THE ANALYST

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Editor: J. H. LANE, B.Sc., F.R.I.C.
7-8, IDOL LANE, LONDON, E.C.3
Telephone: MANsion House 6608

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

ANALYTICAL METHODS COMMITTEE

Sub-Committee on Determination of Egg Yolk Solids in Salad Cream, Mayonnaise and any other Salad Dressing

A Sub-Committee has been appointed to consider the practicability of recommending a standard method of analysis for the determination of egg yolk solids in salad cream, mayonnaise and any other salad dressing.

The Sub-Committee consists of H. E. Cox, D.Sc., F.R.I.C. (Chairman); E. B. Anderson, M.Sc., F.R.I.C.; G. E. Forstner, M.Sc., F.R.I.C.; C. L. Hinton, F.R.I.C.; H. E. Monk, B.Sc., F.R.I.C.; C. G. Daubney, M.Sc., F.R.I.C. (Hon. Secretary).

EXTRAORDINARY GENERAL MEETINGS

Extraordinary General Meetings of the Society were held on September 20th, 1949, and February 1st, 1950, the President occupying the chair on both occasions. Special Resolutions were carried at these meetings by overwhelming majorities altering certain of the Society's Articles of Association.

At the meeting held in September a Resolution was proposed that the name of the Society be changed to "The Society for Analytical Chemistry." A majority of votes was cast in favour of this resolution, but failed to reach the three-fourths majority needed for a Special Resolution, so that the name will remain unchanged.

SCOTTISH SECTION

The Fifteenth Annual General Meeting of the Section was held in Glasgow on January 26th, 1950, and the following office bearers were elected for the year:—*Chairman*—Dr. J. Sword. *Vice-Chairman*—Mr. H. C. Moir. *Hon. Secretary and Treasurer*—Mr. R. S. Watson, City Analyst's Department, 20, Trongate, Glasgow, C.1. *Elected Committee Members*—Messrs. A. Dargie, H. Dryerre, M. Herd, J. M. Leitch, J. M. Malcolm and R. G. Thin. *Hon. Auditors*—Messrs. A. R. Campbell and W. M. Cameron.

MICROCHEMISTRY GROUP

The Sixth Annual General Meeting of the Microchemistry Group was held at Sir John Cass College, London, E.C.3, on Friday, January 27th, 1950. It was reported that the number of members of the Group is now 309, an increase of 51 during 1949. The following Officers and Committee Members were elected for the ensuing year:—*Chairman*—Mr. Ronald Belcher. *Vice-Chairman*—Dr. Cecil L. Wilson. *Hon. Secretary*—Mr. Donald F. Phillips. *Hon. Treasurer*—Mr. Gerald Ingram. *Elected Committee Members*—Messrs. A. E. Heron, R. F. Milton, J. Sandilands, C. E. Spooner, D. W. Wilson and G. H. Wyatt.

An exhibition of new microchemical apparatus was organised by Dr. J. T. Stock, and a series of short papers describing the apparatus were read by Messrs. J. T. Stock, G. Ingram, W. T. Chambers, M. A. Fill, P. Heath, A. J. Lindsey, F. J. McMurray, W. Marshment and A. C. Mason.

PHYSICAL METHODS GROUP

THE Fifth Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 29th 1949, in the Chemistry Lecture Theatre, Imperial College of Science and Technology, London S.W.7. Dr. J. G. A. Griffiths, the Chairman of the Group, presided. The Group Officers and Elected Members of Committee for the forthcoming year are as follows:—*Chairman*—Mr. B. S. Cooper. *Vice-Chairman*—Dr. W. F. Elvidge. *Hon. Secretary*—Mr. R. A. C Isbell, Hilger & Watts Ltd., Hilger Division, 98, St. Pancras Way, London, N.W.1. *Members of Committee*—Messrs. L. A. Haddock, J. Haslam, J. A. C. McClelland, H. E. Monk, J. E Page and A. A. Smales. *Hon. Auditors*—Messrs. Bassett and Garratt (re-appointed).

Votes of thanks were accorded to the three retiring members of the Committee, Messrs J. G. A. Griffiths (Chairman), J. E. Page (Secretary) and W. Cule-Davies, for their services to the Group during the past two years, and to the Hon. Auditors for their work during the past year.

The Annual General Meeting was followed by the Twenty-third Ordinary Meeting of the Group, at which Dr. J. G. A. Griffiths delivered a lecture on "The Mass Spectrometer—A Survey of its Applications in Analysis," and by a demonstration by Dr. E. F. D. Winter of the apparatus used in the Imperial College.

BIOLOGICAL METHODS GROUP

THE Fifth Annual General Meeting of the Biological Methods Group was held at 6.15 p.m. on Tuesday, December 13th, 1949, in the Anatomy Lecture Theatre, University College London, W.C.1. Dr. A. J. Amos was in the chair.

The following were elected as Officers and Members of the Committee for the year 1950:—*Chairman*—Mr. N. T. Gridgeman. *Vice-Chairman*—Dr. H. O. J. Collier. *Hon. Secretary*—Mr. S. A. Price, Vitamins Ltd., 23, Upper Mall, London, W.6. *Elected Committee Members*—Mr. W. A. Broom, Dr. W. F. J. Cuthbertson, Dr. R. L. Edwards, Mr. H. Pritchard, Dr. J. Raventos, Mr. G. A. Stewart and *Ex-officio Members*, the President, Hon. Treasurer and Hon. Secretary of the Society and the Editor of *The Analyst*. *Hon. Auditors*—Mr. D. M. Freeland and Dr. J. H. Hamence.

The Annual General Meeting was followed by an Ordinary Meeting at which the following papers were read and discussed: "The Microbiological Assay of Riboflavine in Yeast and Yeast Products, using *Lactobacillus helveticus* in a 17-hour Titrimetric Method," by A. Jones and S. Morris; "A 24-hour Plate Assay Technique for the Vitamin B_6 Complex of Yeast with a Note on the Possible Presence in Certain Yeasts of a Fourth Member of the B_6 Complex," by A. Jones and S. Morris; "The Assay of Serum Gonadotrophin by the Ovary Weight Method," by G. L. M. Harmer.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held at Manchester on Saturday, November 26th 1949. The Chairman, Mr. J. G. Sherratt, presided, and the attendance was 44, including the President, Mr. G. Taylor, O.B.E. The following papers were read and discussed: "The Standardisation of Hortvet Thermometers," by R. W. Sutton, B.Sc., F.R.I.C., and J. Markland, B.Sc., F.R.I.C., and "Notes on Molasses in Grass Meals and Pellets," by F. Robertson Dodd, F.R.I.C.

INTERNATIONAL MICROCHEMICAL CONGRESS

JULY 2ND TO 5TH, 1950

THIS will be held in Graz, Austria, on the above dates, under the auspices of the Austrian Society for Microchemistry. The provisional programme includes the commemoration of the tenth and twentieth anniversary of the death of Emich and Pregl respectively, both of whom carried out their pioneering work on the development of microchemical techniques in Graz. There will be lectures by well known microchemists covering the whole field of microchemistry, reports, exhibitions, receptions by Government and municipal dignitaries, and bestowal of honorary membership of the Osterreichische Gesellschaft für Mikrochemie to participants.

The Congress President is Professor H. Lieb.

Further information and forms of invitation may be obtained from the Congress Secretary H. Malissa, at the Congress H.Q., Schlögelgasse, 9, Graz, Austria, or from the Hon. Secretary of the Microchemistry Group, Donald F. Phillips, 10, Richmond Road, Blackpool, N.S., Lan

The Examination of Methyl Methacrylate Polymers and Co-Polymers

By J. HASLAM and W. SOPPET

Synopsis—Methods which have proved useful in the examination of methyl methacrylate polymers and co-polymers are described. The method of determination of plasticiser in this type of polymer is described and full details are given of the vacuum depolymerisation of plasticiser-free polymers. Observations are made on the identification of the depolymerisation products with particular reference to polymethyl methacrylate alone, and co-polymers containing polystyrene, polycyclohexyl methacrylate and polyethyl acrylate. The chemical evidence is supported by infra-red data.

Methyl methacrylate polymers and co-polymers are of various types. The preparation may contain plasticiser, colouring agent or filler. It may be co-polymerised, *i.e.*, it may consist of co-polymers of methyl methacrylate and styrene, of methyl methacrylate and ethyl acrylate, methyl methacrylate and cyclohexyl methacrylate or of other possible combinations.

As the most important question which has to be answered about these preparations is the nature of the polymer or co-polymer, the purpose of this paper is to describe some of the methods which have proved to be most valuable in this connection.

When dealing with compositions containing polymethyl methacrylate, the most satisfactory method of identification is to prepare as pure a monomer as possible by depolymerisation of the polymer. The first fact to be realised, however, is that depolymerisation of polymethyl methacrylate preparations in air, without preliminary treatment, yields a monomer which is distinctly unsatisfactory for the purpose of identification. A monomer prepared in this way from polymethyl methacrylate containing small proportions of plasticiser gave the following figures on examination—

Saponification value (mg. of KOH per g.)	=	527
Refractive index, 20° C.	=	1·42
Aldehydes, as H.CHO, per cent.	=	0·11

The recovered monomer was shown, by polarographic and dimedone precipitation methods, to contain formaldehyde.

This monomer was distilled at 20 mm. pressure and the fractions were examined separately with the following results.

	1st Fraction (about 30%)	2nd Fraction (about 60%)	Residue (not volatile at 100° C., 20 mm. pressure)
Saponification value (mg. of KOH per g. sample)	545	548	390
Refractive index, 20° C.	1·4135	1·4135	1·487
Aldehydes, as H.CHO, per cent.	0·064	0·015	—
Carbon, per cent.	—	—	64·9
Hydrogen, per cent.	—	—	7·5

It should be noted that the accepted values for pure methyl methacrylate are as follows—

Carbon, per cent.	60·0
Hydrogen, per cent.	8·0
Refractive index, 20° C.	1·4144
Saponification value (mg. of KOH per g.)	560

It is therefore desirable to remove the plasticiser as a preliminary to any satisfactory depolymerisation process and this is best accomplished by solution of the preparation in acetone followed by precipitation of the polymer with light petroleum. This isolation of the plasticiser-free resin forms part of the ordinary method of determination of plasticiser in polymethyl methacrylate compositions and is as follows—

One gram of the sample, in the form of finely divided drillings, is weighed into a beaker (250 ml.) provided with a glass stirring-rod. Twenty-five ml. of acetone are added and the liquid is brought just to the boil, with continuous stirring on the water-bath. The solution is then allowed to cool for about 1 hour. By this treatment the polymer in all ordinary

samples will be taken into solution, but with abnormal samples it may be necessary to allow the solution to stand for a longer time.

Eighty ml. of light petroleum (b.p. 40° to 60° C.) are now added and the mixture is stirred vigorously and then allowed to settle. After standing for 1 hour the precipitate is filtered on a 1 × G3 sintered-glass Gooch crucible, the filtrate being collected in a flask (250 ml.). The resin is washed three times with 5 ml. of light petroleum on each occasion. The washed resin is then dried to constant weight in the oven at 100° C.

In the determination of the plasticiser, the light petroleum solutions are collected and the solvent removed by evaporation on the water-bath, previous to drying at 100° C.

Carried out in this way the method gives results for the plasticiser that are slightly on the high side, *i.e.*, 0·0 to 0·3 per cent., owing to the solution of small amounts of low molecular weight polymer in the light petroleum extract.

The behaviour of a methacrylate polymer in the plasticiser determination can on occasion yield evidence of the presence of abnormal additives. In the determination of the plasticiser in a recent competitive product, the light petroleum extract of the plasticiser had a strong odour of "oil of wintergreen," and the presence of salicylate was confirmed on hydrolysis of the resulting plasticiser.

Before depolymerisation experiments are carried out, it is often desirable to make exact carbon and hydrogen determinations on the deplasticised resin; in this connection the theoretical figures for the carbon and hydrogen contents of polymethyl methacrylate, polystyrene, polycyclohexyl methacrylate and polyethyl acrylate are of value. They are as follows—

	Carbon %	Hydrogen %
Polymethyl methacrylate	60·0	8·0
Polystyrene	92·26	7·74
Polycyclohexyl methacrylate	71·4	9·5
Polyethyl acrylate	60·0	8·0

It will be noted from the above figures that co-polymerisation of methyl methacrylate with 10 per cent. of polystyrene leads to production of a polymer with the following elementary analysis—

	Per cent.
Carbon	63·2
Hydrogen	7·95

Co-polymerisation with 50 per cent. of cyclohexyl methacrylate produces a polymer with the following elementary analysis—

	Per cent.
Carbon	65·7
Hydrogen	8·75

It is important, however, to realise that co-polymerisation with ethyl acrylate produces a co-polymer with carbon and hydrogen figures exactly similar to those of polymethyl methacrylate.

Depolymerisation in air, *i.e.*, by heating the plasticiser-free resin prepared in the above way in a small flask and subsequent distillation of the monomer, does not yield a very satisfactory monomer for identification purposes, as is shown by the following figures which were obtained by heating plasticiser-free polymethyl methacrylate in a side-arm distillation flask at 350° C., using a Wood's metal bath, for an hour. The distillate had the following characteristics—

Saponification value (mg. of KOH per g.)	540
Refractive index, 20° C.	1·416
Recovery	about 60%
Appearance	Yellow

Our experience with methacrylate polymers goes to show that the only satisfactory method of depolymerisation involves heating *in vacuo* under conditions which ensure the immediate distillation of the monomer as soon as it is formed. We are indebted to Dr. J. W. C. Crawford for details of an apparatus for this purpose, and the method we have found most satisfactory is given below in full.

In this method, as applied to the depolymerisation of polymethyl methacrylate, 1 g. of the plasticiser-free sample is weighed into the tube A of the Pyrex glass vacuum depolymerisation apparatus shown in Fig. 1.

The upper portion of tube A is then sealed off at C in the blowpipe flame, leaving a capillary tip. Tube B is now connected at E to a high vacuum pump and when the pressure on the manometer connected to the pump has been reduced to 1 mm. of mercury and with the pump still connected, the apparatus is sealed off in the blowpipe flame at D.

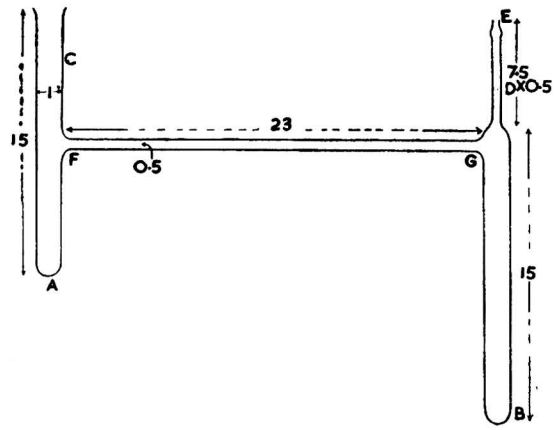


Fig. 1. Vacuum depolymerisation apparatus of pyrex glass.
Dimensions are in centimeters

The apparatus is now assembled with tube A immersed in a bath of Wood's metal at 100° to 120° C. This Wood's metal is contained in a small metal bath, 1-inch internal diameter and 3½ inches long. Tube B is immersed in a solid carbon dioxide - methanol freezing mixture at −70° to −80° C. contained in a Dewar flask (capacity 1 litre) and the levels of A and B are so arranged that the point G is at a higher level than point F. The temperature of the Wood's metal bath is now raised to 340° to 350° C. over a period of 15 minutes and is maintained at this temperature for 1 hour. The apparatus is then withdrawn from the hot Wood's metal bath and cold solid carbon dioxide - methanol mixture, the capillary tip at C broken and the tube B cut off near the top, *i.e.*, at a point G. The analytical examination of the monomer in tube B is carried out without delay in order to avoid polymerisation.

The following figures are typical of those obtained on depolymerisation of polymethyl methacrylate by the above procedure. The figures obtained on examination of pure methyl methacrylate are also given for purposes of comparison.

	Monomer recovered from polymethyl methacrylate by depolymerisation *in vacuo*	Pure methyl methacrylate monomer
Recovery, per cent.	90–97	—
Colour	Water white	—
Refractive index, 20° C.	1·4142–1·4144	1·4144
Saponification value (mg. of KOH per g.)	556–560	560
Acid value	nil	nil

On occasion, valuable evidence about other additives in the polymer may be obtained from the odour of the recovered monomer. A recent competitive sample had a strong odour of mercaptan, presumably added in the polymerisation process. Although the amount present was extremely small it was quite sufficient to mask the very characteristic odour of methyl methacrylate.

Behaviour of methyl methacrylate - polystyrene interpolymers on depolymerisation *in vacuo*

In our experience polymethyl methacrylate - polystyrene interpolymers may be depolymerised by vacuum depolymerisation exactly as indicated above for polymethyl methacrylate alone. The yield of monomer is rather low.

Although it might be expected that because methyl methacrylate boils at 100° C. and styrene at 143° C., preferential depolymerisation of the polymethyl methacrylate would take

place, our analytical evidence hardly supports such a view and the recovered monomer mixture always contains styrene.

The following results were obtained on depolymerisation of a polymethyl methacrylate - polystyrene interpolymer containing 10 per cent. of polystyrene.

Recovery, per cent.	70–75
Colour	Water white
Refractive index, 20° C.	1·4242
Saponification value (mg. of KOH per g.)	510

High carbon figures on the original deplasticised resin, together with figures similar to those given above for the recovered mixed monomer, point to the probable presence of styrene.

It is our practice to confirm the presence of styrene in such samples by heating the monomer recovered from 2 to 2·5 g. of polymer with 5 ml. of 50 per cent. w/v KOH solution for 1 hour under reflux. The mixture is cooled and diluted to 25 ml. with water and the solution then extracted with 5 ml. of ether. The ether layer is washed with 5 ml. of water and then filtered through a dry filter paper into a test tube ($5 \times \frac{1}{2}$ inch). The ether is removed by evaporation on the water-bath, the last traces of the solvent being removed by a stream of compressed air.

Eight to ten drops of the residue are measured into a clean test tube ($5 \times \frac{1}{2}$ inch) and liquid bromine is added dropwise until a definite excess is indicated by the colour of the mixture. The excess of bromine is removed by heating on the water-bath and the mixture cooled.

The solid residue is broken up with a glass rod and dissolved by heating with 5 ml. of 80 per cent. v/v ethyl alcohol. The filtered solution is cooled and the crystalline deposit filtered off and recrystallised from 3 ml. of 80 per cent. v/v ethyl alcohol. The crystallised product is dried in air and its melting-point determined. Styrene dibromide melts at 73° C. (uncorrected).

Behaviour of polymethyl methacrylate - polyethyl acrylate interpolymers on depolymerisation *in vacuo*

Polymethyl methacrylate - polyethyl acrylate interpolymers may be depolymerised by vacuum depolymerisation as indicated above for polymethyl methacrylate alone.

The recovered monomer usually possesses an odour resembling to a certain extent that of ethyl acrylate and is quite different from that of methyl methacrylate alone, but the yield of recovered monomer is low, and the saponification value and refractive index do not differ appreciably from the corresponding figures for pure methyl methacrylate, as the following results indicate—

	Monomer recovered from polymethyl methacrylate with 10 per cent. polyethyl acrylate	Monomer recovered from polymethyl methacrylate with 5 per cent. polyethyl acrylate
Recovery, per cent.	about 60	about 60
Colour	Water white	Water white
Refractive index, 20° C.	1·4141	1·4135
Saponification value (mg. of KOH per g.)	548	557

In material such as this it is desirable to supplement the information by carrying out a test for the detection of the ethyl ester in the recovered monomer as follows—

One ml. of the monomer is heated under reflux for 1 hour with 2·5 ml. of 50 per cent. w/v KOH solution. After cooling, 2 ml. of water are added and the solution is distilled until 2 ml. of distillate have been collected. To this distillate are added 5 ml. dichromate mixture (100 g. of potassium dichromate dissolved in a mixture of 250 ml. of concentrated sulphuric acid and 750 ml. of water) and the solution redistilled into a 15-ml. centrifuge tube until 0·5 ml. of distillate has been collected. One pellet of solid potassium hydroxide is added and the tube placed in a beaker of boiling water.

If an ethyl ester was present in the original recovered monomer, the caustic pellet will turn yellow and the solution will boil, turn yellow and emit the obnoxious odour characteristic of aldehyde resins.

Behaviour of polycyclohexyl methacrylate - polymethyl methacrylate interpolymers on vacuum depolymerisation

The behaviour of polycyclohexyl methacrylate - polymethyl methacrylate interpolymers on depolymerisation *in vacuo* is entirely different from that of polymethyl methacrylate itself.

The procedure is that of the general method except that where this kind of interpolymer is expected the depolymerisation is carried out for a period of 2 hours. With this type of interpolymer the depolymerisation product consists of two layers, the lower one of which is chiefly water. The figures for saponification and refractive index of the product are, therefore, of little diagnostic value.

The following figures, which were obtained on the depolymerisation product of an interpolymer containing 46 per cent. of polycyclohexyl methacrylate and 54 per cent. of polymethyl methacrylate, are typical of the results that may be obtained.

Saponification value (mg. of KOH per g.)	120–200
Refractive index, 20° C.	1·42–1·43
Yield (2 hours), per cent.	40–50
Acid value (mg. of KOH per g.)	3
Water (Fischer), per cent.	7–10

Behaviour similar to that recorded above usually indicates the presence of a co-polymer of cyclohexyl and methyl methacrylates, but it is always necessary to proceed further in order to obtain evidence of this.

Tests which are of great value in this connection involve the production of cyclohexene from the depolymerisation product and its subsequent identification. In this identification the cyclohexene is oxidised to adipic acid, the melting-point of which is determined.

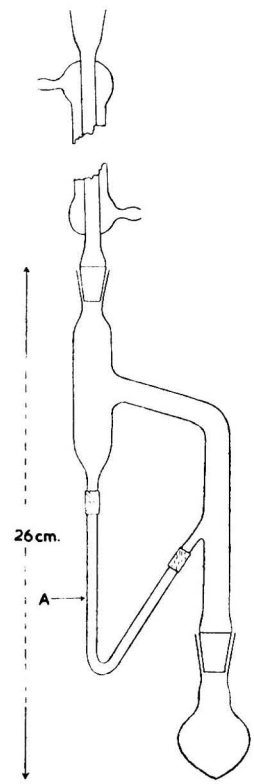


Fig. 2. Apparatus for continuous steam distillation

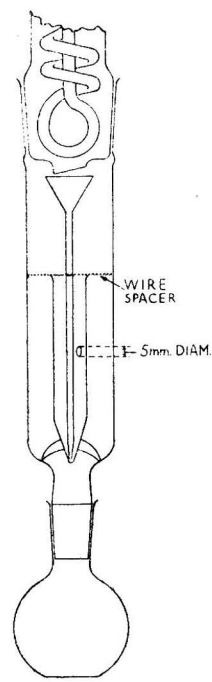


Fig. 3. Continuous ether extractor

The methacrylic acid in the depolymerisation product is isolated and may be identified as its *p*-phenyl phenacyl bromide derivative; the presence of a methyl ester is confirmed by isolation of the corresponding alcohol, oxidation of this to formaldehyde, and the identification of the latter by the chromotropic acid reaction. Full details of the method are given below.

