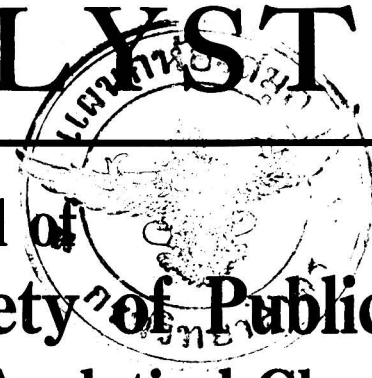
THE ANALYST



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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, May 5th, at The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following papers were presented and discussed: "The Determination of *p,p'*-DDT in Commercial Samples," by A. E. Martin, B.Sc., F.R.I.C., and R. L. Wain, M.Sc., Ph.D., F.R.I.C.; "The Composition of Concentrated Tomato Purée and the Estimation of the Tomato Content of Tomato Ketchup," by J. C. Morpeth, B.Sc., A.R.I.C.

NEW MEMBERS

Kenneth Armstrong Adey; Malcolm Colin Campbell, A.C.G.F.C., A.R.I.C.; Peter Lyndsey Chappell, M.Sc. (N.Z.); Roland Arthur Finch, B.Sc. (Lond.), A.R.I.C.; George Mason Hills, B.Sc., M.A. (Oxon.), F.R.I.C.; James Hallewell St. Johnston, M.A. (Cantab.), F.R.I.C.; Kenneth Dudley Luke; Harold Blythen Stevens, F.R.I.C.; Raymond Vaughan Swann, B.Sc., A.R.I.C.

PHYSICAL METHODS GROUP

The Fourteenth Ordinary Meeting of the Physical Methods Group was held at Mason's College, Birmingham, on Friday, April 2nd, 1948. It was preceded by a visit to the Physics Department of the University, introduced with a short talk on the construction and uses of the 60-inch cyclotron and the proton synchrotron by Professor P. B. Moon and Dr. F. E. Whitmore.

The meeting was presided over by Dr. J. G. A. Griffiths, the Chairman of the Group, and the following papers on The Determination of Radioactive and Stable Tracer Isotopes were read:—"Measurement of β-Activity," by A. G. Maddock, B.Sc., Ph.D.; "Measurement of Radio-active Isotopes," by F. E. Whitmore, B.Sc., Ph.D., A.R.I.C.; "The Mass Spectrometer," by E. R. S. Winter, A.R.C.S., Ph.D., D.I.C.; "Determination of Abundance Ratios of Non-radioactive Isotopes," by E. R. Roberts, A.R.C.S., Ph.D., D.I.C.; "Tracers in Biochemical Investigations," by W. J. Arrol, B.Sc., Ph.D.

The Fifteenth Ordinary Meeting of the Group, organised by the Polarographic Discussion Panel, was held at the University, Leeds. It was preceded by visits to the Brotherton Library and the Physical Chemistry Department of the University, and to the Central Research Department of Brotherton & Co., Ltd. Dr. W. Cule Davies, the Chairman of the Polarographic Discussion Panel, presided, and the following papers were read and discussed:—"Polarography in Germany," by G. W. C. Milner, B.Sc., A.R.I.C., A.Inst.P.; "The Polarographic Analysis of Light Alloys and Metals," by W. Stross, M.D., F.R.I.C.; "The Polarography of Anions," by W. Furness, B.Sc., F.R.I.C.

DEATH

WE regret to have to record the death of Walter George Leach.

Analytical Methods Committee

Metallic Impurities in Foodstuffs Sub-Committee*

REPORT No. 4

The Determination of Zinc in Foodstuffs

THE Analytical Methods Committee has received the following Report from the Metallic Impurities in Metals Sub-Committee and its publication has been duly authorised.

The members of the Sub-Committee are: A. D. Mitchell, D.Sc., F.R.I.C. (*Chairman*), C. G. Daubney, M.Sc., F.R.I.C. (successor to E. G. Kellett), C. L. Hinton, F.R.I.C., R. W. Sutton, B.Sc., F.R.I.C., N. D. Sylvester, M.Sc., A.R.I.C., P. F. Wyatt, B.Sc., and N. L. Allport, F.R.I.C. (*Hon. Secretary*).

INTRODUCTION

1. The Sub-Committee decided that it should endeavour to achieve an accuracy of ±10 per cent. on small quantities of the order of 50 parts per million when 5-g. samples were used. The results recorded later show that this order of accuracy was easily attained and usually exceeded.

2. A review of the existing methods led to the conclusion that the best to use as a basis was that suggested by Norman Strafford to the original Sub-Committee working in 1938, with modifications that had later been found desirable.

3. It was decided to retain purely chemical methods, since expensive physical apparatus might not be readily available to all chemists desiring to use the method.

4. The details given are designed to constitute a referee method: simplifications for less critical work will suggest themselves to the analyst.

5. The method ensures adequate separation of zinc in presence of relatively large quantities of other metals or radicals that might be encountered in foodstuffs. The quantities actually used in testing this separation of zinc were, in micrograms per 5-g. sample: Fe, 500; Ni, 50; Co, 25; Sn, 1000; Cu, 250; Pb, 100; Bi, 25; P_2O_5, 750; Ca, 500; Al, 750; Cd, 125. Later, Hg, 100; Mn, 50; As, 100 were added. None of these constituents, present separately or together, interfered with the clean separation and determination of zinc over a wide range (at least 50 to 500 μg.), and even larger amounts of contaminants can be tolerated, with the single exception of bismuth.

6. Bismuth, if present, must be separated. A tentative method is suggested in an Addendum whereby excessive amounts of bismuth and certain other metals, namely, silver, mercury, copper, most of the lead and part of the iron present, may be removed initially, thus avoiding any risk of incomplete separation in the subsequent extraction processes. (This procedure has not been investigated by the Sub-Committee as a whole.)

7. The method of decomposition was "wet" oxidation. Results presented by one member (see Table) suggest that dry ashing may be equally satisfactory.

METHOD

GENERAL REMARKS—

The solution obtained by wet oxidation is extracted by means of a solution of diethylammonium diethyldithiocarbamate, $(C_2H_5)_2N.CS.S.NH_2(C_2H_5)_2$, in chloroform at *p*H 7·5 to 8·0. The diethyldithiocarbamates of Pb, Cu, Ag, Cd, Hg, Ni, Co, Bi and Zn are extracted, whilst those of Al, Mn, Cr, Mg, alkaline-earth metals and alkali metals remain in the aqueous layer. ($Fe^{'''}$ is only partly extracted.) The extract is then treated with sodium hydroxide - citrate solution, to remove the zinc, and any diethylammonium diethyldithiocarbamate in this extract is destroyed by bromine water. Finally, the zinc is determined by extractive

* The Sub-Committee on the Determination of Arsenic, Lead and other Poisonous Metals in Food-colouring Materials has been re-constituted and renamed as above.

titration with a chloroform solution of diphenylthiocarbazone ("dithizone") at a controlled *p*H value.

The Sub-Committee carried out a series of tests with carbon tetrachloride instead of chloroform as a solvent for dithizone, but decided in favour of chloroform.

The apparatus used must be made of zinc-free resistance glass, such as Pyrex; for many types of glass, particularly soft glass, yield appreciable quantities of zinc to the alkaline solutions employed.

SPECIAL REAGENTS REQUIRED—

I. *Diphenylthiocarbazone (dithizone) stock solution*—A 0·1 per cent. solution in chloroform.

II. *Chloroform redistilled*, containing 1 per cent. of ethyl alcohol. (Chloroform recovered from diethyldithiocarbamate residues must not be used.)

III. *Sodium citrate*, 1 *M*—Dissolve 150 g. of trisodium citrate (A.R.) in water and dilute the solution to 500 ml., remove the zinc from this solution as follows. Transfer it to a large separating funnel, make it slightly ammoniacal and shake it thoroughly with additions of dithizone solution (I) until the last extract remains green and the aqueous layer becomes slightly yellow. Then add 2 ml. of 20 per cent. citric acid (A.R.) solution and extract the whole with successive portions of chloroform until the last extract is colourless.

IV. *Dithizone solution for titration*—Extract 40 ml. of dithizone stock solution (I) with two 50-ml. portions of dilute ammonia solution (50 ml. of water containing 2 ml. of 10 *N* ammonium hydroxide, VIII) and reject the chloroform layer. Acidify the combined ammonia extracts with dilute hydrochloric acid and extract with 100 ml. of redistilled chloroform (II). Wash the extract with two 10-ml. portions of water and filter it through a dry paper. Prepare this solution freshly each day.

It is convenient that 1 ml. of this solution should be equivalent to about 20 to 25 μg. of zinc, and a preliminary standardisation for this purpose may be found desirable.

V. *Diethylammonium diethyldithiocarbamate reagent*—(A) Stock solution. Dilute 1 ml. of redistilled carbon disulphide to 10 ml. with dry redistilled chloroform and add this mixture slowly, with stirring and cooling, to 3 ml. of redistilled diethylamine diluted to 10 ml. with dry redistilled chloroform. Preserve the solution in a glass-stoppered amber-coloured bottle. It should be freshly prepared about once a week.

(B) Extraction solution. Dilute 5 ml. of the stock solution to 100 ml. with chloroform (II) and keep the solution in a glass-stoppered bottle. This solution, referred to hereafter as "carbamate extraction reagent," should be prepared daily as required.

VI. *Standard zinc solution*—(A) Dissolve 1·00 g. of pure zinc in 5 ml. of hydrochloric acid (*d* 1·16) and 10 ml. of water, transfer the solution to a 1-litre measuring flask, dilute it to the mark with water at 20° C. and mix well.

$$1 \text{ ml.} \equiv 0{\cdot}001 \text{ g. } (1000\ \mu\text{g.}) \text{ of Zn.}$$

(B) Transfer 5 ml. of solution A to a 500-ml. measuring flask, add 5 ml. of hydrochloric acid (1 *N*), dilute to the mark at 20° C., and mix well. This solution should be prepared daily as required.

$$1 \text{ ml.} \equiv 0{\cdot}00001 \text{ g. } (10\ \mu\text{g.}) \text{ of Zn.}$$

VII. *Sodium metabisulphite solution*—Five per cent. in water.

VIII. *Ammonium hydroxide*, 10 *N*—Dilute 550 ml. of concentrated solution (A.R.) to 1 litre, or preferably (in order to secure complete freedom from zinc) bubble the gas from a cylinder of liquid ammonia first through a bubbler containing concentrated ammonia solution and then into distilled water until a solution of the required concentration is obtained.

IX. *Bromine water*, saturated.

X. *Buffer solution*—Dissolve 30 g. of borax (A.R.) in water and dilute the solution to 1 litre. Remove interfering metals by extraction with dithizone solution (I) and then remove the last traces of dithizone by repeated extraction with chloroform.

XI. *Alkaline citrate solution*—Mix together 50 ml. of sodium citrate solution (III), 100 ml. of 10 per cent. sodium hydroxide (A.R.) solution and 100 ml. of water.

XII. *Bromothymol-blue indicator*—The commercially available 0·04 per cent. solution.

PROCEDURE—

(1) *Preparation of sample solution*—Weigh out accurately about 5 g. of the well-mixed sample and destroy the organic matter by wet oxidation with sulphuric and nitric acids

(A.R. grades) in the usual manner. After oxidation is practically complete, make small additions (about 1 ml. at a time) of perchloric acid (60 per cent. A.R.) as well as nitric acid, with heating, until the solution is colourless, since it is most important to destroy the organic matter entirely. Heat to fuming for about 15 minutes to drive off most of the excess of perchloric acid, and then evaporate twice with small amounts (10 to 15 ml.) of water. Transfer the solution to a conical flask or beaker, using 40 ml. of water to effect the transfer. Cool the solution and unless it is quite clear filter it. Add 5·0 ml. of *M* sodium citrate solution (III) and 0·2 ml. of bromothymol-blue indicator and almost neutralise with 10 *N* ammonium hydroxide solution.

(2) *Preparation of test solution*—Continue the addition of the 10 *N* ammonium hydroxide solution until the indicator just changes to full blue (*p*H 7·5 to 8·0). (If a precipitate forms, dissolve it by addition of dilute hydrochloric acid; add more citrate solution and then ammonia as before.) Transfer the solution to a separating funnel and extract it with 15 ml. of carbamate extraction reagent (V, B), shaking it vigorously for 40 seconds. Run off the lower layer into a second separating funnel and extract the aqueous layer further with 5 ml. of the extraction reagent and then with 5 ml. of chloroform, shaking for 30 seconds each time; add the chloroform layers to the first extract and reject the aqueous layer.

Add to the combined chloroform extracts 15 ml. of alkaline citrate solution (XI) and shake the mixture for 30 to 40 seconds. Run the chloroform layer into another separating funnel and re-extract it with a further 10 ml. of solution XI. Allow the chloroform layer to separate completely, then carefully run it off and reject it. Combine the aqueous layers and shake the mixture with 5 ml. of chloroform for about 10 seconds. Run off and reject the chloroform layer. Remove the last traces of chloroform-soluble diethyldithiocarbamates from the tap of the funnel with 2 ml. of chloroform (without mixing). Run the aqueous solution into a beaker or conical flask, rinsing the funnel with a little water. Add hydrochloric acid (*d* 1·16) until the colour of the indicator changes from blue to yellow and then 2 ml. in excess. Add 10 ml. of bromine water and boil carefully until the solution is nearly colourless in order to destroy diethylammonium diethyldithiocarbamate. Add 0·5 ml. of sodium metabisulphite solution (VII) and cool to room temperature. Call this the test solution. The whole of the solution may be titrated or it may be made up to a definite volume (say 100 ml.) for titration of a suitable aliquot portion.

Carry out a blank determination on *all* the reagents used from the beginning of the test, including those used in the wet oxidation, and make the blank solution up to the same volume as the test solution.

(3) *Titration of the test solution*—Transfer the whole, or in case of doubt a suitable aliquot portion, of the test solution to a 200- to 250-ml. separating funnel, add 0·2 ml. of bromothymol-blue indicator, 2 ml. of *M* sodium citrate solution (III) and then 10 *N* ammonium hydroxide solution, drop by drop, until the solution just changes in colour through green to a full blue. Add 30 ml. of the buffer solution (X). The *p*H of the solution will now be 8·7 to 9·3. This solution is then titrated with dithizone.

The extractive titration consists in shaking the solution (at about *p*H 9) with controlled additions of dithizone reagent (say 1 ml.) together with about 3 ml. of chloroform—the lower layer containing the red zinc dithizonate being removed as the titration proceeds. As the end-point is approached the reagent is added in smaller portions and eventually a stage is reached when an addition of 0·1 ml. of dithizone solution and 2 ml. chloroform, and shaking, results in a purplish colour in the solvent layer (as distinct from the pure red of the zinc dithizonate previously obtained). This indicates the presence of a trace of free dithizone and is taken as the end-point of the titration. Confirmation of this end-point is obtained by removing the solvent layer and shaking the test solution with a further 0·1 ml. of dithizone and 2 ml. of chloroform, when the extract should remain green or blue.

A sharper end-point is obtained if towards the end of the titration the red extracts are more completely removed with the aid of 1 to 2 ml. of chloroform before the next addition of dithizone is made.

It is important to obtain equilibrium during the extractions and vigorous shaking for 20 seconds is recommended after each addition of dithizone reagent.

All extracts withdrawn during the titration should be pure red and in the titration of a solution containing much zinc the early extracts should be removed sufficiently often to obviate any difficulty in detecting the colour due to excess of dithizone in the presence of the strong red colour of the dithizonate.

To avoid tedious titrations of unknown amounts of zinc several devices can usefully be adopted. For example, the solution may be titrated without a very cautious approach to the end-point and when excess of dithizone is indicated an amount of standard zinc solution (1 ml. ≡ 10 μg.) more than sufficient to react with the excess of dithizone is added. After adequate shaking the presence of excess of zinc will be clearly indicated by the pure red colour of the chloroform layer and the titration with dithizone is completed. A correction is then applied for the volume of standard zinc solution used.

If two or more aliquot portions of the test solution are available, the first portion may be used to get an approximate result and in the titration of further portions it will be possible to add most of the dithizone in the first extraction and to complete the titration of the remaining zinc with smaller additions of dithizone.

If, however, it is not desirable to use aliquot portions, a part of the test solution may be titrated and the remainder may be held in reserve and added as necessary to the main solution in the separating funnel as indications of excess dithizone are obtained.

Let *a* ml. be the volume of dithizone solution required for the aliquot of the test solution titrated.

(4) *Titration of the blank solution*—Measure the same aliquot portion of the "blank" solution as was taken for the titration of the test solution. Add the same reagents indicated under (3) and titrate with dithizone solution. The amount of zinc in this solution will usually be between 10 and 30 μg.

Let *b* ml. be the volume of dithizone solution required for the aliquot of the blank solution titrated.

(5) *Standardisation of the dithizone solution*—For the standardisation of the dithizone solution it is necessary to titrate known amounts of zinc, and these need to be added to a solution entirely free from zinc. It is therefore convenient to make the standardisation by adding standard zinc solution to one of the solutions that has already been titrated (usually the blank) and that has been freed from excess of dithizone by shaking with chloroform.

A small but constant error (equivalent to an over-titration of about 0·15 ml. of dithizone solution) occurs in all titrations and is probably due to an equilibrium in the reaction between the zinc ions and the dithizone at the end-point of the titration. This error is eliminated in the determination itself because similar aliquots are used for the titration of the "blank" and the test solution, and the over-titration of the one is balanced by the same over-titration of the other solution. Special provision, however, needs to be made in the standardisation of the dithizone solution. This is done by titrating 50 and 250 μg. of zinc and recording the difference in volumes of dithizone solution as equivalent to 200 μg. of zinc.

After titration of the "blank" solution, draw off the bottom layer and shake the aqueous layer twice with 5-ml. quantities of chloroform. Reject the chloroform extracts.

Add 5 ml. of standard zinc solution (*i.e.*, 50 μg. of Zn) and titrate with dithizone solution. In standardising, the approximate amount of dithizone solution required will be known and it is permissible to add the greater part of it at once, together with the appropriate amount of chloroform, the titration being then completed by smaller additions of dithizone. Denote the volume of dithizone required for the 50 μg. of zinc by *x* ml.

Shake the titrated solution as before with two 5-ml. quantities of chloroform to remove all traces of excess reagent. Add 25 ml. of standard zinc solution (*i.e.*, 250 μg. of Zn) and titrate the solution again. Denote the volume of dithizone solution required for the 250 μg. of zinc by *y* ml.

If *F* is the number of micrograms of zinc equivalent to 1 ml. of dithizone solution, then $F = 200/(y - x)$.

(6) *Calculation of results*—The amount of zinc in the aliquot portion of the test solution taken for titration is

$$F\ (a - b)\ \mu g.\ Zn.$$

where *a* ml. = the volume of dithizone solution required for the aliquot of the test solution taken for titration

and *b* ml. = the volume of dithizone solution required for the same aliquot of the blank solution.

COLLABORATIVE RESULTS ON CERTAIN FOODSTUFFS

All values are in parts per million of zinc in 5-g. samples

Laboratory*	Syrup 1†	Syrup 2†	Sugared dried egg	Gelatin	Flour (proprietary brand)
1	90·0, 88·0	104, 103	33·0, 30·0	17·5, 17·5	32·5, 32·0
	88·0, 90·0‡	102, 109	31·0,‡ 33·0‡	18·5, 17·5	32·0, 32·5
	91·5‡	110		18·0,‡ 17·5‡	
2	95·5, 90·5	101, 103	31·0, 31·5	15·5, 16·0	31·5, 30·5
	93·0	104, 102	32·5	17·0	
		103			
3	98·0, 101·0	111, 109	34·0, 34·0	16·5, 16·5	30·0, 30·0
	101·5	112, 109		17·5	30·0, 31·0
		108, 108			31·0, 31·5
4	92·0, 93·0	106, 107	32·0, 33·0	17·0, 17·0	31·0, 31·5
	97·0, 97·0	106, 107			
		107, 104			
		105, 109			
		108			
5	98·0, 98·5	109, 108	33·0, 36·5	16·0, 17·0	31·0, 31·5
	98·0	109, 108	33·0, 34·5	15·5, 17·0	32·0
		109, 111	35·0		
		109			

* Most sets of results include determinations by two different workers.

† These are the only samples on which an independent check was available. The original syrup contained 0·4 p.p.m. of zinc as determined by an absorptiometric method, and to this were added 100·5 and 108·9 p.p.m. of zinc for syrups 1 and 2, respectively, so that these actually contained 100·9 and 109·3 p.p.m. respectively.

‡ Results with dry ashing.

Note on results—It will be seen that some laboratories tend to get low results; this is particularly noticeable with the large amounts in the syrups. The workers concerned have been unable to trace the cause of this discrepancy, but have satisfied themselves that incomplete extraction in either of the extractive processes would account for only a small proportion of the difference.

With this reservation, the *precision* of the results is regarded as adequate, and the independent checks afforded by the two syrups show that the *accuracy* is also satisfactory.

ADDENDUM

MODIFIED PROCEDURE IN PRESENCE OF CERTAIN HEAVY METALS, INCLUDING BISMUTH

After wet decomposition and evaporation with water (see "Procedure (1)"), dilute the acid residue with water to give an acidity of not less than 4·5 *N* (preferably 6 *N*) in sulphuric acid. If the acidity is less than this, a little zinc may be extracted; if greater, more lead will be left behind. A fairly safe rule is to add 6 ml. of water for each ml. of concentrated sulphuric acid remaining after the original wet digestion. Then extract the solution, in a separating funnel, with two 10-ml. portions of carbamate extraction reagent, shaking for about 30 seconds each time, and reject the extracts; finally, shake with 5 ml. of chloroform and reject the chloroform wash. The extracts are usually coloured olive-brown, owing to partial extraction of ferric iron with the heavy metals.

Transfer the extracted acid layer to a 100-ml. conical flask, rinsing out the funnel with 2 or 3 ml. of water. Boil for about 1 minute to remove residual chloroform, cool well and proceed as described at the end of "Procedure (1)," beginning "Add 5 ml. of *M* sodium citrate solution . . . ."

Poisons Sub-Committee

REPORT No. 4

The Assay of Yohimba

THE Analytical Methods Committee has received the following Report from the Poisons Sub-Committee and its publication has been duly authorised.

Members of the Sub-Committee—G. Roche Lynch, O.B.E., M.B., B.S., F.R.I.C. (*Chairman*), N. L. Allport, F.R.I.C., W. F. Elvidge, B.Sc., Ph.D., F.R.I.C., G. E. Foster, B.Sc., Ph.D., F.R.I.C., D. C. Garratt, B.Sc., Ph.D., F.R.I.C., C. H. Hampshire, M.B., B.S., B.Sc., F.R.I.C., E. F. Hersant, B.Pharm., Ph.D., W. H. Linnell, D.Sc., Ph.D., F.R.I.C., W. A. N. Markwell, F.R.I.C., W. Mitchell, B.Sc., Ph.D., F.R.I.C., J. R. Nicholls, D.Sc., F.R.I.C., R. A. Stockdale, A.R.I.C., N. Evers, B.Sc., Ph.D., F.R.I.C. (*Hon. Secretary*).

INTRODUCTION

Yohimba (or Yohimbe) bark, from *Pausinystalia yohimba*, contains a complex series of alkaloids, among which are yohimbine, *iso*yohimbine, *allo*yohimbine, yohimbene and others of uncertain constitution.

The drug is valued chiefly for its yohimbine content, and the methods of assay hitherto available depend upon the separation and estimation of this alkaloid alone,[1] but for the purposes of the Poisons Regulations, 1935, estimation of the total alkaloidal content is required.

In the absence of any standard method for the assay of the total alkaloids, the Sub-Committee has studied various procedures with a view to devising a method that would give reasonably consistent and reproducible results in the hands of different workers.

PROCEDURES TESTED

Extraction of the alkaloids by simple maceration and percolation of the powdered bark by various solvents in presence of alkalis was found to be unsatisfactory. The solvents tried were chloroform, ether, benzene, alcohol and various mixtures of these. The alkalis used included ammonia, calcium hydroxide, sodium hydroxide and magnesium oxide. With each one the extraction was found to be slow and the process consequently tedious, resulting in the collection of relatively large volumes of percolate before exhaustion of the drug was complete.

A process of continuous extraction in an apparatus of the Bolton and Revis type or that of the British Pharmacopoeia, 1932, was found to be satisfactory; finally, after various trials, the method described below was evolved.

When the extracted alkaloids were dried at 100° C., they darkened, with the result that the end-point in the subsequent titration was difficult to determine. Drying the residue is therefore not recommended.

RECOMMENDED METHOD

Weigh accurately 2 g. of Yohimba bark in No. 60 to 80 powder. Transfer to a glass mortar and mix with 1 g. of ignited sand (60 to 80 powder). Add 2 ml. of dilute solution of ammonia B.P. (10 per cent. NH_3). Triturate thoroughly for 10 minutes, cover and allow to stand for 2 hours.

Transfer the mixture quantitatively to an apparatus for continuous extraction, either that described in the B.P. or that designed by Bolton and Revis,[2] prepared with a tightly packed plug of cotton wool. Pack the drug in the percolator by means of a glass rod with circular flattened end. Wipe out the mortar with wisps of cotton wool moistened with chloroform and place these on the top of the column of drug. Extract for 1 hour with 100 ml. of vigorously boiling chloroform so that a head of liquid is maintained above the column of drug. Stop the extraction and tamp the drug down more tightly by means of the flat-ended glass rod. Continue the extraction for a further hour. Test for complete extraction by evaporating 2 ml. of the percolate to dryness, dissolving the residue in 5 drops of *N* sulphuric acid and adding Mayer's reagent. Continue extraction until a negative test for alkaloids is obtained. Then transfer the drug from the percolator to the glass mortar. Allow the chloroform held in the drug to evaporate in air, add 0·5 ml. of 10 per cent. ammonia solution,

triturate thoroughly and repack in the percolator as before and extract with 50 ml. of chloroform for 1 hour.

Transfer the combined chloroform extracts to a separator, rinsing the flask of the extraction apparatus with several small portions of chloroform, and extract the alkaloids by shaking with 30 ml. of *N* sulphuric acid. Allow to separate, run off the lower layer into a second separator and continue the extraction of the chloroform by shaking with successive 10-ml. portions of 0·1 *N* sulphuric acid until extraction of the alkaloids is complete, as shown by testing with Mayer's reagent.

Combine the acid extracts and wash by shaking with three successive 5-ml. portions of chloroform. Wash each of the chloroform solutions in succession with the same 10 ml. of 0·1 *N* sulphuric acid and reject the chloroform. Add the acid washing to the combined acid extracts. Make the acid solutions alkaline with ammonia and shake with successive 10-ml. portions of chloroform until the aqueous layer is free from alkaloids. Wash the chloroform extracts in succession with the same 20 ml. of water.

Transfer the combined chloroform extracts to a flask. Distil the chloroform and treat the residue with 2 ml. of neutral absolute alcohol or acetone. Heat on the water-bath until the solvent is evaporated. To the residue add 0·5 ml. of chloroform and 20 ml. 0·02 *N* sulphuric acid. Boil off the chloroform from below the acid by heating on the water-bath. Add 5 drops of methyl red indicator (0·1 per cent. solution in alcohol) and titrate with 0·02 N sodium hydroxide.

Each ml. of 0·02 *N* sulphuric acid is equivalent to 0·00709 g. of alkaloids calculated as yohimbine.

The following results were obtained:

Member	Total alkaloids, per cent.
1	2·50
2	2·41
	2·41
	2·40*
3	2·35
	2·44
	2·59*
	2·56*
4	2·51
	2·51
5	2·62
	2·51
	2·59
6	2·56
	2·59
7	2·44
	2·46
8	2·48
Mean	2·496
S.d. ±	0·078

* Using sodium carbonate instead of ammonia for the preliminary extraction of the drug.

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2. Bolton, E. R., "*Oils, Fats and Fatty Foods*," 2nd Ed., 1928, p. 10.

Poisons Sub-Committee

REPORT No. 5

The Assay of Jaborandi

THE Analytical Methods Committee has received the following Report from the Poisons Sub-Committee and its publication has been duly authorised.

Members of the Sub-Committee—G. Roche Lynch, O.B.E., M.B., B.S., F.R.I.C. (*Chairman*), N. L. Allport, F.R.I.C., W. F. Elvidge, B.Sc., Ph.D., F.R.I.C., G. E. Foster, B.Sc., Ph.D., F.R.I.C., D. C. Garratt, B.Sc., Ph.D., F.R.I.C., C. H. Hampshire, M.B., B.S., B.Sc., F.R.I.C., E. F. Hersant, B.Pharm., Ph.D., W. H. Linnell, D.Sc., Ph.D., F.R.I.C., W. A. N. Markwell, F.R.I.C., W. Mitchell, B.Sc., Ph.D., F.R.I.C., J. R. Nicholls, D.Sc., F.R.I.C., R. A. Stockdale, A.R.I.C., N. Evers, B.Sc., Ph.D., F.R.I.C. (*Hon. Secretary*).

