CONGRESSO INTERNACIONAL DE QUIMICA PURA E APLICADA (*Quimica Analytica*): ACTAS DO CONGRESSO (*8 a 16 de Septembro de* 1956). Volume III. Pp. 978. Lisbon: General Secretary of the XVth International Congress of Pure and Applied Chemistry, Instituto Superior Technico. 1961. Price, together with volumes I and II, 320 Escudos.

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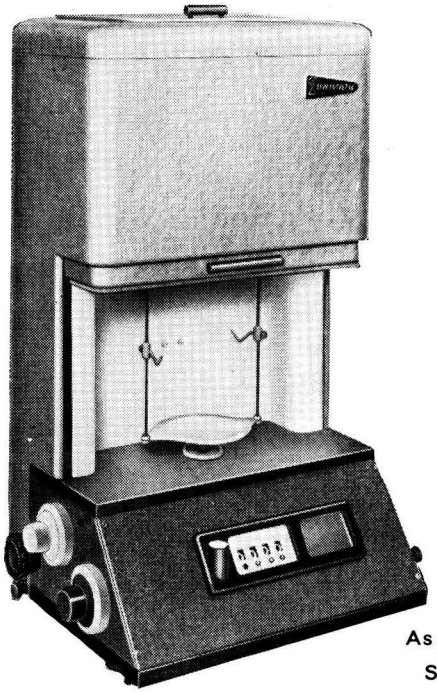

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