One ml. of the depolymerisation product is heated under reflux for an hour with 5 ml. of 50 per cent. w/v potash solution. The mixture is cooled and 5 ml. of water added. The flask containing the solution is then connected to the continuous steam distillation apparatus

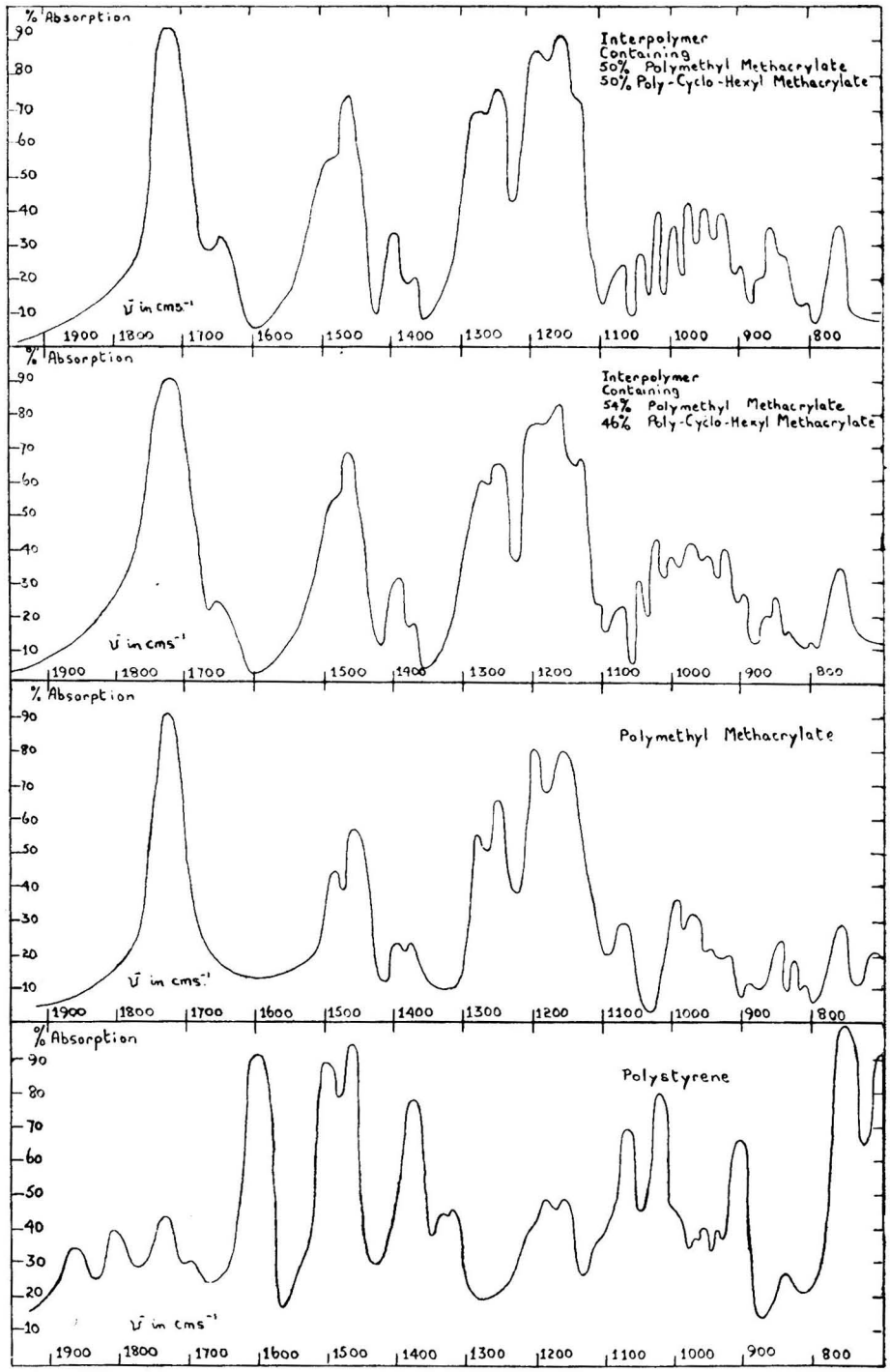
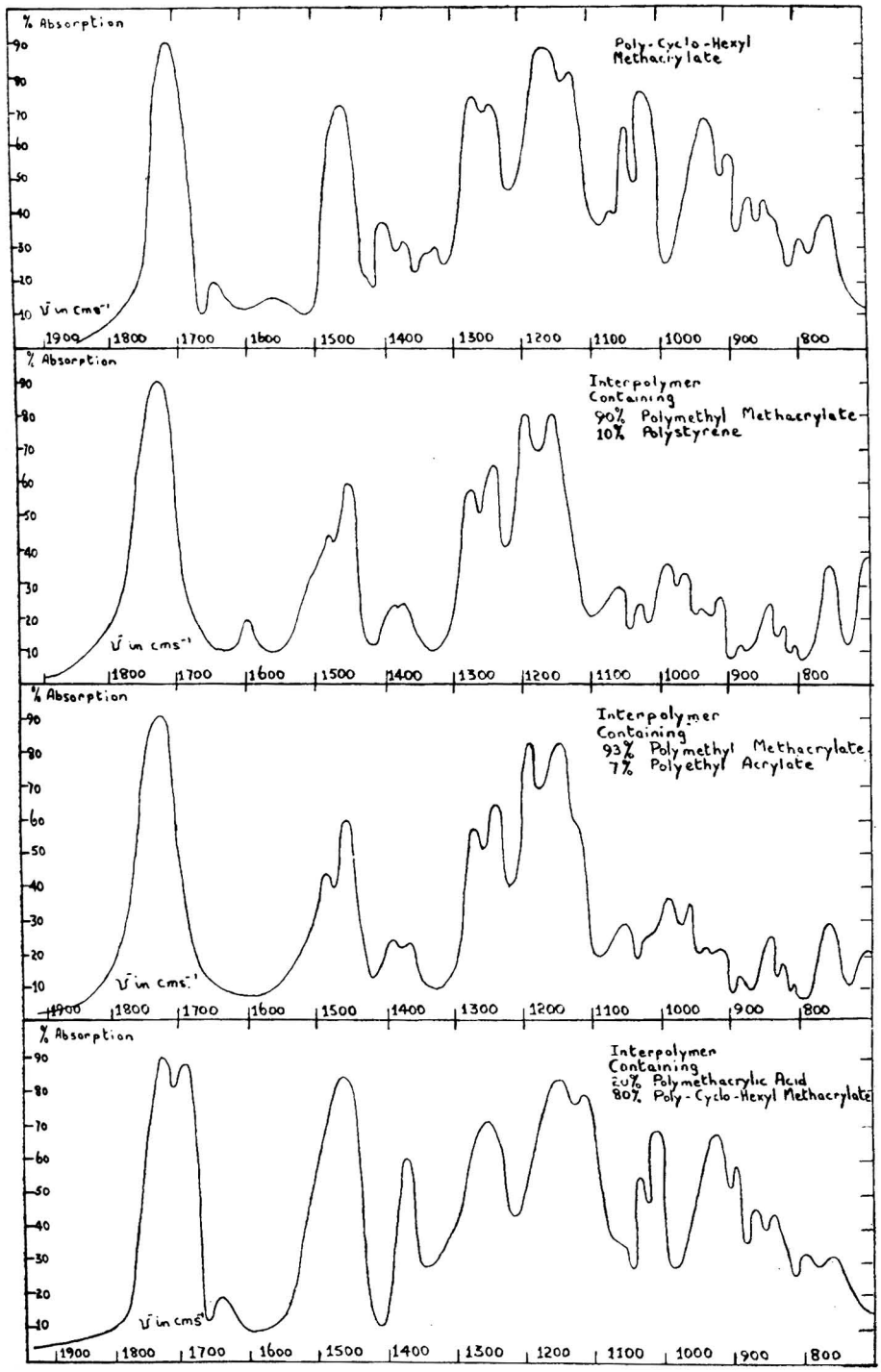


Fig. 4. Infra-red spectra of methyl



methacrylate polymers and co-polymers

illustrated in Fig. 2 and the steam distillation carried out for half an hour, by which time an oily layer will have collected in the detachable tube A. The aqueous layer in the detachable tube is reserved for the detection of methyl alcohol. The oily layer is transferred to a small separating funnel, and after being washed with 5 ml. of water, transferred to a 1-ml. centrifuge tube containing a small quantity of anhydrous potassium carbonate. After the solution has stood overnight, the boiling-point, refractive index and specific gravity of the clear upper layer of cyclohexene are determined. A portion of this cyclohexene is then converted to adipic acid by taking 0·2 ml. of the suspected hydrocarbon and heating it gently under reflux for 10 to 15 minutes with 5 ml. of dichromate - sulphuric acid mixture (100 g. of potassium dichromate dissolved in a mixture of 250 ml. of concentrated sulphuric acid and 750 ml. of water). The product is diluted with 5 ml. of water and then extracted with ether for 15 minutes in a small continuous ether extractor (Fig. 3). The ether is evaporated to low bulk and the solution transferred to a 1-ml. centrifuge tube previous to final evaporation to dryness. The resulting adipic acid is recrystallised from 0·5 ml. of water and the product dried at 100° C. previous to the determination of its melting-point.

For the detection of methyl alcohol, the water in the detachable tube, referred to above and reserved for this test, is transferred back to the original hydrolysis flask containing potash, etc. The mixture is now distilled until 2 ml. of distillate have been collected. To this distillate are added 5 ml. of dichromate oxidation mixture and the solution distilled until 0·5 ml. of distillate has been collected. Three to four drops of this distillate, contained in a 6 × $\frac{3}{8}$ inch test tube, are mixed with 2 ml. of diluted sulphuric acid solution (150 ml. of concentrated sulphuric acid + 100 ml. of water) and a few crystals of the sodium salt of chromotropic acid. The tube is then heated for 10 minutes by immersion in a water-bath at 60° to 70° C. The production of a violet colour indicates the presence of a polymethyl ester in the original polymer.

The presence of methacrylic acid in the depolymerisation products is confirmed as follows—

The alkaline solution obtained in the small flask, after the removal of any methyl alcohol referred to above, is acidified with concentrated hydrochloric acid and the solution saturated with sodium chloride. This saturated solution is then extracted twice with 5-ml. portions of ether. The ether extracts are combined, washed with 2 ml. of saturated sodium chloride solution and transferred to a small flask. One ml. of 10 per cent. w/v sodium hydroxide solution is now added, the ether is evaporated off on the water-bath and the residual solution neutralised with *N* hydrochloric acid solution. *p*-Phenyl phenacyl bromide (0·1 g.) is added and the mixture heated under reflux for 30 minutes. The hot solution is filtered and the filtrate allowed to crystallise. The product is recrystallised from 2 ml. of ethyl alcohol and dried in air previous to determination of its melting-point.

Some idea of the kind of figures that are obtained in this kind of test may be seen from the results of the examination of two polymers, one of which was polycyclohexyl methacrylate itself, whilst the other consisted of an interpolymer containing 46 per cent. polycyclohexyl methacrylate and 54 per cent. of polymethyl methacrylate.

The cyclohexene obtained from the two polymers had the following properties—

	Polycyclohexyl methacrylate	Interpolymer containing 46 per cent. polycyclohexyl methacrylate and 54 per cent. polymethyl methacrylate
Refractive index, 20° C.	1·449	1·451
Boiling-point	84° C.	84° C.
Specific gravity, 25/4° C.	0·838	0·846

In both, the adipic acid prepared by oxidation of the cyclohexene melted at 148° C. The melting-points of the *p*-phenyl phenacyl bromide derivatives of the methacrylic acid obtained from the two depolymerisation products were respectively 121° C. for the interpolymer and 118° C. for the cyclohexyl methacrylate.

It is always desirable to supplement chemical examinations similar to those outlined above with infra-red evidence obtained on known polymers and co-polymers, *e.g.*, a substance submitted for analysis was shown to contain

	Per cent.
Carbon	65·3
Hydrogen	8·9
Oxygen	25·8

On solution in acetone and precipitation with light petroleum followed by evaporation of the light petroleum solution, the substance yielded a residue amounting to 2·2 per cent. This residue was a hard, clear solid which possessed the smell of a monomer. It gave no evidence of the presence of a phthalate and was almost certainly low molecular weight polymer.

Subsequent examination of this polymer by the chemical methods outlined above indicated that it was a co-polymer of polycyclohexyl methacrylate and polymethyl methacrylate and the carbon, hydrogen and oxygen figures indicated that the approximate proportions were 46 per cent. polycyclohexyl methacrylate and 54 per cent. polymethyl methacrylate.

Support for this view was obtained when an interpolymer was prepared containing 50 per cent. of polymethyl methacrylate and 50 per cent. of polycyclohexyl methacrylate. The infra-red spectra of these two specimens, the sample and the comparison mixture were very similar indeed, as is shown in Fig. 4.

On pages 68 and 69 are given the infra-red spectra of the above two specimens, together with those of—

(*a*) Polymethyl methacrylate.
(*b*) Polystyrene.
(*c*) Polycyclohexyl methacrylate.
(*d*) Interpolymer of polymethyl methacrylate containing 10 per cent. of polystyrene.
(*e*) Interpolymer of polymethyl methacrylate containing 7 per cent. of polyethyl acrylate.
(*f*) Interpolymer of polycyclohexyl methacrylate containing 20 per cent. of polymethacrylic acid.

We should like to take this opportunity of expressing our indebtedness to Mr. Willis of our Research Department for the preparation of the infra-red spectra and for his general interest in this investigation.

IMPERIAL CHEMICAL INDUSTRIES LIMITED
PLASTICS DIVISION
WELWYN GARDEN CITY
HERTS.

June, 1949

The Determination of Phenodoxone in Urine

BY J. E. PAGE AND HEATHER KING

SYNOPSIS—A colorimetric method has been devised for determining phenodoxone in urine. Phenodoxone is the official name of a recently available powerful analgesic, DL-6-morpholino-4 : 4-diphenylheptan-3-one, the hydrochloride of which is available under the proprietary name "Heptalgin."

The base from urine samples containing about 0·4 mg. of the drug is liberated with alkali and extracted into toluene; the toluene extract is shaken with an aqueous solution of bromophenol blue buffered at pH 4·0. An amount of dye, equivalent to the weight of drug in the toluene layer, is carried over into the toluene as a yellow compound, and this is decomposed by shaking the toluene layer with aqueous alkali. The sodium salt of the dye then enters the aqueous layer and is determined colorimetrically.

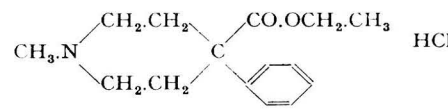
Basic substances that react with bromophenol blue to form a toluene-soluble compound (alkaloids, such as codeine, and synthetic analgesics, such as pethidine and amidone) interfere with the determination.

PHENODOXONE is the official name given to DL-6-morpholino-4 : 4-diphenylheptan-3-one. The hydrochloride of this substance was first studied under the description CB11, which will be used for convenience in the present communication; it is now generally available under the proprietary name "Heptalgin." It is used because of its marked action as an analgesic, which is combined with a relatively low toxicity, so that its therapeutic ratio is higher than that of other similar compounds. Its preparation has been described by Dupré, Elks, Hems,

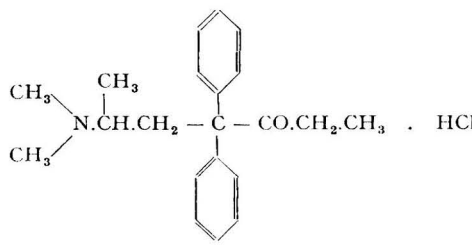
Speyer and Evans[1] and by Attenburrow, Elks, Hems and Speyer,[2] and its pharmacology by Basil, Edge and Somers.[3]

For the determination of CB11 in urine we have used a modification of Lehman and Aitken's procedure[4] for determining pethidine. Scott and Chen[5] and Cronheim and Ware[6] have employed like methods for the estimation of amidone and Hopewell and Page[7] have described a somewhat similar technique for the determination of long-chain aliphatic amines.

Ethyl-1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride. Pethidine.



6-Dimethylamino-4:4-diphenylheptan-3-one hydrochloride. Amidone.



6 Morpholino-4:4-diphenylheptan-3-one hydrochloride. "Heptalgin" (CB11).

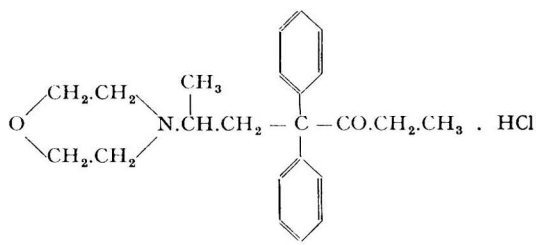


Fig. 1

The method depends on the reaction of equimolecular quantities of the analgesic and bromophenol blue to form a toluene-soluble compound. The free base of the drug is extracted into toluene and the toluene extract is shaken with an aqueous solution of the dye buffered at pH 4·0. An amount of dye, equivalent to the weight of drug in the toluene layer, is carried over into the toluene as a yellow compound, which is decomposed by shaking the toluene with aqueous alkali, whereupon the sodium salt of the dye enters the aqueous layer and is determined colorimetrically. Under these conditions, CB11 gives a more intense colour than when tested by either Lehman and Aitken's bromothymol blue method[4] or Cronheim and Ware's bromocresol purple method.[6]

The polarographic behaviour of CB11 was also studied with a view to finding a suitable analytical method. In 0·1 *N* potassium chloride it gave a polarographic step with a characteristic peak at −1·75 v. (*cf.* Fig. 2). The step height was approximately proportional to concentration over the range 0·0075 to 0·075 per cent. However, the step height was sensitive to small amounts of surface-active material and appeared at a relatively high potential, so that the method was unsuitable for determining CB11 in biological fluids.

Experimental procedure—Our recommended procedure for the determination of CB11 is as follows.

Shake 10 ml. of the solution, containing about 4 mg. of CB11 per 100 ml., with 2 ml. of 10 *N* sodium hydroxide and 30 ml. of redistilled toluene in a separating funnel. Pipette 25 ml. of the supernatant toluene into a second separating funnel and shake for 5 minutes with 20 ml. of a bromophenol blue solution buffered at pH 4·0 (made by mixing 60 ml. of 0·05 *M* potassium hydrogen phthalate with 40 ml. of a 0·08 per cent. bromophenol blue solution). Centrifuge (if necessary) the mixture from the separating funnel, transfer 20 ml. of the supernatant toluene to a third separating funnel, and extract in turn with three 5-ml. portions of 0·1 *N* sodium hydroxide. Dilute the combined alkaline extracts to 20 ml. with 0·1 *N* sodium hydroxide and examine an aliquot portion in a Hilger photo-electric absorptiometer fitted with a yellow gelatin filter (Ilford No. 606) and a heat-resisting Chance glass

filter (No. H503). The concentration of CB11 can be determined from a calibration curve prepared for solutions containing between 1·0 and 10·0 mg. of CB11 per 100 ml., over which range there is a linear relationship between drug concentration and absorptiometer reading. Solutions containing more than 10 mg. of analgesic per 100 ml. should be diluted before testing.

Determination in urine—The bromophenol blue method may be used to determine CB11 in human and rat urine, but because any basic substance capable of reacting with bromophenol blue to form a toluene-soluble compound will respond to the test, the following precautions must be observed.

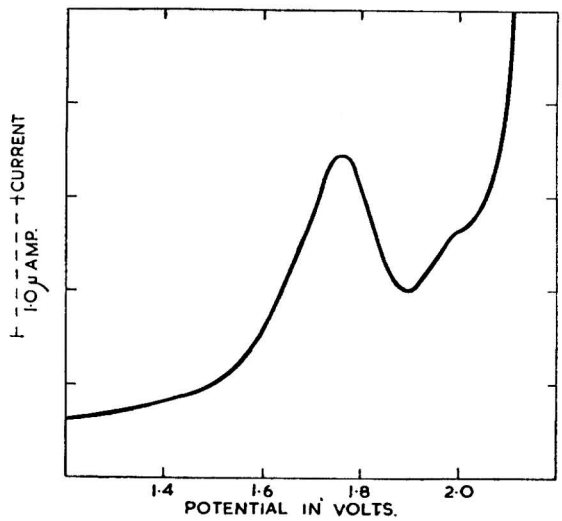


Fig. 2. Polarogram for an 0·025 % solution of CB11 in 0·1 *N* potassium chloride solution. (Drop-time on open circuit in 0·1 *N* potassium chloride solution at 25° C. = 3·13 secs. Weight of mercury dropping per sec. = 1·82 mg.)

Certain alkaloids such as codeine, and synthetic analgesics such as pethidine and amidone, interfere with the determination; the intensity of the colour formed by amidone is greater than that produced by an equivalent quantity of CB11, and is about the same as that formed when amidone is examined by Cronheim and Ware's procedure.[6] Adequate control specimens must therefore be tested and particular care must be exercised in analysing urine samples from subjects who have received special medication. Bacterial growth in the urine leads to the production of basic substances giving the same colour response as CB11; a small amount of an antiseptic (*e.g.*, mercuric chloride or toluene) must therefore be placed in the vessel used for collecting the urine.

During the solvent extraction of urine, the ratio of the volume of toluene to that of the urine must be kept as high as possible in order to prevent the formation of stable emulsions. The calibration curve for the determination of CB11 in urine is about 30 per cent. lower than that for its determination in simple aqueous solution.

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Research Division
Glaxo Laboratories Ltd.
Greenford, Middlesex

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Estimation of the Thickness of Wax "Bloom" on Vulcanised Rubbers

By F. KENDALL and MISS W. M. PHILLIPS

Synopsis—The method consists in cutting a known area of the rubber without disturbing the surface bloom, and wiping the surface with four or five successive twists of de-fatted cotton wool, held by cleaned forceps and moistened with light petroleum. The twists are transferred to a prepared extraction funnel and the fat extracted from them and weighed. The thickness of the bloom is calculated from the area of the specimen and the weight and specific gravity of the wax recovered.

Certain waxes, when incorporated into a rubber mixing, "bloom" to the surface soon after vulcanisation. This bloom forms some protection against the deterioration of strained rubber which takes the form of surface cracking and which is now known to be caused by atmospheric ozone.[1] In an investigation of this protective effect it was important to be able to estimate the thickness of the surface bloom of wax. Several methods for determining the thickness of the bloom have been investigated. They include methods based on removal by scraping, optical interference caused by the thin film, electrical resistivity of the rubber with and without the bloom, difference of focus of a microscope at the top and bottom of the film of bloom, microscopical examination of cut sections of the rubber, and removal of the bloom by wiping with solvent-damped cotton wool and subsequent determination of the wax in the wool. Of these several methods only two, the cutting of sections and the removal of bloom by cotton wool, proved both easy and satisfactory, although, doubtless, optical interference methods could be made satisfactory if adequate apparatus were available.