INTRODUCTION

Jaborandi, as met with in commerce, consists of the dried leaflets of *Pilocarpus Jaborandi* or of *P. microphyllus* and contains a number of associated alkaloids, among which are pilocarpine, *iso*pilocarpine and pilocarpidine. For the purposes of the Poisons Regulations, 1935, a method for estimating the total alkaloidal content is required, but the Sub-Committee has deemed it expedient to describe a procedure whereby the physiologically important alkaloid, pilocarpine, might be isolated if required. The method for the determination of total alkaloids described below is essentially that prepared for inclusion in the forthcoming British Pharmaceutical Codex. The Sub-Committee is indebted to Mr. T. C. Denston, B.Pharm., Ph.C., F.R.I.C., Editor of *The British Pharmaceutical Codex*, for kindly communicating the details of the assay process and for granting permission for its embodiment in this Report.

RECOMMENDED METHOD

Determination of total alkaloids—To about 10 g., accurately weighed, in No. 40 powder and contained in a flask, add 50 ml. of chloroform, shake well and allow to stand for 10 minutes. Add 5 ml. of dilute solution of ammonia B.P. (10 per cent. NH_3) and shake continuously 1 hour; transfer the mixture quantitatively by means of further portions of chloroform to a percolator plugged with cotton wool and continue percolation, using a total of 160 ml.

Member	Total alkaloids as pilocarpine per cent.	Pilocarpine nitrate per cent.
A	Expt. 1 0·83	0·45
	" 2 0·87	0·47
	" 4 (*a*) 0·827 } 0·85	
	" 4 (*b*) 0·026 }	
B	0·84	0·41
C	0·81	0·41
D	0·80	—
	0·81	—
E	0·89 } worker a	0·52
	0·95 }	0·53
	0·87 } worker b	0·46
	0·92 }	0·54
F	0·93 worker c	0·65
	0·92 worker d	0·59
G	0·76	
	0·80	0·51
H	0·82	0·43

In experiment 4 (*a*) by member A, the fourth percolation gave a faint reaction for alkaloid; two further percolations were given and the additional alkaloid was determined separately, the result being 4 (*b*).

of chloroform or until complete extraction is effected as shown by evaporating 2 ml. to dryness, dissolving the residue in 5 drops of 0·1 *N* sulphuric acid and testing with Mayer's reagent. Mix the chloroform solutions and concentrate to a volume of about 40 ml. Transfer to a separator with a little chloroform, add 150 ml. of ether and shake well with 20 ml. of 0·5 *N* sulphuric acid; allow to separate and run off the aqueous lower layer, and continue the extraction with three 10-ml. quantities of 0·1 *N* sulphuric acid. Filter the combined acid extracts into another separator, make alkaline with dilute ammonia solution and shake with four successive 20-ml. portions of chloroform; wash each of the chloroform extracts in succession with the same 10-ml. portion of water. Combine the chloroform extracts, remove the solvent by distillation, add to the residue 2 ml. of dehydrated alcohol and evaporate to dryness. Dry the alkaloidal residue for 5 minutes on a boiling water-bath. Dissolve the residue in 1 or 2 ml. of neutral alcohol, add 15 ml. of 0·05 *N* sulphuric acid and titrate with 0·05 *N* sodium hydroxide, using methyl red solution as indicator; each ml. of 0·05 *N* sulphuric acid is equivalent to 0·0104 g. of alkaloids calculated as pilocarpine.

Since the Poisons Rules, 1935, refer to the alkaloids of jaborandi and not specifically to pilocarpine, the above procedure may be regarded as satisfactorily meeting the requirements under this heading. The results obtained by members of the Sub-Committee upon a circulated sample of powdered jaborandi leaf are shown in the following Table; they indicate the concordance that may reasonably be expected.

Isolation of pilocarpine as nitrate

Although not necessary for the purposes of the Poisons Rules, it is considered that the following procedure for separating pilocarpine nitrate from the total alkaloids of jaborandi may prove to be useful.

Transfer the titrated liquid obtained in the assay for total alkaloids to a separator, render it alkaline with dilute solution of ammonia, shake with four successive 20-ml. portions of chloroform and wash each of the chloroform extracts in succession with the same 10-ml. portion of water. Combine the chloroform extracts, remove the solvent by distillation, add to the residue 2 ml. of dehydrated alcohol and evaporate to dryness. Dry the residue for 5 minutes on a boiling water-bath. Dissolve the alkaloidal residue in about 10 ml. of a saturated solution of pilocarpine nitrate in acetone of analytical purity and add from a capillary tube a freshly made and well-cooled mixture of 9 volumes of acetone and 1 volume of concentrated nitric acid (sp.gr. 1·42) until the mixture is acid as indicated by spotting on a piece of moistened congo red paper. Stir vigorously and leave overnight. Collect the crystals formed in a tared sintered glass crucible of not less than No. 3 porosity. Wash with acetone previously saturated with pilocarpine nitrate, dry at 100° C. and weigh. The crystals should be nearly colourless and should not melt below 170° C. If crystals do not form, the whole assay should be repeated.

Poisons Sub-Committee

REPORT No. 6

The Assay of Ephedra and of Ephedrine in Nasal Sprays

The Analytical Methods Committee has received the following Report from the Poisons Sub-Committee and its publication has been duly authorised.

Members of the Sub-Committee—G. Roche Lynch, O.B.E., M.B., B.S., F.R.I.C. (*Chairman*), N. L. Allport, F.R.I.C., W. F. Elvidge, B.Sc., Ph.D., F.R.I.C., G. E. Foster, B.Sc., Ph.D., F.R.I.C., D. C. Garratt, B.Sc., Ph.D., F.R.I.C., C. H. Hampshire, M.B., B.S., B.Sc., F.R.I.C., E. F. Hersant, B.Pharm., Ph.D., W. H. Linnell, D.Sc., Ph.D., F.R.I.C., W. A. N. Markwell, F.R.I.C., W. Mitchell, B.Sc., Ph.D., F.R.I.C., J. R. Nicholls, D.Sc., F.R.I.C., R. A. Stockdale, A.R.I.C., N. Evers, B.Sc., Ph.D., F.R.I.C. (*Hon. Secretary*).

Introduction

Ephedra of commerce consists of the dried, young branches of perennial herbs of several varieties of *Ephedra* and contains a number of closely allied alkaloids, the most important being ephedrine.

The determination of total alkaloids in ephedra

The British Pharmaceutical Codex, 1934, describes a method for determining the total alkaloids, and the Sub-Committee regards this as satisfactory for the crude drug and, when suitably adapted, for determining, for the purposes of the Poisons Regulations, 1935, the total alkaloids of ephedra in preparations made from the crude drug.

Determination of ephedrine in sprays

The alkaloid ephedrine, which may be isolated from the crude drug or prepared synthetically, has a wide use in nasal sprays and the Sub-Committee has deemed it expedient to investigate its determination in preparations of that type. The recommended method may find application for determining ephedrine in other preparations, provided no other volatile base is present.

Recommended method

Place a suitable weight of the spray containing about 0·15 to 0·2 g. of ephedrine in a steam distillation flask of about 300 ml. capacity, fitted with an efficient splash-head. Add 10 g. of sodium chloride, 15 ml. of 20 per cent. solution of sodium hydroxide and a little pumice or broken glass. Connect the flask to a condenser and steam distil into a receiver containing 25 ml. of 0·05 *N* sulphuric acid. After distillation has begun adjust the volume of the aqueous phase in the flask to between 15 and 30 ml. by the application of heat from a small burner and maintain it at this volume. Some sodium chloride should remain undissolved. When about 700 ml. of distillate have been collected, titrate the excess of acid with 0·05 *N* sodium hydroxide, using methyl red as indicator. Collect a further 50 ml. of distillate in a fresh receiver containing a small quantity of water and 1 ml. of 0·05 *N* sulphuric acid and titrate with 0·05 *N* sodium hydroxide. If necessary, continue the distillation until no further alkaloid is removed. Carry out a blank determination, using the same quantities of reagents and collecting the same volume of distillate, and make the appropriate correction.

Each millilitre of 0·05 *N* sulphuric acid neutralised by the volatile base is equivalent to 0·00826 g. of anhydrous ephedrine.

Results

The following results were obtained by different members of the Sub-Committee on a circulated sample of spray—1·11, 1·06, 1·09, 1·05, 1·00, 1·10, 1·14, 1·12 per cent. w/w of ephedrine. Mean 1·084.

The spray was made up to contain 1·09 per cent. w/w of ephedrine.

Since this work was carried out, the Association of Official Agricultural Chemists have published a report on the determination of ephedrine[1]; this has been adopted as official[2] but the method is not specific for ephedrine, as it depends on direct extraction.

References

1. Welsh, L. H., *J. Assoc. Off. Agric. Chem.*, **1947, 30, 467.**
2. *Ibid.*, **1948, 31, 113.**

Modifications of the Standard Micro-Combustion Procedures found necessary in the Determination of Carbon, Hydrogen and Nitrogen in Aliphatic Nitro Compounds

By A. E. HERON

(*Read at a Meeting of the Microchemistry Group, on May 16th, 1947*)

In recent months several papers on the nitration of olefines have appeared in the journal of the Chemical Society, describing research work carried out by members of the Research Department of the Billingham Division of Imperial Chemical Industries Ltd.[1,2,3,4,5] At all stages the reaction products were submitted for analysis, and for the determinations of carbon, hydrogen and nitrogen micro-analytical methods were used. In general, the well-established methods first described by Pregl[6] have been used, but modifications were found necessary in certain instances and these are described in the following account.

1. Modifications found necessary for the determination of carbon and hydrogen

The first aliphatic compound submitted for analysis was appropriately nitromethane. For this a standard combustion train as described by Pregl[6] was used, the absorbents for the products of combustion being soda asbestos and phosphoric anhydride. The results obtained for both carbon and hydrogen were high (Table I). By combustion of a smaller sample some improvement was effected, but theoretical values could not be obtained although physical tests indicated that the sample was pure.

Lead peroxide is the most critical of the reagents used in the combustion tube. Lead peroxide - asbestos packing as described by Pregl[6] was used for these analyses, the temperature of this section of the combustion tube being maintained by means of a hollow heating mortar containing boiling cymene. In order to confirm that the high results were not due to exhaustion of the lead peroxide, a new combustion tube was prepared and "run in." Results were still high.

The length of the standard heating mortar supplied with our apparatus was 6 cm. and the length of the lead peroxide layer this could accommodate was about 2·5 cm., the remaining length of tube in the mortar being filled by asbestos plugs and silver wool packing. To enable us to use a longer layer of lead peroxide, a solid copper mortar 10 cm. long was made. This was heated by means of a small gas jet and the temperature was controlled by a vapour pressure thermostat capable of maintaining a given temperature to $\pm 1°$ C. When this mortar was used a layer of lead peroxide 6·5 cm. long, almost three times as long as before, could be packed into the combustion tube. A combustion tube with the increased length of lead peroxide - asbestos packing was "run in" and the sample of nitromethane was analysed. The results obtained corroborated the evidence of the physical tests that the sample was pure nitromethane.

The 10-cm. heating mortar was accordingly adopted as standard for our micro-combustion apparatus at Billingham and hundreds of analyses were carried out on nitro compounds, with perfectly satisfactory results. During this time we discontinued the use of lead peroxide - asbestos reagent and used instead the granular type of packing described by Pregl. The life of the former reagent was relatively short when samples containing much nitrogen were analysed, and frequent repacking of the tube was necessary.

Shortage of suitable glass in the war years made it necessary to use silica tubes. It was not long before we discovered that the universal packing described by Pregl was unsatisfactory if the combustion tube was of silica. At the normal temperature of the long furnace, 760° C., there was marked attack of the silica tube wherever it was in contact with the lead chromate and in a few instances the tube became porous after short use. Lead chromate is used to react with the sulphur dioxide and trioxide produced during the combustion of samples that contain sulphur. Metallic silver, heated to a suitable temperature, may be used for the quantitative retention of sulphur oxides,[7] and it was decided to use silver instead

TABLE I

NITROMETHANE

Carbon 19·67 per cent.; Hydrogen 4·92 per cent.; calculated

Description of train	Weight of sample, mg.	C %	H %
Standard Pregl train: 6-*cm. heating mortar*	8·553	23·6	6·7
	7·787	20·5	6·9
	1·812	20·9	6·6
	1·578	21·7	6·6
Standard Pregl train: 6-*cm. heating mortar*— newly packed	4·968	21·3	6·1
	2·505	20·4	5·6
	3·572	20·3	5·6
Train with 10-*cm. heating mortar*	3·742	19·8	5·0
	5·814	19·7	5·1
	4·169	19·8	5·0
	5·535	19·8	5·1

of lead chromate with silica combustion tubes. The length of silver packing used was about 5 cm., and about 2 cm. of this projected into the copper mortar. The main filling of the tube was of copper oxide only. The life of the silica tubing in these circumstances was much improved and the efficiency of the train was not impaired by the substitution of silver for lead chromate.

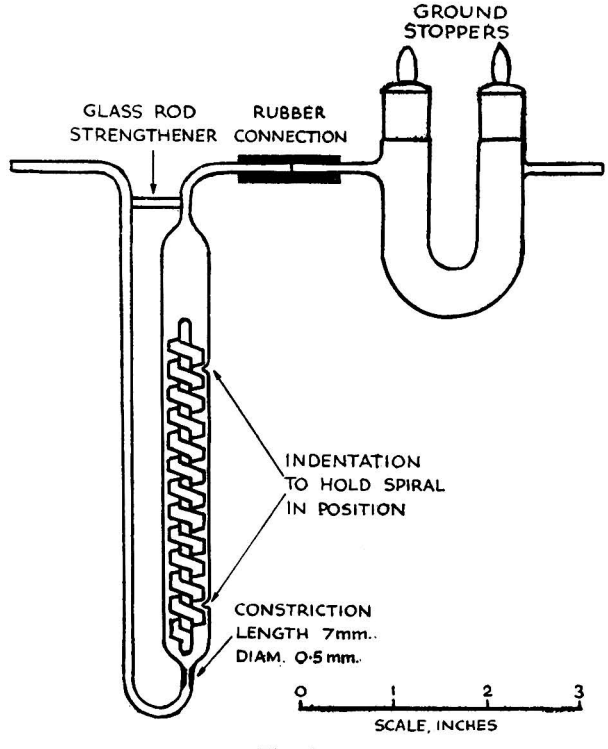

Fig. 1.

The reagent used for absorbing the carbon dioxide produced during combustion was soda asbestos and that for water phosphoric anhydride. The latter reagent was prepared for filling into the absorption tube by being mixed in a stoppered weighing bottle with one-third of its weight of powdered glass, of 20 to 30 mesh; this mixture could be filled readily into the absorption tubes which, in our experience, never choked, for as phosphoric acid was formed by the absorption of water it collected around the powdered glass, leaving a clear

passage for the gas stream. We now use anhydrone (anhydrous magnesium perchlorate) for the absorption of water; for we have found that where removal of oxides of nitrogen from the products of combustion is incomplete, phosphoric acid retains these in part, with consequent error in the hydrogen determination. Anhydrone, which does not become wet in the absorption tube, is free from this disadvantage.

When we first analysed nitro-ethyl nitrate, a liquid of b.p. 74° C. at 1 mm. of mercury, results for carbon and hydrogen were much higher than those expected. Several determinations were made, with different trains, but all the results for carbon and hydrogen were higher than was expected, although the result for nitrogen agreed with the calculated value. After further purification, the sample was, so far as could be ascertained by physical examination and quantitative preparation of derivatives, pure nitro-ethyl nitrate, but it still gave high results for carbon and hydrogen (Table II). The most likely source of error was non-absorption of oxides of nitrogen by the lead peroxide packing of the combustion tube. Two new tubes were packed, one with lead peroxide - asbestos and the other with granular lead peroxide, and after being "run in" were used for analysis of this sample. All the results obtained were high. Examination of the absorption tubes showed that they both contained nitrate, (phosphoric anhydride was being used as desiccant). For some reason, which has not yet been discovered, lead peroxide in the quantities used in our modified train was incapable of completely removing the oxides of nitrogen produced by the combustion of nitro-ethyl nitrate. Decreasing the sample weight and the air rate, and mixing the sample with copper oxide in the weighed capillary tube, gave results no better. Elving and McElroy[8] had recommended the use of liquid absorbents for nitrogen tetroxide and it was decided to investigate their use in this case. After a few preliminary trials a spiral bubbler was designed that would hold about 2 ml. of reagent and allow a reaction time between liquid and gas of about 7 seconds (Fig. 1). Of the reagents suggested by Elving and McElroy we tried (*a*) ceric sulphate and sulphuric acid, (*b*) potassium permanganate and sulphuric acid, (*c*) chromic and sulphuric acids. Ceric sulphate was so slightly soluble in concentrated sulphuric acid that the solution was quickly exhausted and it was necessary to re-charge the absorber for every test. Potassium permanganate in concentrated sulphuric acid was also quickly exhausted, although not so rapidly as the ceric sulphuric acid reagent. Chromic sulphuric

TABLE II

COMBUSTION OF NITRO-ETHYL NITRATE

Carbon 17·65 per cent.; Hydrogen 2·94 per cent.; calculated

	Technique used	Weight of sample, mg.	C %	H %
Sample A.	Silica combustion tube, standard packing	4·753	19·0	3·7
		3·943	22·0	5·6
		3·214	20·8	4·3
		1·771	18·3	3·0
		1·432	20·2	4·4
	As above, but with powdered CuO in capillary tube	2·668	22·2	5·2
		5·141	19·6	4·9
Sample B.	Silica combustion tube, standard packing	5·878	20·6	5·0
		6·177	20·2	4·7
		3·049	20·0	4·0
		2·072	19·6	3·7
		2·380	18·3	2·9
		1·866	22·7	5·5
		1·919	18·1	3·1
		2·688	22·2	5·2
	As above, but with powdered CuO in capillary tube	2·283	18·9	3·9
		5·478	20·0	4·1
		4·297	17·2	3·4
		2·293	20·4	3·7
		2·051	18·4	3·2
Sample B.	With spiral bubbler—Ceric sulphate	6·631	17·7	2·9
		8·434	17·9	2·9
	With spiral bubbler—Chromic-sulphuric acid	6·740	17·8	2·8
		9·540	17·7	3·0
		11·094	17·9	3·1
		7·423	17·9	3·0

acid was much less rapidly exhausted and it was decided to use this reagent. A suitable concentration was obtained by mixing together 20 ml. of 5 *N* chromic acid and 80 ml. of 1·84 sp.gr. sulphuric acid.

The liquid absorption unit consisted of the spiral bubbler containing 2 ml. of chromic-sulphuric acid, connected to a small U-tube containing anhydrone. It was used as a supplement to the usual lead peroxide packing of the tube for the purpose of removing oxides of nitrogen from the products of combustion. The order of the various parts of the absorption train from the outlet of the combustion tube was as follows: (*a*) weighed water absorption tube packed with anhydrone, (*b*) spiral bubbler containing chromic-sulphuric acid, (*c*) the small U-tube containing anhydrone, (*d*) the weighed carbon dioxide absorption tube packed with soda asbestos backed by anhydrone.

Trials on *m*-dinitrobenzene and 2 : 4-dinitrophenylhydrazine indicated that theoretical values for carbon and hydrogen could be obtained when the liquid absorption unit was used; so any absorption of carbon dioxide by the liquid reagent was negligible. With this modification, analysis of the sample of nitro-ethyl nitrate gave results indicating that the sample was pure (Table II). With nitro-propyl nitrate correct results were obtained only when the supplementary absorption unit was used. We now use this modification whenever little-known nitro compounds are submitted for analysis, until comparative tests indicate that it is unnecessary.

The combustion of samples was carried out in air, as it was found that with oxygen many of the compounds burned with violence. Polynitro-ethylene, for example, even in air, burned instantly as soon as the temperature reached a certain critical limit. The burning could, to a small extent, be controlled by covering the sample with finely divided copper oxide. Sample weights were usually of the order of 3 to 6 mg., although, with the bubbler technique, samples of 8 to 10 mg. could be burned satisfactorily with an air rate of 4 to 6 ml. per minute.

2. Modifications employed in the determination of nitrogen

For the determination of nitrogen the gas volumetric method of Dumas was used. In this method the products of combustion obtained by heating the sample with copper oxide in an atmosphere of carbon dioxide are swept forward by a stream of this gas over red-hot copper oxide and reduced copper. The rate of flow of the carbon dioxide is critical and is controlled, in the method described by Pregl, by a long-handled tap in the barrel of which grooves are filed to facilitate fine adjustment. In practice, this tap must be thoroughly greased and there is a tendency for the grooves in it to become filled with lubricant, which then makes it difficult to adjust the rate of flow of the carbon dioxide. In consequence, it becomes necessary to attend to the apparatus during the whole of the determination in order to ensure that no significant change occurs in the rate of flow. We have discarded this method entirely and use a technique in which the rate of flow of carbon dioxide is controlled by a hydrostatic head, which can be varied at will to suit the conditions of test. This has been achieved by using "Drikold" as the source of carbon dioxide in a specially designed generator unit. The system necessitates a modification of the packing of the combustion tube, but the customary reagents copper oxide and reduced copper are used. The generator and packing of the tube will first be described and then the procedure for determining nitrogen in a sample.

The generator—

This consists of four narrow-necked bottles of about 500-ml. capacity, preferably of the tall narrow type, connected to each other as shown in Fig. 2.

A is the generator, which in use is packed to the top with small pieces of "Drikold" (solid carbon dioxide). The stopper is of good quality rubber. The bottle stands in a metal container and is packed round with insulating material.

B is a lute containing a depth of mercury, $\frac{1}{2}$ to $\frac{3}{4}$ inch, sufficient to balance the head of the mercury trap of the azotometer and provide a working pressure.

C is a lute containing mercury to a depth of 3 to 4 inches. When this lute is in operation a rapid stream of carbon dioxide is available for the preliminary sweeping out of the combustion tube, an operation that can be completed in 2 minutes.

D is a lute containing water.

E is the delivery tap of the generator.

F, the tap between C and D, is connected to the dip tube of D by a length of rubber tubing and allows either of the lutes to be used as required. The purpose of lute D is to

facilitate adjustment of the flow of carbon dioxide through the train to a rate of 40 bubbles per minute for the final sweeping out, the azotometer tap being open to the fullest extent. This is a departure from traditional procedure, in which the flow rate is controlled by a grooved azotometer tap.

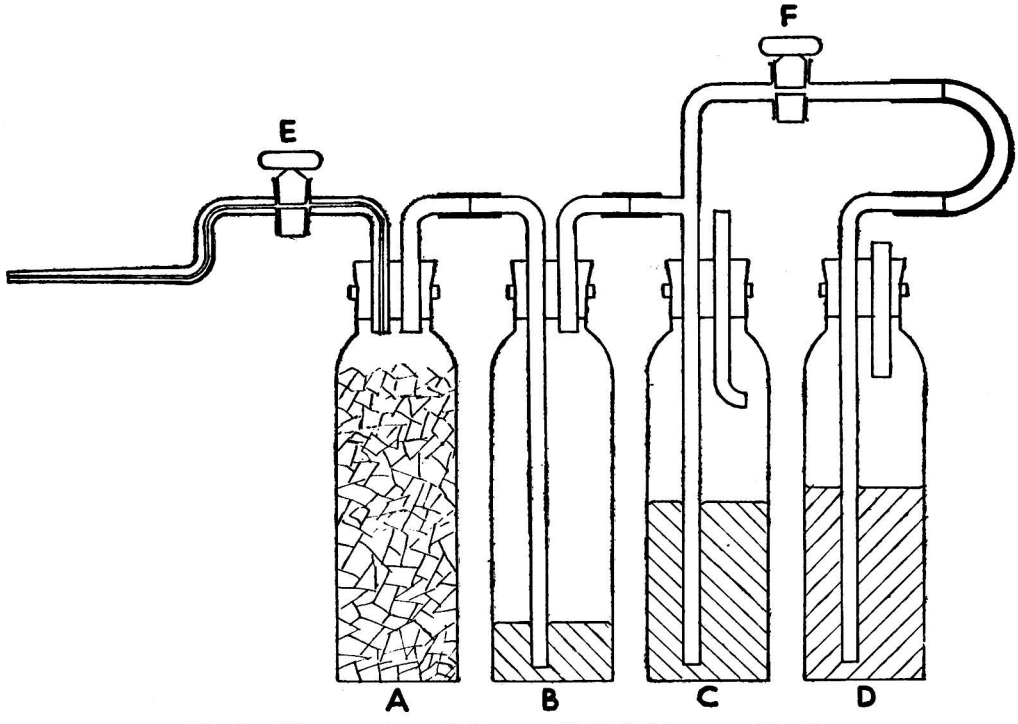

Fig. 2. CO_2 generator unit for use with Drikold; as used for the Dumas nitrogen determination.

COMBUSTION TUBE—

When the combustion tube is being packed for the first time a choking plug is inserted between the copper oxide at the delivery end of the combustion tube and the reduced copper, and is part of the permanent packing of the tube. This choking plug is of such porosity that,

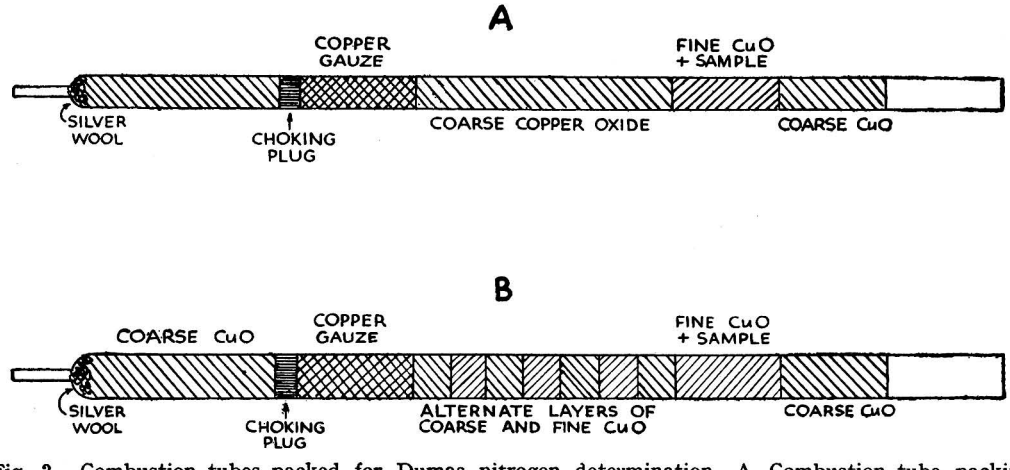

Fig. 3. Combustion tubes packed for Dumas nitrogen determination A. Combustion tube packing for normal micro-samples. B. Combustion tube packing for volatile samples low in nitrogen.

when the generator is charged and connected to the combustion tube and azotometer, lute B being in operation (*i.e.*, with tap F open and no water in D), the rate of flow of carbon dioxide to the azotometer is 40 to 45 bubbles per minute. When this has been achieved a closely fitting roll of reduced copper gauze, 5·5 cm. long, is introduced into the tube and then sufficient coarse and fine copper oxide is added to form the temporary packing.

PROCEDURE—

Charge the generator to the neck with Drikold. Allow to stand for 2 or 3 minutes and insert the bung carrying the delivery tap. After weighing out the sample and mixing it with the fine copper oxide, replace the temporary packing of the tube. Connect the tube to the generator and open tap E. Close tap F and allow carbon dioxide to sweep out the tube for 2 minutes. Connect the azotometer to the tube, open the azotometer tap to its fullest extent and allow carbon dioxide to flow until micro bubbles are obtained. Open tap F of the generator system and adjust the hydrostatic head in D, either by raising or lowering the dip tube or by altering the level of the water, until a rate of flow of 40 bubbles per minute is obtained. Then close delivery tap E of the generator. After the sample has been burned off open the delivery tap E to sweep out the combustion tube. Any slight adjustment in the rate of flow of carbon dioxide can readily be made if necessary.

With this system, one operator can if necessary supervise the operation of three Dumas combustion trains, a matter of great importance when many nitrogen determinations have to be made.

SPECIAL TUBE PACKING FOR VOLATILE SAMPLES LOW IN NITROGEN

Certain samples, which were submitted during the war years, were very volatile and contained small amounts of nitrogen. Results obtained by the usual micro procedure were limit tests rather than precise determinations, so it was decided to increase the weight of sample taken in order to obtain a volume of nitrogen that could be measured with greater precision. It was found necessary to use a wider combustion tube, with internal diameter 12 mm., in order to offer a larger surface of copper oxide to the products of combustion. Results obtained were somewhat erratic until it was decided to use a much larger proportion of fine copper oxide to coarse copper oxide than is usual in the Dumas method. This was packed as alternate bands, 1·5 to 2 cm. long, of coarse and fine copper oxide extending from the reduced copper to the layer of fine copper oxide which contained the capillary and sample. With this modification results could be readily duplicated if care was used in the initial stages of the combustion.

SUMMARY

Modifications of the standard procedures for the determination of carbon, hydrogen and nitrogen are described. It has been found necessary to increase the amount of lead peroxide packing of the combustion tube used in carbon and hydrogen determinations, and in certain circumstances to supplement this reagent by means of a liquid absorbent for complete retention of oxides of nitrogen produced during the combustion of nitro compounds. A generator unit in which "Drikold" is used as a source carbon dioxide is described. By using this generator unit in the Dumas nitrogen determination the adjustment of the flow of carbon dioxide through the apparatus is simplified. A band packing formed by alternate layers of fine and coarse copper oxide is recommended for volatile compounds of low nitrogen content.

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Some Observations on the Semimicro-Determination of Carbon and Hydrogen by the Sucharda and Bobranski Method, using a Macro-Balance

By FRANK GOULDEN

(*Read at the Meeting of the Society on November 5th*, 1947)

The determination of carbon and hydrogen on a semimicro scale, using a macro-balance, has been carried out in this Institute for some time. It has been found in practice that the method given by Sucharda and Bobranski,[1] whilst constituting an excellent foundation, can be improved in speed and accuracy by embodying the modifications outlined below.

Carefully calibrated weights and a reliable balance must be employed, a point not adequately stressed in most books (see, however, Belcher and Godbert[2]).

(*a*) The copper oxide content of the combustion tube was increased by replacing 40 mm. of the pumice mixture by copper oxide wire in order to ensure complete combustion of substances containing 10 per cent. or more of hydrogen.

(*b*) The silver spiral was moved from the vicinity of the boat in order to prevent silver halides from obscuring that portion of the tube. Silver or copper gauze can be rapidly cleaned by passage through the semi-luminous bunsen flame.

(*c*) Oxygen flow was increased to 7 ml. per minute by loosening the choking plug. This reduces back pressure when volatile substances are being burned and shortens the duration of the combustion.

(*d*) All asbestos used in packing the combustion tube was platinised. A small wad of this material completes the filling in place of the silver spiral mentioned under (*b*).

(*e*) The insertion of a piece of 28-gauge silver wire in the beak at the exit end of the combustion tube in conjunction with the hot copper arm ensures that water is completely driven over into the absorption train.

(*f*) The use of an automatic regulator for oxygen flow was abandoned and a positive pressure of oxygen maintained during the combustion by reference to the manometer. This enables electrical heating to be adopted if desired.

(*g*) The frequency of refilling of the oxygen drying tube was minimised by employing a wash bottle of concentrated sulphuric acid followed by a 2-cm. U-tube, one limb of which was filled with 10 to 14 mesh soda asbestos and the other with anhydrone. The *special* U-tube containing small amounts of both absorbents, immediately behind the combustion tube, can then be replaced by a simple U-tube filled with anhydrone only, for the oxygen is purified before entering the manometer.

(*h*) Before each series of analyses 100 ml. of oxygen are passed through the heated combustion furnace and the absorption tubes.

Compounds containing up to 30 per cent. of nitrogen have given satisfactory analytical results and the lead dioxide filling showed no deterioration after 70 consecutive combustions of nitrogenous substances. The results of a few typical analyses are appended in Table I.

TABLE I

Formula of substance	Theoretical C %	Theoretical H %	Found C %	Found H %
$C_{20}H_{22}O_2$	81·60	7·50	81·50	7·40
$C_{34}H_{50}O_3$	80·58	9·95	80·50	10·08
$C_{18}H_{19}O_2N$	76·90	6·80	77·05	6·90
$C_{12}H_9O_4N_3$	55·60	3·50	55·65	3·30
$C_{24}H_{25}O_2N_5$	69·40	6·07	69·65	6·04
$C_{20}H_{21}ONS$	74·29	6·60	74·13	6·70
$C_{14}H_{10}ONBr$	55·26	3·30	55·30	3·39
$C_{14}H_{12}NCl$	73·20	5·27	73·10	5·19

SUMMARY

If a macro-balance is used for semimicro determinations of carbon and hydrogen accurate calibration of weights is essential.

The time required for analysis may be shortened by increasing the copper oxide content of the combustion tube and increasing the rate of flow of the oxygen.

I am indebted to Professor G. A. R. Kon, F.R.S., for his suggestion of the use of silver wire in the beak of the combustion tube. It is a pleasure to thank him and Dr. W. J. Ross for their general advice and encouragement. Dr. W. C. J. Ross originally drew my attention to the necessity for examining the calibration of the weights.

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DISCUSSION

Dr. A. F. COLSON asked, in view of the fact that the amount of oxides of nitrogen produced in the combustion of organic nitro-compounds is greater than in the combustion of substances containing nitrogen linked to nitrogen, carbon or hydrogen, whether the author could give any information as to the number of analyses of nitro-compounds that could be carried out before the lead peroxide filling had to be renewed.

Mr. GOULDEN replied that his experience had been mainly with substances in which the nitrogen was in the ring.

Professor G. A. R. KON said that the principal difficulty with micro and semimicro methods of combustion based on that of Pregl was the lead peroxide. It was impossible to produce a satisfactory preparation by following the method described by Pregl—in particular to obtain a granular form. The activity of different preparations also varied considerably and he would be grateful for suggestions as to how these difficulties might be overcome. In a recent paper by Horning and Horning the use of an external catalyst—sulphuric acid and potassium dichromate—had been suggested and he wondered if anyone present had had any experience of it.

Dr. COLSON observed that chromic-sulphuric acid mixture was efficient for the retention of oxides of nitrogen but its limited absorptive capacity necessitated frequent renewal of the reagent.

Mr. A. BENNETT said he had found the use of external absorbents for oxides of nitrogen unsatisfactory. There was apparently always some absorption in the anhydrone tube and it became more marked as the anhydrone became saturated with water.

Mr. R. F. MILTON suggested that the Nicloux method of determining carbon dioxide be used when the determination was carried out on a semimicro scale. This method avoided the necessity for specially packed tubes to prevent errors due to the presence of nitro-compounds. The method consists of separation of carbonate as the barium salt in strongly alkaline solution, separation of the precipitate by centrifuging, solution of the precipitate in excess of acid and back-titration with standard alkali.

Microchemical Applications of Potentiometry

BY J. T. STOCK

(*Read at a Joint Meeting of the Microchemistry Group and the Physical Methods Group on September 26th*, 1947)

THE fundamental principle of potentiometry as applied to chemical analysis is that the potential of a suitable *indicator electrode* in contact with a solution depends upon, and varies with, the concentration (more strictly, the activity) of the ion to be determined in the solution. In a titration process involving change in the concentration of the ion in question, the potential of the indicator electrode changes most rapidly at the end-point, thus enabling the latter to be detected. Since the potential of a single electrode cannot be measured directly, the e.m.f. of the electrolytic cell comprising the indicator electrode, the solution to be examined and a *reference electrode* of fixed potential (*e.g.*, a calomel electrode) is determined, and the required potential is obtained by difference.

Potentiometry may be used in the determination of substances which, owing to low concentration or to smallness of sample, are present in micro-quantities. The determination

of *p*H is a familiar instance involving low concentrations. Other examples are the determination of boron in plant materials[1] or of furfural in aqueous solution[2]; in both instances a few milligrams of substance contained in a relatively large volume, *e.g.*, 100 ml. or more, of solution are titrated. Such methods will not be considered here, attention being confined to techniques involving the examination of small samples.

Two cases arise: (i) the actual value of an electrode potential is required, (ii) the electrical measurements or observations are used merely to follow the progress of a chemical reaction, such as a titration. The determination of *p*H is an example of the first case and has received much attention. Hydrogen, quinhydrone and glass electrodes suitable for the examination of small samples have been devised; since this field has been recently reviewed[3] it will not be further discussed. Suitable micro-electrodes have also been designed for measurements of potential at various points on a metallographic sample. In this way, Mahin and Brewer[4]

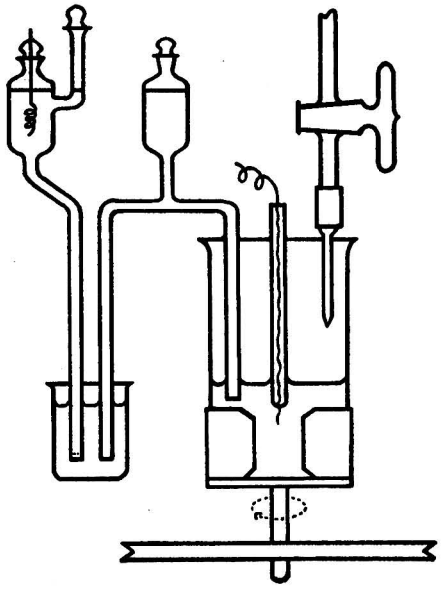

Fig. 1. Stirring by rotation of titration vessel.

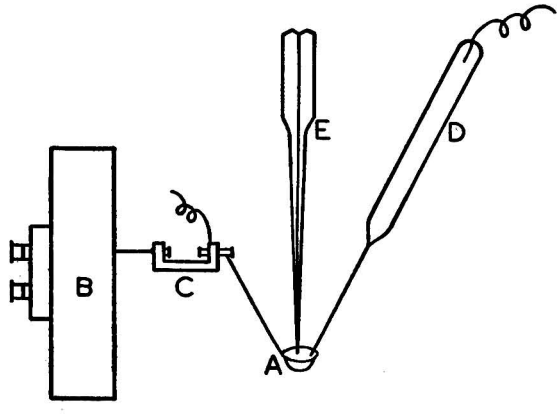

Fig. 2. Schwarz drop-scale titration assembly.

were able to study the segregation of ferrite in steel, caused by alloying elements. The method has also been used for the examination of the alloys zinc - iron, copper - lead and zinc - tin. Under the conditions examined, crystals of the compound $FeZn_7$ were shown to be present in the first of these, but the others consisted of inclusions of one metal in the other.[5]

Owing to the need for satisfactorily observing the colour change, micro-titration using chemical indicators presents special problems, such as the design of a suitable titration vessel.[6] Colour is of no significance in electrochemical methods and very dilute solutions may sometimes be employed.[7]

Adequate stirring is essential, and is often effected by bubbling nitrogen or other suitable gas through the solution. When the solution contains volatile substances a short-stroke micro-stirrer is useful.[8] The flexibility of the fine glass stirrer head prevents damage to the electrodes or the burette tip. Alternatively, the titration vessel may be rotated[9,10]; a titration assembly of this kind, due to Krumholz, is shown in Fig. 1.

For drop-scale titrations, the "vibrating ring" apparatus (Fig. 2) developed by Schwarz may be used.[11] The drop of liquid to be titrated is suspended from a ring-shaped indicator electrode A, which may be of silver wire for argentometry or of platinum for oxidation - reduction work. The electrode is caused to vibrate by attachment to the reed of a loud-speaker unit B, with an insulator C intervening. The loud-speaker unit is energised by the A.C. mains through a controlling resistance. The tips of the reference electrode D and of the micro-burette E dip into the drop of liquid.

An apparatus designed for the drop-scale titration of highly dilute silver solutions is shown in Fig. 3.[12] The "titration vessel" consists of a depression in the upper surface of a small paraffin block A, which is attached to a brass plate B. The latter rests upon the casing, C, of a small electric motor, the shaft of which is triangular in section and passes through a hole in the plate slightly larger in diameter than the shaft. The horizontal vibration thus produced stirs the solution, which is placed in the depression in A. As paraffin is not wetted by the solution, the latter assumes an approximately spherical shape; this facilitates insertion of the tips of the micro-burette D, silver wire reference electrode E and salt-bridge F. With this apparatus a few thousandths of a microgram of silver may be titrated with potassium iodide solution.

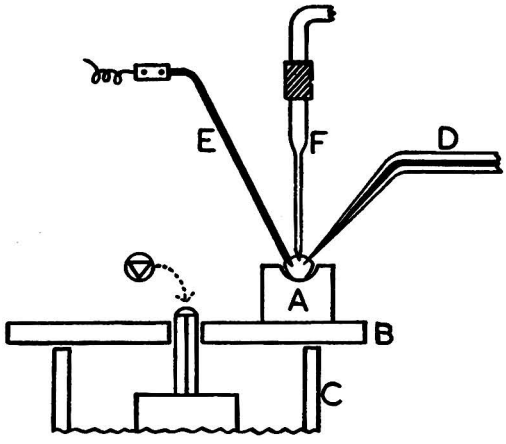

Fig. 3. Apparatus for the micro-titration of highly-dilute silver solutions.

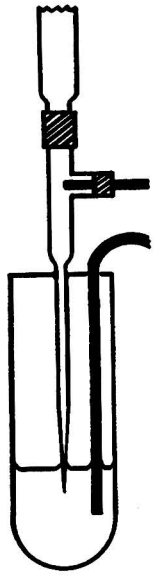

Fig. 4. Apparatus for the determination of microgram quantities of chloride.

In the determination of microgram quantities of chloride in biological samples, destruction of organic material is necessary. Decomposition may be carried out in the heat-resistant glass cell (Fig. 4), and the residue then dissolved and titrated with silver nitrate solution.[13] Two silver electrodes, one inserted in the jet of the micro-burette and one dipping into the solution in the cell, are used. These are connected to the potentiometer system, which is so adjusted that the galvanometer deflection decreases to zero at the end-point and then increases in the opposite direction. No tapping key is used, the circuit being closed and broken by immersion and withdrawal of the burette tip.

Several forms of apparatus for acid - base micro-titration using the glass electrode have been described. A simple cell for use with a standard bulb-type electrode is shown in Fig. 5.[14] To permit connection to the reference electrode the U-shaped capillary is filled with agar - potassium chloride gel. Only 0·1 to 0·2 ml. of solution (sufficient to surround the electrode bulb) is required. Stirring is effected by gently moving the glass electrode up and down.

The glass electrode may also serve as a titration vessel, as in the apparatus described by Ingold[15] and shown in Fig. 6. The vessel has a capacity of about 1·5 ml. A saturated calomel reference electrode is used and has a fine hooked outlet which dips into the solution to be titrated. Another cell of this type, designed for the formol titration of as little as 0·1 ml. of sample[16] is shown in Fig. 7. The exterior of the electrode bulb has a silver coating, A, to which electrical connection is made, and which is covered with varnish layer, B. To prevent damage, the fragile bulb is supported in cotton wool in a small casing and the whole is waterproofed by varnishing. Salt bridge C, which is supported as shown, permits connection to the reference electrode.

Since errors may be caused by electrolysis (*e.g.*, a current of 1 milliampere will deposit about 1 μg. of silver per second), the current drawn from the cell system during observations

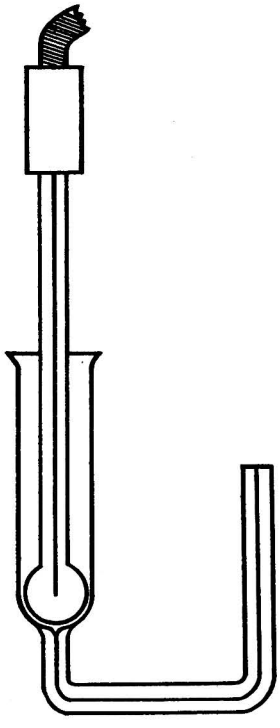

Fig. 5. Titration cell employing a glass electrode.

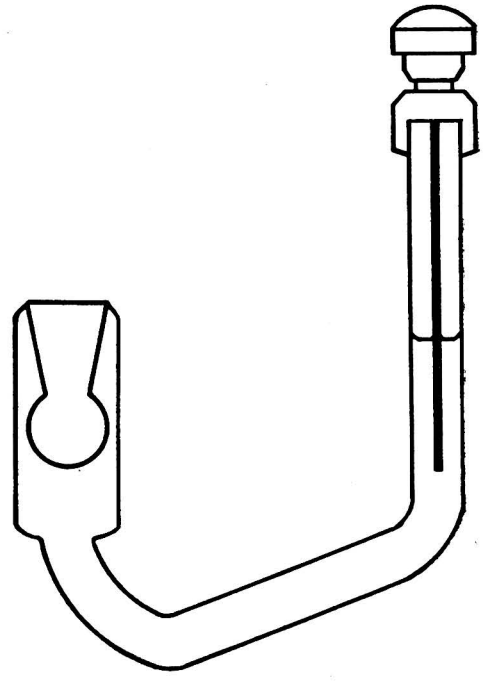

Fig. 6. Combined titration vessel and glass electrode.

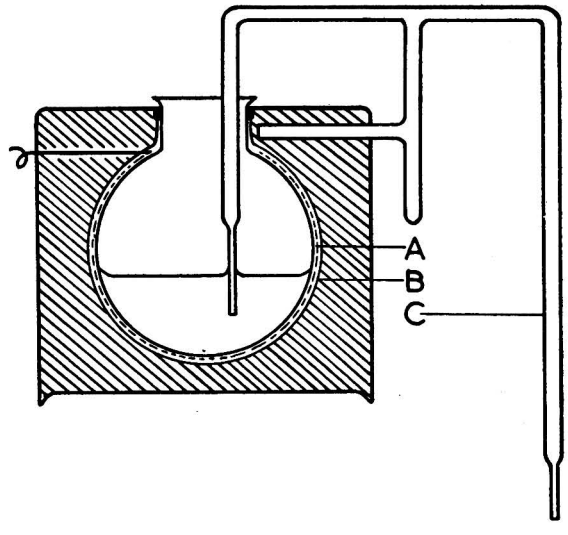

Fig. 7. Cell for formol micro-titration.

must be reduced to a minimum. This precaution is doubly important when the cell system has a high resistance (*e.g.*, when a glass electrode is used); otherwise the great voltage drop may render measurements impossible. A "no-current" device such as a Lindemann electrometer, the needle of which is observed by a reading microscope, may be used,[11] but an electronic

indicator or titrimeter is more convenient. Highly developed devices of this type for use with glass electrodes are available commercially,[17] as is an electronic apparatus designed specially for potentiometric titration.[18] When conditions are not too critical a simpler instrument, constructed from standard radio parts, is suitable.[19,20,21]

The circuit of one of these, due to Garman and Droz[20] is shown in Fig. 8. Since the grid current is only about 10^{-10} ampere, the cell system may be left connected throughout the operation. In practice, the electrode which becomes more negative as the titration proceeds is attached to terminal 1, switch S being in position 1. Potentiometer R_1 having been adjusted until microammeter M reads zero, the titration is begun and its course is followed merely by observing the reading of M. The sensitivity is adjusted by the operation of R_2.

Unless the potential jump near the end-point is large it is usual to make small successive equal additions (ΔV) of reagent near the end-point and to note the corresponding changes in potential (ΔE). If $\Delta E/\Delta V$ is then plotted against V, the end-point volume is indicated by the maximum of the curve.[22] On the macro scale a drop of reagent falling from the burette

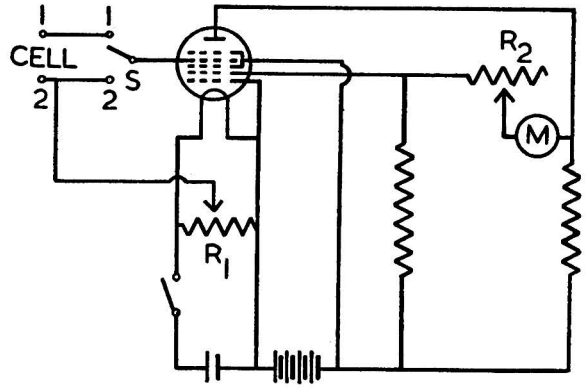

Fig. 8. Battery-operated electronic titrimeter.

is sufficiently reproducible to be taken as ΔV, but in micro-titration the design of the burette needs special attention if a multiplicity of volume readings is to be avoided. A simple device giving drops of about 0·01 ml. has been described by Lochte and Hoover,[23] and the problem was further investigated by Mika.[24] He designed a U-shaped micro-burette operated by drops of mercury; each rotation of the stop-cock caused the delivery of 0·001 ml. of reagent. Other suitable micro-burettes have also been described.[14,25]

Taking advantage of the properties of an electrical condenser, Delahaye has recently developed a method whereby a direct indication of dE/dt (*i.e.*, the rate of change of potential with time) is obtained.[26] If the reagent is added at constant rate, V becomes proportional to t and an indication of dE/dV is obtained. Since minute successive additions of reagent are not required, this technique may prove very useful in micro-titration. A horizontal micro-burette should enable the reagent to be delivered at constant rate.

Besides the examples already referred to, potentiometric titration has found numerous other applications, *e.g.*, in the determination of mercury, lead, selenium and bromide. For bibliography, papers by Ashcraft[27] and by Furman[28] should be consulted.

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August, 1947

DISCUSSION

Mr. A. D. LAUCHLAN asked what was the approximate size of the glass used in the U-tube container shown in Fig. 5 and said that glass electrodes of about 3 mm. diameter were now available.

Mr. NORMAN STRAFFORD enquired whether, in the electrometric titration of minute amounts of chloride, there was equivalence between the amount of halide and the silver nitrate titre, or whether empirical methods of calibration were necessary on account of the solubility of silver chloride.

Mr. J. HASLAM wished to know how the end-point is registered in Delahay's method, in which the titration liquid is delivered at a constant rate.

Mr. J. T. STOCK, in reply, said that the size of the glass used for the U-tube in Fig. 5 was a little larger than the diameter of the electrode bulb, say 1 to 1·5 cm.

In the electrometric titration of chloride the controlling factor was the concentration of chloride and not the actual amount present. The conditions are much the same on the micro as on the macro scale, but interference by traces of impurity often appears to be more marked on the micro scale. In Linderstrøm-Lang's method the zero e.m.f. reading, which marks the end-point, is adjusted to allow for the solubility of silver chloride.

Delahaye's method gives a direct indication of the rate of potential change with time. If the burette delivers at a constant rate, the rate of potential change with the volume of reagent is indicated; and this has a maximum value at the equivalence point.

Micro-Analysis using X-Ray Diffraction Technique*

BY H. P. ROOKSBY

(Read at a Joint Meeting of the Microchemistry Group and the Physical Methods Group on September 26th, 1947)

THERE are two senses in which the term micro-analysis has been used. In one, micro methods of examination may have been imposed upon the investigator because of the minute amount of available sample. It is necessary to develop special techniques by which the very small quantity of material can be handled and an analytical result obtained.

In another sense one may be concerned with the determination of minute amounts or traces of one constituent in another. The total quantity of specimen could be large in this instance and so the more conventional methods of analysis could be applied. There would, in general, then be no need to adopt those special techniques developed for the handling of very small amounts of material.

It is possible for X-ray methods to be applied to particular problems of both kinds. For example, X-ray spectroscopic technique using a standard crystal diffraction grating is very sensitive in the identification of trace elements; sensitivities of the order of 1 part in 10,000 have been claimed in modern applications to the study of rare earth minerals. But X-ray diffraction proper is not widely employed where problems in the second category

* Communication No. 394 from the Staff of the Research Laboratories of The General Electric Company Limited, Wembley, England.

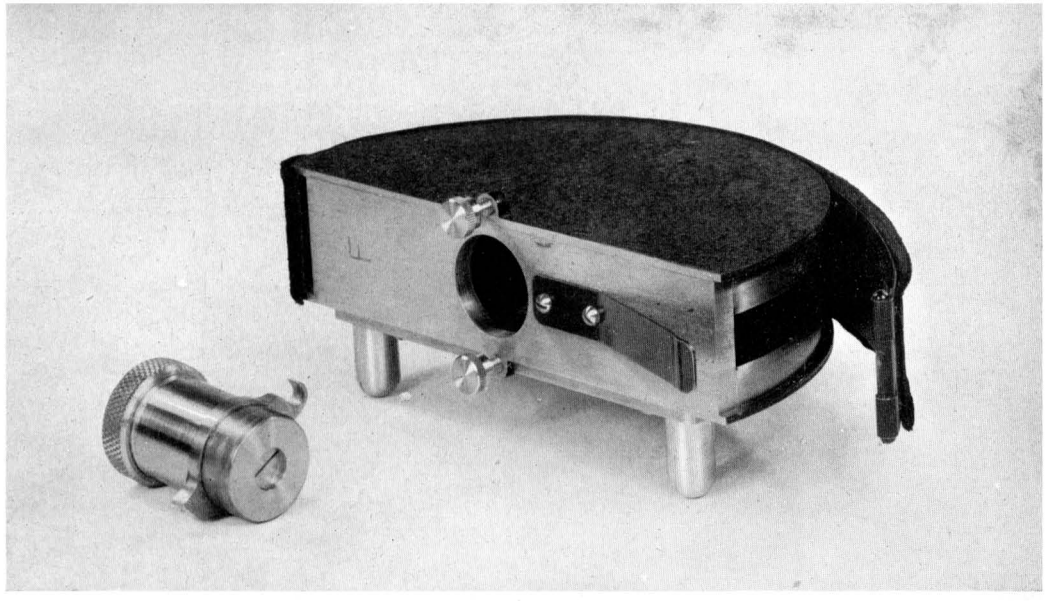

Fig. 1. Six-centimetre radius semi-cylindrical camera for powdered crystal photographs.

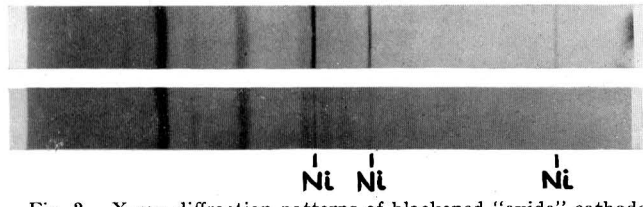

Fig. 3. X-ray diffraction patterns of blackened "oxide" cathode surfaces showing cause of blackening to be metallic nickel.

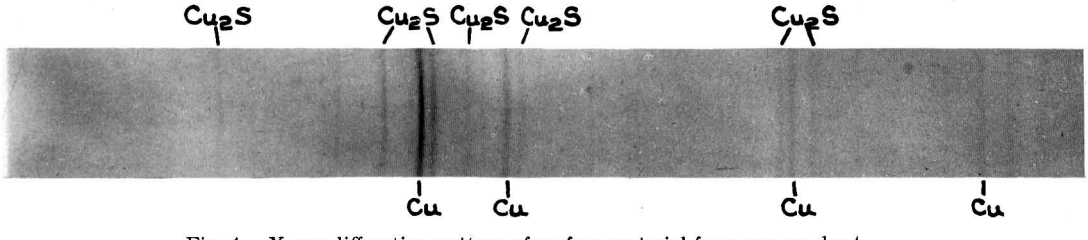

Fig. 4. X-ray diffraction pattern of surface material from copper sheet annealed in sulphur-contaminated atmosphere.

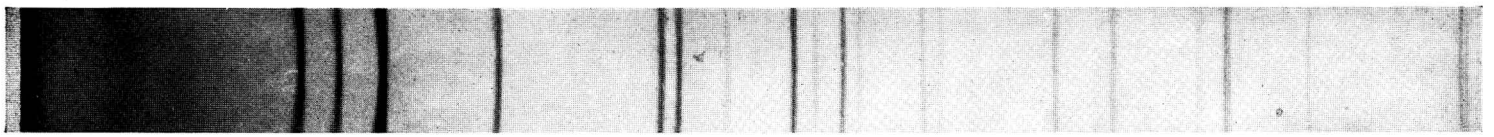

Fig. 2. X-ray diffraction pattern of metallic deposit inside glass exhaust tube of thermionic valve, identifying the deposit as cadmium.

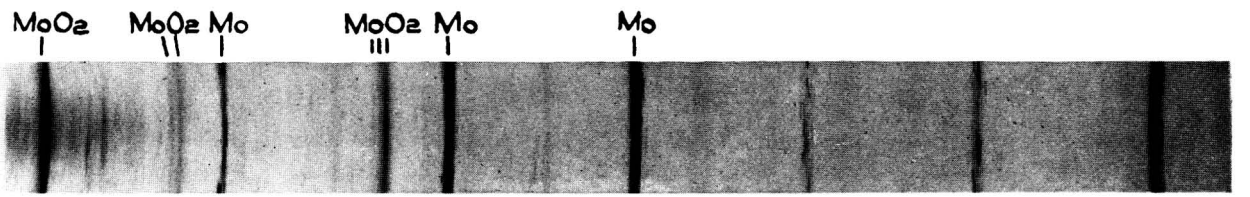

Fig. 5. X-ray "reflection" pattern of discoloured molydenum grid of thermionic valve.

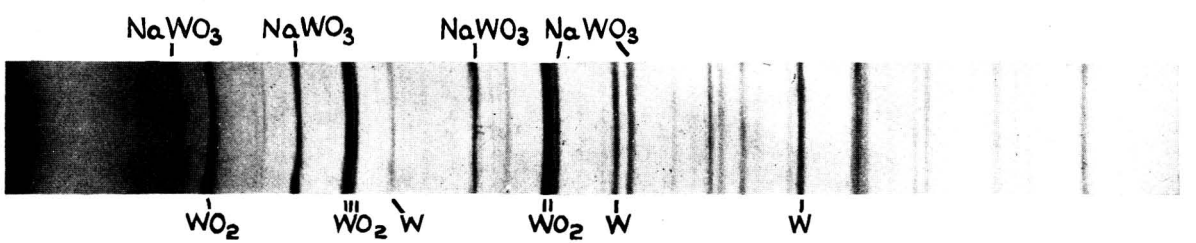

Fig. 6. X-ray "reflection" pattern of interfacial film between glass and metal of tungsten "oxide" seal

are concerned, although instances have been given in THE ANALYST in which a quantitative result was sought and a comparatively high sensitivity obtained.[1]

X-ray diffraction technique is, however, capable of being utilised in the first group of problems, those in which only minute amounts of sample are available. It is with micro-analysis by X-ray methods in this sense that this paper is concerned.