A technique for cutting sections of the rubber was developed so that as far as possible the wax film was cut cleanly without fracture. By microscopical examination of the section the film thickness was measured with an eyepiece scale and a stage micrometer. Polarised light was used to provide greater contrast between wax film and rubber surface. This method gave reproducible results when used for wax blooms not less than 3 microns thick, but was found to be unsuitable for thinner films.

Method adopted for routine use

After trial, the removal of the wax bloom by wiping with cotton wool moistened with a solvent and the subsequent quantitative extraction of the wax from the wool was adopted as the preferred method. Knowledge of the area of rubber involved and the specific gravity* of the wax enables the thickness to be calculated from the weight of wax recovered. It was found convenient to cut disc specimens 5 cm. in diameter from sheet by means of a press cutter, but specimens of other shapes are equally satisfactory provided disturbance of the wax bloom is avoided in cutting.

Procedure—

Cut out a specimen of suitable size and determine its area. Wash the appropriate fingers of the hand with solvent (light petroleum, b.p. 40° to 60° C.) and twist pre-extracted cotton wool round the end of a pair of cleaned forceps. Moisten the cotton wool with solvent and wipe the rubber surface. The wiping action should be rapid but should not involve rubbing or heavy pressure. After wiping transfer the twist of cotton wool to a prepared extraction funnel. Wiping treatments with four successive twists was found sufficient for most blooms, but a fifth twist should be used for heavy blooms. The total amount of cotton wool used for the four or five twists should be such that, when placed in the extraction funnel and extracted with the solvent, it should not expand above the rim of the funnel.

Extraction of wax from cotton wool—The apparatus used was the micro-extraction apparatus described by G. H. Wyatt.[2]

* This is best determined by a flotation method such as is given in *Standard Methods for Testing Petroleum and its Products*, Institute of Petroleum, Ninth Edition, London, 1948.

Weigh a clean extraction cup. (This can be done on an ordinary analytical balance, although in the development of the procedure a micro-balance was used.) Add 2 or 3 ml. of light petroleum (boiling range 40° to 60° C.) and then transfer the cup to the extraction tube. Support the funnel containing the cotton wool to be extracted in the frame immediately above the cup, add a further 1 or 2 ml. of light petroleum and close the tube with the metal condenser. Extract for 2 hours, remove the condenser and funnel and allow the solvent to evaporate to dryness. Transfer the extraction cup to a vacuum desiccator for 15 minutes before weighing.

From the weight of wax, W, and its area, A, and specific gravity, calculate the thickness, t, of the bloom:

$$t \text{ (in cm.)} = \frac{\text{W (in g.)}}{\text{A (in sq. cm.)} \times \text{sp.gr.}}$$

DISCUSSION OF THE METHOD—

Light petroleum (boiling range 40° to 60° C.) was found to be the most suitable solvent as it combines a very high solvent power (11·7 g. of paraffin wax per 100 g. of solvent) with a convenient boiling range.

The standard deviation of replicate determinations was first determined from two sets of three extractions carried out at two different times on the same vulcanisate (Mix 1: pale crêpe 100, sulphur 2, MPC black 5, zinc oxide 0·5, paraffin wax 7, zinc dibutyldithiocarbamate 0·5). The results expressed as the weights determined instead of the thicknesses estimated from them are given in the following table.

Weight of wax extract, mg.	Mean weight, mg.	Standard deviation, mg.
2·62, 2·41, 2·85	2·63	0·22
3·41, 3·93, 3·41	3·58	0·30

On examining the results obtained with other vulcanisates (details of which are omitted) for which duplicate estimations had been made, tabulation of the differences between each pair of figures showed them all to be of the same order, with two exceptions.

Mix No.	2	3	4	5	6	7	8	9	10	11
Difference, mg.	0·37	1·60*	0·65	0·14	1·56*	0·57	0·10	0·02	0·03	0·34
				0·21	0·50	0·36	0·09			
					0·41					
					0·36					

* These figures ignored.

From the mean of these figures, corrected to the nearest tenth of a milligram and omitting the two that were not of the same order as the others, an estimate of the average difference between duplicate results was made. The value obtained was 0·30 mg. It seems highly probable that a manipulative error occurred with one or other of the duplicates marked with an asterisk.

The average difference between the weights of wax obtained in replicate tests is therefore such that an ordinary analytical balance has sufficient discriminating power for the purpose and can be used instead of a micro-balance.

RESULTS OBTAINED—

As an illustration of some typical results, values for a series of transparent rubbers are quoted, where the amount of wax found is shown to be correlated with the amount of wax added to the mixing.

Wax content on 100 parts of rubber hydrocarbon	Weight of bloom, mg.	Thickness of bloom, cm. $\times 10^{-5}$
0·7	nil	nil
1	0·067	3
	0·067	3
3	1·21	6
5	1·43	7
	1·52	8
7	2·28	12
15	3·73	19
	4·07	21

The authors acknowledge with thanks permission from the Research Association of British Rubber Manufacturers to publish this paper.

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RESEARCH ASSOCIATION OF BRITISH RUBBER MANUFACTURERS
105, LANSDOWNE ROAD
CROYDON, SURREY

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Potentiometric Determination of Small Amounts of Procaine, Sulphanilamide and Related Compounds by Bromate - Bromide Titration

BY K. R. SRINIVASAN

SYNOPSIS—Determination of procaine by direct titration with potassium bromate solution, using a pair of polarised platinum electrodes, is described. The method is simple, rapid and yields results of high precision and accuracy. It is applicable to the determination of other local anaesthetics of the *p*-amino-benzoic ester type, and also of some of the sulpha drugs.

SMALL amounts of procaine, in ointments, tablets, blood, etc., have been determined by colorimetric methods[1,10,14] after diazotisation of the primary amino-group and coupling with a suitable reagent to form a coloured compound. These methods are useful only within a limited working range of concentration; they require the use of special equipment and are not rapid and direct, as they need the preparation of standard reference curves or the use of internal standards.

The A.O.A.C. method of determination of procaine by bromination[12] consists in hydrolysis to *p*-aminobenzoic acid, reaction with a large excess of bromine for 2 hours at room temperature and titration of the excess of bromine with thiosulphate solution after addition of potassium iodide, when, under these conditions, tribromoaniline is formed. This method is time-consuming, and unless the conditions are strictly followed, will lead to low recoveries owing to under-bromination.[15] Day and Taggart[3] found that *p*-aminobenzoic acid is not satisfactorily determined by the excess-bromine method because of precipitation of partially brominated products. This is due to the fact that while the velocity constant of formation of tribromoaniline from aniline, or of the dibromo-derivative of *p*-substituted anilines, is very high, being of the order of 10^8 (see[6]), the rate of further substitution of the latter by bromine to form tribromoaniline by elimination of the substituent group in the *p*-position, is relatively low, the extent of this reaction depending on conditions of temperature,[7] time of contact with bromine and residual bromine excess.[15]

Satisfactory results are, however, to be expected in determinations by direct titration with bromate and bromide in acid solution, since the reaction here proceeds to and stops at the stage of dibromo-substitution. But the difficulty with bromometric titration lies in the determination of the end-point. The use of an external indicator such as starch-iodide paper renders the method tedious[3]; with irreversible internal indicators like methyl orange the titration has to be done very slowly and any local concentration of bromine will cause the colour of the indicator to fade before the end-point; other indicators[8] besides being slow, will not be useful in solutions that are coloured. These difficulties are obviated in the present method by the use of a pair of polarised platinum electrodes as described by Foulk and Bawden.[5] A very sharp end-point is obtained, as the slightest excess of bromine in the solution at once depolarises the cathode and causes a large deflection of the galvanometer.

DETERMINATION OF SULPHONAMIDES

Sulphanilamide and its derivatives are determined with equal facility by bromometric titration. The British Pharmacopoeia[2] method consists in titration of the acid solution of the drug at a low temperature (15° C.) with standard sodium nitrite solution, whereby

the NH_2 group is diazotised. The end-point is indicated by the *immediate* formation of a blue colour with starch-iodide paste used as an external indicator. This titration is slow and tedious, and as the reaction slows down towards the end, one has to wait for a few minutes after each addition of the reagent before testing for the end-point. Further, some uncertainty as to the exact end-point is often experienced, for even before the theoretical amount of sodium

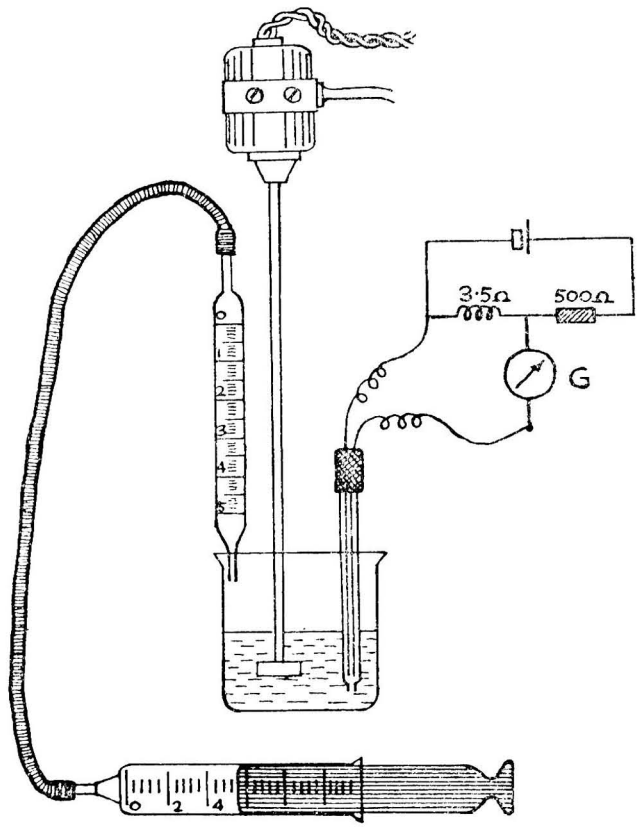


Fig. 1. Titration Assembly

nitrite has been added, a blue colour is formed within a few seconds of placing a drop of the solution on the starch iodide paste.

Determination of sulphanilamide as its dibromo-derivative by the excess bromine method[11] often leads to erratic results owing to over-bromination, the extent of which varies with temperature, time of contact with bromine and residual bromine excess,[9] and check results are obtained only under strictly controlled conditions of the experiment. With sulphathiazole, sulphapyridine, sulphadiazine and sulphaguanidine, Wells[16] found that the excess bromine method cannot be successfully applied for their determination without preliminary hydrolysis to sulphanilic acid by heating with hydrochloric acid under reflux.

The direct method of titration with bromate as outlined here has the advantage of simplicity and rapidity over these methods, and accurate results are obtained even with small amounts (Table I).

REAGENTS AND APPARATUS—

Potassium bromate solution, 0·01 *N*—0·2784 g. of Analytical Reagent grade potassium bromate, dried at 120° C. for 1 hour, is dissolved in a litre of distilled water.

Diluted hydrochloric acid—230 ml. of concentrated acid (sp.gr. 1·18) is made up to a litre with water.

Potassium bromide solution—10 per cent.

The electrode is made by fusing two short pieces (about half an inch) of platinum wire at the end of glass tubing of 3-mm. bore and 10-cm. length. Connections are made by means of copper leads, silver-soldered to the platinum wires. The copper leads are sleeved by two melting-point capillaries. A polarising voltage of about 10 millivolts is obtained from a 500-ohm resistor (I.R.C. $\frac{1}{2}$ watt) and a small spiral of Nichrome wire (No. 26 S.W.G.) having a resistance of about $3\frac{1}{2}$ ohms, connected in series across a $1\frac{1}{2}$-volt dry cell as shown in Fig. 1. G is a Leeds and Northrup type 2420 spot light galvanometer. In titrating very small quantities, a 5-ml. graduated pipette (N.P.L. certified class A accuracy) with a fine tip, operated by a hypodermic syringe and rubber tube, was used in the absence of a precision micro-burette. Stirring is done by any suitable stirrer or even by swirling the beaker by hand.

PROCEDURE—

An aliquot containing about 5 mg. of the substance is taken in a 30-ml. beaker; 1 ml. of the potassium bromide solution and 10 ml. of the diluted hydrochloric acid are added. The electrodes, which have been cleaned by means of hot chromic acid and washed with distilled water, are dipped into the solution and connected up in the circuit. The solution is titrated with the potassium bromate solution, the end-point being indicated by a permanent deflection of the galvanometer from the zero position.

TABLE I

DETERMINATION BY BROMATE TITRATION

Substance	Sample weight, mg.	0·01 *N* $KBrO_3$ required, ml.	Amount found, mg.	Recovery, %
Procaine hydrochloride ..	2·32	3·42	2·33	100·4
	3·25	4·75	3·24	99·6
	4·26	6·27	4·27	100·3
Benzocaine	2·72	6·60	2·725	100·6
	1·86	4·55	1·88	101·0
	3·88	9·42	3·89	100·2
Sulphanilamide	1·70	3·95	1·70	100·0
	1·90	4·42	1·91	100·5
	3·67	8·55	3·675	100·2
Sulphapyridine	3·76	6·0	3·74	99·5
	2·78	4·45	2·775	99·8
	4·56	7·32	4·56	100·0
Sulphaguanidine	2·64	4·52	2·625	99·4
	3·37	5·75	3·34	99·1
	5·45	9·37	5·44	99·8
Sulphathiazole*	2·54	5·95	2·53	99·6
	1·84	4·35	1·85	100·0
	4·59	10·72	4·56	99·3
Sulphadiazine*	3·08	7·40	3·08	100·0
	2·85	6·80	2·835	99·5
	1·89	4·54	1·892	100·1

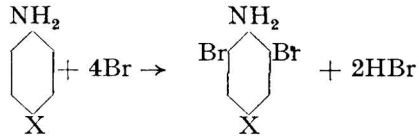
* Equivalent weight is one-sixth of the molecular weight.

DISCUSSION

Table I gives the results of a few of the determinations made and shows the accuracy and reproducibility of the method, which are satisfactory, considering the small size of the sample used. All the samples were purified by recrystallisation from alcohol or water, dried, and weighed out by means of a micro-chemical balance. Only procaine hydrochloride and benzocaine were taken for investigation, although other local anaesthetics of the *p*-amino-benzoic ester type may be determined similarly.

In all cases except sulphathiazole and sulphadiazine the equivalent weight was taken as

one-fourth of the molecular weight, as four equivalents of bromine are taken up according to the equation



where X represents the substituent group in the *p*-position with respect to NH_2. With sulphathiazole and sulphadiazine, six equivalents of bromine are found to be required, presumably owing to bromine substitution in the thiazole and pyrimidine nuclei as well.[4]

The determinations were made at the laboratory temperature of about 30° C. and appreciable deviations in the temperature of the solution were found to have a negligible effect on the final result. An acid concentration of about 2 *N* was maintained.

The limitation of the method is that it is useful only when any of the substances considered is present alone, and in the absence of other easily brominated compounds, *e.g.*, phenols.

Application to procaine penicillin

The method was applied for estimating procaine in procaine benzyl penicillin. As the degradation products of penicillin obtained on treatment with acid consume bromine, it was necessary to separate the procaine from penicillin by extraction with chloroform after liberating the free base with ammonia as described by Shaw.[13] The procaine is taken up in hydrochloric acid from the chloroform solution and the acid solution is titrated with standard bromate. One ml. of 0·01 *N* potassium bromate is equivalent to 0·59 mg. of procaine base. The results are given in Table II.

Table II

Determination of procaine in procaine benzyl penicillin

Sample* weight, mg.	0·01 *N* $KBrO_3$ required, ml.	Procaine, %†
25·0	16·95	39·9
20·7	14·05	40·0
8·3	5·60	39·8

* Squibbs "Crysticillin" brand of procaine penicillin G was used.
† The theoretical value is 40·12 per cent. of procaine.

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Laboratory of the Government Analyst
King Institute, Guindy,
Madras 15, India

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A Colorimetric Method for the Determination of Oxalate

By S. BURROWS

Synopsis—The routine determination of calcium oxalate in mushroom composts by the precipitation of calcium oxalate from a hydrochloric acid extract of the compost is difficult because of the co-precipitation of colouring matter and organo-metallic complexes which interfere in the final determination of oxalate. A number of published colorimetric methods were investigated, but none was found to be satisfactory. An absorptiometric method based on the fading effect of oxalates on various coloured organic complexes of tervalent iron has been investigated and the green complex of iron and 7-iodo-8-hydroxyquinoline-5-sulphonic acid (ferron) shown to be suitable. By removing the colouring matter and phosphates from a compost by preliminary extraction with citric acid and by close control of the acidity, results reproducible to ±4 per cent. have been obtained; but recovery of added oxalate amounts to only 90 per cent. On exposure of iron - ferron solutions containing oxalate to sunlight, further fading, which is related quantitatively to the oxalate present, occurs.

The determination of oxalates in biological materials is usually made by precipitation as calcium oxalate followed by weighing or titration. The method may be expedited and made more sensitive by introducing a centrifuge technique with a cerimetric or iodimetric finish.[1,2,3] Preliminary concentration by acidification and extraction with ether is sometimes required.[4,5]

There arose a demand on this laboratory for a routine method of determining oxalate in mushroom composts; Bau's method, as modified by Myers,[6] with a permanganate titration was first used. Considerable difficulty was experienced in removing colouring matter and metal-organic complexes that were precipitated with the calcium oxalate. Extraction of the compost or the contaminated oxalate precipitate with 1 per cent. citric acid, containing calcium chloride and saturated with calcium oxalate, effected improvement, but reprecipitation was often necessary and this added considerably to the work. A specific colorimetric method which would obviate the necessity for purifying the calcium oxalate was therefore sought.

The method suggested by Mitchell[7] in which a red colour is produced by a sodium vanadate - hydrogen peroxide reagent in the presence of oxalate was unsuitable because the mineral acids necessary to dissolve calcium oxalate also produce a red colour. Calkins[8] gives details of a method by which oxalic acid is reduced to glycollic acid and treated with 2 : 7-dihydroxynaphthalene in sulphuric acid to produce a violet colour, but the numerous compounds that give colours with concentrated sulphuric acid make the method difficult to work.

The fading effect of oxalates on the colours produced by iron and various organic reagents is well known and has been used in a volumetric method for determining oxalate by Erametsa and Parpola.[9] They titrated oxalic acid against a standard ferric chloride - sulphosalicylic acid mixture until a predetermined extinction, measured in the Pulfrich photometer, was reached. Lang[10] also describes a method using the same principle in which the iron thiocyanate colour, partially bleached with oxalate, is matched against standards containing known amounts of oxalate.

Preliminary investigations—Despite the possibility of serious interference from phosphates, these fading methods were examined, and it was found that phosphate interference could be reduced by working in acid solutions below pH 2. The iron reagent eventually chosen was 7-iodo-8-hydroxyquinoline-5-sulphonic acid (ferron) introduced by Yoe[11] and used by Fahey[12] to determine fluorides by their bleaching effect. This reagent was chosen because it could be used in moderately concentrated acid solutions (pH 1 to 3) and the green colour of the iron complex permitted the use of filters that would minimise interference from the brown colouring matter in composts. According to Mellan[13] and Yoe and Hall[14] the ferric ferron complex contains one atom of iron to three ferron residues, but in acid solution an equilibrium exists, and uncombined $Fe^{\cdots}$ ions and ferron are also present, so that Beer's law is not strictly followed. The mechanism of the fading action of oxalate on the colours of ferric complexes is apparently

due to the greater stability of the ferri-oxalate complex and the consequent decomposition of the colour complex. Experiments showed that, with ferron, this also is a balanced reaction, so that a high concentration of iron ferron is desirable to ensure sensitivity to oxalate. This necessitates the use of deep coloured solutions and a sensitive photo-electric measuring instrument. The Hilger Spekker Sensitive Model is satisfactory for the purpose, but it was found necessary to use a neutral screen on the left of the light source in order to obtain a balance when setting the drum at zero with the reagent blank. Interference by phosphate is small at pH 0·9 to 1·0, and the most serious interference is from iron. This difficulty cannot be overcome by using a higher proportion of iron in the reagent since the reagent then becomes relatively insensitive to low amounts of oxalate and an inflection appears in the standard curve. However, when working with composts, in which all the oxalate is present as calcium oxalate, a preliminary extraction with 1 per cent. citric acid containing calcium chloride and saturated with calcium oxalate removes practically all the iron. This reagent also removes most of the phosphate and colouring matter. The hydrochloric acid extract of the residue can then be used directly for the colorimetric finish.

Method

Reagents—

Iron ferron—Dissolve 4·00 g. of ferron in about 500 ml. of hot water containing 1·0 g. of A.R. ferric chloride ($FeCl_3.6H_2O$). Add 300 ml. of 2·0 *N* hydrochloric acid and 68 g. of sodium acetate ($CH_3CO_2Na.3H_2O$), cool and make up to 1 litre.

Citric acid reagent—Dissolve 20 g. of citric acid and 50 g. of anhydrous calcium chloride in water, make up to 1 litre, heat to boiling and add saturated ammonium oxalate solution drop by drop until a permanent turbidity is formed. Boil, set aside overnight and filter.

Procedure—

Extract 1·00 g. of the dried, ground compost sample in a 100-ml. conical flask with 10 ml. of water and 10 ml. of citric acid reagent, by simmering on a hot-plate for half an hour with a small glass funnel in the neck of the flask for condenser. Filter through a No. 30 Whatman filter paper and wash well with cold water. Drain the flask well and return the filter paper and precipitate to the flask after partially drying for an hour at 100° C. Add, from a pipette, 25 ml. or 50 ml. of 0·4 *N* hydrochloric acid, using the former quantity if the calcium oxalate expected is below 1 per cent. or the latter if from 1 to 3 per cent. Bring the liquid just to boiling-point, and then keep below boiling-point for about 5 minutes, swirling occasionally. Filter through a small dry paper, reject the first runnings and pipette 4 ml. of the filtrate into a dry beaker. Add 5 ml. of the iron ferron reagent, protect the solution from direct sunlight and take the drum reading in a 1-cm. cell with the Spekker absorptiometer, using Ilford filters, No. 607, and setting the drum at zero against a reagent blank containing 4 ml. of 0·4 *N* hydrochloric acid and 5 ml. of iron ferron reagent. It will probably be necessary to place a neutral screen on the left of the light source in order to obtain a balance on the galvanometer. The temperature of the blank and sample solution should be the same to within ±0·5° C. Prepare a standard curve using solutions of between 0 and 4 mg. of calcium oxalate ($CaC_2O_4.H_2O$) in 4 ml. of 0·4 *N* hydrochloric acid.

Discussion—Since the method depends on differences in the light absorption between the sample and a highly coloured reference solution, care must be taken that the reference solution (reagent blank) is accurately made up and that the cells used in the absorption measurement are of uniform dimensions. Cells that were satisfactory for ordinary work have been found to give a slight error with the highly absorbing solutions used in this method because of small deviations from the 1-cm. length. As already mentioned, Beer's law is not followed and a non-linear calibration curve is obtained (curve I).