SOME FEATURES OF X-RAY DIFFRACTION TECHNIQUE ADVANTAGEOUS IN THE EXAMINATION OF SMALL AMOUNTS OF MATERIAL

One of the chief advantages of X-ray diffraction methods that comes immediately to mind when one is considering the handling of small quantities of material is that only an extremely small fragment of a crystalline substance is required to give a satisfactory X-ray pattern. Indeed, the problem in dealing with most objects is that of ensuring that the specimen actually X-rayed is a representative one. The problem is not, of course, peculiar to the X-ray technique; any method in which the specimen examined is a very small part of the whole will involve similar difficulties.

Single-crystal photographs can be obtained with extremely small fragments of crystal. The lower limit of size of crystal is set more by the manipulative difficulties of mounting it in a suitable orientation than by those of actually photographing the X-ray diffraction pattern. Useful photographs of mineral fragments have been obtained when the fragment weighs only a few millionths of a gram.[2] Fankuchen[3] claims that crystals in the form of needles need only be some $0{\cdot}1 \times 0{\cdot}01 \times 0{\cdot}01$ mm. in size to give good X-ray patterns.

In general, micro-analysis is required on multi-crystalline samples rather than on individual crystal fragments, so that the single-crystal methods are not applicable, and the better known Hull - Debye - Scherrer powder technique is adopted. Details of the technique itself can be found in the literature and it is proposed to mention one or two aspects that are particularly relevant to the question of micro-analysis.

The most important feature is that concerning the form of specimen required. There is the problem of preparing and mounting the specimen in the camera. The conventional arrangement is for a short cylinder approximately 0·3 mm. in diameter and a few millimetres in length to be made up and held in a special specimen holder along the axis of the camera. This cylinder may be formed in one of several ways. The powdered material may be attached to a fine glass or hair thread with the aid of a little adhesive such as starch paste, liquid glue or Canada balsam, and a cylinder of suitable diameter built up by successive applications. Alternatively, a thin-walled glass capillary tube of appropriate internal diameter is used as container.[4] A third method is to form the specimen by extruding it from glass barometer tubing of suitable bore after mixing the powder with a small amount of plastic binder.

The weight of powder will, of course, vary to some extent with the atomic weight and density of the elements of which it is composed, but on an average the actual specimen X-rayed will not weigh more than about half a milligram.

Naturally there must usually be a larger quantity of sample from which the X-ray specimens can be made up. For either the glass capillary method or the extruded rod technique the available sample must not be extremely small in amount. On the other hand, a few fragments of powder may be picked up by the glass fibre method and identifiable X-ray patterns obtained from specimens that are only just visible to the naked eye.

There is, however, yet another method of mounting the specimen by which still smaller quantities of material may be examined, but this involves the use of an unconventional form of camera. In this method the specimen forms a thin narrow band or layer on tissue paper as a backing medium. The X-ray camera is designed so that the collimated X-ray beam passes through the tissue paper specimen, which is attached to the back of the collimator with a smear of tap grease. The film forms a half cylinder, instead of the complete cylinder of the conventional Debye - Scherrer camera, because no reflections of Bragg angle greater than 45° can occur with the arrangement indicated. This limitation is not serious for general identification work; in very few instances is ambiguity likely to arise because of the absence of high-order lines.

A photograph of the semi-cylindrical camera with collimator withdrawn is shown in Fig. 1.

In order to impregnate the tissue paper with the material to be subjected to examination it is only necessary to smooth out a narrow band of the powder with a clean blade of a pen-knife. A high proportion of specimens will be robust enough to suffer transfer to the collimator

support without disintegration, but if any difficulty is found a little dilute collodion may be applied to act as a binder. The band of powder need only be a millimetre or so wide, just wide enough to ensure that it intercepts the narrowly defined X-ray beam. It must of necessity be very thin, because it must not absorb an undue proportion of the characteristic X-rays from which the useful X-ray pattern arises.

Excellent X-ray patterns have been obtained from the speck of active coating on the cathode in a cathode ray tube. This coating in its initial condition weighs only a fraction of a milligram, and at the end of the life of the device this fraction may have been still further reduced. Some of the specimens from which good X-ray diagrams have been obtained have been weighed and the weights are of the order of a hundredth of a milligram.

A survey of technique would not be complete without reference to the fact that in some problems an analysis is desirable of a specimen that cannot conveniently be removed from its base support or the base on which it has been formed. The identifications of thin films formed in vacuum devices or of oxide films on metals are legitimately regarded as micro-analytical problems, and it is not invariably practicable or indeed desirable to remove them for handling by one of the techniques mentioned above. But we can adopt what is best described as "reflection" technique, whereby the X-ray diagram is obtained by using a rectangular specimen cut out of the untouched subject, mounting this so that its plane passes through the axis of the circular camera and inclining it at an angle of a few degrees towards the incident X-ray beam. If during the exposure the specimen can be given a small rocking motion through an angular range of say 10°, so much the better, but in many applications this is not an indispensable refinement. Although special cameras have been designed for the application of the surface reflection technique, and carefully collimated X-ray beams are often desirable, much of the work has been carried out by adaptation of the conventional circular cameras already described.

Examples of application of X-ray technique to micro-analytical problems

Some specific practical examples in which X-ray technique has proved valuable can now be discussed. These have been chosen to be illustrative of the various methods that have been indicated above.

We are often called upon to examine deposits in vacuum devices, formed perhaps during pumping operations or during life. In experimental work on lamps and thermionic valves for instance, occasions arise on which the pumping conditions may have been inadequate and when deposits are found an identification will help to trace the cause of trouble. Often conventional chemical methods or spectrographic analysis will be sufficient, but sometimes it is desirable to establish the exact nature of the specimen, whether it be metal, oxide, nitride or sulphide, for example. The X-ray method will provide this information.

The first example is a very simple one. A deposit of metallic appearance was formed inside a glass exhaust tube during the pumping of a thermionic valve. A little difficulty was experienced in reaching a decisive result by chemical means, partly because the specimen was so thin and small in area, and so the specimen was submitted for X-ray examination. It was found that by lightly scraping the glass with a knife sufficient of the deposit could be collected to attach to the specimen support for the conventional circular camera. The X-ray diffraction pattern is reproduced in Fig. 2. This immediately identifies the deposit as metallic cadmium, an entirely unsuspected element. It was evident from this result that some of the soldered parts of the valves being pumped were giving trouble, a cadmium-bearing solder having been used.

Sometimes the film or deposit in question is not so easily removed from its associated material so that it can be studied separately. If the film occurs on the cathode coating of a valve device for example, it is impracticable to free it from the material of the coating. The best that can be done is to prepare a specimen of coating with the contaminant in as concentrated a proportion as possible, usually by removing a very thin layer from the coating surface. The semi-cylindrical camera with its tissue paper specimen support is often employed because the very minimum of sample is required. Typical instances of X-ray diffraction patterns of blackened cathode surfaces obtained in such a way are reproduced in Fig. 3. The main part of the X-ray pattern consists of lines for which the coating itself is responsible, but over and above this some extra lines are distinguished, revealing the presence of metallic nickel. The X-ray analysis proves decisively that the blackening of the cathode coating is associated with the presence of nickel.

Occasionally one is forced to adopt a more elaborate procedure than the one just described. The discolouring agent may be in too low a concentration to be detected; in this event it has been found possible, after detaching the coating from its base support, to dissolve the alkaline earth constituent in 10 per cent. acetic acid. The operations, including acid treatment and washing, can be carried out on a cover glass with the aid of a small pipette, or in other suitable microchemical apparatus. With coatings discoloured by a metal or metallic oxide, residues weighing perhaps a tenth of a milligram have been handled, the X-ray examination being done on the semi-cylindrical camera.

A problem of different character arises during investigations connected with the heat treatment of metals. Annealing of sheet copper for example may be conducted in electric furnaces using controlled atmospheres, and there are sometimes difficulties in maintaining the appropriate atmospheric conditions to produce a bright finish, especially if burnt town's gas is being used. It is important to be able to determine the compositions of any deposits or discoloured surfaces that may form during a run of the furnace, as a knowledge of the nature of the deposit helps to indicate in what direction modifications of the atmosphere should be made.

In the example chosen the copper sheet had an obviously blackened surface, and it was possible to prepare an X-ray specimen by lightly scraping the surface. The X-ray diffraction pattern is shown in Fig. 4. Strong reflections for metallic copper are distinguishable, but a large number of weaker lines occur, which represent the black deposit. They can all be accounted for by a mixture of cuprous sulphide and cuprous oxide, thus proving that blackening did not by any means result only from oxidation but was also associated with the presence of sulphur not removed sufficiently completely from the gases employed in the furnace. The significant factor that had to be ascertained was the nature of the compound of copper responsible for the black film, whether it was oxide, sulphide, nitride or a more complex mixture.

In the next two instances the X-ray "reflection" technique has been applied. The first is that of the discoloration of a fine molybdenum valve grid, taking place during the process of sealing the thermionic valve assembly into its glass envelope. It was clearly impracticable to collect a specimen of the deposit sufficient in quantity for examination by conventional technique, so the grid wires were examined as they stood. The complete grid had an area of less than a square centimetre and was mounted, as it stood, in the appropriate position in the X-ray camera. The X-ray pattern is reproduced in Fig. 5. In addition to lines for metallic molybdenum, a pattern of lines occurs which is readily recognisable as that of molybdenum dioxide, MoO_2. This identification of the cause of discoloration leads to the conclusion that the grid had become sufficiently hot during the sealing-in process for superficial oxidation of the molybdenum to take place.

The second example is taken from a series of examinations carried out with the object of detecting and identifying interfacial films formed when various glasses and metals are sealed together. It typifies those glass to metal joints made between tungsten and glass and usually described as "oxide" seals. The film can be observed as a yellowish-brown interlayer, which on breaking the joint adheres very firmly to the glass surface. It is quite impracticable to separate the interlayer from the glass, and the X-ray diffraction pattern shown in Fig. 6 was obtained by "reflecting" from a selected fragment of the glass, with the interlayer in question uppermost towards the incident X-rays.

The X-ray diagram reveals two principal constituents. One is tungsten dioxide, WO_2, as might be suspected, but the other is a soda - tungsten oxide compound, $NaWO_3$. The identification of this compound is of some practical interest, because it demonstrates that with the glass employed, which contains a small percentage of soda, reaction occurs between the oxide first formed on the metal and a constituent of the glass. This strengthens the bond between oxide and glass and explains why on breaking this type of seal the interlayer invariably remains firmly stuck to the glass.

So far we have been considering examples of an essentially qualitative nature, but it should not be thought that quantitative analysis is ruled out. Many instances could be given in which quantitative results are sought and obtained, and the example now to be mentioned is a micro-analytical one.

The example selected utilises a well recognised feature of X-ray diffraction patterns, the systematic alterations occuring as a result of mixed crystal or solid solution formation. The

changes that take place are used to provide quantitative information concerning the individual constituents involved in solid solution formation.

We have already referred to the analysis of the "oxide" type thermionic cathode coating. The problem is to determine the ratio of barium to strontium oxide. Not only is this a difficult problem for conventional chemical technique, but the analysis is often required on the minute amount of coating on a very small cathode, such as that employed in a cathode ray tube. This speck of material can be examined by X-rays by the semi-cylindrical camera technique. It is necessary to ensure that the barium and strontium constituents are in a suitable chemical and structural condition. A mixed carbonate is formed by controlled heat treatment in carbon dioxide before the coating is removed from the cathode.

Then an X-ray diagram of a specimen that may weigh only a few hundredths of a milligram is compared with standard patterns of the individual pure carbonates. Measurements are carried out to estimate the molecular ratio of barium carbonate to strontium carbonate.

The technique has been utilised for a considerable number of years now, and experience shows that the numerical values obtained do not differ from the true values by more than 2 or 3 parts in 100.

Conclusion

Many other examples besides those given could have been cited, but the ones chosen illustrate sufficiently well the kind of problem in the inorganic field to which the X-ray method may be applicable.

Naturally the application of X-rays, like the use of other techniques, does not invariably give a successful result. One of the limitations, so far as micro-analytical problems are concerned, is that already mentioned, *viz.*, the manipulative problem of moulding a minute sample into a form that will give a readable X-ray diffraction pattern. On the other hand, there is little room for doubt that the X-ray record obtained is that of a specimen of truly micro-analytical dimensions.

The identity of crystalline phases and an approximate estimate of concentration in minute specimens are subjects well suited to investigation by X-ray methods. It should not be forgotten too, as has been discussed elsewhere,[5] that X-ray technique may supplement this information with details of texture and other structural features, details that are quite properly embraced by a broadly conceived analysis. Were the potentialities in the field of microchemical analysis more fully realised, the X-ray method would be much more widely adopted than it is at present.

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A Modified Method for the Microbiological Assay of Tryptophan, Methionine, Cystine and Tyrosine

By E. C. BARTON-WRIGHT and N. S. CURTIS

(*Read at the Annual General Meeting of the Biological Methods Group, December 16th,* 1947)

It was shown in a previous communication[1] that it is possible to assay all the so-called "essential" amino acids of Rose[2] with the three lactic acid-forming organisms, *Lactobacillus arabinosus* 17/5, *Leuconostoc mesenteroides* P.60 and *Streptococcus faecalis* R. Although microbiological assays of amino acids have the great advantages of rapidity, specificity and accuracy, they are nevertheless distinctly expensive to carry out. At the present time the cost of a number of these acids, especially the synthetic products, is very high. If, therefore, some method or methods could be devised for removing completely some one particular amino acid from either an acid casein hydrolysate or peptone, the high cost of its assay would be materially reduced, because the treated casein hydrolysate or peptone could be used in the basal medium to replace a large number of individual amino acids. Moreover, the tedious task of weighing out a small amount of each acid in the preparation of the basal medium would be avoided.

It has been known for some time that hydrogen peroxide will destroy the amino acids, cystine, methionine, tryptophan and tyrosine in peptone. Lyman *et al.*[3] have described a method of treating peptone with hydrogen peroxide to eliminate these four acids and have suggested the use of this product in microbiological assays. Their method for the preparation of the peptone has been modified in this investigation and the product has proved to be very satisfactory for the assay of the four acids mentioned above.

Preparation of hydrogen peroxide-treated peptone—50 g. of Difco Bacto Peptone are dissolved in 500 ml. of *N* hydrochloric acid and 32 ml. of 20 vols. hydrogen peroxide (6 per cent.) are added. The mixture is allowed to stand overnight at room temperature. The solution is then heated in a steam steriliser at atmospheric pressure for 30 minutes, cooled, neutralised with 30 per cent. sodium hydroxide solution and steamed again for 1 hour to remove the last traces of hydrogen peroxide. It is then diluted to 1000 ml. with glass-distilled water and is ready for use. It will keep for several months in a refrigerator (4° C.) under sulphur-free toluene. It was found that the most satisfactory assay results were obtained when the treated peptone was used at a concentration of 0·75 per cent. in the basal medium.

Assay of tryptophan—The organism recommended for this assay is *Lactobacillus arabinosus* 17/5. The basal medium has the following composition.

TABLE I

COMPOSITION OF BASAL MEDIUM FOR THE ESTIMATION OF TRYPTOPHAN

Substance	Amount
H_2O_2-treated peptone	0·75 per cent.
L-Cystine	0·02 »
DL-Methionine	0·01 »
L-Tyrosine	0·004 »
Glycine	0·002 »
Glucose (anhydrous)	2·0 »
Sodium acetate (anhydrous)	2·0 »
Xylose	0·1 »
Ca D-pantothenate	0·1 p.p.m.
Aneurine	0·1 »
Riboflavine	0·2 »
Pyridoxine	0·1 »
Nicotinic acid	0·4 »
p-Aminobenzoic acid	0·1 »
Biotin	0·0005 p.p.m.
Adenine	10·0 p.p.m.
Guanine	10·0 »
Uracil	10·0 »
Xanthine	10·0 »
Ammonium sulphate	0·3 per cent.
Inorganic salt solution A*	0·5 ml. per 100 ml.
» » » B*	0·5 » »

* See Barton-Wright.[1]

It is unnecessary to add sodium chloride to this or any of the other basal media described below. There will be sufficient concentration of sodium chloride (0·5 per cent.) in the medium if the recommended addition, 0·75 per cent., of hydrogen peroxide treated peptone be made. Any great excess of sodium chloride over 0·5 per cent. acts as a growth depressant on many lactic bacteria. Assay procedure is exactly the same as previously described. It must, however, be emphasised that incubation of the assay tubes should be carried out at 30° C. and not 37° C. The range of tryptophan to establish a standard curve is 2 to 10 μg.

Assay of L-*methionine,* L-*cystine and* L-*tyrosine*—The organism recommended for the assay of these three acids is *Leuconostoc mesenteroides* P.60. It was previously suggested that *L. arabinosus* should be used for the assay of cystine. For various reasons, however, that organism proved to be unsatisfactory, whereas *L. mesenteroides* has proved to be satisfactory in every way for this particular assay.

The basal medium has the following composition.

Assay procedure is again similar to that previously described. As in the assay of tryptophan, incubation temperature should be 30° C. and not 37° C. The range of L-methionine to establish a standard curve is 15 to 40 μg., that of cystine 5 to 35 μg. and that of tyrosine 10 to 50 μg.

TABLE II

COMPOSITION OF BASAL MEDIUM FOR THE ESTIMATION OF L-METHIONINE, L-CYSTINE AND L-TYROSINE

Substance	Amount
H_2O_2-treated peptone	0·75 per cent.
L-*Methionine**	0·01 "
L-*Cystine**	0·01 "
L-*Tyrosine**	0·01 "
DL-Tryptophan	0·02 "
Glycine	0·01 "
Glucose (anhydrous)	2·0 "
Sodium acetate (anhydrous)	1·2 "
Ammonium chloride	0·6 "
Adenine	12·0 p.p.m.
Guanine	12·0 "
Uracil	12·0 "
Xanthine	12·0 "
Aneurine	1·0 "
Pyridoxine	1·6 "
Ca D-pantothenate	1·0 "
Riboflavine	2·0 "
Nicotinic acid	2·0 "
Biotin	0·005 "
p-Aminobenzoic acid	0·05 "
Inorganic salt solution A†	0·5 ml. per 100 ml.
" " " B†	0·5 " "

* The amino acid to be assayed is omitted from the medium.
† See Barton-Wright.[1]

Assay of DL-*methionine*—It is possible to assay DL-methionine using *Lactobacillus fermenti* 36. This organism and brewers' yeasts are unique in that they can utilise either enantiomorph of methionine.

The basal medium recommended has the following composition.

TABLE III

COMPOSITION OF BASAL MEDIUM FOR THE ESTIMATION OF DL-METHIONINE

Substance	Amount
H_2O_2-treated peptone	0·75 per cent.
L-Cystine	0·01 "
L-Tyrosine	0·01 "
DL-Tryptophan	0·02 "
Glycine	0·01 "
Glucose (anhydrous)	2·0 "
Sodium acetate (anhydrous)	1·2 "
Ammonium chloride	0·6 "
Adenine	10·0 p.p.m.
Guanine	10·0 "
Uracil	10·0 "
Xanthine	10·0 "
Aneurine	1·0 "
Pyridoxine	1·6 "
Ca D-pantothenate	1·0 "
Riboflavine	2·0 "
Nicotinic acid	2·0 "
Folic acid	0·002 "
Biotin	0·005 "
p-Aminobenzoic acid	0·05 "
Inorganic salt solution A*	0·5 ml. per 100 ml.
" " " B*	0·5 " "

* See Barton-Wright.[1]

Although the general plan of this assay is the same as for the others described above, it differs in one or two small respects. For instance, the *p*H of the basal medium is adjusted to 6·4 and not 6·8, folic acid is an essential nutrient for this organism and a heavy inoculum is used for the tubes. The best results are obtained when, after centrifuging the inoculum, the organisms are taken up in 10 ml. of 0·9 per cent. sterile saline solution and no further dilution of the suspension is made. The tubes are inoculated with 1 drop of this suspension per tube from a sterile pipette. They are incubated for 72 hours at 37° C.

Preparation of hydrolysates—Strong acids cannot be used for the hydrolysis of material for the assay of tryptophan and tyrosine. Tryptophan is completely and tyrosine partially destroyed by them. Alkali hydrolysis must therefore be used. It was previously stated that barium hydroxide should be used as an hydrolysing agent for tryptophan, but it has since been found more convenient to use sodium hydroxide. Dry materials are finely ground and heated for 8 to 10 hours with 5 *N* sodium hydroxide solution at 15 lb. pressure in an autoclave. The hydrolysate is adjusted to *p*H 4 with hydrochloric acid and in the assay of tryptophan extracted successively with ether and toluene to remove indole and anthranilic acid. Any precipitate that is formed is centrifuged off and the solution is adjusted to *p*H 6·8 and made up to volume. Since in prolonged alkaline hydrolysis the amino acid undergoes complete racemisation and the D-enantiomorph is inactive, the figure found by assay will have to be doubled to give the correct value.

Material for the assay of methionine and cystine is hydrolysed with 2·5 *N* hydrochloric acid for 6 hours at 15 lb. pressure in an autoclave. After cooling, 2 ml. of 2·5 *M* sodium acetate solution are added and the *p*H is adjusted to 4·5 with sodium hydroxide solution. The liquid is then made up to volume and filtered. Experience has shown that *all* hydrolysates, after the *p*H has been adjusted to 4·5, should be twice vigorously shaken with light petroleum or ethyl ether to remove lipide material. The presence of fatty acids, unsaponifiable matter, etc., will invalidate an assay.

Method of computation—The older method of calculating assay results by direct reading from the standard curve should now be abandoned in favour of the statistical method devised by Wood[4] for the computation of microbiological assays in which there is no strictly linear portion to the standard curve. On this method of computation the logarithm of the dose is plotted against the logarithm of the response (here ml. of 0·1 *N* sodium hydroxide), and a straight line is obtained for the standard doses of pure amino acid or vitamin. The test preparation is treated in the same way; if the assay is valid, a straight line will be obtained parallel to the standard line. Any significant lack of parallelism is evidence of invalidity. From the two parallel lines it is a simple matter to calculate the results of an assay.

The protocols of typical standard curves for tryptophan, L-methionine, DL-methionine, cystine and tyrosine are given in Table IV.

TABLE IV

TYPICAL STANDARD CURVES FOR THE ASSAY OF TRYPTOPHAN, L-METHIONINE, DL-METHIONINE, CYSTINE AND TYROSINE

Tryptophan		L-Methionine		DL-Methionine		Cystine		Tyrosine	
Dose μg.	Response 0·1 *N* NaOH ml.	Dose μg.	Response 0·1 *N* NaOH ml.	Dose μg.	Response 0·1 *N* NaOH ml.	Dose μg.	Response 0·1 *N* NaOH ml.	Dose μg.	Response 0·1 *N* NaOH ml.
0	1·2	0	0·35	0	0·4	0	0·25	0	0·7
2	4·45	15	3·0	10	1·5	5	3·2	10	3·15
4	6·45	20	5·65	20	3·2	10	5·3	20	5·3
6	8·45	30	7·65	30	4·4	15	6·57	30	7·6
8	10·55	40	9·35	40	6·25	20	7·7	40	9·25
10	12·45	50	10·95	50	7·1	25	8·8	50	10·5
				60	8·25	30	10·0	60	11·15
				80	9·2	35	11·2		
				100	10·0				

SUMMARY

A modified method for the microbiological assay of tryptophan, methionine, cystine and tyrosine is described. Peptone, after treatment with hydrogen peroxide, which destroys these four amino acids, is substituted in the basal medium for the usual series of individual amino acids.

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The Cup-Plate Method in Microbiological Assay, with Special Reference to Riboflavine and Aneurine

By A. L. BACHARACH and W. F. J. CUTHBERTSON

(*Read at the Annual General Meeting of the Biological Methods Group, December 16th, 1947*)

During a recent discussion[1] one of us suggested the possibility that the cup-plate method, as used in the bio-assay of penicillin, a modification of Heatley's original cylinder-plate method, might be adapted to the microbiological assay of vitamins and amino acids. A note published elsewhere[2] indicated that the possibility would be worth exploring further, preliminary experiments having given encouraging results. We wish here to report details of those and subsequent experiments and to attempt an assessment of the limitations, advantages and potentialities of the procedure.

In assaying penicillin by this technique the test organism is grown on a suitable complete agar medium in Petri dishes. "Cups" cut out of the agar are filled with appropriate dilutions of a standard penicillin solution and of the test material. After incubation the cups are found to be surrounded by circular "zones of inhibition," in which the organism has failed to grow, owing to the anti-bacterial action of the penicillin, which diffuses into the medium. The transparent zones are sufficiently clearly demarcated from the rest of the medium, opalescent or opaque from growth of the test organism, for measurement of their diameters to be made with considerable precision. These diameters are found to bear, over a range of penicillin concentrations wide enough for practical assay purposes, a linear relationship to the amounts of penicillin in the cup.

When the method is applied to the assay of certain vitamins the medium is made deficient only in the single substance to be assayed and the organism chosen is one that will not grow in its absence. If solutions containing the missing substance are put into the cup and the plate is then incubated under suitable conditions of time and temperature, growth of the organism may occur in sharply defined circular "zones of exhibition" round the cups. The diameters of these zones have been found to be related to the concentration of the added nutrient and may be measured by means of callipers, as in the penicillin method. The appropriate range of concentrations, the nature of the algebraic function relating zone diameter to concentration and the precision of the method must be established empirically and by suitable statistical analysis.

Method

Media are made up to the final concentrations used in the usual methods of microbiological assay, but with addition of 1·5 per cent. of agar. Eleven-ml. portions of medium are placed in Pyrex bacteriological test tubes, autoclaved at 10 lb. for 10 minutes and stored in the refrigerator until required. Just before use the tubes are heated in the boiling water-bath to melt the medium and then held in a bath at 45° C. until needed. The tubes are now inoculated with 1 ml. of a suitable suspension of organism; after thorough mixing by several inversions, the medium is poured into Petri dishes, which are allowed to cool on a flat bench. It is essential to use dishes of which the circular inner surface is plane and level.

Five holes are cut in the agar by means of a sharp 10-mm. cork borer, the underside of the plates being marked to permit subsequent identification of the cups. Three drops of test or standard solutions are now added to the appropriate cups from a pipette, of the type used in penicillin estimations, in which the dropper has a platinum tube, of 0·0365 inch external and 0·0295 inch internal diameter and about 12 mm. long, fused into the tip at an angle of about 130° to take up a position normal to the Petri dish during delivery. A pipette of this type has been found to give drops highly uniform in size. The plates are incubated at a temperature suitable for the organism used. The width of the zones of growth may be measured after 18 hours' incubation, but it has been found that, under the conditions so far investigated, the diameters are not altered by longer incubation.

During preparation of the plates strict asepsis is unnecessary; normal cleanliness and the use of oven-dried apparatus have so far avoided all trouble from contaminating organisms and it has also been found unnecessary to sterilise either test or standard solutions.

The medium of Roberts and Snell[3] has been used, with the appropriate omissions, for the estimation of riboflavine, using *Lactobacillus casei* ϵ and of biotin or nicotinic acid, using *Lactobacillus arabinosus*. *Lactobacillus fermentum* P.36 has been used with the medium of Sarett and Cheldelin[4] for aneurine estimations. These media were selected because they have been shown to be capable of supporting the very fast growth essential for a rapid assay technique and particularly important with this procedure, in which diffusion of the growth factor leads to a decrease in its concentration round the cups.

The *L. casei* and *L. arabinosus* cultures used were maintained on the liver - tryptone medium of Nymon and Gortner,[5] inocula being prepared from 18- to 24-hour cultures in liver - tryptone broth. *L. fermentum* was maintained and inocula were grown in media rich in aneurine, as suggested by Cheldelin, Bennett, and Kornberg.[6] We have found, in agreement with these workers, that the aneurine requirement of *L. fermentum* is only apparent in cultures maintained on media containing large amounts of the vitamin. We have also found that a series of about 6 to 10 passages through media rich in aneurine fully restores the aneurine requirements of cultures that have become nutritionally independent of it.

EFFECT OF INOCULUM DENSITY AND VITAMIN CONCENTRATION—

The width of the zones of growth and the sharpness of their edges are determined by the concentration of growth factor in the cups and the density of the inoculum used in preparing the plates. Table I shows the effects of inoculum size and concentration of the vitamin on the diameter of the growth zones. The inoculum density is given as ml. of suspension used, the suspensions having been prepared as follows. Thirty-two ml. of 20- to 24-hour cultures of *L. casei* or *L. arabinosus* were centrifuged, washed once with saline and re-suspended in 12 ml. of saline; 8 ml. of 20- to 24-hour cultures of *L. fermentum* were centrifuged, washed once with saline and re-suspended in 80 ml. of saline.