Interfering substances—Table I shows the principal interfering substances met with in composts. The effect of fluorides has not been investigated since they have not so far been found in mushroom composts.

Table I

Interfering substance	Limits permissible
Phosphate	100 p.p.m. P
Ferric iron	3 p.p.m. $Fe^{...}$
Citrate	1000 p.p.m.
Hydrochloric acid	±1 per cent. of the amount in the reagent blank

Close control of acidity is necessary because sodium acetate is a poor buffer at the pH used (0·95). Other buffer systems have not been tried. Dextrose, sucrose and the following acids or their calcium salts do not interfere: formic, acetic, propionic, succinic, malonic, tartaric, glutaric, gluconic, fumaric and maleic. Yellow colorations are tolerated up to a deep straw-yellow.

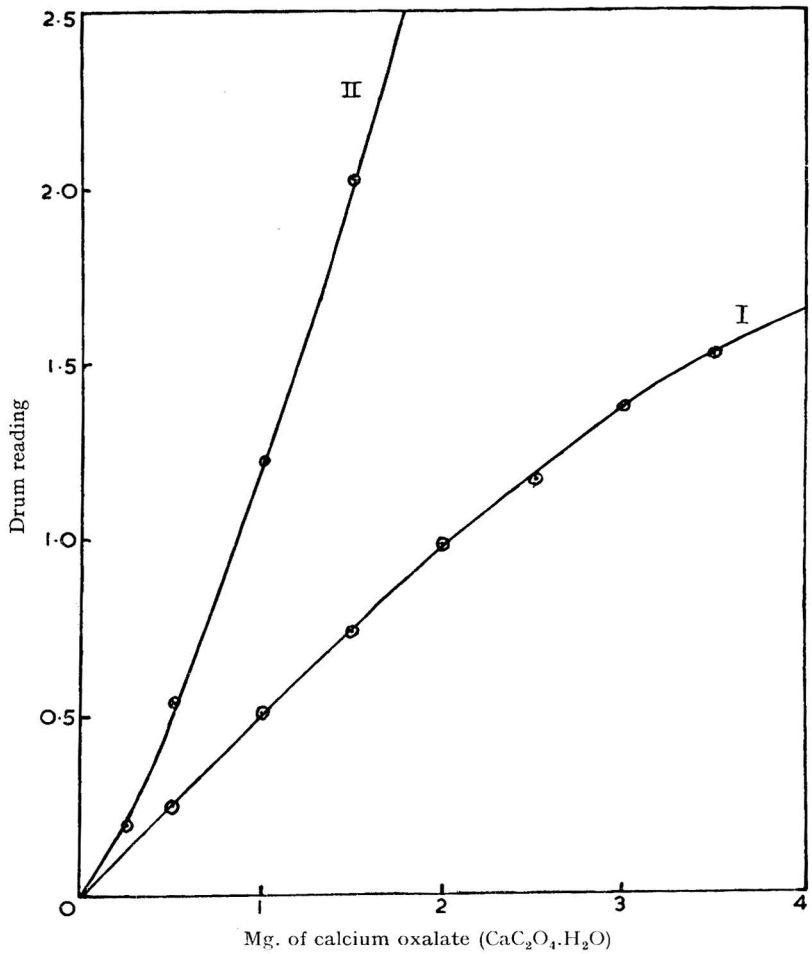

Fig. 1 Curve I. Calibration curve. Curve II. Calibration solution after irradiation

Results—Table II gives the mean of three determinations on separate 1-g. sub-samples from three typical composts dried and ground to pass the 1-mm. screen. In addition, 2-ml. aliquots of a suspension of calcium oxalate containing 8·10 mg. of $CaC_2O_4.H_2O$ per ml. were added to three sub-samples of sample No. 2 and oxalate was determined in the usual way. Oxalate was also determined by the method of Myers as mentioned in the introduction.

TABLE II

Sample	Calcium oxalate found, mg.	
	Myers' method	Ferron method
1	29·4	28·2 ± 0·4
2	12·0	13·8 ± 0·6
3	2·7	2·3 ± 0·5
2 + 16·2 mg.	—	28·6 ± 0·5

Calcium oxalate recovered = 14·8 mg. = 91 per cent.

The reproducibility obtained (±4 per cent. in samples containing about 1 per cent. of calcium oxalate) is satisfactory in view of the rapidity of the method. The low recovery of added calcium oxalate, which has been confirmed a number of times, may be due to adsorption of oxalate on the compost during the hydrochloric acid extraction. In addition to this source of error the results are likely to be low because the citric acid reagent, although it is saturated with calcium oxalate in the cold, dissolves another 0·5 mg. per 10 ml. on boiling. A factor to correct for these errors has not been used, as only comparative values have so far been required.

The effect of sunlight—The test solutions, when ready for absorption measurements are stable in the dark for a number of hours, but fading occurs on exposure to sunlight; this is a function of the amount of oxalate added and leads to a drum reading of two to three times the original figure. The reagent blank is unaffected. Curve II, obtained from these drum readings, shows a slight upward curvature as against the downward curvature of the original calibration curve, I. A number of compounds are known to reduce ferric chloride when irradiated with ultra-violet light, but in the presence of ferron the concentration of ferric ions is much diminished and it appears that complex formation with the iron is necessary before the photo-reaction can take place. Thus, dextrose and all the non-interfering acids already mentioned have no effect on the ferron complex in sunlight; whereas tannic, gallic, pyrogallic and, to a lesser extent, citric and lactic acids behave in a manner similar to oxalic acid.

The following equations represent the reactions that are likely to take place with oxalic acid (Q = ferron residue in the colour complex)—

$$FeQ_3 \rightleftharpoons Fe''' + 3Q' \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$2Fe''' + 3C_2O_4'' \rightleftharpoons Fe_2(C_2O_4)_3 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$Fe_2(C_2O_4)_3 \xrightarrow{u.v.l.} 2Fe'' + 2C_2O_4'' + 2CO_2 \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The tendency to form a ferro-oxalate complex is probably slight at the low pH employed, so that the oxalate ions liberated according to equation (3) will combine with more ferric ions from the ferron complex and further oxidation of oxalate will occur until decomposition is complete, so that the concentration of ferron complex will be determined solely by equation (1), and the equilibrium will not be affected by oxalate (equation (2)) as it is in the dark reaction. Thus, at the completion of the light reaction, equation (1) predominates, giving an upward curvature to curve II, whereas in the dark reaction equation (2) controls the concentration of the ferron complex, causing a downward curvature in the calibration curve I.

The approximately three-fold increase in drum reading on irradiation may be explained by comparing equations (2) and (3). In equation (2) (dark reaction), three oxalate ions combine with two ferric ions, whereas in equation (3) (light reaction), only one oxalate radical is required to reduce two ferric atoms.

In bright sunlight the maximum drum reading is reached in 20 to 40 minutes, but thereafter the drum reading begins to decrease owing to re-oxidation of the iron, so that the conditions of irradiation need to be standardised in order to obtain reproducible drum readings. As an ultra-violet lamp was not available, the possibility of using the light reaction to determine oxalates was not studied further, although there is some likelihood that interference due to phosphates and iron may be reduced by this means.

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An Improved Micro-Method for the Determination of Fluorine Based on an Examination of the Fluoride - Fluosilicate Equilibrium

BY J. B. RICKSON

SYNOPSIS—A study of the fluoride - fluosilicate equilibrium system indicates that low results in the micro-determination of fluorine by titrating with thorium nitrate after separation of fluorine from interfering ions by distillation may be caused by some of the fluorine being present as the fluosilicate ion SiF_6''. The fluosilicate ion does not form an un-ionised compound with thorium as does fluoride.

This error can be avoided by carrying out the titration in a 50 per cent. alcoholic system buffered at pH 5·3 with gallocyanine as indicator. The method can be used for the determination of 20 to 400 μg. of fluorine. The precision is approximately 1 μg. up to 100 μg. of fluorine, but the experimental error increases with larger quantities of fluorine up to 5 per cent. with 400 μg.

The effect of chloride ions and varying pH on the method is discussed.

THE determination of small amounts of fluorine is a problem which has received a considerable amount of attention over a long period. The thorium nitrate titration described by Willard and Winter,[1] with modifications which appear to be based more on personal choice than on any fundamental reasoning, is now widely used for this determination. An essential preliminary when determining fluorine in insoluble fluorides, such as are found in soil, fertilisers, plant ash and similar material, is the separation of the fluorine from ions which would interfere in the subsequent titration. This separation may be carried out by distillation from either sulphuric or perchloric acid, water or steam being added to the distillation flask to maintain the boiling-point of the liquid at a temperature low enough to prevent sulphuric or perchloric acid from appearing in the distillate.

One of the most disturbing features of the literature is that no adequate explanation has been offered for the slightly low result which is usually obtained when the distillate is titrated with thorium nitrate. Clifford[2] showed that, in general, complete recovery of fluorine is never obtained on distillation from either sulphuric or perchloric acid, although the former acid appears to be the more efficient. The maximum recovery varies from 93 to 97 per cent. If the distillation flasks are freshly boiled with sulphuric acid before distillation, a recovery of 98 per cent. can be attained, but this is not increased by collecting larger quantities of distillate. A possible explanation of this fact is that a non-volatile silicon oxyfluoride may be formed in the presence of hydrated silica.[3,4,5] Although there appears to be no independent confirmation of the existence of such a compound, there is evidence that, in the presence of gelatinous silica (*e.g.*, as silica gel) the amount of fluorine recovered may be reduced to as little as 10 per cent. of the quantity present.[5] This decrease does not occur if the silica is dehydrated before being added to the distilling flask. No explanation is offered here for this effect of hydrated silica, but the results reported by Clifford indicate that the slightly low recoveries obtained in the absence of hydrated silica are not wholly due to the distillation technique, some error being inherent in the titration procedure; proposals are made in this paper for altering the conditions of the titration so as to reduce the error involved.

In order to understand how this error arises in the thorium nitrate titration now in common use, it is necessary briefly to review the literature since 1933, when Willard and Winter published their paper. According to the original publication the titration system contained equal volumes of water and alcohol, the acidity being adjusted with hydrochloric acid to a

point which was judged by the colour of the zirconium - alizarin indicator. No measurements of the pH values of the solutions were recorded. Reynolds and Hill[6] showed that the addition of zirconium to the alizarin was unnecessary if the solution is buffered, and that a more clearly defined end-point may be obtained by omitting alcohol. The buffer solution used by these workers was half-neutralised monochloroacetic acid, as recommended by Hoskins and Ferris.[7] It is to be noted here that the pH of the aqueous alcohol system used by Hoskins and Ferris was thereby maintained at 3·5, whereas the pH of the aqueous system used by Reynolds and Hill was approximately 2·8. Hammond and MacIntyre[8] made a comparison of the thorium nitrate titration in aqueous and alcoholic systems using the chloroacetic acid buffer, and showed that, in the range 0 to 50 μg. of fluorine, a linear relationship, approximating to stoicheiometric requirements, was obtained between fluorine and thorium nitrate in 50 per cent. alcohol; in aqueous systems the relationship was non-linear. With larger quantities of fluorine, linear relationships were obtained in both aqueous and alcoholic systems.

In later papers on this problem more emphasis was given to the importance of the pH at which the determination was carried out. It was shown[9] that below pH 3 the amount of thorium nitrate solution required to cause a colour change of the indicator in a blank titration rapidly increases with decreasing pH, and that above pH 3·5 the end-point becomes indistinct. Consequently it was considered that the optimum pH was between 3·0 and 3·5. Williams,[10] in an analysis of the factors influencing the titration, suggested that the ionisation of ThF_4 depended on the hydrogen ion concentration, and recommended adding hydrochloric acid to control the dissociation at an optimum level. Milton, Liddell and Chivers[11] investigated the possibility of using some indicator other than alizarin S, and recommended the use of Solochrome Brilliant Blue B.S., which gives a colour change from pink to blue. This indicator was stated to be preferable to alizarin S in that it is more sensitive and the reaction is immediate. These workers also adopted the chloroacetic acid buffer at about pH 3·0 in an aqueous system.

The fluoride - fluosilicate equilibrium—It has generally been supposed that the pH in the titration must be controlled in order to obtain a sharp end-point with the indicator used and to restrict the ionisation of thorium fluoride. The titration of fluorine with thorium nitrate is generally represented by—

$$4F' + Th'''' \rightleftharpoons ThF_4 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

In acid solutions the thorium fluoride is only slightly ionised, but as the acidity decreases the amount of fluoride ion in solution increases, presumably owing to the precipitation of basic thorium salts or hydrous thorium oxide. There is, however, yet another factor which may be influenced by the pH. During the separation of fluorine from interfering ions, the silicon tetrafluoride that is distilled off reacts with steam in the condenser to produce a distillate containing fluosilicic acid with some precipitated silica.

$$3SiF_4 + 2H_2O \rightleftharpoons 4H^{\cdot} + 2SiF_6'' + SiO_2 \quad .. \quad .. \quad .. \quad .. \quad (2)$$

When a buffer is added before titration, the fluosilicate ions will be hydrolysed in accordance with the equation—

$$SiF_6'' + 4OH' \rightleftharpoons H_2SiO_3 + 6F' + 2H_2O \quad .. \quad .. \quad .. \quad .. \quad (3)$$

It follows that the proportion of fluorine present as fluoride ion will be greater at higher pH values. If it is assumed that the fluosilicate ion does not form a slightly ionised compound with thorium, it becomes necessary to find an optimal pH range to balance two opposing factors; with increasing pH values the amount of thorium fluoride present would decrease owing to the removal of thorium ions by precipitation, whilst the concentration of fluoride ions would increase through more complete hydrolysis of the fluosilicate. The problem therefore reduces to one of finding a convenient balance between the equilibria of equations (1) and (3), both reactions occurring in the same system.

If this reasoning is correct, it would be expected that, when working in solutions of about pH 3, the proportion of fluorine titrated in a distillate would decrease as the absolute amount of fluorine present increased; this would be so because the amount of silicon in the distillate necessarily follows the amount of fluorine, and increasing amounts of silicon will tend to increase the proportion of fluorine in the fluosilicate form under any defined equilibrium conditions.

This was confirmed by titrating the fluorine distilled from sodium fluoride and sulphuric acid; it was then found that the percentage of fluorine apparently recovered was lower with

5 mg. fluorine present than with 2 mg. (Fig. 1). In both experiments the distillation was continued until titration of aliquots of successive portions of distillate showed that no more fluorine was distilling over. The titrations were carried out in aqueous systems buffered at pH 2·8 with half-neutralised chloroacetic acid, that is, under conditions similar to those recommended by Reynolds and Hill.[6] Had any fluorine been retained in the distilling flask its amount might have been expected to be independent of the amount of fluorine originally in the flask. On the other hand, any discrepancy which arose in the titration might be expected to vary with the amount of fluorine present, especially if any complex fluorine ions were involved.

Further evidence for the adverse effect of the formation of fluosilicate on the thorium titration of fluorine was afforded by an attempt to determine the end-point of the titration by a conductimetric method. As would be expected from equation (1), a marked break in the conductance curve is observed when titrating sodium fluoride with thorium nitrate.

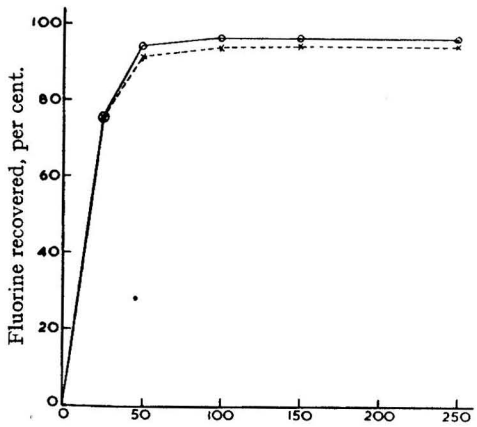

Fig. 1. Recovery of fluorine from the distillation of sodium fluoride. Titrated with thorium nitrate, using chloroacetate buffer and alizarin S indicator. O = 2 mg. of fluorine, × = 5 mg. of fluorine.

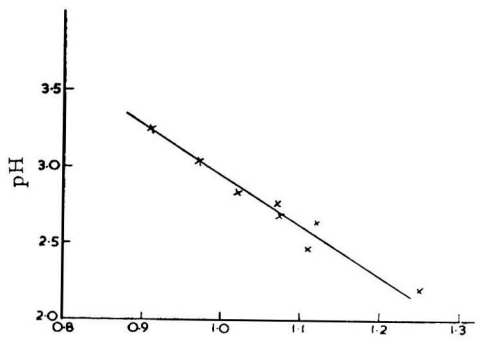

Fig. 2. The variation in the thorium nitrate titre of 500 μg. of fluorine with the pH value, using alizarin S indicator.

However, with the distillate from an equal amount of fluorine, a curve of completely different shape was obtained, suggesting that there was no close similarity between the ionic compositions of the two solutions. This dissimilarity was too great to be explained by the fact that one contained sodium ions and the other hydrogen ions in equivalent quantities. Similar results were obtained from conductimetric titrations using silver nitrate instead of thorium nitrate; as silver fluoride is a co-ordinated compound it would be expected to behave in a similar way to thorium fluoride.

The apparent deterioration on storage of sodium fluoride solutions used for standardising thorium nitrate solutions, which has been reported by several workers, can also be explained by this view of the fluoride - fluosilicate - thorium system. Ryss and Bakina[12] showed that solutions containing sodium fluoride, sodium fluosilicate and silica show a definite change in pH value with time. The direction in which the pH changes depends on the initial concentration of sodium fluoride, and in all but the highest concentration used by these workers the time taken to reach equilibrium was more than 24 days. These results indicate that the system

$$6NaF + SiO_2 + 2H_2O = Na_2SiF_6 + 4NaOH \quad .. \quad .. \quad .. \quad (4)$$

reaches equilibrium slowly, so that solutions of sodium fluoride stored in unprotected glass vessels will show a slow increase in fluosilicate content and a consequent decrease in thorium nitrate titer.

In view of this evidence, information was sought on the conditions of equilibrium of the system represented by equation (3) above. A number of papers have been published on the decomposition of the fluosilicate ion in aqueous systems.[12,13,14,15] Unfortunately the data given in these papers were all obtained with fluosilicic acid solutions partly neutralised with

sodium hydroxide, so that the equilibrium constants calculated from such data are applicable only to systems of a much lower hydrogen ion concentration than those with which we are concerned. However, Malaprade[16] has shown, from the neutralisation curve of fluosilicic acid, that the neutralisation takes place in two stages:

$$H_2SiF_6 + 2NaOH = Na_2SiF_6 + 2H_2O \quad .. \quad .. \quad .. \quad .. \quad (5)$$

$$Na_2SiF_6 + 4NaOH = 6NaF + SiO_2 + 2H_2O \quad .. \quad .. \quad .. \quad (6)$$

The curve also shows that SiF_6'' occurs in appreciable proportions only in solutions with pH below 3. If it is desired to isolate quantitatively either fluorine or silicon from a fluosilicate the pH must be greater than this value.

From the evidence given it follows that disturbance of the titration by fluosilicate will be minimised by working at as high a pH as possible, and that the use of hydrochloric acid or of a chloroacetic acid buffer gives pH values that are definitely too low.

The necessary conditions for the thorium nitrate titration of fluorine—Before adopting a buffer solution which would give a higher pH value than those previously used, it was necessary to investigate the behaviour of the alizarin S indicator in solutions of varying acidity. This

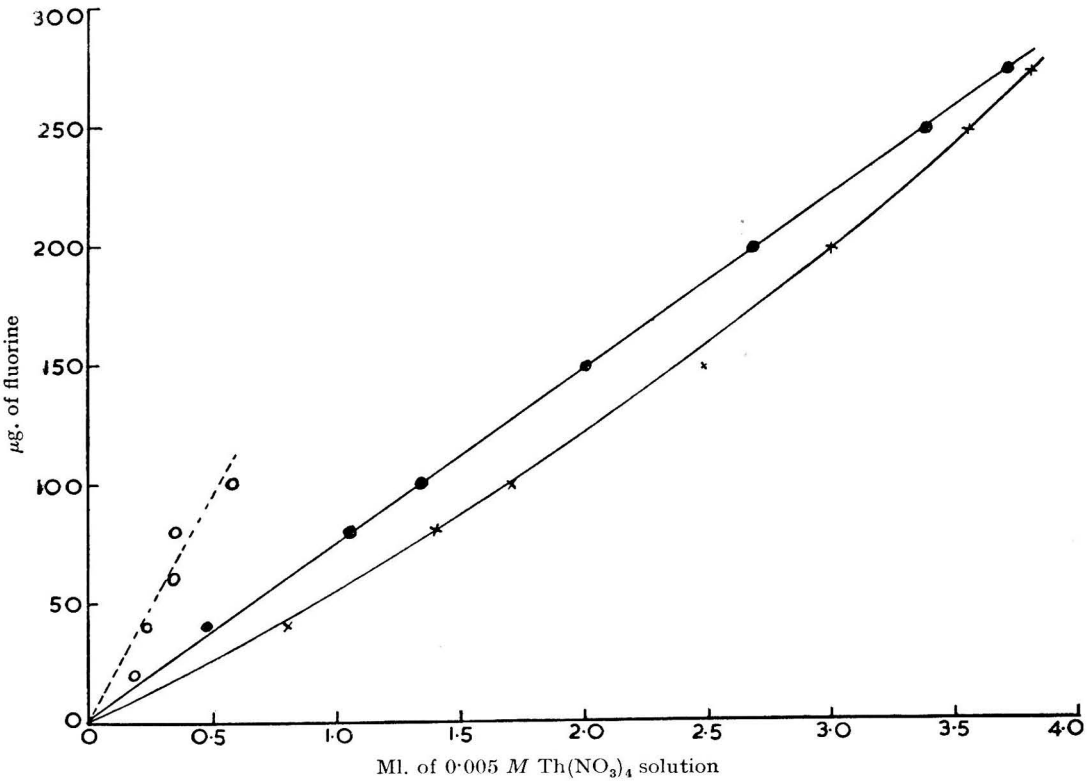

Fig. 3. The fluorine equivalent of 0·005 *M* thorium nitrate solution. X = at pH 2·8 in aqueous solution, O = at pH 5·3 in aqueous solution, ● = at pH 5·3 in 50 per cent. alcohol solution.

was done by titrating 0·5-mg. quantities of fluorine with thorium nitrate, the pH being adjusted by the addition of either hydrochloric acid or acetic acid. The fluorine was added as a solution of sodium fluoride, the volume of solution being kept constant at 20 ml. The results obtained are plotted in Fig. 2. It will be seen that below pH 2·8 the points are scattered, indicating the difficulty of recognising the end-point. Above pH 3·5 the indicator becomes very sensitive to pH changes, as it behaves as an acid - alkali indicator over the pH range 4 to 5. Hence it appears that the useful pH range of this indicator in the thorium nitrate titration is 2·8 to 3·5 approximately; Matuszak and Brown[9] give the optimum pH as 3·0 to 3·5. It was therefore necessary to find an indicator for the titration reaction which would be suitable at higher pH values. The only other indicator which appears to have

been used for this titration is Solochrome Brilliant Blue B.S.[11] This is not now commercially available, but two other dyestuffs of similar type were obtained, Solochrome Azurine B150 and Solochrome Cyanine R200. Of these the former appeared promising, showing a colour change from pink to blue-violet with an excess of thorium. Unfortunately the colour change was too slow for this substance to be useful as an indicator. Solochrome Cyanine R200 gave a similar colour change, but only in solutions more alkaline than pH 6; after trying a large number of other substances which might behave as adsorption indicators, it was found that gallocyanine behaves as an adsorption indicator above pH 4, the colour change in 0·1 *M* acetic acid - sodium acetate buffer at pH 4·12 being only just discernible, whilst in 0·01 *M* acetate buffer (pH 4·10) the colour change is quite good. It was also observed that the speed of the colour change increased as the pH is increased, so the buffer solution finally adopted for use in the titration was 0·1 *M* acetate in which the molar ratio of sodium acetate to acetic acid was 2 : 1. In the titration system the buffer is diluted to 0·01 *M*, giving pH 5·3 in aqueous solution. (The pH values throughout this work were determined with a valve potentiometer, using a glass electrode and a saturated calomel half-cell.)

One consequence of titrating at this high pH in aqueous solution is that the degree of dissociation of the thorium fluoride is quite high, so that as the amount of fluorine present increases, the volume of titrant required for a given increment of fluorine decreases. This results in a non-linear standardisation curve, the sensitivity of the titration decreasing as the amount of fluorine increases. The dissociation of thorium fluoride can be decreased by carrying out the titration in a mixture of water and ethyl alcohol; calibration curves for thorium nitrate in aqueous and in 50 per cent. alcoholic systems are shown in Fig. 3. The effect of pH on the dissociation of thorium fluoride is also illustrated here by a comparison of the calibration curves at pH 2·8 and pH 5·3 in aqueous solution.

Method

Reagents—

Thorium nitrate, 0·005 *M*—2·76 g. of $Th(NO_3)_4.4H_2O$ in 1 litre.

Buffer - indicator solution—3·4 ml. of acetic acid (1 *M*) + 6·6 ml. sodium acetate (1 *M*), diluted to 100 ml. with a 0·01 per cent. aqueous solution of gallocyanine. This gives a solution which is 0·1 *M* with respect to acetate and approximately 0·01 per cent. with respect to the indicator. The mixed solution deteriorates on keeping, and should be not more than 3 days old when used.

Ethyl alcohol—98 per cent.

Sodium fluoride, for standardisation—To prepare the standard solution, dissolve 0·2211 g. of sodium fluoride in 500 ml. of water. Ten ml. of this stock solution are diluted to 100 ml. when required; the diluted solution contains 0·020 mg. of fluorine per ml. (It is not possible to obtain "Analytical Reagent" grade sodium fluoride, the best material that is easily obtainable being "Laboratory Reagent" grade. It is advisable to recrystallise this from water, although little difference in titer has been noticed when this has been done. Through the kindness of the Government Chemist's Laboratory, a sample of pure sodium fluoride, used as a standard, was obtained, and it was found that the recrystallised material was as good as this sample.)

Procedure—

Pipette a 5-ml. aliquot of the solution containing fluorine (which may be a distillate from a fluorine separation) into a 25-ml. beaker or specimen tube, and add 5 ml. of alcohol. Add 1 ml. of the buffer - indicator solution. Prepare a blank in a similar way, using 5 ml. of water, 5 ml. of alcohol and 1 ml. of buffer - indicator solution. Using the thorium nitrate solution from a micro-burette (2-ml. capacity graduated in 0·01 ml.), titrate the blank dropwise until a marked change of colour occurs from the original blue to a reddish-purple shade. This should require only 1 or 2 drops (approx. 0·05 ml.) of thorium nitrate. Titrate the fluorine solution, adding the titrant dropwise until the colour of the solution matches the colour of the titrated blank. Deduct the blank titer from that of the fluorine solution to obtain the volume of thorium nitrate equivalent to the fluorine present. With each batch of determinations, carry out titrations with known amounts of fluorine as sodium fluoride, and prepare a calibration curve, which should be linear. From this curve the amount of fluorine present in the unknown sample is obtained. This frequent standardisation is necessary as the strength of the thorium nitrate may decrease on standing; a 0·005 *N* solution

deteriorated to 95 per cent. of its initial strength in 6 days. In view of earlier opinions on the stability of sodium fluoride solutions, it is of interest that, on titrating with thorium nitrate by the method described above, it was found that the fluorine content of a standard solution of sodium fluoride stored in an unprotected glass bottle, had not changed over a period of 4 months. This is in marked contrast with the apparent deterioration in fluorine content met with when using the older methods of determination.

It is not essential to carry out all titrations in 10 ml. of solution, and indeed it may be inconvenient. Provided that (*a*) the concentration of alcohol is 50 per cent. by volume in the solution before adding the buffer, (*b*) the amount of indicator - buffer solution is 1 ml. per 10 ml. of the 50 per cent. alcohol solution, and (*c*) the standardisation and blank titrations are carried out under similar conditions, then the absolute volume is not critical. The effect of titrating in a larger volume of solution is to decrease the thorium nitrate titer for a given amount of fluorine; for example, in 10 ml. of solution, 50 μg. of fluorine are equivalent to 0·68 ml. of titrant (0·005 *M*), and in 20 ml., are equivalent to 0·50 ml. It follows that it is necessary to standardise the thorium nitrate solution under the same conditions as the determination, and that it is advisable to carry out the titration in as small a volume as possible.

RESULTS—

The amounts of fluorine determined on titrating aliquots of the distillates from known amounts of sodium fluoride are shown in Table I, which also shows the results obtained

TABLE I

RECOVERY OF FLUORINE FROM SODIUM FLUORIDE ON DISTILLATION

Fluorine added to distilling flask	Fluorine in titrated aliquot	Fluorine determined at pH 2·8	Fluorine determined at pH 5·3
μg.	μg.	μg.	μg.
200	10	11	10
400	20	21	21
600	30	29	32
800	40	38	40
1000	50	48	50
1000	100	90	100
2000	200	160	205
3000	300	280	285
4000	400	360	385

on titrating similar aliquots with thorium nitrate using chloroacetic buffer and alizarin indicator. It is seen that the accuracy obtainable by the proposed method is better than that obtained when titrating under the conditions which have been used previously for this titration. The results also indicate that titration at pH 5·3 tends to give low results when more than 200 μg. fluorine are titrated. Under such conditions the end-point of the titration is not sharp owing to the formation of a precipitate, probably thorium fluoride. It is recommended, therefore, that the volume of the aliquot titrated should be such that the amount of fluorine present is less than 200 μg., although up to 400 μg. may be determined with an error of about 5 per cent.

INTERFERING ANIONS—

The effect of other anions on the determination has been considered. It is known that PO_4''' and SO_4'' react with thorium nitrate and so lead to erroneously high results in the fluorine determination. However, the separation of fluorine by distillation from sulphuric acid is effective in that no phosphate appears in the distillate, and if the apparatus contains an efficient spray trap no trouble arises from contamination of the distillate by sulphuric acid spray. The presence of organic matter in the parent material can give rise to sulphurous acid in the distillate, which is also undesirable. The results of experiments with soil, carried out at this laboratory, have shown that the effect of organic matter can be eliminated by mixing the soil with an equal weight of calcium oxide or hydroxide and igniting at 550° to 600° C. for 1 hour. MacIntyre[17] has recently published results confirming the value of this treatment; as an alternative he suggests that the fluorine be first distilled off from sulphuric acid, and then the distillate redistilled from perchloric acid. It is considered that this procedure, involving two distillations, is tedious and unnecessary.

Salts which give rise to volatile acids on heating with sulphuric acid may interfere in the determination; of such substances, soil may contain nitrates, nitrites, sulphides and chlorides. The first two of these are decomposed by the ignition treatment with lime. If sulphides are present they may be oxidised in the distilling flask by adding potassium permanganate.[6] Chlorides, however, cannot be removed easily and completely, so the effect of hydrochloric acid in the distillate was determined by titrating a standard fluoride solution with thorium nitrate in the presence of varying amounts of sodium chloride (Table II).

TABLE II

EFFECT OF CHLORIDE ION ON THE DETERMINATION OF FLUORINE

Fluorine added μg.	Chlorine added μg.	Fluorine determined μg.
20	10	19
20	100	20
20	1000	20
40	10	41
40	100	40
40	1000	40
80	10	78
80	100	78
80	1000	78

It is seen that there is no adverse effect caused by chloride in any of the concentrations used; the maximum concentration of Cl′ was 1 mg. per 10 ml., and the maximum value of the Cl′/F′ ratio was 50/1.

Another point which required investigation was the effect of altering the pH at which the titration was carried out. This was done by altering the sodium acetate - acetic acid ratio in the buffer solution, whilst maintaining the concentration of acetate at 0·01 *M* in the titrated solution. The results are shown in Table III, and it is seen that there is no significant difference in titer over the pH range 4·5 to 5·3. These pH values are not absolute, owing to the unreliable behaviour of the glass electrode in alcoholic solutions, but the range of 0·75 pH units is a good indication of the flexibility of this factor.

TABLE III

EFFECT OF VARIATION OF pH ON THORIUM NITRATE TITER OF FLUORINE

(50 μg. fluorine present)

pH	$Th(NO_3)_4$, 0·005 *M*, ml.
5·30	0·535
4·90	0·540
4·70	0·540
4·55	0·535

From this information it can be shown that it is not necessary to neutralise the fluosilicic acid with sodium hydroxide before adding the buffer solution, because addition of 0·5 mg. of fluorine as fluosilicic acid lowers the pH of the buffered system by only 0·3 pH unit, which is well inside the pH range given in Table II. As noted previously this amount of fluorine is too large to be determined with accuracy, owing to the precipitation of thorium fluoride, so that in practice the amount of fluorine present will never cause a fall in pH of as much as 0·3 unit. If hydrochloric acid is present in quantities comparable with the maximum amount of chloride used in Table II, the pH of the system will be reduced thereby to about 4·1. At this pH the colour of the indicator itself will show that the pH has been lowered appreciably. Conditions of this type might be met when working with saline soils, and for these it would be necessary to remove the bulk of the chloride from the material before distillation. With soil containing 0·02 per cent. of fluorine this would be necessary if the chlorine content were greater than 1·0 per cent.

Precision—At the end-point of the titration the addition of 0·01 ml. of 0·005 *M* thorium nitrate produces little recognisable change in the colour of the indicator, so that this amount must be accepted as the precision of the method (equivalent to approx. 1 μg. of fluorine).

Thus the accuracy required sets a lower limit to the amount of fluorine which can be determined; for example, if as little as 20 μg. of fluorine is titrated, the error may be 5 per cent. The upper limit is set by the precipitation of thorium fluoride as mentioned earlier, which occurs at concentrations of 400 to 500 μg. of fluorine per 10 ml. of 50 per cent. alcohol. The precision at this end of the range is 0·2 per cent., a figure which is negligible when compared with the accuracy of the method.

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Rothamsted Experimental Station
Harpenden, Herts.

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An Absorptiometric Method for the Determination of Magnesium

By J. G. HUNTER

Synopsis—The method is based on the formation of coloured complexes of magnesium hydroxide with certain dyes of the Thiazol Yellow class. A constant quantity of the dye, more than is necessary for the formation of the complex, is taken, and the quantity of magnesium present is ascertained from the excess of unused dye, which is extracted from the reaction mixture by means of *n*-butyl alcohol and read as a solution in that solvent, on a Spekker absorptiometer. A calibration curve relates quantities of magnesium directly with drum readings of the unused dye solution.

The method was developed for the determination of magnesium in plant tissues and soil extracts, and can be adapted also to trichloroacetic acid filtrates of milk and blood serum. It determines from 0·02 to 0·20 mg. of magnesium with an accuracy satisfactory for this type of determination. Permissible concentration limits of certain interfering ions and means for controlling their interference are given where necessary. Data on the concentrations of magnesium, iron, calcium, manganese and aluminium in extracts of a number of soils, prepared with (*a*) *N* ammonium acetate solution, (*b*) Morgan's solution and (*c*) 0·5 *N* acetic acid, are tabulated.

Introduction—Methods previously published for the determination of magnesium on a semi-micro scale by means of the red-coloured co-ordination complex of magnesium hydroxide and a dye such as Thiazol Yellow, have been based on direct absorptiometric measurement of the complex itself. In the method now proposed a constant quantity of dye is used and the excess over and above that required for forming the complex is separated and determined absorptiometrically in true solution. The method described can be used for the determination of magnesium in plant tissue, soil, milk and blood.

The use of the magnesium hydroxide - dye complex for the determination of magnesium is subject to certain fundamental difficulties. When the pH of a solution containing magnesium ions and an appropriate dye is adjusted to about 12·5, the complex is formed very rapidly and is not consistent in physical type and colour intensity. The complex in the solution is not constant in degree of dispersion and may be anything from a suspensoid

to a sol. Errors may therefore arise from inconsistency in colour intensity and from difficulty in measuring it accurately. Errors may also be introduced by the presence of other substances (for example, calcium, iron and manganese ions) which may affect the complex or form precipitates in the alkaline medium.

In numerous published methods[1,2,3,4,5,6,7,8,9,10,11] attempts to overcome these difficulties are usually made by incorporating reagents to stabilise the colloids or to prevent or standardise interferences. Peech and English[9] in a paper on soil tests, discuss these measures and describe a method that they consider to be a significant advance, although not so satisfactory as the methods they use for the determination of other ions.

In the method now to be described the sources of error are substantially controlled. The complex is formed slowly and is more consistent physically, a non-colloidal solution is used for the colour intensity measurement, and interference from other ions is materially reduced. The slow rate of complex formation is obtained by means of a tartrate. The true solution for colour intensity measurement is obtained by producing an alcoholic solution of that part of the dye that has not reacted with the magnesium hydroxide; it is the upper layer of the two-phase system which forms after shaking an organic solvent with the mixture containing the complex and the excess of dye. Prevention of interference by other ions is attained by introducing certain reagents, which are discussed more fully in the section on interference.

The dyes that can be used in this method are fundamentally diazoamino compounds of derivatives of dehydrothio-*p*-toluidine, and among the names used rather indeterminately for them are Titan Yellow, Clayton Yellow, Thiazol Yellow, Tourmarine and Mimosa. There are considerable differences in sensitivity among the dyes and although samples may have the same name and even be from the same source, their sensitivities may not be identical. This difference in sensitivity has been emphasised by Mikkelsen and Toth,[12] who show that the most satisfactory dye is Thiazol Yellow (General Aniline Works, Rensselaer, New York). In the present investigation a sample of this dye was compared in equivalent quantities with samples of Titan Yellow (British Drug Houses) and Chlorazol Yellow (Imperial Chemical Industries) and its superiority for the present purpose confirmed.

The method is suitable for determining between 0·02 and 0·20 mg. of magnesium, and over this range the degree of accuracy is satisfactory. The method is relatively rapid and simple, although not in the "quick test" category. A hundred determinations, including the measurement of aliquots, can be completed in $1\frac{1}{2}$ days.

Method

Preparation of the sample—

The aim of any preliminary treatment of a sample to be used for the determination of magnesium by this method is to produce a solution which does not contain excessive amounts of organic matter or interfering ions and in which the magnesium concentration is such that 0·02 to 0·20 mg. of magnesium can be obtained in easily handled aliquots. In addition, the magnesium extractant should be easily removable so that it can be replaced by Morgan's reagent[4]; if Morgan's reagent is itself the extractant, the required aliquot should be not more than 15 ml. Some satisfactory methods of preparing solutions for analysis are outlined below.

Plants—The usual technique of ashing followed by solution in dilute hydrochloric acid is suitable for plant tissues; thereafter, a convenient aliquot is transferred to the reaction flask, evaporated to dryness and the residue dissolved in exactly 15 ml. of Morgan's reagent.

Plant tissues extracted with Morgan's reagent are usually used without treatment other than removal of excessive amounts of organic matter by activated carbon (Hester[24]). It is essential that the carbon used shall contain no extractable magnesium; ordinary grades can be purified by acid washing.

Soils—Solutions prepared by extracting the soil with Morgan's reagent as described by Peech and English[9] are used as indicated above.

If the extract has been prepared by using neutral *N* ammonium acetate (Piper[18]) or 0·5 *N* acetic acid (Williams[19] and Williams and Stewart[25]) it is evaporated to dryness and the residue is dissolved in a convenient volume of dilute hydrochloric acid, to give a solution from which the aliquot is taken. Alternatively, a convenient aliquot of the original extract is evaporated to dryness in the reaction flask and the residue dissolved in 15 ml. of Morgan's

reagent. In either technique, excessive amounts of organic matter can be removed by repeated addition of 20-volume hydrogen peroxide as the evaporation nears completion.

Milk and blood—Suitable solutions can be made from these by an ashing procedure similar to that for plants. Magnesium is then determined as described in the section on procedure.

These solutions can be prepared more easily by means of trichloroacetic acid (for example, Sanders[26]).

In analysing milk, a convenient aliquot (usually 10 ml.) is diluted to 100 ml. with trichloroacetic acid reagent (200 g. of trichloroacetic acid dissolved in water and diluted to 1 litre). The mixture is shaken occasionally during 30 minutes and then filtered through a dry No. 30 Whatman filter-paper. Ten ml. of the filtrate are pipetted into the reaction flask, 5 ml. of Morgan's reagent are added and the magnesium is determined as in the section on procedure—with a slight alteration in the method of calibration, which is also described.

A similar method can be used for blood. For this, however, the sample should be diluted with an equal volume of the trichloroacetic acid solution, and the resulting precipitate removed by centrifuging rather than by filtering.

REAGENTS—

Morgan's reagent[4]—100 g. of hydrated sodium acetate and 30 ml. of glacial acetic acid are dissolved in water and diluted to 1 litre.

Oxalic acid reagent—15 g. of hydrated oxalic acid are dissolved in water and diluted to 1 litre.

Tartrate reagent—10 g. of sodium hydrogen tartrate, 10 g. of mannitol and 2·5 g. of hydrazine sulphate are dissolved separately in water, and the mixed solutions diluted to 1 litre.

Sodium hydroxide reagent—150 g. of sodium hydroxide are dissolved in water and diluted to 1 litre.

Dye reagent—The concentration of dye required will depend on the particular specimen of dye used and should be chosen to give a convenient range of Spekker readings. The following may prove satisfactory: 0·09 g. of Thiazol Yellow (or 0·15 g. of Titan Yellow or 0·25 g. of Chlorazol Yellow) dissolved in water, diluted to 1 litre, mixed with 2 drops of sodium hydroxide reagent, and filtered after 24 hours. The reagent is stable for several months.

Dye solvent—600 ml. of butan-1-ol (pure technical *n*-butyl alcohol) mixed with 400 ml. of ethanol (rectified spirits).

Magnesium stock reagent (400 p.p.m. magnesium)—4·054 g. of A.R. magnesium sulphate ($MgSO_4.7H_2O$) are dissolved in Morgan's reagent and diluted accurately to 1 litre with Morgan's reagent.

Magnesium standard solution (20 p.p.m. magnesium)—50 ml. of magnesium stock reagent are accurately diluted to 1 litre with Morgan's reagent.

Calcium compensating reagent (200 p.p.m. calcium)—0·50 g. of A.R. calcium carbonate is dissolved in Morgan's reagent and diluted to 1 litre with Morgan's reagent.

PROCEDURE—

An aliquot containing from 0·02 to 0·20 mg. of magnesium is put in the reaction flask (100-ml. conical) and treated as already described in the section on Preparation of the Sample, so that ultimately the magnesium is present dissolved in 15 ml. of Morgan's reagent—except where a trichloroacetic acid filtrate from milk or blood is concerned. Five ml. of the oxalic acid reagent are added to the solution, which is mixed and left for at least 1 hour at room temperature. Five ml. of the tartrate reagent are then added followed in succession by exactly 5 ml. of the dye reagent and 20 ml. of the sodium hydroxide reagent. The contents of the flask are mixed after each addition and the final mixture is left overnight.

Fifty ml. of the dye solvent are then added and shaken with the mixture for about 30 seconds. After standing for 1 minute two phases will have separated. Within 30 minutes as much of the upper layer as can be conveniently decanted off is transferred to a small conical flask containing 0·5 ml. of acetone. The contents of this flask are then mixed.

The colour intensity of this solution is determined within 30 minutes; a Spekker photoelectric absorptiometer with 4-cm. cells and glass violet filters can be used.

The graph required for the conversion of the Spekker readings is constructed by diluting 0, 1, 2, . . . 10 ml. of magnesium standard solution to 10 ml. with Morgan's reagent and

adding 5 ml. of the calcium compensating reagent. The oxalic acid reagent is then added and the determination completed as above.

Typical calibration readings are given below. They were obtained at 16° C. with Thiazol Yellow, on a Spekker absorptiometer, with 4-cm. cells and water setting of 1·00.

Magnesium, mg.	nil	0·02	0·04	0·06	0·08	0·10	0·12	0·14	0·16	0·18	0·20
Spekker reading	0·070	0·150	0·220	0·295	0·380	0·465	0·555	0·635	0·705	0·770	0·810

NOTES ON THE METHOD—

If it is desired to obtain results more quickly, the procedure can be accelerated by heating the solution to boiling after the addition of the oxalic acid reagent, then cooling, and allowing the colour to develop for 1 hour; any such changes must, of course, be incorporated also in the calibration procedure. It should be noted that this modified method effects no saving in the time required for the manipulations, although the results are obtained sooner; moreover, results obtained in this way tend to be less accurate.

The sodium hydroxide reagent must be added immediately after the dye reagent and in such a way that it washes all the dye from the sides of the flask into the solution. It is important that the 5 ml. of dye reagent should be measured accurately.

The contents of all the reaction flasks during the colour development period (especially in the initial stage) and the dye extraction period, should be maintained at the same temperature, which must lie between 10° and 35° C. The temperature of the sodium hydroxide reagent should also be within that range. A constant temperature can be maintained thermostatically, but it is simpler to work at room temperature (provided it is within the required range) and to prepare a calibration graph with each set.

While the mixture is being shaken with the dye solvent, the flask should be closed by a glass or rubber stopper.

The phases which separate consist of a clear orange-coloured alcoholic upper layer containing the excess of dye, and a turbid aqueous lower layer containing the magnesium hydroxide - dye complex and other insoluble substances. The upper layer has a volume of about 50 ml. and it is an easy matter to pour off approximately the same amount each time; although slight differences in the decanted volume do occur, the dilution error introduced in adding the 0·5 ml. of acetone is small and in any case is within the limits of accuracy. Acetone is added to prevent the alcoholic solution becoming turbid; it is ineffective if incorporated with the mixture before separation. Once the solution is poured into the Spekker cell the reading should be made without delay. Any adjustment necessary to keep the process within time limits may suitably be made by dividing sets into groups of not more than 15 immediately before extracting the excess of dye, and completing the determinations on one group before proceeding with the next.