TABLE I

EFFECT OF INOCULUM SIZE ON DIAMETER (IN MM.) OF GROWTH ZONES

(*a*) Aneurine

L. fermentum Amount of inoculum, ml.	Aneurine concentration (μg./ml.) 0·0125 mm.	0·025 mm.	0·05 mm.	0·1 mm.	0·2 mm.
0·1	13	16	17	—	24
0·3	—	12	15	18	22
1·0	—	11	13	15	17

(*b*) Riboflavine

L. casei ϵ Amount of inoculum, ml.	Riboflavine concentration (μg./ml.) 0·01 mm.	0·03 mm.	0·1 mm.	0·3 mm.	1·0 mm.	3·0 mm.	10·0 mm.
0·3	11	12	13	19	28	40	45
1·0	—	—	14	18	25	28	40
3·0	—	—	14	17	22	—	—

(*c*) Nicotinic acid

L. arabinosus Amount of inoculum, ml.	Nicotinic acid concentration (μg./ml.) 1·0 mm.	5·0 mm.	25·0 mm.
0·2	19	23	33
0·5	—	20	30
1·0	—	18	26

(*d*) Biotin

L. arabinosus Amount of inoculum, ml.	Biotin concentration (μg./ml.) 0·001 mm.	0·005 mm.	0·025 mm.	0·125 mm.
0·2	12	16	21·3	25
0·5	—	12·5	18·0	21·5
1·0	—	11·0	15·5	19·5

In general, the zone diameters decrease with increasing inoculum density, whilst the zone boundaries become more sharply defined as the inoculum density is increased. The width of the zones obtained with riboflavine, aneurine, nicotinic acid and biotin, using the organisms already named, increased with increasing concentrations of the vitamins. So did the sharpness of the zones for the first three vitamins, but with biotin, on the contrary, the sharpness of the edges decreased with increasing concentration, although the organism used, *L. arabinosus*, was the same as for nicotinic acid. The difference is clearly not due to the very different solubilities of riboflavine and biotin, for both are used in these tests at concentrations far below those of their saturated solutions. An explanation in terms of relative diffusibilities is thus difficult to accept, since the molecular weight of biotin (244), which behaves in the one way, is intermediate between those of nicotinic acid (123) and of aneurine (306·5) and riboflavine (376), which all behave in the other. Some phenomena connected with the biological utilisation of the four different vitamins by the three different organisms must presumably lie behind the differences in appearance of the zones.

It can be seen from Table I that the doses used to give the responses held to be high enough for accurate measurement are much above those used in microbiological assays involving acidimetric, turbidimetric or gravimetric measurements. Nevertheless, the difficulties frequently found in applying these techniques to the assay of riboflavine and aneurine seemed to us to justify attempts at estimating these vitamins by the cup-plate technique.

Riboflavine estimation

Inoculum size—In deciding on the inoculum size a compromise has to be made between the size of zone obtained and the sharpness with which its edges are defined. We have found 1 ml. of a suspension of *L. casei*, prepared as described, to be satisfactory and to give sufficiently well-defined zones of 20- to 30-mm. diameter with riboflavine concentrations of 0·5 to 4·0 μg. of riboflavine per ml.

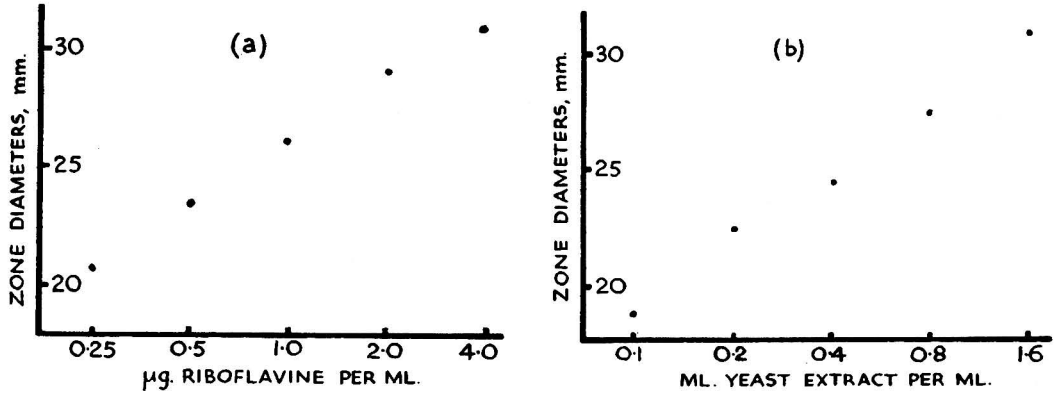

Fig. 1. Response of *Lactobacillus casei* ϵ to riboflavine.
Ordinates: mean zone diameters, in mm.
Abcissae: logarithmic plot.
Each point represents the average of four zone diameters.

Relation between riboflavine concentration and zone diameter—The diameters of the growth zones have been found to be proportional to the logarithms of the concentrations of riboflavine used, over a wide range, as shown in Fig. 1 (*a*), and liver and yeast extracts were also found to give a like relation over a similar wide range of concentrations. A typical result obtained with a yeast extract is shown in Fig. 1 (*b*). It follows, therefore, that the estimation of riboflavine by this method and of the error of the estimate—and the same is true of the aneurine assays, as reported below—can be made by means of a "four-point assay," as applicable to many types of macro-biological assay, but not by the "slope-ratio" method[7] necessary with those techniques involving a linear relation between the dose itself and the measured response. In a "four-point assay" the levels of dose must lie well within the linear portion of the log-dose response curve, and the dose-ratio, which is best made identical for standard and unknown, should be as large as is possible over that range. But it is

necessary to have an approximate idea of the concentration of active substance in the test material if one is to depend on only two dose levels for comparison with two of standard.

TABLE II

DIAMETERS OF GROWTH ZONES MEASURED IN MILLIMETRES

Four plates each with 4 cups

Concentration of solutions placed in cups

Plate	1·0 μg. of riboflavine per ml. mm.	4·0 μg. of riboflavine per ml. mm.	0·04 ml. of yeast extract per ml. mm.	0·16 ml. of yeast extract per ml. mm.	Totals
1	22·0	25·0	20·0	23·1	90·1
2	21·5	25·5	19·5	23·2	89·7
3	21·4	25·1	20·0	23·3	89·8
4	21·5	25·0	19·5	23·1	89·1
Totals ..	86·4	100·6	79·0	92·7	
		187·0		171·7	

Table II gives the results obtained in a four-point assay of riboflavine in liver extract, of which the approximate riboflavine content had been satisfactorily forecast, giving reasonably close mean responses for standard and test. The dose-ratio for each was 4 : 1. The 16 observations on which the assay is based were submitted to the usual variance analysis (Table III), which confirmed the lack of any significant differences between the four plates used, whether in mean responses, or in the interactions with slope, with test *versus* standard or with parallelism differences. There is also no significant difference in parallelism between the slopes for test and standard, confirming the validity of the assay technique. The slope of the regression of diameter on log concentration is very highly significant. The results of the analysis given in Table III lead to a value of 0·058 for s^2, the residual variance. From this the fiducial limits of the estimated potency were calculated by a formula based on Finney's equation,[8] giving a result of 11·7 μg. per g., with limits of 10·3 to 13·4 (88·5 to 115 per cent.) at a probability level of 5 per cent. These limits seem to us very satisfactory for an assay based on 16 observations and using only four Petri dishes. This may be compared with the fiducial limits, 94 to 105·5 per cent. at the same 5 per cent. level of probability of a typical riboflavine assay on a sample of malt with *L. casei* ϵ, with 3 levels of standard including the blank, 2 levels of test material, 4 tubes at each level and the usual acidimetric technique—a total of 20 observations.[7]

TABLE III

VARIANCE ANALYSIS OF FOUR-POINT CUP-PLATE ASSAY (RIBOFLAVINE) AS IN TABLE II

Treatment:	Degrees of freedom	Sum of squares	Variance
S Slope	1	48·65	48·65
T Test *versus* standard	1 } 3	14·63 } 63·30	14·63
U Unparallelism	1	0·02	0·02
D Plates	3	0·13	0·04
Interactions:			
SL	3	0·33	0·11
TL	3 } 9	0·16 } 0·56	0·05 } 0·06
UL	3	0·07	0·02
Total	15	63·96	

The fiducial limits of a biological assay are a measure of its precision, that is, its reproducibility. If the figures given for the two assays above are representative, it would seem that, for a given number of observations, the acidimetric method evaluated by slope ratio is more precise than the plate cup method evaluated on the usual logarithmic basis. E. C. Wood (private communication) has pointed out that there are theoretical reasons for this, connected with the different ways of distributing observations over doses by the two methods.

ANEURINE ASSAY

The method is similar to that of the riboflavine determination. Inocula are prepared from cultures of *L. fermentum*, as described above, and 1 ml. is used per plate. We have found that inocula of *L. fermentum* grow much less vigorously on freshly prepared than on "matured" media; with the former it may be necessary to use a 1/2 or 1/5 instead of a 1/10 dilution of the 20- to 24-hour growth.

Relation between aneurine concentration and zone diameter—Over a wide range of concentration the widths of the zone diameters were found to be proportional to the logarithms of the concentrations of aneurine. This was found to be true for solution of the pure vitamins and also of yeast and liver extracts. Here, however, valid assays were not possible, for the

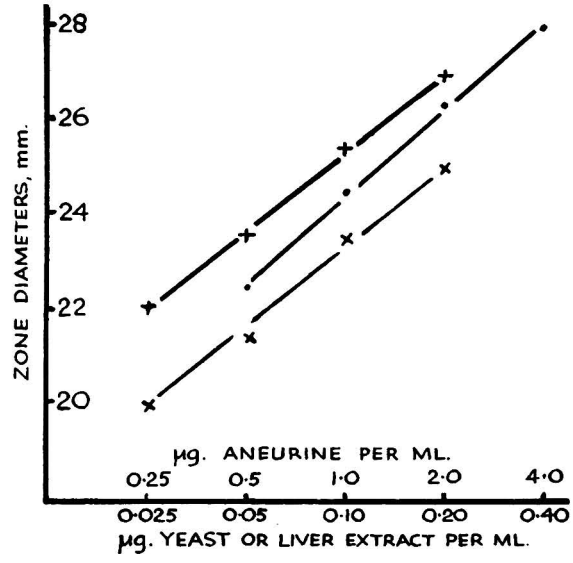

Fig. 2. Response of *Lactobacillus fermentum* P36 to aneurine. On Sarett and Cheldelin medium.
Ordinates: mean zone diameters in mm.
Abcissae: logarithmic plot of
μg. of aneurine per ml.
ml. of yeast extract or liver extract per ml.
Dosage response curves:
upper line — liver extract
lower line — yeast extract
middle line — aneurine solution
Each point represents the mean of four diameters.

response lines from yeast and liver extracts were not parallel to that from pure aneurine solutions (see Fig. 2). It was thought that this might be due to some deficiency in the medium. Some experiments were performed with the Roberts and Snell medium, as in the riboflavine assay, but omitting aneurine. This medium also was not completely satisfactory. There was again a significant departure from parallelism between the dosage - response curves for sample and standard; this was presumably due to the presence in the sample of a growth factor, or more than one, absent from the basal medium. Addition to the Sarett and Cheldelin medium of small amounts of yeast extract treated with sulphite failed to improve matters. Media that are partially deficient may give rise to very misleading results; the zones given by yeast extract on such media may hardly differ at all from those on a complete medium. Fortunately, they are also much less opaque and therefore easily detected.

A further attempt to adapt the method to the assay of yeast extracts was made by adding to both standard and test solution a yeast extract treated with sulphite to destroy aneurine. The amount so added to the standard solutions gave a concentration of 50 per cent. and enough was added to the test solutions (diluted yeast extracts) as well to give in all solutions a final 50 per cent. concentration of sulphite-treated plus untreated yeast extract.

This procedure was found to give log dose - response curves for yeast and aneurine that were not only linear but also parallel. An assay of aneurine carried out on this assumption is illustrated in Fig. 3. Statistical evaluation of the results, by the same procedure as for

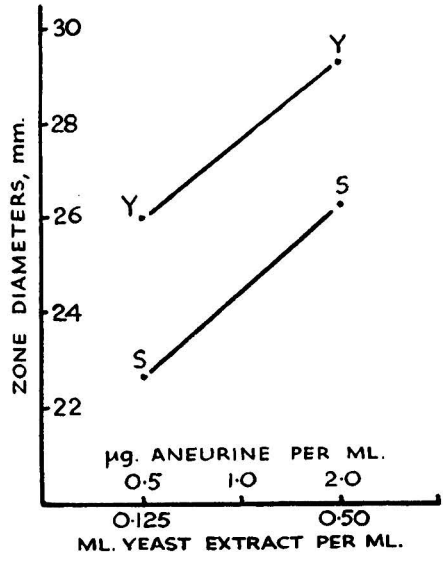

Fig. 3. Assay of aneurine in yeast extract. On Sarett and Cheldelin medium (sulphited yeast extract added to samples).
Ordinates: mean zone diameters, in mm.
Abcissae: logarithmic plot of
ml. of yeast extract per ml.
μg. of aneurine per ml.
Y—Y: yeast extract. S—S: aneurine solution.

the riboflavine assay recorded above, gave a value of 17·8 μg. per ml. for the aneurine content of the extract, and the fiducial limits of the estimate ($P = 0{\cdot}05$) were 14·0 to 23·6 (79 to 133 per cent.). The error is a little higher than was found for the riboflavine assay, though there were 4 more observations (20 on 5 plates). The reason, however, is not far to seek. We had over-estimated the potency of the extract and consequently tested it at too low a level. The mean response indicated that the doses of standard were about twice as high as those of the extracts. In spite of this highly significant difference in response and a just significant variance due to interaction of "plate" with "test *versus* standard," there was no significant departure from parallelism—an indication of the extended range over which there is a linear relation between log dose and zone diameter in this particular assay. Here also there was no significant difference between the mean responses for the individual plates.

Discussion

Although these assay methods employing the cup-plate technique are somewhat lacking in sensitivity, the speed with which they may be carried out can be of value and should at least provide a method for checking the efficacy of normal microbiological assay determinations with certain materials, for it may well be that the effect of non-specific growth stimulants and inhibitors may be different for this and established procedures of microbiological assay. Differences in results obtained in internally consistent assays by this and other techniques might lead to the discovery of otherwise unsuspected sources of error, while visual inspection of growth obtained in the zones may often indicate whether or not basal media are lacking in growth factors or stimulatory substances.

It must, moreover, be emphasised that we do not claim to have done more than open up this particular field for exploration. The organisms used by us were simply those under our hands for use in the ordinary microbiological assays involving acidimetry. Amongst the myriad bacterial strains recognised by the micro-taxonomist, there must surely be dozens of non-exacting ones requiring at most one or two essential organic nutrients—amino acid or vitamin—besides the usual basal pabulum of carbohydrate and salts. These should be

worth examining for their response under the assay conditions we have described; some might well prove to be sufficiently sensitive, without sacrifice of rapidity in growth, to eliminate the main disadvantage of the method. Furthermore, even if no suitable species is found among known organisms, there remains the possibility, nowadays not to be ignored, of producing suitable artificial mutants.

A fundamental study of the conditions existing in a "zone of exhibition" during active growth of an organism under the stimulus of sub-maximal amounts of an essential nutrilite might throw light not only on the basic principles underlying this procedure, but also on those of the cup-plate test for antibiotic action. Such a study would involve considerations of both a biological and a physico-chemical nature. It seems clear, however, that the assay of antibiotics and the estimation of vitamins by the cup-plate procedure differ in one fundamental respect. In the former there is diffusion of the active substance, but, probably even at the lowest concentration, little loss of it by utilisation or destruction. In the latter, on the contrary, the nutrient being assayed may well be utilised just to the extent that it stimulates growth of the test organism: utilisation and diffusion proceed simultaneously in an outward direction and doubtless jointly affect both zone diameter and intensity of bacterial growth.

SUMMARY

1. Conditions have been described for producing sharply defined zones of growth on addition of certain vitamins to deficient media inoculated with suitable organisms.
2. The relations between inoculum density, concentration of test solution and diameter of growth zone have been investigated.
3. An assay method suitable for determination of riboflavine has been developed and a method of estimating aneurine has been tried.
4. The method employed by Cheldelin *et al.* to maintain the aneurine requirement of *Lactobacillus fermentum* has been tested and confirmed.
5. The relative insensitivity of the cup-plate assay method has been discussed, as well as its potentialities.

We wish to acknowledge the valuable technical assistance of Mr. N. McLeod in the experimental work described here and also to thank Dr. E. C. Wood for helpful discussions of certain methodological problems.

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The Teaching and Applications of Microchemistry in Great Britain

THREE years ago Dr. Cecil L. Wilson conducted a survey, under the auspices of the Committee of the Microchemistry Group of the Society, of the facilities afforded for studying microchemistry by university colleges and technical schools in this country. The result of this investigation, which showed that the general situation, although by no means discouraging and in many ways better than had been anticipated, still left room for improvement in many directions, was published in *Nature*, 1945, **156**, 588.

A second report by Dr. Wilson, covering the results of a survey designed to co-ordinate courses of study in microchemistry with the uses made of microchemical methods in industry, was published in *Chemistry and Industry*, 1947, 353–355. In this, 53 replies to a circular letter, received from a total of 160 that were sent to the members of the Group, are classified and arranged in six tables showing the types and numbers of the laboratories that use microchemical methods, the type and distribution of the methods used and the advantages and disadvantages that accompany their use.

Amongst the various types of laboratories using microchemical methods, those of research institutions (this term including Government establishments, university research laboratories and the like) head the

list, and are followed by chemical manufacturers, consultants and public analysts. In the lists showing the types and distribution of the various methods in use, colorimetric methods are those most favoured, and then follow inorganic volumetric, organic quantitative, inorganic gravimetric, physical, inorganic qualitative, chromatographic, methods of gas analysis and miscellaneous.

In addition to supplying specified information, the members of the Group were asked to express their opinion upon the subject of the enquiry in general. The replies received were favourably inclined towards the use of microchemical methods and to the Group's activities in encouraging facilities for the promotion of the technique.

The general results of the enquiry conducted by Dr. Wilson may be summarised, briefly, as follows. (*a*) Colorimetric methods are the most favoured of microchemical methods. (*b*) In the laboratories that use microchemical methods they are of general rather than of special application and, having been once introduced, their scope has a tendency to widen, although a micro-method is not adopted unless it shows some advantage compared with an existing full-scale method. (*c*) A significant number of the replies emphasised the necessity for close collaboration between the teaching of microchemical technique and its industrial application.

The results of the enquiry, as a whole, although admittedly confined to chemists with a bias towards microchemistry, is considered to be of sufficient interest to be extended to cover a wider field, one wide enough to include chemists who are not, as yet, confessedly "micro-minded" as well as those whose work in hospital laboratories involves microchemical routine but who were not specially included in this enquiry. With that object in view Dr. Wilson wishes the publication of the report to be taken as an invitation to the chemists of this country, whether at present users of microchemistry or not, to supply any information, in general terms, without detailed descriptions of specific methods, that would give a fuller picture of its uses. Communications should be addressed to Dr. Cecil L. Wilson, Microchemical Laboratory, Chemistry Department, The Queen's University of Belfast, Northern Ireland. F.L.O.

Ministry of Food

STATUTORY INSTRUMENTS

1948—No. 1509. The Meat Products and Canned Meat (Control and Maximum Prices) Order 1948. Dated July 4th, 1948. Pp. 18. Price 5d.

This Order replaces the Meat Products and Canned Meat (Control and Maximum Prices) Order, 1947, as amended (S.R. & O., No. 162 of 1947; ANALYST, 1947, 72, 111). With the exception of items removed from price control and changes in maximum prices, the following are the principal changes in the Order—Ravioli and imported salmi sausages are made excepted products, i.e., there is no restriction on their manufacture or sale. The substance of the first schedule is now as follows—

Minimum meat content of specified foods (not canned).

Pork sausages, pork sausage meat, pork slicing sausage: 50 per cent. of which at least 80 per cent. shall consist of pork.

Beef sausages, beef sausage meat, beef slicing sausage: 50 per cent.

Cooked sausages of the following descriptions:—luncheon sausage, breakfast sausage, and polony: 30 per cent.*

Meat roll or galantine: 30 per cent.

Liver sausage: 45 per cent.

Ministry of Health

FOOD AND DRUGS ACTS, 1938 AND 1944

Amendment Regulations transferring certain Functions to the Minister of Food

IN Circular 81/48, dated May 31st, 1948, the Ministry of Health directs attention to certain amendments consequent on the Transfer of Functions (Food and Drugs) Order, 1948 (see this vol., pp. 99–100), in Regulations made under or kept in operation by the Food and Drugs Act, 1938. The amendments are embodied in seven Amendment Regulations (Statutory Instruments, 1948, Nos. 1117 to 1123†) issued jointly by the Minister of Health and the Minister of Food, after consultation with the Local Authorities' Associations. They came into operation on June 1st, 1948. The Circular gives the following information about these Amendment Regulations.

Milk (Special Designations) Amendment Regulations, 1948 (S.I., 1948, 1117). In accordance with Article 6 (2) of the Transfer of Functions (Food and Drugs) Order, 1948, these Amendment Regulations provide that any licence granted under the Regulations to a local authority, if not a producer's licence, shall be granted by the Minister of Food, and that the Minister of Food shall determine any appeal against a local authority's decision to refuse, suspend or revoke any licence not being a producer's licence. Where

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* *This paragraph differs from the corresponding paragraph of S.R. & O., No. 162 of 1947.*

† Obtainable at H.M. Stationery Office, price 1d. each.

a producer's licence is concerned, both these functions will remain with the Minister of Health until the Food and Drugs (Milk and Dairies) Act, 1944, comes into operation.

By the remaining Amendment Regulations, functions hitherto given in the relevant Regulations to the Minister of Health have in almost every case been transferred to the Minister of Food. Among these changes, attention is called more particularly to the following—

Amendment Regulations.	*Functions transferred to the Minister of Food.*
Public Health (Condensed Milk) Amendment Regulations, 1948 (S.I., 1948, 1122).	Allowing modification of declaration on labels of tins or other receptacles containing condensed or dried milk. Public Health (Condensed Milk) Amendment Regulations, 1927, Regulation 4, and Public Health (Dried Milk) Amendment Regulations, 1927, Regulation 4.
Public Health (Dried Milk) Amendment Regulations, 1948 (S.I., 1948, 1123).	Receipt of information from the local authority in whose district a consignment of condensed or dried milk is deposited which does not comply with the Regulations, when it has been ascertained that the consignment was manufactured or labelled at a place not in England or Wales. Public Health (Condensed Milk) Regulations, 1923, Regulation 7, and Public Health (Dried Milk) Regulations, 1923, Regulation 7.
Public Health (Preservatives, etc., in Food) Amendment Regulations, 1948 (S.I., 1948, 1118).	Allowing modification of declaration upon labels required for certain foods containing preservative, and for an article sold as a preservative. Public Health (Preservatives, etc., in Food) Regulations, Second Schedule, paragraphs 2 and 3.
Public Health (Imported Food) (Amendment No. 2) Regulations, 1948 (S.I., 1948, 1121).	Recognition of an Official Certificate. Public Health (Imported Food) Regulations, 1937, Regulation 2 (1).
Public Health (Meat) Amendment Regulations, 1948 (S.I., 1948, 1119).	Authorisation of Meat Marking Schemes and approval of marks. Public Health (Meat) Regulations, 1924, Regulations 15 and 16.

Public Health (Shell-fish) Amendment Regulations, 1948 (S.I., 1948, 1120). Local Authorities are required under Regulations 7 and 10 (1) of the Public Health (Shell-fish) Regulations, 1934, to inform the Minister of Health and the Minister of Agriculture and Fisheries of action taken by them under those Regulations. The Amendment Regulations require this information to be given also to the Minister of Food.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Estimation of Solanine in the Potato. M. J. Wolf and B. M. Duggar (*J. Agric. Res.*, 1946, 73, 1–32)—The method for the assay of potato tubers is a modification of that of Bömer and Mattis (*Z. Unters. Nahr. Genussm.*, 1924, **47**, 97; ANALYST, 1924, **49**, 284).

Procedure—Finely grind four or more tubers, according to size, in a Nixtamal mill, thoroughly mix the resulting pulp for about 5 min., and then quickly pour off two portions of about 250 g. each. Add an equal weight of 0·25 per cent. acetic acid, allow the mixture to stand at room temperature with occasional stirring for 0·5 to 1 hr., and then squeeze it out through three thicknesses of cheese cloth in a hydraulic press at 5,000 lb. per sq. in. Extract the pad of ground tissue three more times in the same manner. To prevent enzymic activity, make the combined extracts slightly ammoniacal as soon as they are prepared and, finally, add 10 g. of diatomaceous earth and stir the mixture well.

Evaporate the extract at 85° C. and, when only a small amount of liquid remains, rinse down the sides of the container with a small quantity of 0·25 per cent. acetic acid, make the liquid slightly ammoniacal, stir well, and complete the evaporation. Grind the residue in a mortar and extract it with alcohol in a Soxhlet extractor for 18 hr. Re-grind the residue and repeat the extraction for a further 18 hr. Evaporate the combined extracts to dryness, take up the residue quantitatively with several 25-ml. portions of 0·25 per cent. acetic acid, filter, adjust the filtrate to *p*H 10 to 10·4 with aqueous ammonia solution, and precipitate the solanine by digesting the liquid on the water-bath for 20 to 30 min. Collect the alkaloid on filter paper, purify it by dissolution in 0·25 per cent. acetic acid (100 ml.) adjusting the solution to *p*H 10 to 10·4, and precipitating it as before, repeating this purification three times until a perfectly white precipitate is produced. Collect this on a tared filter paper, wash it with three 15-ml. portions of 2 per cent. aqueous ammonia solution, and dry to constant weight. The product is free from ash but may contain small amounts of solanidine and solanthrene if any are present in the tissues. Numerous tests indicated a decomposing point of about 240° C. (the m.p. of pure solanine is 285° C., with decomposition). The standard error of the method was 0·120 mg. of solanine per 100 g. of fresh tissue.

With tissue other than tubers, dry the material at 85° to 90° C. After 48 hr., grind the dried tissues in a Nixtamal mill or in a mortar, and store the powdered material in a stoppered bottle, weighing

re-dried portions as required. Extract three times by boiling for 0·5 to 1 min. with 50 ml. of 1 per cent. acetic acid, filtering each extract under moderate suction and washing the residue with 1 per cent. acetic acid. Adjust the *p*H of the combined extracts to 10 to 10·4 with aqueous ammonia solution, and digest on the water-bath for 30 min. Collect the flocculent precipitate on Whatman No. 42 paper with light suction, and extract the paper and residue three times by boiling in 25-ml. portions of absolute alcohol. Evaporate the combined alcoholic extracts to dryness on the water-bath, heat the residue with about 50 ml. of 0·5 per cent. acetic acid (or sulphuric acid), and make the combined filtrates and washings up to 100 ml. (50 ml. for stem tissues, which are of low solanine content). Estimate the solanine in these colorimetrically by the method employed semi-quantitatively by Alberti (*Z. Unters. Lebensm.*, 1932, **64**, 260; ANALYST, 1932, **57**, 726) and adapted to the quantitative determination of solanine in potato tuber extracts by Pfankuch (*Biochem. Z.*, 1937, **295**, 44). Add 6 ml. of concentrated sulphuric acid drop by drop from a burette to a 3-ml. aliquot of the acid extract while it is being rotated vigorously in an ice-bath. Allow the yellow solution to stand for 1 min. and add, drop by drop, 3 ml. of 1 per cent. formaldehyde solution, prepared by careful dilution of commercial formalin assayed by the A.O.A.C. method. The yellow colour changes through orange and red to violet-red. Estimate the optical density of this solution, which becomes stable after about an hour and a half, in an Evelyn photo-electric colorimeter with filter No. 540. The molar extinction coefficients of solanine and solanidine coincide very closely at all wavelengths, indicating that the production of the red-violet colour is independent of the sugars on the solanidine nucleus. Solanthrene, however, has a much lower absorption in the green, yellow, and orange regions of the spectrum, but the general form of the absorption curve is the same in all three compounds exhibiting a maximum at 560 to 570 mμ. A filter for this region was not available, and therefore the 540 filter, in which about 95 per cent. of the light transmitted is between 515 and 570 mμ., was used.

To prepare the calibration curve, plot the optical densities of known solanine solutions against the solanine concentration. The values obtained conform to Beer's law, particularly those from 0·05 to 0·8. From the curve the value of the combined constant *kl* (the slope) in the equation $\log.I_o/I = Klc$ is 4·7, so that the concentration of solanine (*c*, in mg. per ml.) in unknown solutions may be obtained from the curve or may be calculated by dividing the optical density by 4·7. The most accurate results are obtained at concentrations of 0·01 to 0·09 mg. per ml. and readings should generally be taken within this range, the solutions being diluted if necessary.

In normal tubers, solanine occurs in highest concentration in the peripheral zone comprising the periderm and cortical parenchyma. Little or none occurs in the pith and only small amounts in the intermediate region. In 32 varieties, the concentration ranged from 2 to 13 mg. per 100 g. of fresh tuber, but tubers of *Solanum commersonii* had as much as 520 mg. per 100 g. of tissue. The concentration falls as the season advances.