Although oxalic acid reagent effects control of calcium interference within satisfactory limits, there is a tendency for solutions that contain no calcium to give rather high results; for this reason some calcium is incorporated in the standards used to construct the calibration curves.

When trichloroacetic acid is present in the solution, approximately the same amount should be included in the calibration solutions; this can be easily arranged by incorporating the appropriate amount in the calcium compensating reagent.

INTERFERENCES—

The effect of certain ions on the method was investigated and it was found that some of these influenced the results when present in amounts outside the tolerance limits given in

TABLE I

ION TOLERANCE LIMITS

Permissible amounts (milligrams) of ions in the aliquot taken for analysis

Magnesium .. .. .. 0·02–0·20

Calcium	0–4·0	Sodium	0–20·0*
Iron	0–0·6	Silicate†	0–2·0
Manganese	0–0·6	Carbonate	0–10·0*
Aluminium	0–0·6	Chloride	0–1300*
Titanium	0–2·0	Nitrate	0–10·0*
Ammonium	0–700*	Phosphate	0–10·0*
Potassium	0–20·0*	Sulphate	0–10·0*

* The effect of greater amounts was not investigated. † Expressed as silica.

Table I. From Table II it is seen that variable magnesium values result from excessive amounts of calcium or iron in the aliquot and high values from excessive manganese. Low values were obtained when the amounts of aluminium, titanium and silicate exceeded the limits.

TABLE II

ION INTERFERENCE

Effect of calcium, iron and manganese on the determination of magnesium

(Amounts as milligrams per determination)

Magnesium present	Calcium added	Iron added	Manganese added	Magnesium found	Percentage error
0·10	—	—	—	0·10	0
0·10	2·00	—	—	0·10	0
0·10	4·00	—	—	0·099	− 1
0·10	6·00	—	—	0·08	−20
0·18	4·00	—	—	0·184	+ 2
0·18	6·00	—	—	0·206	+14
0·18	10·00	—	—	0·10	−44
0·10	1·00	0·60	—	0·10	0
0·10	1·00	1·00	—	0·094	− 6
0·10	1·00	2·00	—	0·08	−20
0·10	1·00	—	0·60	0·10	0
0·10	1·00	—	1·00	0·113	+13
0·10	1·00	—	2·00	0·133	+33

Control within the established limits is obtained for calcium by oxalic acid, for manganese by a tartrate, and for iron and aluminium by a reagent containing a tartrate, hydrazine sulphate and mannitol.

The tolerance limits are not often exceeded in the solutions as usually prepared for analysis, but excessive amounts of calcium, iron, manganese and aluminium do sometimes occur. The possibility of interference is discussed below.

Few of the several thousand plant analyses enumerated by Beeson[13] show excessive calcium and even fewer show excessive iron or manganese. The aluminium values given for ordinary plants by Winter and Bird[14] and Hutchinson[15] indicate that excessive amounts are not present. However, plants grown on some very acid soils have very high manganese contents (Hale and Heintze[16] and Hunter and McGregor[17]), and a few plant species are recognised aluminium accumulators (Hutchinson[15]); interference may be found in these. In general then, calcium interference is seldom encountered in analysing plant tissue and interference from iron, manganese or aluminium is exceptional.

In soil analysis, the concentration of interfering ions in an extract will depend not only on their content and form in the sample, but also on the method used to prepare the extract. To obtain some idea of the possible occurrence of interfering substances in extracts, twelve soils of various types, mostly very acid, were extracted with (*a*) neutral *N* ammonium acetate solution (Piper[18]), (*b*) Morgan's reagent (see section on reagents, p. 93) pH 4·8 (as described by Peech and English[9] but extraction period increased to 2 hours), and (*c*) 0·5 *N* acetic acid, pH 2·5 (Williams[19]). The concentrations of calcium, iron, manganese and aluminium in the extracts were determined as described by Peech and English,[9] and magnesium by the method described in the section on procedure, interfering ions being removed where necessary. The results are given in Table III. It will be seen that calcium was extracted in excessive amounts from two of the soils by ammonium acetate and Morgan's reagent, and from three by acetic acid; this extraction of excessive calcium from some soils by all three solutions was not unexpected and if more calcium-rich soils had been examined a higher proportion of extracts containing excessive calcium would have been obtained. Ammonium acetate extracted little iron, manganese and aluminium, and although Morgan's reagent removed more, the extracts did not contain excessive amounts; it is possible, however, that large amounts of iron, manganese and aluminium, especially the last, will be extracted from some soils, and if the magnesium content of these soils is low and the maximum permissible aliquot for Morgan's reagent (15 ml.) is taken, interference may occur. The acetic acid extracts contained more iron, manganese and aluminium than the other extracts, interfering amounts of iron being present in two, manganese in one, and aluminium in seven; this is characteristic of the more acid extractants.

TABLE III

ION INTERFERENCE

Magnesium, calcium, iron, manganese and aluminium in soil extracts

(Concentrations expressed as parts per million of air-dry soil)

Nature of soil			Per-centage loss on ignition	Magnesium			Calcium			Iron			Manganese			Aluminium		
Parent material	Texture	pH		(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
Raised beach	Sand	4·9	4	21	19	22	160	153	180	5	30	30	0	0	5	0	50	200*
Fluvio-glacial sand and gravel	Loamy sand	4·7	7	38	39	41	230	187	300	5	30	30	12	25	50	10	75	500*
Till (quartzose schist)	Loamy sand	4·8	9	44	44	49	45	33	130	15	45	150*	0	0	0	0	60	75
Till (acid igneous)	Coarse sandy loam	4·6	8	41	43	45	600	550	800	5	15	35	5	25	30	0	50	75
Till (Old Red Sandstone)	Loam	4·3	8	13	12	14	800*	900*	1400*	5	10	10	0	5	25	0	25	75*
Till (basic igneous)	Loam	4·9	7	69	63	66	1000	750	1500*	5	5	25	10	20	45	5	150	750*
Till (quartzose schist)	Organic loamy sand	4·4	18	26	24	23	130	75	185	5	30	45	25	35	120*	5	150	750*
Till (serpentine)	Organic loamy sand	4·6	54	281	225	306	1500	1500	2500	175	300	400	75	250	500	10	75	750
Till (basic igneous)	Organic sandy loam	4·8	21	125	125	115	650	650	900	25	150	500*	13	25	45	5	75	1000*
Till (Old Red Sandstone)	Clay	5·5	9	340	310	335	1850	1800	1850	5	15	45	0	5	25	0	20	25
Basaltic	Organic sandy loam	5·3	26	220	200	229	400	400	450	5	5	90	15	15	80	40	250	1500*
Raised beach	Loam	7·0	9	105	140	150	3400*	5000*	7500*	5	5	25	7	10	25	5	10	100

(*a*) Extracted by *N* ammonium acetate.
(*b*) Extracted by Morgan's reagent.
(*c*) Extracted by 0·5 *N* acetic acid.
* Likely to interfere with determination of magnesium.

In general then, it can be taken that with ammonium acetate and Morgan's reagent interference may frequently arise from excessive extraction of calcium, but seldom of other ions. On the other hand, excessive calcium, iron, manganese and aluminium occur commonly in acetic acid extracts (and in solutions from alkaline fusion and from relatively concentrated acid extraction).

The composition of normal cow's milk (Bushill and Rooke[20]) is such that interference from calcium, iron, manganese and aluminium does not arise. However, calcium interference may occur in the analysis of milk deficient in magnesium.

The calcium in normal human whole blood and serum, and cattle serum (Evans[21] and Sjollema[22]), does not interfere in the determination of magnesium, but when the magnesium content of blood is low, as it is in cattle affected by grass tetany, calcium may be in excess. The iron content of normal human whole blood, plasma and corpuscles is given by Evans[21]; in plasma (or serum) the content is small and does not interfere, but in whole blood or corpuscles the content is very large and interference occurs. The amounts of manganese and aluminium in blood serum (Kehoe, Cholak and Story[23]) are small and unlikely to cause interference.

When an excessive amount of calcium, iron, manganese or aluminium does occur in a solution in which magnesium is to be determined, a removal procedure such as described

TABLE IV

DEGREE OF ACCURACY OF THE METHOD

Statistical analysis of replicate determinations

Milligrams of magnesium per determination	Number of determinations	Standard deviation	Standard error	Percentage error A*	Percentage error B*
0·0200	10	0·00292	0·000923	15·0	10·4
0·0200	10	0·00266	0·000841	13·7	9·52
0·0300	10	0·00220	0·000696	7·54	5·24
0·0300	10	0·00162	0·000512	5·55	3·86
0·0406	10	0·00196	0·000620	4·96	3·45
0·0404	10	0·00148	0·000468	3·76	2·62
0·100	10	0·00238	0·000752	2·45	1·70
0·100	10	0·00196	0·000620	3·00	1·39
0·160	10	0·00262	0·000820	1·67	1·16
0·160	10	0·00288	0·000911	1·85	1·29
0·200	10	0·00322	0·00102	1·66	1·15
0·200	10	0·00220	0·000696	1·13	0·78

* *Percentage error A*. This will not be exceeded 99 times out of 100.
Percentage error B. This will not be exceeded 19 times out of 20.

immediately below must be introduced. The presence of excessive calcium is readily detected in the initial stage of a magnesium determination by the excessively large precipitate produced on adding the oxalic acid reagent; excessive iron is detected by the yellow-brown colour of the original solution, and excessive manganese by the production of an orange-red instead of a red complex.

Removal of excessive calcium, iron, manganese and aluminium—When excessive amounts of calcium are indicated by the size of the precipitate on adding oxalic acid, a further 15 ml. of Morgan's reagent and 5 ml. of the oxalic acid reagent are added and after about an hour the mixture is filtered through a dry No. 40 Whatman filter-paper. Twenty ml. of the filtrate, which will be slightly turbid, are then transferred to a reaction flask and the colour is developed in the usual way. The magnesium value found must be adjusted by the dilution factor of 2.

Excessive iron, manganese and aluminium can be simply removed from solution in Morgan's reagent or dilute hydrochloric acid as described below, but when in other extractants, such as ammonium acetate solution or acetic acid, it is usually better to evaporate the solution to dryness and dissolve the residue in dilute hydrochloric acid. When the extractant is Morgan's reagent, the iron, manganese and aluminium are precipitated in presence of about 10 ml. of 20-volume hydrogen peroxide per 100 ml. of solution by adding sodium hydroxide solution dropwise until the liquid is alkaline (litmus paper). This is boiled for 5 minutes, cooled and filtered. The precipitate, etc., is washed with water and the filtrate and washings are evaporated until the volume is less than the original. Five ml. of glacial acetic acid are added per 100 ml. of the original solution and the whole is diluted with water to the original volume. When the solvent is dilute hydrochloric acid, the procedure is similar except that

the filtrate and washings are made up to any convenient volume with water; a suitable aliquot is then transferred to a reaction flask and evaporated to dryness, the residue being dissolved in 15 ml. of Morgan's reagent. It is unlikely that the residue will contain ammonium salts in interfering amounts, but where necessary these may be removed by gentle ignition.

ACCURACY—

To determine the degree of accuracy of the method, solutions containing known amounts of magnesium were prepared and their magnesium contents determined. The statistical analysis of these determinations is given in Table IV.

It will be seen that the standard deviation and standard error for each set of determinations is relatively constant. In order to estimate on a percentage basis the degree of accuracy at different levels of magnesium concentration, the product of each standard error and the appropriate t value is expressed as a percentage of the mean determination. In this way the percentage accuracy that will be obtained 99 times out of 100, and the percentage accuracy

TABLE V

DETERMINATION OF MAGNESIUM IN PLANTS

Material	Method of preparing solution for analysis	Milligrams magnesium per 100 g. dry matter: Determined gravimetrically	Determined absorptiometrically	Percentage difference
Tomato leaf-laminae	Dry matter ashed and extracted by hydrochloric acid	260	267	+ 2·7
Oat leaves		612	600	− 2·0
Swede leaf-petioles		212	216	+ 1·9
Tomato stems	Dry matter extracted by Morgan's reagent	162	156	− 3·7
Oat leaves		426	424	− 0·5

obtained 19 times out of 20 were estimated and are recorded in Table IV. These show that for 0·04 to 0·20 mg. of magnesium the result will be within 5 per cent. of the true value 99 times out of 100, and for 0·03 to 0·04 mg. of magnesium, 19 times out of 20. The errors are relatively greater for smaller amounts of magnesium and the same level of accuracy is not reached. Thus, for 0·02 mg. of magnesium, 10 determinations will give a mean value within 10 per cent. of the true value in 19 cases out of 20. These results indicate that the degree of accuracy of the method can be considered satisfactory by the standards usually recognised for this type of determination.

The magnesium contents of numerous samples of plant tissue, soil, milk and blood were determined by the method described. For some of the results the degree of accuracy was estimated by comparison with results obtained by established methods. The remainder were examined by repeating their analysis, using aliquots of different volume as the replicates; in such cases, satisfactory agreement between replicates would not be obtained if interfering ions were present (since no other ion examined affects the dye as does magnesium). Both methods of examination gave satisfactory results, some of which are shown in Tables V, VI and VII.

TABLE VI

DETERMINATION OF MAGNESIUM IN SOILS

(N ammonium acetate extraction)

pH	Percentage loss on ignition	Milligrams of exchangeable magnesium per 100 g. soil: Determined gravimetrically	Determined absorptiometrically	Percentage difference
8	5·6	206	197	− 4·4
6·7	5·5	40·6	42·6	+ 4·9
6·1	11·4	6·80	6·56	− 3·5
5·7	3·6	12·5	12·6	+ 0·8
4·1	16·8	10·3	10·6	+ 2·9

TABLE VII

DETERMINATION OF MAGNESIUM IN MILK AND BLOOD SERUM

Solution prepared for analysis by trichloroacetic acid method

	Milligrams magnesium per 100 ml.		
		Determined absorptiometrically	
Material	Determined gravimetrically	5-ml. aliquot	10-ml. aliquot
Milk	12·4	12·5	12·4
Milk	—	10·9	10·8
Milk	—	10·8	11·2
Blood (serum)	2·70	2·72	2·76
Blood (serum)	—	2·12	2·08

The preliminary work on this method was carried out in the Chemistry Department of the West of Scotland Agricultural College.

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THE MACAULAY INSTITUTE FOR SOIL RESEARCH
CRAIGIEBUCKLER, ABERDEEN

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The Effect of Bicarbonate on the Colorimetric Determination of Uranium

By T. R. SCOTT

Synopsis—When peruranate colours are to be developed in sodium carbonate solutions in the hydrogen peroxide method of determining uranium, the presence of bicarbonate in the solution causes errors. Their magnitude and means of avoiding them are considered. They may be eliminated by conducting the preliminary neutralisation of the solution with sodium hydroxide and measuring the peruranate colour within a few hours of making the solution alkaline with sodium carbonate. If readings are made at 445 mμ., no error occurs even in presence of bicarbonate, but the sensitivity is much less than at 360 mμ. The decrease in colour at this lower wavelength is attributed to the conversion of higher to lower peruranates, the latter increasing in amount as the pH of the solutions is decreased.

The intense yellow colour developed on addition of hydrogen peroxide to alkaline solutions containing uranium has been used for many years in the colorimetric estimation of that element. Precautions must be taken, however, when the colour is developed in the customary fashion[1] in sodium carbonate solution, since considerable errors may be introduced if sodium bicarbonate is present. The magnitude and variability of the error is demonstrated below and has not been previously reported except for a preliminary note[2] by the writer. The purpose of this paper is to amplify and supplement the original short note.

Experimental

Formation of bicarbonate in the analytical procedure—

Uranium is conveniently separated from most other elements by ether extraction,[3] the ether extract being allowed to evaporate in presence of water, leaving an aqueous solution of uranyl nitrate and nitric acid. When this solution is made alkaline with sodium carbonate, considerable quantities of sodium bicarbonate are formed, which cause a marked reduction in the intensity of the peruranate colour subsequently formed with hydrogen peroxide.

The results of experiments with synthetic ether extracts (containing 30 ml. of ethyl ether, 4 mg. of U_3O_8 and 2 ml. of concentrated nitric acid) are given in Table I. These extracts were poured into different volumes of sodium carbonate solution, the ether was evaporated and the resulting aqueous solutions were made up to 50 ml. The peruranate colours were developed by addition of 0·1 ml. of 30 per cent. hydrogen peroxide per 10 ml. of solution and the extinction values determined at 360 mμ. with a Coleman spectrophotometer. In an attempt to eliminate bicarbonate, the solutions were boiled vigorously for 45 minutes, and the rise in pH shown in Table I is evidence that considerable amounts of carbon dioxide were lost, but the extinction values, although increased by this procedure, were still considerably lower than the value for solutions free from bicarbonate.

Table I

Formation of bicarbonate from ether extract

Volume of 10 per cent. Na_2CO_3 solution used, ml.	Before boiling		After boiling	
	pH	E*	pH	E
16†	7·46	0·10	9·65	0·46
20	8·23	0·28	9·93	0·45
30	9·09	0·35	9·97	0·41

* E = 0·554, in absence of bicarbonate.

† 15·2 ml. required to neutralise the acid present, according to the equation $2HNO_3 + Na_2CO_3 = 2NaNO_3 + H_2O + CO_2$.

Carbon dioxide is also absorbed from the air by solutions of sodium carbonate, so that solutions initially free from bicarbonate may give erroneous extinction values if several days are allowed to elapse (as over a week-end) before the colours are developed. In Table II details are given of the decrease in pH (measured with an "Alki" glass electrode) in solutions

of sodium carbonate, kept in beakers covered with watch glasses at room temperature. Each solution contained in addition 80 mg. of U_3O_8 per litre, and the extinction values given in the last column show the effect of bicarbonate formation after 2 and 6 days.

TABLE II

EFFECT OF ABSORPTION OF CARBON DIOXIDE BY SODIUM CARBONATE SOLUTIONS

Solution	pH					*Extinction (E)	
	Initial	After 1 day	After 2 days	After 3 days	After 6 days	After 2 days	After 6 days
9 per cent. Na_2CO_3 ..	11·50	11·38	11·32	11·20	10·93	0·552	0·553
2·5 " " ..	11·39	11·25	11·16	11·04	10·74	0·541	0·530
0·5 " " ..	11·24	10·89	10·67	10·48	10·08	0·503	0·458

* In absence of bicarbonate, E = 0·554.

It is evident that no detectable error occurs with concentrated sodium carbonate solutions, even after 6 days, but that errors appear with relatively dilute solutions within 2 days. When the beakers are uncovered, uptake of carbon dioxide is much more rapid and, for 2·5 per cent. solutions, the extinction value is in error by 3 per cent. after only 7 hours' exposure. It is thus advisable to eliminate this source of error either by determining extinction values within a few hours of making the solutions alkaline, or by keeping the solutions in stoppered flasks.

MAGNITUDE OF THE BICARBONATE ERROR—

To solutions containing 6 mg. of U_3O_8 per 100 ml. and known amounts of sodium carbonate and bicarbonate, 1 ml. of 30 per cent. hydrogen peroxide was added per 100 ml.

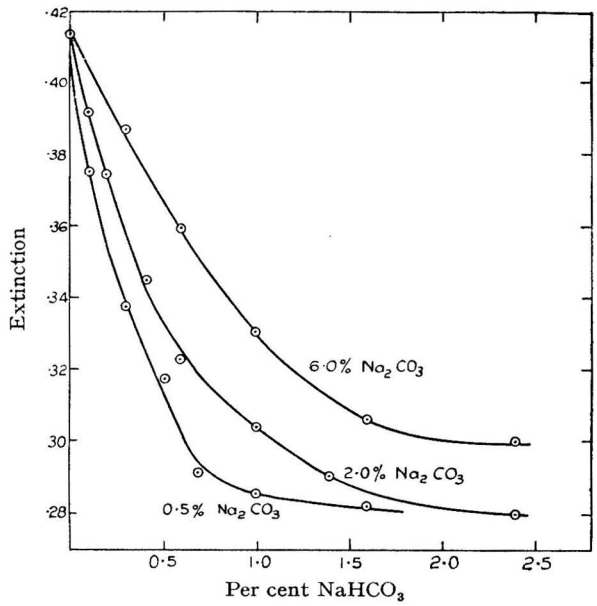

Fig. 1. Effect of bicarbonate on extinction values

and the extinction values were determined at 360 mμ. Fig. 1 shows that, with a fixed amount of bicarbonate, the effect is more pronounced as the concentration of sodium carbonate becomes less. On the other hand, the decrease in extinction becomes larger for a given carbonate/bicarbonate ratio as the carbonate concentration increases. This non-linearity of the relationship between extinction and bicarbonate concentration leads to considerable difficulty in correcting results once bicarbonate has been introduced into the solutions.

Most of the extinction values shown in Fig. 1 fall approximately on a straight line when plotted against pH, except at pH values below 9·8, where the decrease in extinction only varies slightly with increased bicarbonate concentration. It must be stressed, however,

that points do not necessarily fall on or near this line when colours are developed in media other than the carbonate - bicarbonate solutions under consideration.

ELIMINATION OF THE BICARBONATE ERROR—

According to Stumper[4] the decomposition of sodium bicarbonate in aqueous solution is a first-order reaction and prolonged boiling is therefore needed to destroy all the bicarbonate. In presence of excess of sodium carbonate the decomposition is further retarded. This evidence, taken in conjunction with the findings shown in Table I, suggests that errors may still persist despite prolonged boiling of carbonate - bicarbonate solutions, and indicates that alternative methods of avoiding the error are to be preferred.

The addition of sodium hydroxide to convert bicarbonate to carbonate is not recommended, for excess of the reagent will augment the colour intensity beyond that produced in pure sodium carbonate solutions.

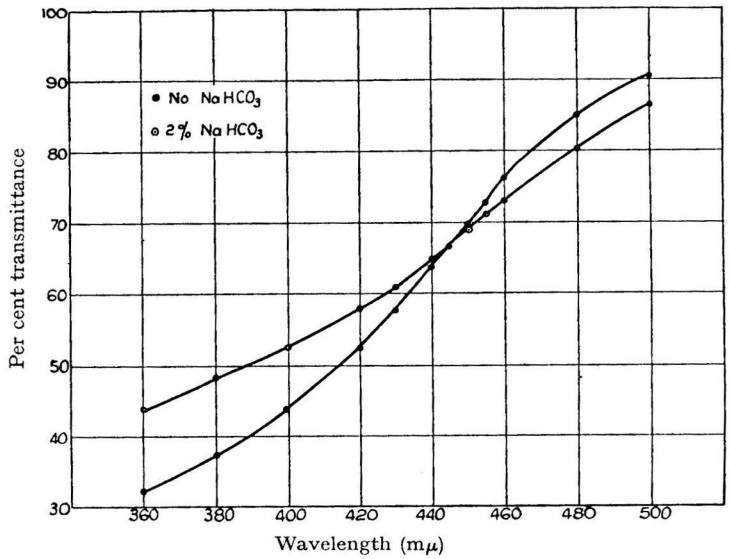

Fig. 2. Transmittance curves for sodium peruranate solutions (using Beckman spectrophotometer)

As shown in Fig. 2, the bicarbonate effect becomes less marked at longer wavelengths, and is non-existent at 445 mμ. No error will be caused if readings are made at this wavelength, but the extinction for a given concentration of uranium is barely one-third of that at 360 mμ. As the ratio of bicarbonate to carbonate decreases, the wavelength at which the two curves cross becomes slightly less, but the error introduced by using 445 mμ. for all ratios should never exceed 2 per cent.