A. O. JONES

Amperometric Determination of Dissolved Oxygen in Orange Juice. V. M. Lewis and H. A. McKenzie (*Anal. Chem.*, 1947, **19**, 643–646)—The oxygen content of orange juice is sufficient to give a polarographic step suitable for analytical purposes. Owing to the presence of citric acid, the second step is absent. As the polarogram shows a steady diffusion current in the range from −0·3 to −0·7 v. *versus* the saturated calomel electrode, an amperometric method can be used, and the current flowing between a dropping mercury electrode as cathode and a saturated calomel electrode at an applied potential of −0·4 v. has been shown to be proportional to the oxygen concentration, as determined by the Van Slyke manometric method. Since the solubility of oxygen in orange juice is affected by the total solids present, it is necessary to determine the latter. The diffusion coefficient is affected slightly by the solids present owing to the variation in the viscosity of the medium; temperature has a small effect.

Procedure—Collect the sample and transfer it to a polarographic cell, carefully avoiding any contact with air. After measuring the diffusion current at −0·4 v. *versus* the saturated calomel electrode, remove the dissolved oxygen by passing hydrogen through the liquid for 30 min., and then measure the residual current. Record the temperature of the sample and determine the total solids with a Brix hydrometer or a refractometer. The oxygen concentration is calculated from a table of values calculated by means of the manometric method.

Amounts of about 0·02 ml. of oxygen per 100 ml. of orange juice can be determined with an accuracy to within ±5 per cent., and amounts as low as 0·002 ml. of oxygen per 100 ml. of sample can be detected. Dissolved metals in canned samples do not interfere.

J. G. WALLER

Component Acids and Glycerides of Australian Lumbang Oil. F. D. Gunstone and T. P. Hilditch (*J. Soc. Chem. Ind.*, 1947, **66**, 205–208)—Kernels of the lumbang (candlenut) tree from North Queensland yielded 62 per cent. of oil, with the following characteristics and constants: iodine value, 164·3 per cent.; free fatty acid (as oleic), 0·3 per cent.; n_D^{25}, 1·4771; saponification value, 190·5; unsaponifiable matter, 0·4 per cent. Its component glycerides include about 32 per cent. containing three linoleic or linolenic groups and about 53 per cent. containing two of these and an oleic or a saturated acid group: all 85 per cent. of mixed glycerides probably contain one or two linolenic groups. The oil yields the following weight percentages of component acids: palmitic, 5·5; stearic, 6·7; arachidic, 0·3; oleic, 10·5; linoleic, 48·5; and linolenic, 28·5.

E. B. DAW

Component Acids of West Indian Ben and Mango Seed Oils. H. C. Dunn and T. P. Hilditch (*J. Soc. Chem. Ind.*, 1947, **66**, 209–211)—Ben (Behen) oil, the seed fat of *Moringa oleifera*,

obtained from the kernels of Trinidad seed, had the following characteristics: iodine value, 67·1 per cent.; free fatty acid (as oleic), 6·5 per cent.; unsaponifiable matter, 1·1 per cent.; n_D^{25}, 1·4650. Weight percentages of its component acids were calculated as: palmitic, 5·5; stearic, 7·8; arachidic plus behenic plus lignoceric, 9·2; hexadecenoic, 0·9; and linoleic, 0·8. Oil in kernels, 30 per cent.; in whole seeds, 21 per cent. Its composition suggests that, if available in suitable quantity and quality, it would be an excellent substitute for edible olive oil. Kernels of Jamaican mango seeds yielded 6 per cent. of fat; the Jamaican fruit-coat (rind and pulp) contained only traces (about 0·2 per cent.) (iodine value, 91 per cent.). Indian mango seed recently examined (Pathak, Gunde and Gudhole, in the press) contained 10 per cent. of fat. The constants of Jamaican (*a*) and Indian (*b*) fat also differ, being: iodine value (*a*) 49·1 per cent., (*b*) 39·2 per cent.; free fatty acids (as oleic) (*a*) 3·3 per cent., (*b*) 10·0 per cent.; unsaponifiable matter (*a*) 2·3 per cent., (*b*) 2·9 per cent. The weight percentages of component acids of the West Indian mango fat are: palmitic, 4·4; stearic, 42·5; arachidic, 3·0; oleic, 44·7; and linoleic, 5·4. Linoleic glycerides appear to be absent from the Indian fat. The high stearic acid content is unusual for fats from this botanical family. E. B. DAW

Determination of Calomel in Pills. G. J. W. Ferrey (*Quart. J. Pharm.*, 1947, **20**, 237–240)—This method for determining calomel in presence of a large proportion of vegetable matter is dependent on the destruction of the latter by bromine in acetic acid solution, and the subsequent conversion of the calomel to mercury metal, which is then estimated in the usual manner.

Procedure—Weigh 20 pills, reduce them to powder, and heat a quantity equivalent to 8 pills with 30 ml. of glacial acetic acid and 2 ml. of nitric acid under a reflux condenser until the mass is disintegrated. Cool, add 15 ml. of bromate-bromide reagent (2·5 g. of potassium bromate and 10 g. of potassium bromide in 100 ml. of water), and boil under a reflux condenser until the calomel is dissolved. Filter the hot solution and wash the flask and filter with about 30 ml. of a mixture of 2 volumes of glacial acetic acid and 1 volume of water. To the combined filtrate and washings add 3 g. of zinc filings, boil gently under a reflux condenser for 15 min., wash the condenser with 25 ml. of warm water, and boil for a further 5 min. Filter while hot, retaining the amalgam as far as possible in the flask, wash the amalgam first with the hot acetic acid-water mixture and then with hot water until the washings are free from halogen. Transfer the filter paper and its contents to the flask containing the amalgam, add 20 ml. of water and 20 ml. of nitric acid, and boil gently until the amalgam is dissolved and nitrous fumes are removed. Cool, add dropwise sufficient potassium permanganate solution to give a permanent pink colour, decolorise with a drop of hydrogen peroxide, and titrate with 0·1 *N* ammonium thiocyanate using ferric alum as indicator. A. H. A. ABBOTT

Assay of Fuller's Earth for Montmorillonite Content. W. Smith (*Quart. J. Pharm.*, 1947, **20**, 367–372)—Clays contain many distinct materials the most important of which, pharmaceutically, are kaolinite $Al_2O_3,2SiO_2,2H_2O$ and montmorillonite $Al_2O_3,4SiO_2,H_2O$. Montmorillonite is the principal constituent of bentonite and fuller's earth, but it does not occur in kaolin. Because of the difference in crystalline structure of montmorillonite and kaolin the former shows a greater capacity for base exchange, and this property is used to distinguish between the two minerals. By thorough standardisation of all conditions, the method is capable of producing trustworthy results, expressed as grams of barium sulphate per gram of dried clay material.

Procedure—Stir mechanically for 1 hr., 1 g. of the fuller's earth, previously dried at 105° C., with 100 ml. of *N* barium chloride adjusted to *p*H 8·2 with triethanolamine. Filter, and wash the residue with 150 ml. of *N* barium chloride, adjusted to *p*H 8·2, and then with water until the filtrate is free from chloride. Wash the residue from the filter into a beaker with water, dilute to 250 ml. with water, add 12·5 g. of ammonium chloride, stir mechanically for 1 hr., and filter. Wash the residue with 0·05 *N* ammonium chloride until 1 drop of the washings gives no reaction for barium with 1 drop of freshly prepared, 0·1 per cent. aqueous solution of sodium rhodizonate. Dilute the filtrate and washings to about 500 ml., add 5 ml. of concentrated hydrochloric acid, boil, and add a slight excess, about 5 ml., of dilute sulphuric acid. Boil to coagulate the precipitate and allow to stand overnight. Then (1) filter through a filter paper, wash well until free from chloride, ignite and weigh the barium sulphate, or (2) filter through a sintered-glass crucible, wash well until free from chloride, then with alcohol, and finally with anhydrous ether, dry, and weigh.

Results—Results may be recorded as grams of barium sulphate obtained from 1 g. of the dried sample by the above method. Sometimes it is more convenient to express the results as mg.-equivalents of barium per 100 g. of the dried sample, and for this purpose the weight of barium sulphate obtained

TABLE I

Material	Barium sulphate obtained g.
Kaolin, heavy B.P.	0·0042
Kaolin, heavy B.P.	0·0050
Kaolin, light B.P.	0·0050
Osmo Kaolin	0·0060
Floridin filtrol	0·0250
Floridin earth XS grade	0·0227
Floridin earth XXS grade	0·0245
Kaogel	0·1050
Decalso, fine powder	0·2572

from 1 g. of the dried material should be divided by 0·001167. The approximate montmorillonite content may be calculated from the weight of barium adsorbed by the use of the arbitrary factor 153, and this method is advocated by the Fuller's

Earth Union. Results obtained by the proposed method on various types of clays, zeolites, adsorbing materials, etc., other than fuller's earth are given in Table I, whilst corresponding figures for various grades of fuller's earth, activated fuller's earth, and bentonite are shown in Table II.

TABLE II

Grade	Barium sulphate obtained g.
White earth A	0·1116
White earth B	0·0762
Surrey powder	0·1087
Redhill natural earth	0·0932
Fulbent 182	0·2806
Fulbent 150	0·3220
Fuller's earth 249	0·0700
Fuller's earth KN11	0·0646
Fuller's earth TB20	0·1402
Fuller's earth CT200	0·0800
Fuller's earth SP4	0·0834
Lloyds reagent (1)	0·1134
Lloyds reagent (2)	0·1062
Bentonite (clay spur) (1)	0·1170
Bentonite (clay spur) (2)	0·1174
North African bentonite	0·0888

A. H. A. ABBOTT

Colorimetric Method for Jaborandi Alkaloids. W. F. Elvidge (*Quart. J. Pharm.*, 1947, **20**, 234–237)—The qualitative colour test published by Ekkert (*Pharm. Zentr.*, 1925, **66**, 36) has been suitably modified to adapt it as a quantitative colorimetric method for 1 to 5 mg. of pilocarpine. The alkaloids present in jaborandi consist mainly of pilocarpine and *iso*pilocarpine, together with smaller quantities of pilocarpidine and pilosine. All the alkaloids except pilosine give the same colour as pilocarpine and the colorimetric method is therefore recommended as giving reasonably accurate results in the determination of total alkaloids of jaborandi, calculated as pilocarpine.

Procedure—Introduce an aliquot of the sample solution, containing from 1 to 5 mg. of pilocarpine, into a 25-ml. volumetric flask, add 1 ml. of 2 per cent. sodium nitroprusside solution, 1 ml. of *N* sodium hydroxide, allow to stand for at least 3 min., and add 5 ml. of 0·01 *N* potassium permanganate. Immediately add 3 ml. of 3 *N* sulphuric acid, make up to 25 ml. with distilled water, and read in a suitable colorimeter (for the Spekker absorptiometer use 4-cm. cells and Ilford Spectrum green filter 604). At the same time conduct a blank and a series of determinations on solutions containing 1, 2, 3, 4, and 5 mg. of pilocarpine; the standard pilocarpine solution, 1 mg. per ml., is prepared by making a 0·13 per cent. solution of pilocarpine nitrate B.P. From the figures so obtained prepare a calibration curve and from it calculate the pilocarpine content of the sample.

Although the full colour develops immediately and only slowly loses its intensity, readings should as a precautionary measure be taken within 5 to 10 min. of colour development. A. H. A. ABBOTT

Catalytic Evolution of Hydrogen at the Dropping Mercury Cathode due to the Amide of Nicotinic Acid and Other Pyridine Derivatives. E. Knobloch (*Coll. Czech. Chem. Comm.*, 1947, **12**, 407–421)—Pyridine-carbonic acids and their derivatives give rise to polarographic reduction waves that can be used for analytical purposes. Determinations can be made in neutral or alkaline solutions, 0·1 *N* potassium chloride or 0·1 *N* sodium hydroxide being used as supporting electrolyte. The method is applicable to the determination of nicotinamide in medicinal preparations.

In addition to the reduction wave, nicotinamide produces a catalytic wave at low *p*H values. Similar catalytic effects are shown by pyridine, piperidine, quinoline, nicotinic acid and its methyl ester, picolinic acid, γ-aminopyridine, and aniline.

J. G. WALLER

Biochemical

Quantitative Determination of Amphetamine. W. D. McNally, W. L. Bergman, and J. F. Polli (*J. Lab. Clin. Med.*, 1947, **32**, 913)—Methods for estimating amphetamine in organic material have been investigated. A shortened, more satisfactory technique employing a combined method of purification, distillation, and diazotisation is given.

Diazo reagent—To 5 ml. of a solution of *p*-nitroaniline hydrochloride in 3 per cent. hydrochloric acid, add 1 ml. of concentrated hydrochloric acid, place the mixture in ice for 10 min., and add 3 ml. of a 0·7 per cent. aqueous solution of sodium nitrite. Place the mixture in the ice-bath for 6 min., dilute to 100 ml. with distilled water, mix, and return to the ice-bath for 10 min. If stored at 0° to 4° C., this reagent will keep for 2 weeks, but it must be standardised against a solution of amphetamine sulphate containing 0·03 mg. per ml.

Procedure—Add 150 ml. of distilled water to 25 g. of blood, urine, or finely minced organs, shake well for 5 min., and allow to stand for 10 min. Add 10 ml. of 10 per cent. sodium hydroxide solution, shake well, and add 30 ml. of 10 per cent. sodium tungstate solution, and shake thoroughly again. Add slowly 30 ml. of *N* sulphuric acid, shaking continuously, acidify with 18 *N* sulphuric acid, and add 5 ml. in excess. Allow to stand for 15 min., filter, transfer the filtrate to a litre round-bottomed flask, neutralise with 10 per cent. sodium hydroxide solution, and add 1 ml. in excess. Steam-distil and collect 200 ml. of distillate in a flask containing 10 ml. of 4 *N* sulphuric acid. Transfer the distillate to a separating funnel, make alkaline with 10 per cent. sodium hydroxide solution and extract with four successive 20-ml. portions of ether. Combine the ether extracts, wash with water, extract the ether with three successive 10-ml. quantities of 0·5 per cent. hydrochloric acid, evaporate the acid extract to dryness on a steam-bath, and diazotise in the following way. Dissolve the residue in 1 ml. of water, add 5 ml. of cold diazotising reagent, and 5 ml. of 1·1 per cent. sodium carbonate solution, drop by drop, and allow

to stand for 15 min. Add 1 ml. of 10 per cent. sodium hydroxide solution dropwise, and set aside for 10 min. Dilute to 40 ml. with water, mix, add 10 ml. of *n*-butanol, and mix by inversion. Allow the coloured layer to clear, read the transmission in a spectrophotomer at 530 mμ., and calculate the amount of amphetamine present from a standard curve.

Amphetamine may be identified in an aliquot part of the distillate by preparing the picrate (m.p. 142° to 143° C.) or the picrolonate (m.p. 195° to 196° C.).

Neither nicotine nor pyridine interferes with the above technique. A. H. A. ABBOTT

Graff Modification of the Vickery - White Procedure for the Estimation of Cystine. J. Schultz and H. M. Vars (*J. Biol. Chem.*, 1947, **167**, 715–719)—The Vickery - White estimation of cystine is based on the determination of the sulphur content of the copper - cysteine precipitate produced with cuprous oxide. The Graff modification (Graff, Maculla, and Graff, *Ibid.*, 1937, **121**, 71) is based on the determination of nitrogen in the precipitate by the micro-Kjeldahl method; the method used for forming the precipitate (reduction with zinc, precipitation at *p*H 4·0 in presence of acetate, and washing with dilute citrate - acetate buffer at *p*H 3·6) gives such a pure product that a nitrogen estimation can be substituted for the sulphur analysis previously recommended. Whereas Graff found that no non-cysteine nitrogen was precipitated by cuprous oxide from the reduced hydrolysates of the various proteins studied, it has been reported recently that hydrolysates of nucleoproteins, keratin, and gelatin precipitated more nitrogen in the cuprous mercaptide than could be accounted for from the sulphur present in the mercaptide, or from the cysteine originally present.

Accordingly, various proportions of a series of standard solutions of cystine, xanthine, guanine, and gelatin were analysed for (1) purine-nitrogen by the method of Graff and Maculla (*Ibid.*, 1935, **110**, 71); (2) cystine-nitrogen; and (3) cystine-sulphur both as described by Graff, Maculla, and Graff (*loc. cit.*). The methods gave satisfactory results on pure solutions of single substances, and the determination of purines was not affected by the presence of cystine. The copper - cysteine precipitate, however, contained an amount of nitrogen equal to the cystine-nitrogen plus the purine-nitrogen of the original solution; furthermore, there was little destruction of purine after 12 hr. hydrolysis with 21 per cent. hydrochloric acid in presence of gelatin, and the purine of hepatic protein was not completely destroyed even after 30 hr.

In order to confirm that the Graff modification of the Vickery - White procedure estimates non-cysteine nitrogen, and that this nitrogen is purine-nitrogen, a series of analyses was carried out on the precipitates obtained from the test solutions used previously. The precipitates were analysed for total nitrogen, and total sulphur; they were also treated with trichloroacetic acid and alkali, and re-precipitated; nitrogen was determined in the new precipitate, which was found to contain less nitrogen than the original precipitate by an amount corresponding to the cystine-nitrogen originally added. Further, the difference between the total nitrogen in the original precipitate and the cystine-nitrogen calculated from the sulphur content corresponded to the purine-nitrogen originally added. Similar agreements were obtained in analyses of the cuprous oxide precipitates from hepatic nucleoprotein hydrolysates.

On the basis of these results it is recommended that, until conditions in which purines are destroyed in the presence of cystine are found, only the sulphur content of the cuprous oxide precipitate from reduced protein hydrolysates be taken as a measure of the cystine present.

G. R. PRIMAVESI

Determination of Guanidoacetic Acid and Arginine in Human Urine and Serum. H. D. Hoberman (*J. Biol. Chem.*, 1947, **167**, 721–727)—Arginine and guanidoacetic acid were estimated in biological materials by developing the Sakaguchi colour before and after treatment with arginase. Guanidoacetic acid was used as the colorimetric standard. Arginase specifically destroys L-(+)-arginine, whereas adsorbents such as permutit may also adsorb guanidoacetic acid. The method was tested on synthetic solutions prepared to simulate human urine in so far as the concentrations of urea, creatinine, and guanidoacetic acid are concerned, with the addition of L-(+)-arginine. The arginine was quantitatively hydrolysed and the guanidoacetic acid was quantitatively recovered. The presence of relatively large amounts of creatine did not affect either the hydrolysis of arginine or the recovery of guanidoacetic acid.

The application of the test to normal male human urine, both fresh and 24-hr. samples, showed that the intensity of the Sakaguchi colour was unaffected by arginase treatment. Although quantitative recovery of arginine was incomplete at low concentrations, the results indicated that normal male urine contains less than 15 mg. of L-(+)-arginine per litre. Guanidoacetic acid can be quantitatively recovered from urine to which L-(+)-arginine has been added, when the solution has been treated with arginase before estimation.

Dubnoff (*Ibid.*, 1941, **141**, 711) and Albanese and Frankston (*Ibid.*, 1945, **159**, 185) have reported excretion of arginine, by normal adult males, of from 38 to 108 mg. per day, and have found no urine free from arginine. The author suggests that the discrepancy between these results and his may be due to the removal of guanidoacetic acid, as well as arginine, by Permutit. Neither beef nor calf arginase hydrolyses D-(−)-arginine, but the presence of such amounts of this isomeride in urine seems unlikely. It still remains to be proved whether or not the Sakaguchi-positive compound estimated was methylguanidine.

The arginase method was applied to serum dialysates. The amount of arginine found agreed well with the results of Hier and Bergeim (*Ibid.*, 1946, **163**, 129), who used a microbiological method

for arginine. Added guanidoacetic acid was quantitatively recovered from serum after arginase treatment. The concentration of L-(+)-arginine in five normal male sera during the post-absorptive period was 2·0 to 2·4 mg. per 100 ml. The concentration of guanidoacetic acid in the same sera was 0·24 to 0·28 mg. per 100 ml. The analysis of whole blood (the blood being laked during dialysis) gave values for guanidoacetic acid equal to those obtained on the serum. The concentration of arginine found, however, indicated that more than 90 per cent. of the arginine present in the serum must have been hydrolysed by the arginase, the presence of which in the red cells has recently been demonstrated by van Slyke and Archibald (*Ibid.*, 1946, **165**, 293).

Preparation of arginase—Take fresh beef or calf liver, strip off connective tissue and fat, and cut it into small pieces. Add an equal weight of finely chopped ice, and convert to a fine suspension in a Waring blendor. Press the ice-cold suspension through cheese-cloth and add 0·4 volume of cold acetone. Filter by gravity in an ice-box overnight. To the slightly turbid filtrate add 0·23 volume of cold acetone, and allow the precipitate to form for 1 to 2 hr. Remove the supernatant liquor by siphoning and centrifuging in the cold. Dissolve the precipitate in an equal volume of tap-water and dialyse overnight against cold, running tap-water. Make the dialysed solution up to 500 ml. per lb. of liver used. Quickly heat to 56° to 58° C. for 3 min. and chill promptly. Clarify by centrifuging in the cold. One preparation was found to require further dilution before use owing to contamination by enzymes that produced sufficient ammonia to inhibit the development of the Sakaguchi colour. Dilution reduced the amount of ammonia to a level tolerated by the reagent and still left enough arginase to hydrolyse a 2·5-ml. portion of urine containing 0·5 g. of L-(+)-arginine monohydrochloride per litre.

Stored in an ice-box, stock solutions of arginase remained useful for as long as 6 weeks. The arginase need not be activated by either cobalt or manganese. No attempt was made to work out the optimum *p*H range for arginase activity.

Procedure for urine—Put 2·5 ml. of urine in a 25-ml. volumetric flask. Add 2·5 ml. of 0·25 *M* phosphate buffer, prepared according to van Slyke and Cullen (*Ibid.*, 1916, **24**, 117), 1 drop of capryl alcohol, and 1 ml. of arginase solution. Incubate at 37° C. for 1 hr., cool to room temperature, and dilute to 25 ml. Transfer an 8-ml. portion to a 15-ml. conical centrifuge tube and add 1 ml. of water-saturated chloroform. Stopper, wrap the tube in a towel, and shake vigorously in a bottle shaker for 10 min. Centrifuge for 5 min. and withdraw 4 ml. of the clear, supernatant liquid with a pipette. Carry out the Sakaguchi reaction as described by Dubnoff and Borsook (*Ibid.*, 1941, **138**, 381) with Sims's modifications (*Ibid.*, 1945, **158**, 239). Use an Evelyn photometer with the 6-mm. aperture and 540 M filter.

Serum analysis—Allow venous blood, obtained under oil, to clot for 20 to 30 min., and then centrifuge at low speed for 20 to 30 min. Dialyse 2 ml. of the serum against 5 ml. of distilled water for 3 hr. at room temperature in the apparatus of Hamilton and Archibald (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 136). At the same time, dialyse a 4-ml. portion to which 0·1 ml. of arginase solution has been added. At the end of the dialysis, pipette 4-ml. portions from the outer chamber and analyse directly by the Sakaguchi test as above. (*Note*—In one preparation of arginase the blank correction was equivalent to 0·018 mg. of guanidoacetic acid per 100 g. in the solution taken from the outer chamber.) G. R. PRIMAVESI

Colorimetric Determination of Sulphur Dioxide. W. M. Grant (*Anal. Chem.*, 1947, **19**, 345–346)—The sensitive colour reaction described by Steigmann (*J. Soc. Chem. Ind.*, 1942, **61**, 18; ANALYST, 1942, **67**, 240) can be used for determining sulphur dioxide in concentrations up to 10 μg. per ml. in solution or in an alkaline extract or distillate of certain biological materials. The method is simple and sensitive. The reaction, in which a red colour is formed by the action of sulphur dioxide on fuchsin in the presence of formaldehyde and a strong mineral acid, is relatively insensitive to aldehydes other than formaldehyde. Steigmann (*loc. cit.*) showed that although a similar colour is formed with sulphhydryl compounds and with thiosulphate, the test could be made specific for sulphur dioxide by preliminary removal of interfering substances with mercuric chloride; this treatment with mercuric chloride reduces blank determinations for several biological animal materials to reasonably low values, and appropriate methods of application have been devised for determining sulphur dioxide in such materials, *e.g.*, cornea, conjunctiva, aqueous humour, and blood. For the ocular tissues the colour reaction can be applied directly to a mercuric-chloride-treated extract. For blood, a modification of the procedure removed the large amount of soluble protein present, and then the colour reaction could be applied directly. The natural colouring matter of fruits and vegetables is not removed by treatment with mercuric chloride and the determination of sulphur dioxide in fruit has to be made by a distillation procedure.

Procedure—Prepare the reagent daily by diluting 11 ml. of concentrated sulphuric acid with 234 ml. of water and adding 4 ml. of a 3 per cent. solution of fuchsin (basic indicator) in alcohol, and then 1 ml. of 40 per cent. formaldehyde solution until the brownish liquid has a faint pink colour. The fine dark sediment appearing after several hours has little effect on the sensitivity of the supernatant liquid. Prepare the standard sulphur dioxide solution from iodimetrically standardised aqueous solutions of the gas, or, more conveniently, from sodium bisulphite. To calibrate the colorimeter, mix 4 ml. of colour reagent with 1 ml. of each of a series of dilutions of standard sulphur dioxide solution or, for the blank experiment, with 1 ml. of water. When mercuric chloride is to be used to remove interfering substances, include an equal amount of mercuric chloride in the standard solutions. After 5 min. at room temperature,

measure the colours with a Cenco Photelometer, using the green filter and setting the galvanometer at 100 with the blank mixture. The colour concentration relation follows Beer's law over most of the range 0 to 10 μg.

For the analysis of aqueous solutions containing no protein or coloured substances, *e.g.*, the alkaline solutions of sulphur dioxide obtained in air analysis, apply the procedure described for the treatment of standard solutions, diluting the samples to contain up to 10 μg. of sulphur dioxide per ml. When sulphhydryl compounds or thiosulphates are present, mix the sample with an equal volume of saturated mercuric chloride solution and centrifuge before applying the colour reagent, and compare with standards containing the same amount of mercuric chloride. Treat animal tissues containing little soluble protein, *e.g.*, cornea, conjunctiva, or aqueous humour, with a 1 per cent. solution of potassium hydroxide in 10 per cent. alcohol, using about 1 ml. per 20 mg. of cornea or conjunctiva, or per 0·15 ml. of aqueous humour. By a single soaking of the solid tissue in the extractant for 10 min. with occasional stirring, over 95 per cent. of the total sulphur dioxide extractable by two successive treatments is removed. Make the mixture neutral to phenolphthalein with *N* hydrochloric or acetic acid and add 1 ml. of saturated mercuric chloride solution per ml. of extractant. Centrifuge for 2 or 3 min. to promote precipitation of proteins, mix 1 ml. of the supernatant liquid, or an appropriate dilution thereof with 4 ml. of colour reagent and after 5 min. compare the mixture with standards containing the same amount of mercuric chloride. With blood, add 0·5 ml. of 1 per cent. alcoholic potassium hydroxide solution (*supra*) and 2·35 ml. of water to 0·15 ml. of the sample. Then, without neutralisation, mix the solution with 1 ml. of saturated mercuric chloride solution and centrifuge. Mix 1 ml. of the supernatant liquid, or of an appropriate dilution thereof, with 4 ml. of colour reagent and measure the colour in the usual way. With fruit, macerate about 250 mg. with 3 ml. of 1 per cent. potassium hydroxide in 10 per cent. alcohol. Place a 2-ml. aliquot in the side bulb of the vacuum distillation apparatus previously described (Grant, *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 729), and place a 50-mg. pellet of sodium hydroxide in the condenser portion. Freeze the material in the bulb by immersion in a mixture of "dry-ice" and acetone, and add about 0·05 ml. of concentrated sulphuric acid to it. Evacuate and seal the apparatus and then allow it to attain room temperature to permit the fruit extract to melt and react with the sulphuric acid. Re-freeze the mixture in the side bulb to prevent bumping and immerse the condenser portion in the dry-ice and acetone mixture for distillation. Allow the volatile constituents to distil without heating and, when distillation is complete, allow the distillate to melt and dissolve the sodium hydroxide pellet, but do not admit air for 30 min. so that residual gaseous sulphur dioxide may be absorbed. Examine the distillate by the procedure described for sulphur dioxide solutions free from interfering substances.

The effective removal of sulphhydryl by mercuric chloride was confirmed by analysis of samples containing up to 8 μg. of sulphur dioxide per ml. of 0·01 *M* cysteine hydrochloride solution. The time between the addition of mercuric chloride and the colour reagent must be a minimum, since there is progressive loss of sulphur dioxide at the rate of about 1 per cent. per min. when 5 μg. are originally present. The procedure described for fruit, although simple and specific, is not comparable in sensitivity with the methods given for animal tissue.

A. O. JONES

Gas Analysis

New Method of Determining Tetra-ethyl Lead in Air. M. S. Bikhovskaya (*Gigiena i Sanitariya, Moscow*, 1945, **10**, Part 9, 17–21; *Chem. Age*, 1947, **57**, 505–506)—Silver nitrate reacts with tetra-ethyl lead to give metallic silver and other products. In alcoholic solutions a black precipitate or a yellowish-brown colour is observed. The intensity of the colour is proportional to the amount of tetra-ethyl lead and may be used for its determination. Tetra-ethyl lead in air may be determined by absorbing it in silica gel impregnated with silver nitrate and comparing the coloration of the gel with standards.