The simplest method for completely eliminating the source of error is to neutralise solutions with sodium hydroxide, with addition of sodium carbonate at a later stage. In this way no bicarbonate is formed.

Although more intense colours are produced when sodium hydroxide is used instead of sodium carbonate to make the solutions alkaline, the method has several disadvantages. Care must be taken to ensure that uptake of carbon dioxide from the air, or the presence of sodium carbonate as an impurity, does not lead to errors similar to those occurring with solutions made alkaline with sodium carbonate. Moreover, when alkaline solutions are coloured with compounds other than uranium salts, suitable blanks cannot be produced with sodium hydroxide, since sodium uranate is precipitated: with sodium carbonate, on the other hand, the uranium in the blank remains in solution as the double carbonate.

DISCUSSION

At first sight it may appear that the decrease in colour in the presence of sodium bicarbonate can be explained by the reversible reaction—

$$2Na_2UO_6 + 8NaHCO_3 = 2Na_4(UO_2)(CO_3)_3 + 2Na_2CO_3 + 4H_2O_2 \quad .. \quad .. \quad (1)$$

In the absence of sodium bicarbonate, the equilibrium in carbonate solutions with excess of hydrogen peroxide would be displaced to the left with formation of a peruranate (Na_2UO_6 is only one of several possibilities) and the minor amounts of bicarbonate formed by the reaction with milligram quantities of uranium would have no significant effect. With gross amounts of bicarbonate, however, the equilibrium would be displaced to the right and the intense peruranate colour would be partly replaced by the relatively weak colour of sodium uranyl tricarbonate.

This simple explanation can be, at best, only partly true, because inspection of Fig. 2 shows that the transmittance curve for bicarbonate solutions is quite different from that for carbonate solutions, whereas it should be similar (but with decreased absorption) if a diminution in peruranate concentration has taken place. The change in the shape of the curve cannot be ascribed to sodium uranyl tricarbonate, which shows comparatively little absorption above 350 mμ. at the concentrations involved. Hence it must be concluded that the colour-producing compound has changed in whole or part because of the presence of sodium bicarbonate.

In the three peruranates, $Na_2U_2O_{10}$, Na_2UO_6 and Na_4UO_8, described by Rosenheim and Daehr,[5] the ratio of active oxygen to uranium increases in the order given. Relationships between these compounds are shown in equations (2) and (3) and indicate that the formation of higher peruranates (*i.e.*, those containing a greater proportion of active oxygen) is favoured by increase in pH and hydrogen peroxide concentration.

$$2Na_4UO_8 + 4H_2O = 2Na_2UO_6 + 4NaOH + 2H_2O_2 \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$2Na_2UO_6 + 2H_2O = Na_2U_2O_{10} + 2NaOH + H_2O_2 \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Reduction of pH by addition of sodium bicarbonate can thus be expected to favour of the formation of lower peruranates, as shown in equation (3), which may be rewritten, to compare with equation (1), in the form—

$$2Na_2UO_6 + 2NaHCO_3 = Na_2U_2O_{10} + 2Na_2CO_3 + H_2O_2 \quad .. \quad .. \quad .. \quad (4)$$

In view of the high concentrations of hydrogen peroxide and sodium carbonate in the solutions studied, it is understandable that reduction in pH would not cause complete displacement of equilibrium to the right-hand side of equation (4) and the transmittance curves shown in Fig. 2 more probably represent the effect of mixtures of two (or more) peruranates, with the lower peruranate preponderating in the curve for the solution containing sodium bicarbonate.

If the lower peruranate has a smaller extinction coefficient than the peruranate that predominates in the absence of bicarbonate, the decrease in the extinction values in the presence of bicarbonate can be readily understood. An alternative that cannot be overlooked is that the lower peruranate has a higher extinction coefficient, in which instance a low extinction value in the presence of bicarbonate must be ascribed to the formation of sodium uranyl carbonate (with a very small extinction coefficient at 360 mμ.) as well as a lower peruranate. Insufficient data are available to make it possible to choose between these alternatives.

It should be emphasised that the equations given above are merely illustrative and the actual peruranates responsible for the colours may be different from those mentioned. In the course of work in hand, however, it has been found that the transmittance curve obtained in presence of bicarbonate can be duplicated in absence of bicarbonate by appropriate reduction of the concentration of hydrogen peroxide. This fact might have been predicted from a study of equation (4) and confirms the belief that a lower peruranate is formed under these conditions.

Conclusions

When peruranate colours are to be developed in sodium carbonate solutions, errors due to bicarbonate may be eliminated by conducting the preliminary neutralisation of the solution with sodium hydroxide and measuring the intensity of the peruranate colour within a few hours of making the solution alkaline. If readings are made at 445 mμ., no error occurs even in presence of bicarbonate, but the sensitivity is much less than at 360 mμ. The decrease in colour at this lower wavelength is attributed to the conversion of higher to lower peruranates, the latter increasing in amount as the pH of the solutions is decreased.

The work described in this paper was carried out as part of the research programme of the Division of Industrial Chemistry, Council for Scientific and Industrial Research, Melbourne, Australia.

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COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH
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The Quantitative Electro-deposition of Tin from Chloride Solutions

Part II. Stannous Tin Solutions

BY A. J. LINDSEY

SYNOPSIS—The electro-deposition of tin from hydrochloric acid solutions has been described by several authors, but until Part I of this work was published, *Analyst*, 1940, **65**, 498, no explanation had been given of occasional failure to recover all the tin from solution. It was shown in Part I that losses previously noted by various authors were probably due to re-solution of the tin deposit during the processes of washing and disconnection.

In the present part, the electrolysis of stannous and mixtures of stannous and stannic tin solutions are studied. Strict adherence to the technique of Sand is shown to be essential and by observing the precautions described, satisfactory results are obtained. In the range 0·1 to 0·3 g. the recoveries show an average error of ±0·05 mg.

THE advantages of using hydrochloric acid solutions for the rapid electrolytic analysis of metals have been set forth in previous communications from this laboratory[1,2,3,4] and in Part I of the present researches.[5] Prior to 1940 no adequate explanation had been given of the difficulties which various analysts had found in recovering residual quantities of tin in the electrolysis of chloride solutions[1,6,7,8] and the present investigation is a completion of work that was interrupted by the war.

In Part I it was established that losses caused by volatilisation of stannic chloride, cathodic formation of stannane or mechanical loss of crystals of tin were unlikely to have accounted for the deficiences which had been noted. When using stannic solutions it was shown that during washing there is a considerable risk of losing a portion of a tin deposit by electrolytic action between the deposit and the platinum electrode. Strict adherence to the details of the method reduced the average error to 0·2 mg. with deposits weighing from 0·13 to 0·25 g.

The deposition of tin from stannic solutions causes very little reduction to the stannous state, but if other metals are deposited first, partial reduction to the stannous state occurs. It is therefore important to test the method for stannous tin solutions and for mixtures of both valency states in solution.

As in Part I of this work, depositions were made on copper-coated cathodes and on plain platinum cathodes. The solutions for analysis were prepared by dissolving "Chempur" tin in 10 to 12 ml. of hydrochloric acid with addition of 5 ml. of water. As depolariser, 2 g. of either hydrazine sulphate or hydroxylamine hydrochloride were then added and the solutions electrolysed at 40° to 45° C. and with the cathode at −0·6 to −0·75 volt with respect to the saturated calomel auxiliary electrode of Sand.[9] The apparatus and electrodes of Sand[9,10] were used in some of the experiments, and those of Fischer[11] in a commercially made electrolysis stand were used for others. The results are given in Table I.

Further experiments were carried out as above, but immediately after dissolving the tin, measured quantities of a standard solution of potassium chlorate were added and the

solution warmed to oxidise some of the tin to the stannic condition. The electrolyses were completed in the manner described above. The results are given in Table II.

SUMMARY—

Good analytical results are obtained in the quantitative deposition of tin from either stannous or stannic chloride solutions or from mixtures containing the two valency states when potential control is exercised. Ammonium salts present in considerable amount (up to 2 g.) cause errors due to partial re-solution of deposited tin, but these errors are reduced

TABLE I

DETERMINATION OF STANNOUS TIN

Tin taken, g.	Tin found, g.	Error, g.
0·1077	0·1076	− 0·0001
0·1115	0·1114	− 0·0001
0·1274	0·1276	+ 0·0002
0·1441	0·1444	+ 0·0003
0·1486	0·1489	+ 0·0003
0·1613	0·1618	+ 0·0005
0·1958	0·1954	− 0·0004
0·2212	0·2209	− 0·0003

to a minimum if the electrolyte is neutralised at the end of a determination, and if the platinum electrode is previously coated with copper. The considerable losses reported by earlier workers were almost certainly due to re-solution of the deposit during washing. Rapid washing and drying and strict adherence to the technique of Sand are recommended. The increased reliability which is now attainable in the deposition of tin from hydrochloric acid solutions improves the methods previously described for the electro-analysis of bearing

TABLE II

DETERMINATION OF STANNOUS AND STANNIC TIN

Tin taken, g.	Stannic, g.	Tin found, g.	Error, g.
0·0930	0·050	0·0933	+ 0·0003
0·1419	0·050	0·1413	− 0·0006
0·1491	0·025	0·1489	− 0·0002
0·2106	0·100	0·2102	− 0·0004
0·2114	0·100	0·2110	− 0·0004
0·3079	0·100	0·3080	+ 0·0001

metals, solders and bronzes. These two papers make it clear that equally accurate results can be obtained in the electro-deposition of tin from hydrochloric acid solutions by the method described in Part I, whether the tin is present in the stannic or stannous condition.

The author wishes to acknowledge with thanks the loan of apparatus by Imperial Chemical Industries Ltd. He also wishes to thank his former co-workers, Messrs. F. G. Kny-Jones and A. C. Penney, for their co-operation and for agreeing to his wish to proceed with work which was brought to a halt in the autumn of 1939.

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SIR JOHN CASS TECHNICAL INSTITUTE
JEWRY STREET
LONDON, E.C.3

May, 1949

Notes

A CONFIRMATORY TEST FOR THE PRESENCE OF DIPHENYLAMINE IN COMMERCIAL PETROL

FOR some time we have sought a satisfactory confirmatory test for the presence of diphenylamine in commercial petrol. The following chromatographic test has been found helpful in distinguishing diphenylamine from other compounds giving a positive reaction in the official test. The method is based on the circular filter-paper technique described by Rutter.[1]

PROCEDURE

Carry out the official test as described in the Ministry of Fuel and Power Memorandum RP 1/8/7/3 to the final stage at which the diphenylamine is extracted with light petroleum spirit. Drain off as much of the aqueous phase as possible and evaporate the petroleum spirit (avoiding drops of water) in a 50-ml. flask to about 5 ml. Divide the petroleum spirit extract that remains approximately equally between two small 7 × 1 cm. test tubes. To one tube add 0·1 ml. of 0·05 per cent. solution of diphenylamine in light petroleum. Evaporate the petroleum spirit in the tubes by placing them in a beaker of hot water until only 0·1 ml. of residue remains in each tube. Transfer the residues with the aid of micro-pipettes to the centres of two 11-cm. Whatman No. 2 filter-papers, controlling the rate of flow from the pipettes so that the central spots do not exceed 1·5 cm. in diameter. In each of the filter-papers, make two parallel cuts 3 mm. apart, starting from the outer edge and extending to the centre of the petroleum deposit, thus forming a "tail" or tongue, which is then folded downwards at right angles to the paper. Place each of the filter papers between two small sheets of glass, one of which has a hole 4 mm. in diameter drilled in the centre, through which the filter paper "tail," cut as described above, is placed. The glass sheets are then placed on top of a dish containing light petroleum, boiling range 40° to 60° C., into which the filter paper "tail" dips. Allow the chromatograph to develop until the circular solvent front has a diameter of about 8 cm. Remove the chromatograph from between the glass plates and cut out a suitable segment for testing. Dab the segment with a piece of cotton wool wetted with the official ammonium vanadate reagent in hydrochloric acid.

When diphenylamine is present a bright blue ring is developed near the edge of the "solvent front" and is usually adjacent to a red colour ring formed by traces of dye remaining in the extracting solvent. With pure diphenylamine, no other rings or discs are obtained near the original "central spot" on the filter-paper.

The chromatograph obtained with the added diphenylamine is the more important test. In this test only one blue band is obtained if the blue colour in the official test is due to diphenylamine. Other nuclear substituted compounds such as diphenyl benzidine and the amino compounds of diphenylamine yield blue rings of various shades of blue near the original central spot.

If suitable sheets of glass are not available, the test may be conducted without them provided precautions are taken to avoid evaporation of the petroleum spirit during development of the chromatograph.

The small central hole in the glass plate is easily made by the aid of a drill and emery powder.

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DR. B. DYER AND PARTNERS
LONDON, E.C.3

J. HUBERT HAMENCE
December, 1949

THE DETERMINATION OF PENICILLINASE ACTIVITY

THE action of the enzyme penicillinase on penicillin is well known.[1] It converts it to a compound, possibly penicilloic acid, which has no antibiotic activity and has one extra acidic group for each molecule of penicillin originally present. Penicillinase activity is measured by the rate of decrease in penicillin concentration or else by the rate of production of the acidic group. McQuarrie and Liebmann[2] used the former method, measuring the penicillin concentration by means of a biological assay. Henry and Housewright[3] followed the production of the acidic group from penicillin by measuring the volume of carbon dioxide evolved from a mixture of penicillin, penicillinase and bicarbonate buffer in a Warburg apparatus.

The chief objection to these methods is that they are rather tedious to use. A simpler method of estimating penicillinase has been developed, based on the observation by Murtaugh and Levy[4] that the total acid produced from the penicillin by the action of penicillinase can be titrated with

sodium hydroxide. The rate of production of the acidic group is measured by titrating with sodium hydroxide at a constant pH. It has been found that there is an optimum pH value for the maximum rate of reaction; the titration is therefore carried out electrometrically so as to maintain the pH continuously at this value. The penicillinase activity is then measured by the rate of addition of alkali at this pH and at a definite temperature.

Procedure—Use a preparation of penicillinase, diluted if necessary, such that the reaction takes at least 3 minutes. Adjust the pH of the penicillinase solution to 7·8 just before use. Place about 50 ml. of a penicillin solution containing 200 to 300 units per ml. in a beaker kept in a thermostat at 25° ± 0·2° C. Insert the electrodes of a pH meter into the contents of the beaker, above which is placed a burette to deliver 0·01 *N* sodium hydroxide solution. The contents of the beaker should be stirred; a stream of carbon dioxide-free air is convenient for this purpose.

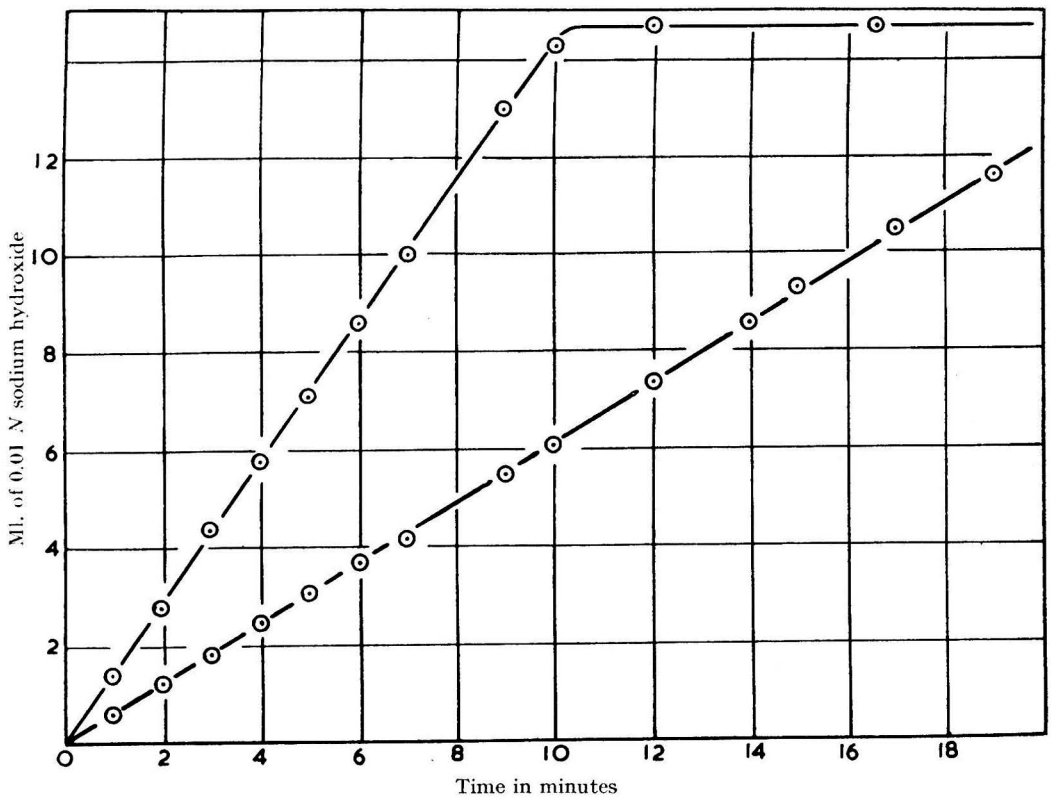

Fig. 1. Burette readings against time at two enzyme concentrations

Adjust the pH of the penicillin solution to 7·8 and then add a known volume of the penicillinase solution. In order to prevent a lowering of pH, add sodium hydroxide continuously and at such a rate that the pH is constant at 7·8. Note a number of burette readings together with the corresponding times. The slope of a graph of burette readings plotted against time, in ml. of 0·01 *N* sodium hydroxide per min., is a measure of penicillinase activity.

Since the rate of reaction is proportional to the total quantity of enzyme present in the solution, and independent of the penicillin concentration, it remains constant throughout, despite dilution by the titration. We have thus taken as a convenient unit of penicillinase activity the volume in ml. of 0·01 *N* sodium hydroxide per min. per 1 ml. of the original enzyme preparation. All values are taken at the optimum pH of the enzyme, in this case 7·8, and at a temperature of 25° C.

Results—Typical experimental results are given in Fig. 1, which shows clearly that the rate of reaction is constant with respect to time, *i.e.*, independent of the penicillin concentration. Thus the quantity of penicillin used in the estimation is not critical. The dependence of rate of reaction on enzyme concentration is shown in Fig. 2. It will be seen that the rate of reaction is proportional to the enzyme concentration over a wide range.

The rate of reaction was found to depend on the purity of the penicillin used, very impure samples giving lower rates. In general, it was found that commercial penicillins, of potency greater than about 1200 units per mg. were satisfactory, especially when comparative results were needed, for example, in studying the up-grading of a penicillinase-containing broth. The purity of the penicillinase used did not affect the determination unless it was so highly buffered that the change in pH on formation of the acid group from penicillin was too small to be measured easily. In these circumstances, the penicillinase preparation was dialysed before the activity was determined.

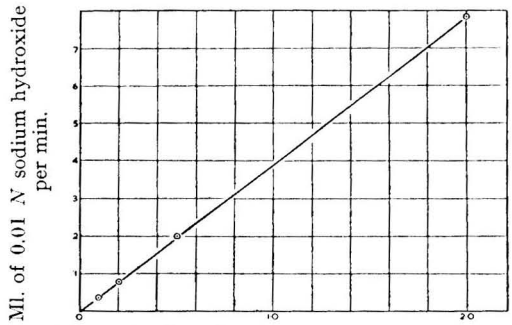

Fig. 2. Rate of reaction against enzyme concentration

In general, the determined kinetics of the reaction confirm the results of Henry and Housewright. The optimal pH, however, was found to be 7·8, which differs somewhat from the American value of 7·2 (which, however, was for a temperature of 36° C.). These investigators found that at high enzyme concentrations the rate of reaction at 36° C. was independent of the enzyme concentration, the limiting rate being 0·08 ml. of 0·01 *N* sodium hydroxide per min. Fig 2 shows that the rate of reaction varies directly with enzyme concentration up to a rate of at least 8 ml. per min. at 23° C. Unfortunately, it has not been possible to investigate this point further. The explanation may be that the enzymes have different characteristics, that the difference in temperature was large enough to affect the kinetics in this way, or that the Warburg apparatus gave anomalous results at fast rates of carbon dioxide evolution.

We are indebted to the Directors of the Distillers Company Limited for permission to publish this note.

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THE DISTILLERS COMPANY LIMITED
RESEARCH AND DEVELOPMENT DEPARTMENT
GREAT BURGH, EPSOM, SURREY

W. S. WISE
G. H. TWIGG

March, 1949

DETECTION OF PHENYL BARBITURATES

PESEZ[1] describes a method for the detection of phenyl barbiturates based on the nitration of the benzene nucleus and the subsequent identification of the nitro-compound by its colour reaction with sodium hydroxide in acetone solution. He uses about 50 mg. of the barbiturate for the test. The method described below is sensitive to 0·5 mg. The three phenyl barbiturates examined were—

Luminal: 5-ethyl-5-phenyl barbituric acid.
Rutonal: 5-methyl-5-phenyl barbituric acid.
Prominal: 1-methyl-5-phenyl-5-ethyl barbituric acid.

Procedure—Heat about 1·0 mg. of the barbiturate in a test tube with 200 mg. of potassium nitrate and 20 drops of concentrated sulphuric acid in boiling water for about 20 minutes. Cool and dilute with water to about 30 ml. Transfer to a separating funnel and extract, first with

30 ml. of chloroform, and then with 30 ml. of ethyl ether. Wash each extract separately with a little water. Evaporate the solvents, dissolve each residue in 2 ml. of acetone and transfer to two test tubes. Add 1 drop of a 50 per cent. solution of sodium hydroxide to each and shake vigorously. Allow to stand and observe the colour of the acetone layers.

The following colour changes were observed with 0·5 mg. of barbiturate—

	Chloroform extract	Ether extract
Luminal	purple	light purple
Rutonal	—	purple
Prominal..	blue changing slowly to a dark purple	

With luminal and rutonal the colour fades quickly.

Ethyl ether should be washed with sodium hydroxide solution before use, as some samples of ether give a pinkish colour with aqueous sodium hydroxide.

Reference

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Government Analyst's Laboratory
Colombo, Ceylon

E. Rathenasinkam
May, 1949

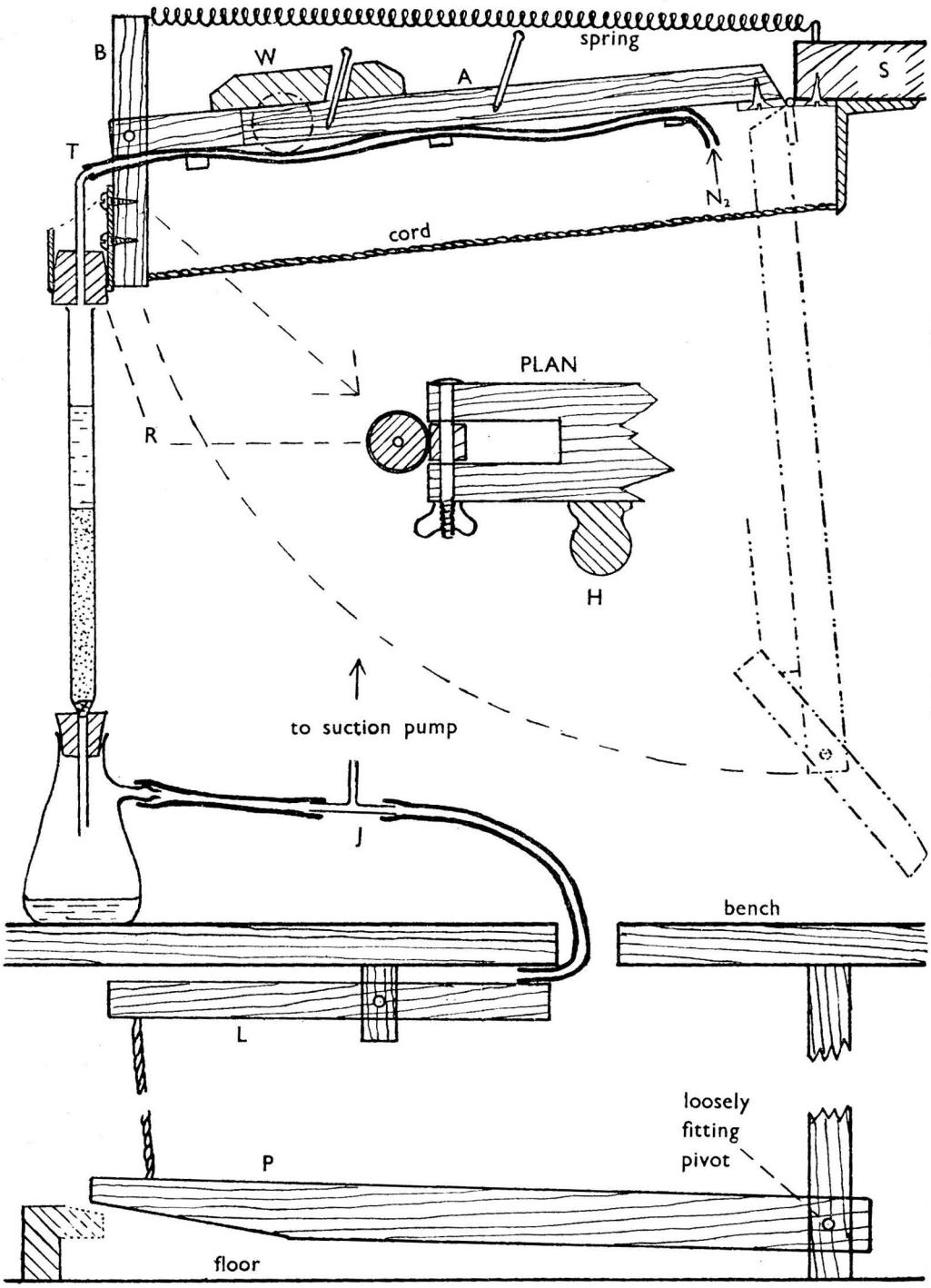
APPARATUS FOR PRESSURE AND SUCTION REGULATION IN CHROMATOGRAPHIC COLUMNS

In the course of chromatographic adsorption analysis trouble is often experienced with columns having a low percolation rate. An extreme example is that of a long column of very fine powder, which may take some hours for complete development. Filter aids may be mixed with dense powders, but the extent to which such dilution can be tolerated is limited. By applying suction to the bottom of the column percolation may be hastened, but the maximum effective pressure available for driving the liquid through the column cannot exceed 1 atmosphere and is usually much less. Pressure can be applied to the liquid on the top of the column, but most types of apparatus so far described for achieving this are complicated. The simplest method is to lead a gas under pressure through a bung in the top of the chromatographic tube. This method is illustrated by Williams,[1] by Gridgeman, Gibson and Savage[2] and by others. The pressure that can be applied by this means is limited by the tenacity with which the bung can be held in the tube. Williams, *loc. cit.*, describes an apparatus for overcoming this disadvantage, but each apparatus can be used only on tubes of one diameter and it takes several minutes every time to replenish the liquid. The chromatofuge of Hopf[3] is at its best when used on a large scale.

An apparatus for applying pressure has therefore been made and this can be brought into action in a few seconds, can be applied to ordinary adsorption tubes of different diameters and length, and is instantly disconnectable for replenishing the solvent. The apparatus is shown in use in the figure. The part of it above the laboratory bench is concerned with the application of gas pressure above the column. The part below the bench is concerned with the control of suction.

For the pressure apparatus, a board A (shown both in elevation and in plan in the figure), 50 cm. long by 2½ cm. thick by 8 cm. wide, is hinged at one end to a shelf, S, 65 cm. above the bench. A bar of wood, B, of 2½ cm. square section by 20 cm. long, is pivoted in a slot at the end of the board farthest from the hinge. This bar is constrained to remain vertical by a spring at its top end and a cord at its foot. The other end of the cord is tied to an inverted shelf bracket. The board, the bracket, the cord and the vertical bar form a rough parallelogram. When not in use the apparatus is allowed to swing down under the shelf; the bar is drawn inwards by the spring until it stops against the weight, W, and the cord slackens. The weight is a block of lead 15 cm. in diameter weighing 5 kg. The weight is held by a slanting nail and its force can be varied by moving it to a different nail. A rubber bung, R, 4 cm. in diameter, is pushed up into a brass tube, which is so cut away that it can be screwed to the vertical bar. Through the bung passes a tube, T, which can be connected to a source of compressed gas. A pressure reducing valve attached to a cylinder of nitrogen is convenient but not essential.

To bring the pressure apparatus into use it is swung up by grasping the handle H with one hand; the chromatographic tube is centred under the rubber bung and the nitrogen is turned on. The gas pressure that is applied may be regulated at the source. The maximum pressure on the liquid in the column is limited by the downward thrust of the apparatus. This is regulated by the mass and position of the lead weight. The spring also adds a little to the thrust. If the



Apparatus for applying pressure over, and suction below, a chromatographic column. The suction apparatus, which is shown in the idle position, is independent of the pressure apparatus, which is shown in use (firm lines) and out of use (broken lines). The inset is a plan of the bung-holder, etc.

gas pressure exceeds the thrust pressure some gas escapes between the rubber bung and the top of the chromatographic tube. In this way the junction between the bung and the tube acts as a safety valve. Moreover, the pressure can be released at once by simply lifting the apparatus.

The principal parts of the suction control apparatus below the bench have already been briefly mentioned (Booth[4]), but without a diagram. A T-join, J, is inserted in the vacuum pump line. The T opens to the air through a pressure rubber tube which passes under a lever, L, fitted beneath the bench. The lever is joined by a cord to a pedal, P, depression of which clamps the tube. Suction is thereby applied to the flask. Release of the pedal admits air and suction ceases at once. This arrangement permits immediate control of the suction and leaves both hands free.

The pivot securing the idle end of the pedal fits loosely. This enables the pedal to be drawn back and held under the bracket for prolonged suction. The pedal is released from the bracket by a kick.

Either device may be used independently of the other. The pedal-controlled suction apparatus is used first and the pressure apparatus is brought into use if the percolation rate with suction is still too slow.

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DUNN NUTRITIONAL LABORATORY
UNIVERSITY OF CAMBRIDGE AND MEDICAL RESEARCH COUNCIL

V. H. BOOTH

May, 1949

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1950, **75**, 57).

Public Analyst	*Appointments*
ALLEN, David George (Deputy) ..	Metropolitan Borough of Bermondsey.
ALLEN, David George (Deputy) ..	County Borough of Southampton.
CHALMERS, Frederick Grant Ducan (Deputy)	County Borough of Walsall.
CREGEEN, William Allan (Deputy) ..	County of Somerset.
DALLEY, Richard Arthur (Deputy) ..	County Borough of Leeds.
JAMES, George Vaughton	County Borough of Bournemouth.
JENKINS, Daniel Ceiriog	County of Warwickshire.
JENKINS, Daniel Ceiriog	County Borough of Walsall.
MINOR, Roland Gordon (Deputy) ..	Metropolitan Borough of Islington.
SMITH, Arthur (Deputy)	East Riding of Yorkshire.
WATSON, Robert Sinclair (Additional)	City of Glasgow.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 604).

Agricultural Analyst	*Appointments*
DALLEY, Richard Arthur (Deputy) ..	County Borough of Leeds.
JAMES, George Vaughton	County Borough of Bournemouth.
JONES, Daniel Evans	County Borough of Merthyr Tydfil.

Ministry of Food

STATUTORY INSTRUMENTS*

1949.—No. 1816. The Food Standards (Curry Powder) Order, 1949. Price 1d.

This Order, which comes into force (a) *as respects sales by the manufacturer of curry powder, on the 1st day of April,* 1950, (b) *as respects sales by wholesale, on the 1st day of July,* 1950, (c) *as respects sales by retail on the 1st day of October,* 1950, *is to be read with the Food Standards (General Provisions) Order (as amended). It prescribes the following standards for curry powder:*

(*a*) Curry powder shall contain not less than 85 per cent. spices, aromatic seeds and aromatic herbs.

(*b*) No curry powder shall contain lead in excess of 10 parts of lead per million parts of curry powder.

— No. 1817. The Food Standards (Tomato Ketchup) Order, 1949. Price 1d.

This Order, which comes into force (a) *as respects sales by the manufacturer of tomato ketchup, catsup, sauce or relish, on the 1st day of April,* 1950, (b) *as respects sales by wholesale on the 1st day of July,* 1950, (c) *as respects sales by retail on the 1st day of October,* 1950, *and which should be read with the Food Standards (General Provisions) Order,* 1944 *(as amended), prescribes a standard for tomato ketchup, catsup, sauce and relish and prescribes a maximum limit for traces of copper therein as follows—*

(*a*) Tomato ketchup, catsup, sauce and relish shall contain not less than six per cent. by weight of tomato solids derived from clean and wholesome tomatoes or from tomato puree, or its equivalent, made from clean and wholesome tomatoes.

(*b*) The tomatoes, tomato puree or its equivalent or the tomato ketchup, catsup, sauce or relish shall be so strained, with or without heating, as to exclude seeds or other coarse or hard substances.

(*c*) Tomato ketchup, catsup, sauce and relish shall contain no fruit or vegetables other than tomatoes except onions, garlic and spices added for flavouring purposes.

No tomato ketchup, catsup, sauce or relish shall contain copper in excess of 50 parts of copper per million parts of the dried total solids.

Proceedings in England and Northern Ireland for an infringement of Article 1 of the Food Standards (General Provisions) Order, 1944, *as amended by S.R. & O.,* 1944 *(Nos. 42 and 654), II, pp. 505 and 508, in respect of tomato ketchup, catsup, sauce or relish may be brought by a Food and Drugs Authority without the consent of the Minister.*

— No. 1893. The Food Standards (Preserves) (Amendment) Order, 1949. Price 1d.

This Order, which came into force October 16th, 1949, *amends the Food Standards (Preserves) Order,* 1944, *as amended by S.R. & O.,* 1944 *(No. 842). It prescribes standards for fruit curd and mincemeat that were previously controlled administratively under the Preserves Order,* 1947. *This is effected by substituting for paragraph 2 of Part I of the Schedule to the Order of* 1944 *(as amended) the following paragraphs—*

"2. The standard for fruit curd (including fruit flavour curd) shall be as follows, that is to say:—

(i) Each 100 parts of fruit curd shall contain not less than—

(*a*) 4 parts of fat,

(*b*) 0·33 part of citric acid,

(*c*) 0·125 part of oil of lemon (or 0·25 part of oil of orange),

(*d*) 1 part of dried whole egg
or 1½ parts of sugar dried whole egg
or 3¾ parts of liquid or frozen whole egg
or 4½ parts of shell egg;

(ii) The percentage of soluble solids contained in fruit curd shall be not less than 65 per cent.

2A. The standard for mincemeat shall be as follows, that is to say:—

(i) Each 100 parts of mincemeat shall contain—

(*a*) not more than 0·5 part of acetic acid (80 per cent. or glacial),

(*b*) not less than 30 parts of added sugar,

(*c*) not less than 30 parts of dried fruit and peel,

(*d*) not less than 2·5 parts of suet or equivalent fat;

(ii) The percentage of soluble solids contained in mincemeat shall be not less than 65 per cent."

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee DAC/2—Freezing-Point of Milk.

CK(DAC) 9298—Draft on the Method for the Determination of the Freezing-Point Depression of Milk (Hortvet Method).

Reviews

ISOTOPIC CARBON: TECHNIQUES IN ITS MEASUREMENT AND CHEMICAL MANIPULATION. By M. CALVIN, C. HEIDELBERGER, J. C. REID, B. M. TOLBERT and P. F. YANKWICH. Pp. xiii + 376. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1949. Price $5.50; 44s.

The significance of carbon isotopes for tracer studies in chemistry and biology cannot be overestimated. The usual definition of organic chemistry as the chemistry of carbon compounds indicates the unique importance of these isotopes.

Five carbon isotopes with atomic weights ranging from 10 to 14 are known. Ordinary stable carbon is a mixture of 98·9 per cent. of carbon-12 and 1·1 per cent. of carbon-13. The latter can be separated from carbon-12 by either chemical exchange or thermal diffusion and is available for experiments requiring a stable carbon isotope. The three other isotopes are radioactive. Carbon-10 and -11 emit energetic positrons and have half-lives of 8·8 seconds and 20·5 minutes respectively. Carbon-14 is produced in the nuclear pile: it emits slow electrons and has a half-life of about 5000 years. Before carbon-14 was discovered, cyclotron-produced carbon-11 was used in tracer experiments, but it has now been largely superseded by the heavier isotope. The decay of carbon-10 is too rapid for tracer work.

Practically all the early tracer work with carbon isotopes was carried out in the United States, but recently, thanks to the activities of the Atomic Energy Research Establishment at Harwell, supplies of carbon-14 have become available to British research institutes, and the subject of this excellent monograph by Professor Calvin and his colleagues has thus become of more than academic interest to British analysts. In this volume, both the methods available for the determination of carbon-11, -13 and -14 and the chemical manipulations needed for preparing carbon-labelled compounds, by direct synthesis or biosynthesis, are described in some detail.

The volume is essential to all chemists using carbon-14 and is also of considerable value to chemists working with other isotopes emitting electrons of low energy. Many of the experimental procedures were developed in the authors' laboratories at the University of California and are described in greater detail than in ordinary publications. Since carbon-14 is a very weak electron-emitter, particular care must be taken in preparing samples for radioactivity measurement and special detection devices must be employed. These techniques are equally suitable for other isotopes emitting electrons of low energy, such as sulphur-35. However, although synthese involving stable carbon-13 are presented along with those involving radioactive carbon-11 and -14, the measurement of carbon-13 is not described so fully as that of carbon-14, and anyone proposing to use the stable isotope would have to go elsewhere for detailed information. Some of the appendixes, such as those dealing with isotope dilution methods, statistical treatment of counting data and the determination of counter efficiency, are of particular interest to the analyst.

Carbon-14 is normally supplied as barium carbonate and consequently the greatest ingenuity has had to be exercised in using the methods of classical organic chemistry for introducing labelled carbon atoms into organic molecules ranging in complexity from acetone to testosterone. Labelled glucose and sucrose can be prepared by use of biosynthetic methods. All these operations must be carried out on a micro-scale. The authors believe that it will soon be possible to label almost any carbon compound. In this volume every isotopic carbon synthesis reported in the literature up to April, 1948, has been described in sufficient detail for the procedure to be used in the laboratory.

The style is generally clear and easy to follow. The book is well-printed and bound but, as is now inevitable with American books, the price is high. This is a serious matter for monographs that discuss rapidly developing subjects and may therefore be nearly out-of-date before they are printed.

J. E. PAGE

CHEMISCHE SPEKTRALANALYSE. EINE ANLEITUNG ZUR ERLERNUNG UND AUSFÜHRUNG VON SPEKTRALANALYSEN IM CHEMISCHEN LABORATORIUM. By W. SEITH and K. RUTHARDT. Fourth Edition. Pp. vii + 173. Berlin: Springer-Verlag. 1949. Price DM.16.50.

Seith and Ruthardt have produced a new edition of their earlier handbook of spectrochemical analysis, an essentially practical textbook for those working in laboratories of industrial and teaching organisations.

As a work on spectrochemical analysis in Germany the volume is comprehensive, but the authors have admittedly confined the scope almost exclusively to the apparatus and technique employed there. They have avoided all but passing reference to developments elsewhere, both in instrumentation and applications; the volume thereby loses almost entirely any wider appeal.

The book is liberally illustrated with diagrams and photographs of apparatus, some of which are inadequately described in the text. It seems pointless to devote valuable space to the description of apparatus that is obsolete in comparison with present-day standards. An early micro-photometer is described in some detail, followed by an account of an excellent up-to-date model.

An adequate theoretical treatment of the subject is given together with a wealth of practical detail covering all branches of emission analysis. Some details of absorption spectra in the visible and ultra-violet regions of the spectrum are also included.

This volume, though a useful handbook on the subject in its country of origin, could have been of much greater value in Britain had the authors not restricted their field to German products and methods.

J. A. C. MCCLELLAND

CHEMICAL ANALYSIS. Vol. V, AQUAMETRY. APPLICATION OF THE KARL FISCHER REAGENT TO QUANTITATIVE ANALYSES INVOLVING WATER. By J. MITCHELL jun. and DONALD M. SMITH. Pp. xi + 444. New York and London: Interscience Publishers. 1948. Price $8.00; 48s.

The authors apparently intend their newly coined word *aquametry* to signify the determination of water in any substance by any method. The word is no doubt etymologically correct, but is not particularly euphonious, and the reviewer, for one, is doubtful whether it will find wide acceptance. Having created a perfectly general term and used it as the title of their work, the authors proceed, after an introductory chapter on miscellaneous methods of water determination, to describe one particular method in minute detail. In other words, the sub-title more correctly describes the contents of the book than does the main title.

It is now widely known that the Fischer technique involves the use of a reagent containing iodine and sulphur dioxide in pyridine solution. It is only some fifteen years since the method was introduced, yet it has been the subject of widespread study, and its popularity and outstanding value are evident from the large number of literature references quoted in the book. The authors (members of the duPont organisation) are well equipped for their task, for they themselves have contributed much to the application of the Fischer method in various directions.

The book is in two main parts, the first dealing with the determination of water present as such in both organic and inorganic substances, and the second with an extension of the method to the determination of organic functional groups, in which the water is produced (or consumed) by a reaction between the sample and an appropriate reagent.

Part I describes, with wealth of detail, the stoicheiometry of the reaction, the nature of the parasitic side reactions, the specifications of the components of the Fischer reagent, and the preparation and standardisation of the reagent and of the standard water-in-methanol solution.

There are next given detailed instructions for carrying out the titration, by both visual and by electrical (potentiometric and "dead-stop end-point") methods, followed by instructions for determining moisture in a very wide variety of substances, the organic ones, of course, predominating.

It is clearly indicated that, in general, attention must be paid to (1) methods for getting the water in the sample into a form suitable for titrating, *e.g.*, by dissolving in, or extracting with, anhydrous methanol, (2) avoiding undesired reactions in which iodine is absorbed by reacting with the substance under analysis. In this connection many ingenious devices have been used, *e.g.*, the —CHO group may be rendered inert to the unmodified Fischer reagent by prior reaction with hydrocyanic acid to form the cyanohydrin. By such devices and, in some cases, modification of the composition of the Fischer reagent, water may be determined in a very large variety of organic substances, such as acids, alcohols, esters, acetals, acyl halides, amines, amides, carbonyl compounds, hydrazine derivatives, hydrocarbons, mercaptans and peroxides.

Part II of the book deals with the application of the Fischer method to the determination of functional groups in organic substances, *i.e.*, to such determinations as aliphatic alcohols (alone or in presence of phenols), amines, carboxylic acids, acid anhydrides, carbonyl compounds, amides, nitriles, peroxides. As always with a new technique, there are doubtless many cases where its application may not possess any advantage over older alternative methods; there must be many others, however, where it will supply information which would not otherwise be readily obtainable.

In most of the applications of the method the authors quote detailed results which have been obtained in establishing the method; these give a very impressive idea of both the accuracy and the extreme sensitivity of the method.

The book has clearly been compiled with much care, and it will undoubtedly be found of great value in organic research and analytical laboratories. N. STRAFFORD

LABORATORY AND WORKSHOP NOTES. Compiled and edited by RUTH LANG, Ph.D., A.Inst.P. Pp. xii + 272. London: Edward Arnold & Co. 1949. Price 21s.

During the past 25 years the Laboratory and Workshop Notes published in the *Journal of Scientific Instruments* have provided many beautiful examples of the simple and ingenious solution of experimental problems. Feeling that the contents of these Notes should be more widely known, the Board of the Institute of Physics invited Dr. Lang to make a selection from those published in the *Journal*. This book, the royalties from the sale of which are to be placed to the credit of the Institute's Benevolent Fund, is the outcome.

In an undertaking of this kind, omission of subject-matter rather than its inclusion is the difficulty. In choosing the 181 Notes for this book (less than half of those selected for publication in the *Journal*), Dr. Lang must have had many moments of sore trial. The highly-assorted nature of the subjects included renders classification no easy matter and the grouping is necessarily somewhat arbitrary. The arrangement adopted is the division of the Notes into eight sections: laboratory and workshop tools, processes and devices; clamps, supports and agitators; soldering, brazing and welding; technique of glass manipulation and silvering; vacuum and pressure techniques; electrical and magnetic devices and techniques; optical devices and techniques and devices for liquids and gases. A useful index supplements the full list of titles.

An enormous amount of information is packed between the covers of this book. There are numerous examples of the construction of special tools and of the solution of really difficult problems. The reviewer, who has never ceased to marvel at the never-ending uses of an ordinary T-piece, was particularly gratified to find, in addition, a host of items having simplicity as the keynote.

Apart from its principal function as a source of ideas and information, the whole work makes fascinating reading. The galaxy of novel ideas is neatly prevented from causing mental indigestion by the inclusion of several longer Notes on more general topics such as cements, soldering, glass-working, etc. In dealing with such subjects, the older well-tried techniques are naturally introduced along with much that is new, thereby affording the reader just the right degree of relief.

It appears from the foreword that a further volume of these Notes may be published. If the standard of the first volume can be maintained, this cannot take place too soon.

J. T. STOCK

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.) *To be reprinted.*

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *To be reprinted.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajaput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 1. Determination of Arsenic. *Out of print.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

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Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

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