Tetra-ethyl lead in solution—Procedure—To the solution in ethyl or butyl alcohol, add 0·05 ml. of 1 per cent. aqueous or alcoholic silver nitrate solution per 5 ml., and compare with standards. The reaction is sensitive to 0·05 mg. of tetra-ethyl lead. A sensitivity of 0·01 mg. is attained if a small amount of ammonia solution or sodium hydroxide solution, insufficient to precipitate any silver oxide, is added.

Tetra-ethyl lead in air—With a rate of flow of air of not more than 40 litres per hr., and two absorption vessels containing ethyl or butyl alcohol, complete absorption of the tetra-ethyl lead is possible but, because of the volatility of the solvents and the difficulty of finding a less volatile solvent suitable also as a medium for the reaction, absorption in silica gel is preferred. *Procedure*—Soak silica gel in 1 per cent. alcoholic silver nitrate solution, dry in the dark at room temperature, pack into a glass tube 4 mm. in diameter and 70 to 80 mm. long, and insert cotton wool at the ends. Such tubes should be prepared fresh and used the same day. Pass a known volume of the air to be tested through the tube, moisten the stain with alcohol, and compare the coloration with artificial standards previously prepared on paper. The treatment with alcohol intensifies the colour of the stain considerably. As little as 0·001 mg. of tetra-ethyl lead may be detected. The maximum amount capable of quantitative comparison is 0·07 mg. The test takes from 5 min. to 2 hr. according to the concentration of tetra-ethyl lead in the air and the volume of air required to give a suitable stain.

The method is inapplicable in presence of carbon monoxide, but substances like ethyl chloride that may accompany the tetra-ethyl lead have no effect.

G. S. SMITH

Organic

Quantitative Estimation as Acetic Acid of Acetyl, Ethylidene, Ethoxy, and α-Hydroxyethyl Groups. R. U. Lemieux and C. B. Purves (*Canad. J. Res., Sect. B.*, 1947, **25**, 485–489)—Kuhn and L'Orsa's method (*Z. angew. Chem.*, 1931, **44**, 847) for estimating terminal methyl and similar groups, based on the recovery of the acetic acid formed when such units are oxidised by hot chromic acid solution, has been improved to a point where results in close agreement with calculated values for acetyl, ethylidene, ethoxy, and α-hydroxyethyl groups in a variety of substances are obtained on a semi-micro scale. Acetylated triphenylmethyl cellulose ethers give good results by the new procedure.

Procedure—Weigh from 15 to 50 mg. of the sample into a 100-ml., round-bottomed, distillation flask fitted with a ground-glass joint, add 10 ml. of 30 per cent. aqueous chromium trioxide solution, and immerse two-thirds of the flask in an oil-bath. Connect the distillation arm to a water condenser, 20 cm. long, leading vertically down to a receiver graduated in increments of 5 ml., and assemble a dropping-funnel and a gas-bubbler, the latter dipping below the surface of the chromic acid, in the ground-glass neck-joint. Adjust a supply of nitrogen gas to a rate of 1 or 2 bubbles per sec., raise the temperature of the oil-bath to 155° C. during 0·5 hr., and maintain this temperature until the end of the determination. Add 5 ml. of distilled water to the still from the dropping funnel for each 5 ml. of distillate collected until 50 ml. of water have been added and 55 ml. of a pale yellow distillate collected in the receiver. Disconnect the condenser, wash out with distilled water, mix the washings and distillate, and titrate the combined solution with 0·02 *N* sodium hydroxide free from carbon dioxide, until the pink colour of the phenolphthalein indicator only just fades. Bring the solution to the boil, cool rapidly, and complete the titration to an end-point where the pink colour is stable for 10 sec. Add approximately 0·5 g. of sodium bicarbonate, 10 ml. of 10 per cent. sulphuric acid and, after effervescence has ceased, 1 g. of potassium iodide. Stopper the flask, shake, set aside in the dark for 5 min., and titrate the liberated iodine with 0·02 *N* sodium thiosulphate. Determine the ratio of the blanks in terms of the number of millilitres of 0·02 *N* sodium hydroxide to 1 ml. of 0·02 *N* sodium thiosulphate, which is constant for any given apparatus and set of reagents and is equal to the empirical factor *K*. The acetic acid equivalent for each estimation is given by $(x-Ky)$ ml. of 0·02 *N* alkali, where x = ml. of 0·02 *N* sodium hydroxide and y = ml. of 0·02 *N* sodium thiosulphate. A. H. A. ABBOTT

Determination of the Total Solids in Sulphate Pulp Mill Evaporator Feed Liquor. B. B. Edmonds, Jun. (*Anal. Chem.*, 1947, **19**, 820)—*Procedure*—This is suitable for routine tests, and is quicker and simpler than heating with xylene under refluxing conditions. Transfer 1 ml. of sample to each of seven pieces of bleached blotting paper (2 × 4 in.; caliper, 0·022 in.) that have previously been oven-dried at approx. 230° F. and cooled in a desiccator. Replace the blotters in the oven, and after 15 min. cool and re-weigh them. If the weighing pan of the balance is suspended freely from the balance arm inside the oven, then the step of cooling in a desiccator is eliminated. Results for 10 samples (sp.gr. 1·047 to 1·087) were in close agreement with those obtained by the xylene and overnight oven-drying methods for sp.gr. above approx. 1·055; below these, the xylene method gave results high in comparison with the other two, which agreed closely. J. GRANT

Transmission of Odour through Packaging Materials. L. W. Elder (*Paper Trade J.*, 1947, **125**, Nov. 6, 128, 130)—Methods of evaluating the transmission of odours in terms of the quantity of volatile chemical substance principally responsible for the odour (as determined analytically) are summarised.

Procedure—The sample is fixed over a shallow cup containing the volatile compound, and its outer side is swept by an inert gas. The rate of transmission may be determined from the loss in weight of the test cell, or by determining the amount of substance actually transmitted in a given time, gravimetrically, volumetrically, spectrophotometrically, polarimetrically, or refractometrically after absorption in a suitable reagent; or in the sweeping gas, by gas refractometry. Typical test substances are vanillin, citral, benzaldehyde, *d*-limonene, butyric acid, heptaldehyde, and allyl mustard oil. J. GRANT

Application of Synthetic Resins and Polymers to Paper. A. F. Tout (*Proc. Tech. Sect. Paper Makers' Assoc.*, 1946, **27**, 151–159)—Melamine-formaldehyde (M.-F.) and urea-formaldehyde (U.-F.) resins in paper are detected as follows:—Dye the paper with Solway Blue BNS under (*a*) neutral, and (*b*) acid conditions, and (*c*) after alkaline hydrolysis. With (*a*), papers containing either resin are dyed. With (*b*), the M.-F. gives the heavier shade, and sometimes the U.-F. gives no colour at all, since it is hydrolysed; the acid aids the dyeing action. With (*c*), the results are similar to those of (*b*), but protein matter not decomposed by the acid is decomposed. Glacial acetic acid is preferable to the usual mixture of dioxan and water for the recrystallisation, prior to identification, of the dixanthyl urea formed (as long, thin needles) by the action of xanthydrol on the hydrolysis products of the U.-F. resin. J. GRANT

Identification of Unsulphonated Azonaphthol AS Dyes. L. Koch and R. F. Milligan (*Anal. Chem.*, 1947, **19**, 312–313)—Battegay *et al.* (*Chim. et Ind.*, 1924, **11**, 453) have synthesised 1-aminonaphthol AS, m.p. 180° to 181° C., by the sodium dithionite (hydrosulphite, $Na_2S_2O_4$) scission of phenylazonaphthol AS. The following simple, rapid method, based on this procedure, is proposed for the identification of unsulphonated azonaphthol AS dyes.

Procedure—To 50 ml. of boiling dioxan in which 2 g. of finely ground dye, previously purified by recrystallisation from dioxan, are suspended, add 50 ml. of water followed at once by 20 g. of dry sodium dithionite. Heat gently, stirring continuously to decolorise the solution; pour the reduction product into 350 ml. of water, and heat this on a steam-bath or hot-plate to coagulate the precipitated 1-aminonaphthol AS. Filter, wash with 50 ml. of water, dry at 60° C., crystallise from alcohol, and determine the m.p. of the dye. To prepare the benzoyl derivative of the reduction product of the diazo-component, add 10 g. of sodium hydroxide and 5 ml. of benzoyl chloride to the filtrate, shake, and leave overnight. Collect on a small Buchner funnel, wash, crystallise from a suitable solvent, and determine the melting point.

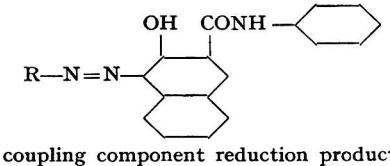
All melting points were determined with a Thiele tube, filled with dioctyl phthalate; a standardised, 360° C. thermometer was used, and all precautions for rate of heating and depth of thermometer immersion for this apparatus were carefully followed. Comparisons of the observed melting points (uncorr.) of 26 diazo-components with those given in the literature are quoted in the table; a few differ considerably. Results (m.p. of benzoyl derivatives) for the reduction products of diazo-components are also presented. E. B. DAW

Pectin. O. Wurz and O. Swoboda (*Papier-Fabrikant*, 1939, **37**, 125–127)—*Detection*—The sample (*e.g.*, wood pulp) is treated with 1 per cent. hydrochloric acid for 30 hr. at 20° C. and washed, the pectic acid is extracted from the residue in

IDENTIFICATION OF UNSULPHONATED AZONAPHTHOL AS DYES*†

	Diazo component	Melting point of dye‡ observed: Uncorrected °C.	Melting point of dye‡ observed: Literature °C.	Reduction product of diazo component	Melting point of benzoyl derivative of reduction product of diazo component (uncorrected) °C.
1.	*p*-Anisidine	210–212	213	*p*-Anisidine	155–156
2.	*p*-Phenetidine	216–217	218	*p*-Phenetidine	172–173
3.	*o*-Phenetidine	220–221	201	*o*-Phenetidine	Oil
4.	*m*-Toluidine	220–221	221	*m*-Toluidine	123–124
5.	2 : 4-Dimethylaniline	221–223	220	2 : 4-Dimethylaniline	193–194
6.	*o*-Toluidine	222–223	220	*o*-Toluidine	144–145
7.	*p*-Toluidine	230–232	225	*p*-Toluidine	157–158
8.	2-Methyl-4-chloroaniline	237–239	233	2-Methyl-4-chloroaniline	142–143
9.	*o*-Anisidine	239–241	229	*o*-Anisidine	Oil
10.	2-Naphthylamine	244–246	211	2-Naphthylamine	160–161§
11.	Aniline	245–246	237	Aniline	162–163§
12.	*o*-Chloroaniline	245–247	241	*o*-Chloroaniline	104–105
13.	*p*-Chloroaniline	251–253	249	*p*-Chloroaniline	191–192
14.	*m*-Chloroaniline	256–258	—	*m*-Chloroaniline	120–121
15.	*m*-Nitroaniline	270–271	271	*m*-Phenylenediamine	241–242
16.	3-Nitro-4-aminoanisole	270–272	—	4-Methoxy-*o*-phenylenediamine	251–252
17.	1-Naphthylamine	276–278	270	1-Naphthylamine	158–159
18.	2-Methyl-5-chloroaniline	277–279	—	2-Methyl-5-chloroaniline	171–172
19.	4-Nitro-2-methylaniline	281–282	268	2-Methyl-*p*-phenylenediamine	301–302
20.	2-Nitro-4-methylaniline	282–283	284	4-Methyl-*o*-phenylenediamine	263–264
21.	*o*-Nitroaniline	283–285	279	*o*-Phenylenediamine	303–304
22.	*p*-Nitroaniline	285–287	290	*p*-Phenylenediamine	338–339
23.	5-Nitro-2-methylaniline	297–298	304	1-Methyl-*m*-phenylenediamine	225–226
24.	2,5-Dichloroaniline	310–311	—	2 : 5-Dichloroaniline	119–120
25.	4-Chloro-2-nitroaniline	310–312	304	4-Chloro-*o*-phenylenediamine	226–227
26.	2-Chloro-4-nitroaniline	318–319	313	2-Chloro-*p*-phenylenediamine	239–240

* Naphthol AS is 2-hydroxynaphthoic acid anilide, and the dyes have the general structure



† 1-Aminonaphthol AS, the coupling component reduction product, was crystallised from ethanol; m.p. observed 188° to 190° C. (uncorrected); m.p. literature 180° to 181° C.

‡ All literature melting-point data are from tables of Rowe and Levin (*J. Soc. Dyers Colourists*, 1924, **40**, 218).

§ To differentiate further between dyes 10 and 11, run a mixed melting point with an authentic sample of benzanilide.

0·2 per cent. aqueous ammonia for 3 hr. at 20° C., and the extract is concentrated in a vacuum. The resulting clear yellow liquid gives a white, voluminous, flocculent precipitate with calcium chloride solution or hydrochloric acid, and the naphthoresorcinol test for uronic acid is positive, if pectin was present originally.

Determination—Two g. of air-dry sample are heated with 12 per cent. hydrochloric acid for 8 hr. in a stream of nitrogen-free carbon dioxide, on a glycerin-bath at 125° to 130° C., under reflux. The distillation products that do not condense are passed successively through concentrated calcium chloride solution and dry calcium chloride, two weighed U-tubes containing soda-lime, and a control bubbler (2 bubbles per sec.). The percentage of galacturonic acid is obtainable from the carbon dioxide given by the increase in weight (x), by means of the expression 22,000 x/y, where y is the percentage of dry substance in the sample; the error is ± 5 per cent. Results for various types of wood pulp and other substances are tabulated, and the influence of pectin on their properties is discussed. J. GRANT

Determination of the Methoxyl Group in Woody Substances. K. Kürschner and K. Wittenberger (*Papier-Fabrikant*, 1939, **37**, 165–167)—The chemistry of the micro-Zeisel method and its modifications is discussed, and the following improved method is recommended:—Weigh 14 to 45 mg. of the finely-divided sample into the closed glass tube used in the Pregl - Zeisel micro-apparatus (fitted with a ground glass joint enabling interchangeable flasks to be used), and add 1·5 to 2 ml. of hydriodic acid. The wash-vessel is half-filled with an aqueous emulsion of finely-powdered red phosphorus, and the receiver contains 3 to 4 ml. of a solution of 10 g. of potassium acetate in 100 ml. of acetic acid containing 4 to 5 drops of bromine. Heat to the boiling point in 20 to 25 min., passing carbon dioxide through so that only 1 bubble is visible at a time in the receiver. After the appropriate boiling time, wash the contents of the receiver into a flask containing a solution of 1 to 2 g. of sodium acetate, remove the excess of bromine by adding formic acid, a drop at a time, and shaking for 10 sec. after each addition; 2 to 3 drops are required, and absence of bromine is indicated by the permanence of the colour produced on adding 1 drop of methyl red solution. After 5 min., add 1 crystal of potassium iodide and 3 ml. of diluted sulphuric acid (1 : 10), and after a further 5 min., titrate the liberated iodine with $N/30$ sodium thiosulphate (starch indicator). Results obtained with 13·0 to 16·6 mg. of vanillin, anisic acid, or *p*-hydroxybenzoic acid methyl ester gave a maximum deviation of −0·12 per cent. from the calculated values. J. GRANT

Rapid Methods of Micro-Elementary Analysis. I. Determination of Carbon and Hydrogen in Substances containing Carbon, Hydrogen, and Oxygen. M. O. Korshun and V. A. Klimova (*J. Anal. Chem. Russ.*, 1947, **2**, 274–280)—The rapid micro-method of combustion in an empty tube as described by Belcher and Spooner (*J. Chem. Soc.*, 1943, 313) gives results for carbon that are 1 to 5 per cent. too low. Satisfactory results for carbon and hydrogen are obtained with solid substances when a small silica tube, closed at one end, and lying on its side, with its closed end towards the oxygen-inlet end of the combustion tube, is used instead of a micro-boat as the sample container. Heating by a burner is started at the open end of the sample tube, and the vapours, partly oxidised, then pass, together with oxygen, through a strongly-heated zone of the combustion tube at 850° to 900° C. The total time of combustion is about 10 min.

With volatile liquids, the sample is obtained in a spiral capillary and is made to vaporise into a tube, similar to that used for solids, and similarly placed in the combustion tube.

As in Belcher and Spooner's method, no filling is used in the combustion tube, and the rate of flow of oxygen is about 50 ml. per min. With substances containing from 60 to 94 per cent. of carbon, results are correct to 1 or 2 parts per 1000.

This method, as well as the normal Pregl method, gives, with highly volatile hydrocarbons, figures over 100 per cent. for carbon plus hydrogen. The error is traced to the effect of displacement of air in the capillary sample container by vapour. A correction amounting to about 50 μg. with the spiral and about 25 μg. with the Pregl capillary, should be made to the sample weight.

G. S. SMITH

Method for the Determination of the Quality of Emulsifying Media of the Type of Fatty Acid Esters of Poly-Alcohols. H. P. den Otter (*Chem. Weekblad*, 1947, **43**, 345–349)—*Procedure*—Dissolve about 40 g. of the crude material in 200 ml. of lukewarm ethyl acetate, and shake out three or four times with water to remove alcohols. Run off the ethyl acetate solution, remove the solvent by evaporation, and dry the residue to constant weight. Take a suitable weight (about 0·3 g. for compounds of glycerol, 0·55 g. for those of propylene glycol, or 0·6 g. for those of ethylene glycol) of this residue in an Erlenmeyer flask with standard ground neck, add 20 ml. of 0·5 N alcoholic potassium hydroxide, and saponify under refluxing conditions for 1 hr. Then add 25 ml. of water through the reflux condenser, followed by 50 ml. of periodic acid solution (5·5 g. of periodic acid and 35 ml. of 50 per cent. sulphuric acid in 500 ml.). Allow the reaction mixture to stand for 15 min. at room temperature, and add 20 ml. of 25 per cent. potassium iodide solution. Titrate the liberated iodine with 0·2 N sodium thiosulphate. Carry out a blank test at the same time. Calculate the percentage of combined glycerol (equivalent = 92/4) or of glycol (equivalent = 62/2). Determine also, in the purified ester mixture, the proportions of free fatty acids, unsaponifiable matter, and moisture, and calculate therefrom the "corrected" percentage of glycerol or of glycol, *i.e.*, the percentage in the ester fraction, by multiplying by the factor $100/(100 - F - G - H)$, where F, G, and H are the percentages, respectively, of free fatty

acids, unsaponifiable matter, and water in the latter fraction. Determine also, by the periodic acid method of Pohle *et al.* (*Oil and Soap*, **1945**, **22**, 115), the percentage of mono-glyceride. Finally, calculate the proportion of esters from the following equations:—

For glycerol

$$K = 92{\cdot}09\left[\frac{100-(L+X)}{O}+\frac{X}{N}+\frac{L}{M}\right]$$

where K is the "corrected" percentage of combined-glycerol and L the "corrected" percentage of mono-ester, M is the molecular weight of the mono-ester, N that of the di-ester, and O that of the tri-ester. and X is the (unknown) percentage of di-fatty acid ester of glycerol.

For glycol the formula is

$$K' = P\left[\frac{(100-X)}{N}+\frac{X}{M}\right],$$

where K' is the "corrected" percentage of combined glycol, M is the molecular weight of the mono-ester, N that of the di-ester, and P that of glycol, and X is the (unknown) percentage of mono-fatty acid ester of glycol. G. MIDDLETON

Inorganic

Method of Qualitative Analysis for Cations without Systematic Separations. Part I. Characterisation of Arsenic, Tin, Antimony, and Bismuth. G. Charlot and D. Bézier (*Analyt. Chim. Acta*, 1947, **1**, 113–123)—In the system of qualitative analysis previously described by Charlot and Bézier (*Ann. Chim. Analyt.*, **1943**, **25**, 90, 114) an attempt was made to reduce the number of separations to a minimum, emphasis being placed on the selectivity of the reactions used. The present paper reports an extension of that work. Tests for arsenic, tin, antimony, and bismuth in accordance with the criterion that each element shall be detected by the corresponding test when the total amount of the other three elements present is 100 times greater, are given. It is presumed that the elements are in hydrochloric acid solution and that silver, thallium, silica, fluoride, nitric, and organic acids, and very rare elements are absent. The chosen tests are: arsenic, ammonium molybdate test and formation of arsine by reduction in alkaline solution (C. J. van Nieuwenburg *et al.*, "*Tableaux des reactifs pour l'analyse minérale*," Leipzig, 1938; "*Réactifs pour l'analyse qualitative minérale*," Basle, 1945); tin, reduction of the iodine - starch complex; antimony, rhodamine-B; and bismuth, reduction by stannite. Details of the sensitivity and selectivity of the tests are given in the paper. The test for tin is new and is therefore described below.

Test for tin—Reagent—To 1 ml. of 0·1 *N* iodine (12·7 g. of iodine and 40 g. of potassium iodide per litre) add starch solution until the blue colour reaches its maximum intensity and then add a few millilitres in excess. Dilute the liquid to 100 ml.

Procedure—Stannous tin—Place a drop of the reagent on a drop-reaction plate and add a drop of the test solution, which should be 1 to 6 *N* in respect of hydrochloric acid. If the solution contains tin the drop is immediately decolorised.

Stannic tin—Boil a little of the test solution with iron powder for 1 min., filter, and allow to stand. Using a drop of the supernatant liquid, perform the test as described above.

The limit of sensitivity of the reagent is 5×10^{-4} g.-ion of tin per litre, but by diluting it four-fold the sensitivity is increased to 10^{-4} g.-ion per litre. Ferrous iron, quadrivalent vanadium, tervalent chromium, tervalent antimony, and tervalent arsenic do not interfere. As the univalent ions of copper and mercury do interfere, reduction of solutions containing cupric or mercuric ions must be prolonged, to complete the reduction of these ions to the metallic state. Quadrivalent uranium and tervalent titanium react only very slowly when the test solution is strongly acid. To a drop of the test solution reduced by iron add 1 drop of concentrated hydrochloric acid and add the mixture to the drop of reagent. Tungsten, molybdenum, and vanadium interfere and must be removed by precipitation. Precipitate tungsten by cinchonine and sexavalent molybdenum and quinquevalent vanadium by α-benzoinoxime.

B. ATKINSON

Formazylcarboxylic Acid as an Analytical Reagent [for Silver]. L. M. Kulberg and A. M. Ledneva (*J. Anal. Chem. Russ.*, 1947, **2**, 131–134)—"Formazylcarboxylic acid," obtained by the condensation of benzene-diazonium chloride and ethyl acetoacetate in presence of aqueous potassium hydroxide solution (*cf.* Bamberger and Wheelwright, *J. prakt. Chem.*, 1902, **65**, 123), is shown to contain the same grouping as diphenyl-thiocarbazone. Its formula is probably

$$\begin{matrix} C_6H_5.N:N \\ C_6H_5.NH.N \end{matrix}\!\!>\!C.COOH$$

The acid, cherry-red needles, m.p. 162° to 163° C. (decomp.), is insoluble in water, difficultly soluble in alcohol, and ether, and very easily soluble in chloroform, benzene, or acetone. Its solutions (orange) turn blood-red with caustic alkali and claret with mineral acids, but show no change of colour with acetic acid. With neutral solutions, or solutions acid with acetic acid, silver nitrate gives a claret-coloured precipitate. Under similar conditions, salts of barium, calcium, magnesium, beryllium, aluminium, ferrous iron, ferric iron, manganese, nickel, cobalt, thorium, thallium, indium, zinc, cadmium, lead, mercury, copper, bismuth, uranium, tin, and antimony, are apparently without effect.

The reagent is a specific and sensitive one for silver. The silver salt is insoluble in water, and almost insoluble in alcohol, difficultly soluble in benzene (orange), more easily soluble in ether (bright yellow), and easily soluble in chloroform (orange).

Detection of silver—Procedure—Impregnate filter paper with a saturated alcoholic solution of the reagent, and place a drop of a neutral solution

containing silver ion on the orange-coloured paper. In presence of silver, a claret-red spot appears. The intensity of the colour is proportional to the amount of silver present. The sensitivity is 0·0023 μg. of silver, and the limiting dilution 1 in 136,000.

To detect silver in a mineral acid solution containing other ions, add an excess of dry sodium acetate before placing a drop on the reagent paper. Filtration from any precipitate given by the sodium acetate is unnecessary. It is possible to detect 0·3 μg. of silver in presence of considerably greater amounts of other cations, *e.g.*, 2500 times as much cadmium, or 1500 times as much copper.

G. S. SMITH

Volumetric Determination of Aluminium. S. Lacroix (*Analyt. Chim. Acta*, 1947, **1**, 3–12)—When an acid solution of an aluminium salt is titrated with sodium hydroxide solution, there is no sharp change in the *p*H of the solution corresponding to the start of precipitation, and, owing to the adsorption of anions, the final point of inflexion occurs before an amount of sodium hydroxide equivalent to the aluminium has been added. The direct titration of the acid aluminium solution with sodium hydroxide solution when indicators to detect the two points of inflexion are used gives results accurate to ±1 per cent. Results of higher accuracy can be obtained by following the procedure given below. The free acid in the aluminium solution is determined by titration to *p*H 7·1 in presence of potassium oxalate, which prevents precipitation of aluminium. The total acidity is determined by adding an excess of sodium hydroxide solution to the boiling aluminium solution and titrating back with diluted hydrochloric acid to *p*H 7·0. If ferric iron is present, potassium fluoride is added in the first titration instead of the oxalate, as the ferric oxalate complex is not sufficiently stable. The end-point of the titration is then at *p*H 8·5. The second titration is similar to that performed in the absence of iron, but the end-point is at *p*H 7·2. The iron is determined separately by oxidation - reduction titration, and the acidity due to the iron allowed for in calculating the aluminium content of the solution.

METHOD WHEN IRON IS ABSENT—*Indicator*—A 0·1 per cent. solution of bromothymol blue in 20 per cent. ethyl alcohol.

Procedure—Determination of free acid—Take 10 ml. of an approximately 0·1 *M* solution of the aluminium salt, dilute to 100 ml. in a 250-ml. beaker, and add 5 g. of pure potassium oxalate. Boil the solution for a few minutes, cool, add 5 drops of the bromothymol blue solution, and titrate with 0·1 *N* sodium hydroxide to *p*H 7·d as shown by comparing the colour of the solution with a colour scale.

Determination of total acidity—Dilute 10 ml. of the 0·1 *M* aluminium solution to 100 ml. in a 250-ml. beaker and boil for a few minutes. Add 5 drops of the bromothymol blue solution, titrate immediately with 0·1 *N* sodium hydroxide to a faint blue colour (*p*H 7·4), and add 0·5 ml. in excess. Boil the solution again and titrate with 0·1 *N* hydrochloric acid to *p*H 7·0.

Determination of basic aluminium salts—To solutions of aluminates or basic salts of aluminium add a known excess of hydrochloric acid before proceeding as above.

METHOD WHEN FERRIC IRON IS PRESENT—*Indicator*—A 0·1 per cent. solution of thymol blue in 20 per cent. ethyl alcohol. *Potassium fluoride solution*—To a solution of 200 g. of potassium fluoride in 1 litre of water add a few drops of thymol blue solution and adjust the *p*H to 8·5 by adding drops of sodium hydroxide solution.

Procedure—Determination of free acid—To 10 ml. of a 0·1 *M* solution of the aluminium salt, in a 250-ml. beaker, add 25 ml. of the potassium fluoride solution and dilute to 100 ml. with boiled water. Add 5 drops of thymol blue solution and titrate with 0·1 *N* sodium hydroxide to *p*H 8·5.

Determination of total acidity—Proceed as for iron-free solutions but titrate to *p*H 7·2. To observe the colour of the indicator it is necessary to decant the upper portion of the solution.

By both methods of procedure aluminium is determined to within ±3 parts in 1000. Ammonium ions by their buffering effect, and ions forming complexes with aluminium interfere, as do titanium, chromium, and vanadyl ions, which are precipitated at the same time as the aluminium. Calcium, magnesium, and moderate amounts of sulphate ions do not interfere.

B. ATKINSON

Rapid Determination of Chromium and Vanadium. L. Ducret (*Analyt. Chim. Acta*, 1947, **1**, 135–139)—The sum of the chromium and vanadium, oxidised to their highest valency states, is determined by titration with ferrous sulphate in presence of 3 *N* sulphuric acid and phosphoric acid. Although there is a slow reaction between diphenylamine sulphonate and chromate this indicator can be used if the titration is performed without delay. For the determination of vanadium the solution resulting from the first titration is oxidised by an excess of permanganate. Because of the extremely slow rate of oxidation of chromium by small concentrations of permanganate only vanadium is oxidised. Excess of permanganate is destroyed by sodium azide and the quinquevalent vanadium is titrated with ferrous sulphate as in the first titration.

Procedure—Sum of chromium and vanadium—Render 20 or 30 ml. of the solution 3 *N* in respect of sulphuric acid by adding diluted sulphuric acid (1 + 4). Add 5 ml. of 85 per cent. phosphoric acid and 4 to 5 drops of a 0·3 per cent. solution of barium diphenylamine sulphonate solution and titrate immediately with 0·1 *N* ferrous sulphate made 1 *N* in respect of sulphuric acid.

Vanadium—While keeping the temperature of the solution at about 25° C. add, at intervals of a few seconds, 1-ml. portions of approximately 0·1 *N* potassium permanganate until a faint pink coloration persists; then add another millilitre if the colour fades after 2 to 3 min. standing. Add 2 ml. of a 1 per cent. solution of sodium azide, shake, wait 5 min., and then add 5 ml. of 85 per cent. phosphoric acid, 20 ml. of diluted

sulphuric acid (1 + 4), and 3 to 4 drops of the indicator solution. Titrate immediately with 0·1 *N* ferrous sulphate.

The method gives results accurate to within about ±1 part in 500. B. ATKINSON

Polarographic Determination of Tin in High-purity Zinc and Zinc Die-Casting Alloys. R. C. Hawkings, D. Simpson and H. G. Thode (*Canad. J. Res.*, 1947, **25**, 322–330)—The polarographic determination of tin in alloys is complicated by the fact that the reduction wave of the stannous ion occurs at the same potential as that of lead. This difficulty has been overcome by dissolving the alloy in sulphuric acid, and precipitating the tin with cupferron after adding hydrochloric acid to keep the lead in solution. The precipitate is re-dissolved and the tin determined polarographically.

Procedure—Add 50 ml. of distilled water and 15 ml. of concentrated sulphuric acid to 10 g. of the zinc or zinc alloy in a 150-ml. beaker and, when the reaction has subsided, heat until the zinc is dissolved. Dilute the solution to 100 ml., add 5 ml. of 30 per cent. hydrogen peroxide and boil the solution until clear, adding more peroxide if necessary. Continue to boil the liquid to destroy the excess of peroxide and dilute to 100 ml. After adding 5 ml. of concentrated hydrochloric acid, cool to 15° C. or less and then add 3 ml. of cupferron solution drop by drop, stirring the liquid at the same time. (The cupferron solution is prepared by dissolving 5 g. of cupferron and 0·25 g. of acetophenetidine in 100 ml. of water.) Stir the liquid to coagulate the precipitate and filter it within 30 min. on a Jena No. 3 sintered-glass crucible, washing the beaker and precipitate with 20 ml. of 0·05 per cent. cupferron solution containing 2 ml. of concentrated sulphuric acid per 100 ml. Dissolve the precipitate in 10 ml. of concentrated nitric acid, added in small portions, and wash the filter with a small volume of water, collecting the solution in a 150-ml. beaker. Add 4 ml. of concentrated sulphuric acid and evaporate to fuming. Cool, add 5 ml. of concentrated nitric acid, and evaporate again to fuming. Wash down with 10 ml. of water and warm till the solids are dissolved. Transfer the solution quantitatively to a 50-ml. volumetric flask and, after adding 4 ml. of concentrated hydrochloric acid and 0·1 g. of pure aluminium foil, heat gently in a stream of carbon dioxide until the solution clears; continue for 15 to 30 min. After cooling the solution, add 1·25 ml. of 0·1 per cent. gelatin solution, dilute to 50 ml. with freshly boiled distilled water, and transfer to a polarographic cell through which nitrogen is passing. Record a polarogram immediately between −0·3 and −0·7 v. *versus* the saturated calomel electrode. The tin content of the sample, calculated from a calibration curve, is accurate to within ±0·0001 per cent. for samples containing about 0·0005 per cent. of tin. None of the metallic elements present in zinc or zinc die-casting alloys interferes with the determination when the above procedure is followed.

J. G. WALLER

Colour Reaction for Antimony with Methyl Violet. V. I. Kuznetsov (*J. Anal. Chem. Russ.*, 1947, **2**, 179–181)—Because of the scarcity of Rhodamine B the mechanism of its colour reaction with antimony was studied to find a more common reagent with a similar effect. The author has already shown (*Compt. rend. Acad. Sci. U.S.S.R.*, 1946, **52**, 231) that the reaction is not one of oxidation, as supposed by Eegriwe (*Z. anal. Chem.*, 1927, **70**, 400) and Feigl ("*Qualitative Analysis by means of Drop Reactions*"), but a simple formation of a difficultly soluble salt of Rhodamine B with the acid $HSbCl_6$, accompanied by a change of colour when the suspension is observed by transmitted light. Difficulties sometimes found in making the reaction work are due to the great ease with which the anion $SbCl_6'$ is hydrolysed, even in strongly acid medium, to give other ions containing quinquevalent antimony but incapable of reacting with the dyestuff.

It is now shown that methyl violet may be used instead of Rhodamine B.

Procedure—With tervalent antimony, add to 1 drop of the solution 3 to 5 drops of concentrated hydrochloric acid, and 1 drop of *N* sodium nitrite to oxidise antimony to $SbCl_6'$ (*cf.* Eegriwe, *loc. cit.*), wait for not less than 1 min., then add a crystal or 1 drop of a saturated solution of urea, dilute to 3 to 5 ml., and finally add 1 drop of 0·2 per cent. aqueous methyl violet solution. In presence of antimony, the methyl violet at once gives a fine crystalline suspension, appearing violet or blue in transmitted light, and showing a strong greenish-gold iridescence in reflected light. In absence of antimony, the solution is yellowish-green. If the amount of antimony is less than 4 μg. a blank should be carried out under the same conditions. Nitrates are without effect. Excessive acid is harmful. The nitrite may be destroyed with sodium sulphite instead of with urea. In this case, add a small crystal of sodium sulphite or a drop of its saturated solution after dilution of the test solution with water. If nitrite is not destroyed, the yellow-green colour of methyl violet gradually changes to violet and, after a few minutes, the solution is completely decolorised, but, if not too great an excess of nitrite is used, the excess need not be destroyed since the reaction of methyl violet with antimony is instantaneous, and the colour change can be seen before the slower reaction with nitrite is completed.

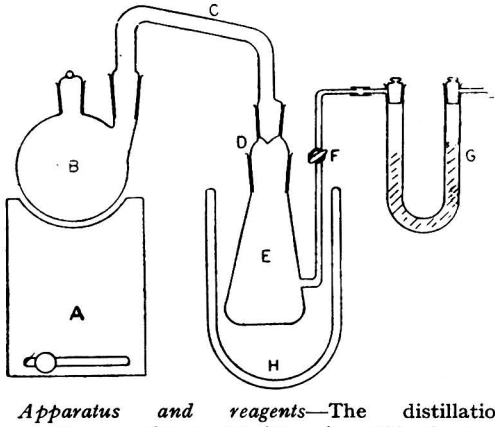
With quinquevalent antimony, *e.g.*, in solutions of alloys and minerals in *aqua regia*, the ion $SbCl_6'$ can be obtained only after preliminary reduction of antimony to the tervalent state, followed by re-oxidation with nitrite. Heat the solution containing hydrochloric and nitric acids to remove most of the nitric acid, leaving the hydrochloric acid concentration about 6 to 7 *N*. Cool, and add 1 or 2 drops of 15 per cent. stannous chloride solution. In the cold, the residual nitric acid is scarcely affected by the stannous chloride, whilst quinquevalent antimony is immediately reduced. Then add sufficient nitrite to oxidise both antimony and tin and proceed as described above.

The sensitivity equals that with Rhodamine B. In 5 ml. the minimum amount detectable is 0·5 μg., showing that the ion $SbCl_6'$ is precipitated at a dilution of 1 in 10,000,000. Antimony can be detected in presence of more than 1000 times its amount of bismuth, with tin, iron, copper, zinc, and many other elements also present.

Similar colours are given by tungstates (*cf.* Kuznetsov, *Compt. rend. Acad. Sci. U.S.S.R.*, **1943, 41**, 113), gold, tervalent thallium, mercury, and heteropolyacids.

The colour given by quinquevalent antimony is immediately destroyed by addition of stannous chloride, and the solution becomes yellowish-green. Tervalent thallium reacts similarly, but the colour given by tungstates is unchanged. The colours given by gold and mercury are indeed changed by addition of stannous chloride, but the effect is clearly distinguishable from that obtained with antimony. Thus, interference by tungstates, gold, and mercury may be prevented by carrying out the test as described above, and then observing the effect of stannous chloride. G. S. SMITH

Determination of Water in Caustic Soda and other Alkaline Materials. H. R. Suter (*Anal. Chem.*, 1947, **19**, 326–329)—The water content of caustic soda cannot be determined by oven-drying methods, and gravimetric determinations carried out by fusing the alkali in dry, carbon dioxide-free air yield erratic results. Direct determination using Fischer reagent is also not applicable owing to reaction between the alkali and the reagent. In the method described, the water is first separated by distillation with xylene, and subsequently titrated potentiometrically with the Fischer reagent, methyl alcohol being added to render the water, xylene, and reagent mutually soluble. Twenty to 200 mg. of water may be determined accurately to within ±5 per cent. The method has also been carried out successfully on alkali silicates and calcium carbonate and is suggested for inorganic substances generally where ordinary methods are not applicable.



Apparatus and reagents—The distillation apparatus as shown consists of a 500-ml., two-necked distillation flask, B, and a 250-ml. flask with a side-arm as receiver, E. The receiver is immersed in a "dry-ice" - acetone freezing-bath, H, and the side-arm is connected to a drying tube, G, to prevent entry of atmospheric moisture. The preparation of the Fischer reagent and of standard water in methyl alcohol solution and the potentiometric titration are carried out as recommended by Wernimont and Hopkinson (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 272). To eliminate interference from water in the methyl alcohol, a slight excess of Fischer reagent is added and titrated to the equivalence point with standard water in methyl alcohol solution. The xylene is dehydrated by distilling and storing over sodium. In this way, the blank is reduced to less than 5 mg. of water.

Procedure—Dry the distillation apparatus in an oven and assemble while hot. To ensure dryness, distil a few millilitres of xylene from the flask and reject. Allow to cool, permitting air to enter through the drying tube. Grind the weighed sample under xylene and transfer to the distilling flask by means of a wash-bottle containing xylene, adding a total of 100 ml. of xylene to the flask. The transference should be carried out as quickly as possible.

Start the distillation and adjust the heat so that the xylene refluxes from the short vertical leg of the delivery tube. Continue refluxing for 4 hr., finally distilling over all the xylene. Remove and stopper the receiver, closing the tap in the side-arm, and allow to stand until room temperature is reached. Add 50 ml. of methyl alcohol that has previously been adjusted to the equivalence point with Fischer reagent and standard water in methyl alcohol solution. Add an excess of the Fischer reagent and titrate back with standard water in methyl alcohol solution. Carry out blank determinations in the same manner and correct the results accordingly. H. J. CLULEY

Sulphite Waste Liquor Analysis. Determination of Sulphate by a Conductometric Titration Method. Q. P. Peniston, V. F. Felicetta, and J. L. McCarthy (*Anal. Chem.*, 1947, **19**, 332–334)—The gravimetric determination of sulphate in sulphite waste liquor is subject to errors due to adsorption of impurities by the barium sulphate precipitate, and is not suitable for control purposes. A rapid method has been evolved in which the liquor is titrated conductometrically with barium chloride solution. The presence of calcium and bisulphite ions was found to be undesirable, as the relationship between conductivity and volume of titrant added ceased to be linear, and an extended time was required after the addition of titrant before steady resistance readings were obtained. In the recommended method, calcium is removed by cation exchange with sodium, and interference of bisulphite is prevented by addition of formaldehyde.

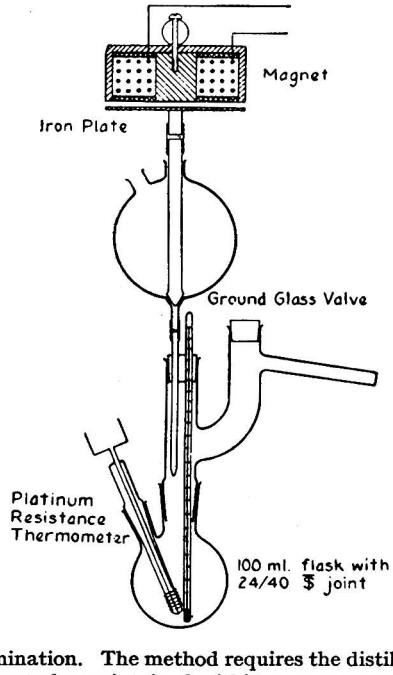
Apparatus—The ion-exchange column is a 35-cm. long, 13-mm. diameter tube attached to a syphon tube at the bottom and containing a 20-cm. column of Amberlite IR-100 or similar cation-exchange resin. The resin is regenerated by 200 ml. of 10 per cent. sodium chloride solution, and washed with water until free from chloride.

The conductometric titration is carried out in a 400-ml. beaker, a mechanical stirrer and a "dipping-type" conductivity cell being used. The titration beaker is immersed in a larger beaker of water at room temperature to minimise temperature fluctuations.

Procedure—Pass a 10-ml. sample through the exchange column at a rate of about 5 ml. per min., followed by four, 20-ml. portions of water. Dilute the total effluent to 100 ml., add 5 ml. of 5 per cent. formaldehyde solution, dilute to 300 ml. with 75 per cent. *iso*propyl alcohol in water, and cool to within 1° C. of room temperature. Transfer to the titration assembly and stir for 2 min. before taking the initial resistance reading. Add 0·1 *N* barium chloride in 0·5-ml. increments, allowing 60 sec. after each addition before determining the resistance. Continue the titration to obtain 3 or 4 points beyond the end-point, which is indicated by a more rapid fall in resistance. Convert the resistance readings to conductivity values and determine the end-point by the usual graphical method.

The 0·1 *N* barium chloride should be standardised conductometrically against weighed samples of anhydrous sodium sulphate. H. J. CLULEY

Electronically Controlled Apparatus for Distillation of Fluoride as Hydrofluosilicic Acid. H. H. Willard, T. Y. Toribara, and L. N. Holland (*Anal. Chem.*, 1947, **19**, 343–344)—The distillation of hydrofluosilicic acid serves to separate fluorine from interfering ions prior to its



determination. The method requires the distillation mixture to be maintained within a narrow temperature range, and for this purpose an automatic device that replenishes the mixture with water as fast as it distils off has been evolved. By this means, the boiling point may be confined to a range of about 3° C.

The apparatus for the automatic water feed is as shown. When the temperature rises above a pre-determined value the magnet is energised, causing the valve to open and water to flow into the distillation flask. A reduction in temperature de-energises the magnet and the valve closes. The thermo-regulator is a platinum resistance thermometer encased in a glass envelope, and used as one arm of a bridge circuit, the magnet being operated by the off-balance potential of the mid-points of the bridge. The operating temperature range may be regulated by varying the resistance of one arm of the bridge circuit by means of a potentiometer. For details of the circuit the original paper must be consulted. H. J. CLULEY

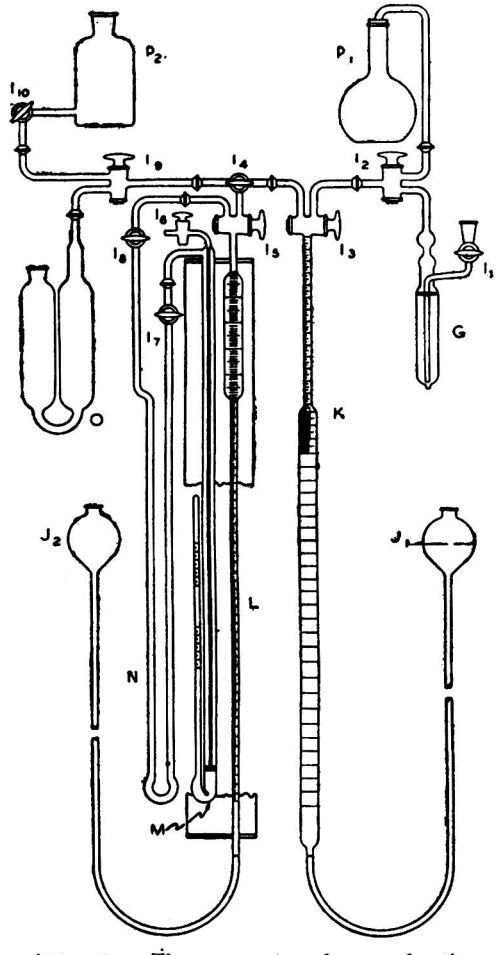
Determination of Phosphorus in Phosphate Rock. Separation from Cations by Ion-Exchange Resin. K. Helrich and W. Rieman, 3rd (*Anal. Chem.*, 1947, **19**, 651–652)—Prepare the ion-exchange column by suspending 170 ml. of Amberlite IR-100-H A.G. in water and pouring the suspension into a filter tube, 40 cm. long, with a stop-cock at the bottom. Wash thoroughly with tap-water entering slowly at the cock, and re-generate the column by pouring through it 500 ml. of *N* hydrochloric acid and then 300 ml. of distilled water, maintaining the liquid level 2 cm. above the top of the column; the operation should take about 20 min. Back wash and re-generate the column before each determination.

Procedure—Boil a 450- to 550-mg. sample in a covered 150-ml. beaker with 15 ml. of 12 *N* hydrochloric acid for 30 min.; evaporate to dryness on a steam-bath and bake there for 1 hr. Add 2 ml. of 6 *N* hydrochloric acid and 98 ml. of water. Pass the solution through the column, followed by 300 ml. of water, and collect the filtrate and washings in a 1-litre casserole; this should take about 10 min. Add 3 drops of 0·1 per cent. methyl red solution and 18 *N* sodium hydroxide until the solution is alkaline and then add immediately 1·0 *N* hydrochloric acid dropwise until the solution is slightly acid. Adjust the *p*H to 4·63 with 0·1 *N* hydrochloric acid, a comparison buffer of 17 ml. of 1·3 *M* sodium acetate, 25 ml. of 1·0 *M* acetic acid, 360 ml. of water, and 3 drops of methyl red being used. Add 1 drop of 1 per cent. phenolphthalein solution and titrate to *p*H 8·98 with 0·1 *N* sodium hydroxide, a comparison buffer of 130 ml. of 0·1 *M* borax, 40 ml. of 0·1 *M* hydrochloric acid, 230 ml. of water, 3 drops of methyl red, and 1 drop of phenolphthalein being used.

Removal of most of the fluoride and dehydration of the silica by evaporation greatly reduce the buffering action, so that the titration corresponds to the conversion of primary phosphate, formed at *p*H 4·63, to secondary phosphate at *p*H 8·98.

Two rocks were analysed by this method and the mean of four determinations on each sample agreed to within 1 in 1600 on the Bureau of Standards value. M. E. DALZIEL

Determination of Carbon in Low-Carbon Steel. Low-Pressure Gasometric Method. C. E. Nesbitt and J. Henderson (*Anal. Chem.*, 1947, **19**, 401–404)—Apparatus and procedure are described for the determination of up to 0·05 per cent. of carbon in steel accurately to within ± 0·0003 per cent. After combustion of the sample in oxygen, the carbon dioxide formed is absorbed in carbonate-free sodium hydroxide solution, and then evolved into the gasometric apparatus by means of acid. The carbon dioxide is determined by measuring the total gas volume under reduced pressure, and re-measuring the volume after absorption of the carbon dioxide in sodium hydroxide solution.



Apparatus—The apparatus for combustion of the sample is of the type normally used for direct combustion methods. For elimination of the oxides of sulphur an absorbent of activated manganese dioxide packed between layers of ironised asbestos is recommended. The carbon dioxide absorber, which is packed with helical glass rings, has a stop-cock and a cup-shaped opening on the inlet side to permit the introduction of liquids into the absorber. The carbonate-free sodium hydroxide solution for the absorber is prepared from selected sodium and from carbonate-free water in an atmosphere of nitrogen. The gasometric apparatus, as shown, consists essentially of an evacuating burette, a measuring burette, and an absorption pipette containing 20 per cent. sodium hydroxide solution. The measuring burette is connected through a water manometer to a compensator that is used to adjust the gas to a standard pressure, the water manometer being used in equalising pressures in compensator and measuring burette. The compensator and measuring burette are enclosed in a water jacket maintained at 25° C. Two reservoirs of wash liquid are connected to the apparatus, one containing an acid wash (*p*H 4) to flush out the apparatus after a determination and remove traces of hydroxide, and the other containing an alkali wash (*p*H 11·5) used to remove acid from the apparatus during the absorption of carbon dioxide. The apparatus is filled with mercury and levelling bulbs are provided for each burette.

Procedure—The combustion of a 2-g. sample is carried out by the usual technique. While pre-heating the boat just inside the combustion tube, introduce 2 ml. of 40 per cent., carbonate-free sodium hydroxide solution into the carbon dioxide absorber, using 2 ml. of carbonate-free water as a rinse. Thrust the boat into the hot zone of the furnace and complete the combustion as usual.

Flush out the tubing of the gasometric apparatus where necessary with the acid wash, de-gassify the mercury, expel the air recovered, and fill up with mercury. Connect the absorber to the apparatus and withdraw as much air as possible into the evacuating burette. Expel the air from the apparatus and repeat the operation twice. Introduce 4 ml. of carbonate-free, diluted sulphuric acid (1 + 1) into the absorber, rinsing three times with carbonate-free water. With the levelling bulb of the evacuating burette lowered as much as possible, heat the bottom of the absorber and allow the contents to boil gently until vapours begin to condense at the top of the burette. Cease heating and pass the collected gas from the evacuating burette into the measuring burette. Boil the contents of the absorber again under reduced pressure, passing about 20 ml. of carbonate-free air through the absorber to remove last traces of carbon dioxide, and collecting the gas in the evacuating burette. Again pass the collected gas into the measuring burette.

Lower the levelling bulb of the measuring burette until the pressure is approximately 334 mm. of mercury. Adjust the pressure to exactly 334 mm., using the water manometer to indicate equality of pressures in the compensator and burette, and observe the volume. Pass the gas into the absorption pipette, and flush out the measuring burette with the alkaline wash to remove all traces of acid water. Pass the gas back from pipette to burette two or three times to ensure complete absorption of carbon dioxide. Finally, withdraw the gas slowly into the measuring burette, adjust the pressure as before and observe the volume. From the difference between the two observed volumes subtract the blank, determined in the same manner, and calculate the carbon content of the sample.

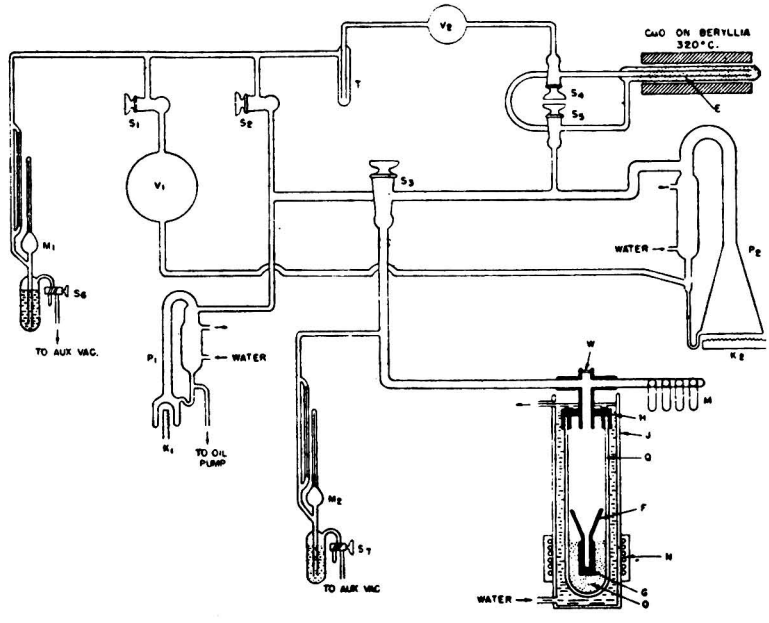
At 334 mm. pressure and 25° C., 1 ml. of carbon dioxide ≡ 0·01 per cent. of carbon on a 2-g. sample.

H. J. CLULEY

Determination of Oxygen in Steel by the Vacuum Fusion Method. L. Alexander, W. M. Murray, and S. E. Q. Ashley (*Anal. Chem.*, 1947, **19**, 417–422)—A simplified vacuum fusion apparatus is described for the determination of oxygen, hydrogen, and nitrogen in steel. The fusion is carried out in a graphite crucible which converts the oxygen into carbon monoxide, hydrogen and nitrogen being evolved unchanged. Successive fusions of a large number of samples loaded into the apparatus permit almost continuous operation. A method for determining surface oxygen and hydrogen is also described.

Apparatus—A schematic diagram of the vacuum fusion apparatus is shown. The sample is melted in a graphite crucible, G, fitted with a graphite funnel, F, to minimise trouble from splashing of molten metal. The crucible is seated in fine graphite powder, which insulates it from the outer quartz tube, Q. The top of the quartz tube is sealed to a non-magnetic, stainless steel head, H, to which is welded a cross-shaped dropping tube, W, and the quartz tube and head are immersed in a water jacket, J. Heating is provided by a 550-kc. high-frequency induction coil, N, on the outside of the water jacket. The cross-shaped dropping tube has a glass window at the top for observation purposes and to permit determination of temperature with an optical pyrometer. The horizontal arms of the dropping tube are sealed on the one side to the analysis system and on the other side to the loading arm, M. The loading arm, of Pyrex, has a number of side-arms and as many as 30 samples may conveniently be loaded provided that they are stamped for identification and that a strong magnet is available for moving them singly into the dropping position. The analysis system consists essentially of two storage bulbs, V_1 and V_2, of 5000-ml. and 500-ml. capacity, respectively, a freezing-out trap, T, a circulating mercury diffusion pump, P_2, a McLeod gauge, M_1, and a furnace, E, maintained at 320° C., containing copper oxide on beryllia for oxidation of carbon monoxide and hydrogen. A second McLeod gauge, M_2, is used for the observation of pressure in the fusion furnace, and a second mercury diffusion pump, P_1, is required for the removal of gases from the system after analysis. All parts of the analysis system are of Pyrex.

Preparation of sample—Use a sample weight of 3 to 10 g., depending on gas content. Remove scale by grinding with rough emery, and, using



rubber gloves, polish with fine emery. Stamp the sample for identification and remove emery particles by brushing. Rinse in benzene and allow to dry in air. Weigh and store in a vacuum desiccator until the sample can be loaded. After loading samples, the loading arm should be sealed and evacuated as soon as possible.

Procedure—Using both pumps, evacuate the whole analysis system, and evacuate the fusion furnace at a slow rate so that the graphite powder is not carried out of the furnace. If a new crucible is being used, raise the temperature slowly to 2300° C. and evacuate for 2 hr. If the crucible contains steel residues from previous determinations raise to 1750° C. and evacuate for 30 min. Cool to 1650° C., the working temperature, and determine the rate of evolution of blank gases by collecting the output of gas in the 5000-ml. bulb and observing the pressure at 10-min. intervals on the McLeod gauge. Continue until a satisfactorily low rate of evolution is reached. (Typical blanks expressed as

ml. of gas at N.T.P. evolved per hr., are 0·033 ml. for an empty crucible and 0·058 ml. for a crucible containing steel residues.) Evacuate the blank gases from the analysis system.

Transfer a steel sample to the dropping position and evacuate the evolved gases into the 5000-ml. bulb, the time required for gas extraction varying with samples, from 10 to 30 min. Observe the pressure of the gas mixture on the McLeod gauge. Circulate the gases through the cupric oxide furnace for 25 min. and freeze out the water produced by placing a "dry-ice" - acetone bath round the trap. Enclose the uncondensed gases, carbon dioxide and nitrogen, in the 5000-ml. bulb, remove the freezing-bath and withdraw the water vapour from the system. Observe the pressure of the carbon dioxide - nitrogen mixture and from the difference between this and the previously observed pressure calculate the hydrogen content of the sample. With the trap immersed in liquid air, circulate the residual gas mixture for 5 min. to condense the carbon dioxide. Enclose the uncondensed gas in the 5000-ml. bulb as before, remove the liquid air-bath and allow the carbon dioxide to expand into the 500-ml. bulb. Observe the pressure of the carbon dioxide and calculate the oxygen content of the sample. Finally, measure the pressure of the uncondensed gas, assumed to be nitrogen, in the 5000-ml. bulb and calculate the nitrogen content of the sample.

To determine surface oxygen and hydrogen in a steel, cut a section into a number of samples and cold-roll half the samples to produce a considerable increase in surface area. Prepare the samples and determine the oxygen and hydrogen contents as above. The surface oxygen (or hydrogen) may then be calculated by dividing the increase of oxygen (or hydrogen) per gram by the increase of surface area per gram. It is advisable to take a number of samples to minimise errors due to non-uniformity of the steel. H. J. CLULEY

Colorimetric Determination of Phosphorus in Steel. U. T. Hill (*Anal. Chem.*, 1947, **19**, 318–319)—Two methods, based on the formation of the yellow molybdovanadophosphoric acid complex, are described for the determination of phosphorus in steel. The complex is stable over a wide range of temperature, and the variation of the absorption of sample solutions with temperature, as reported by Center and Willard (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 287) and others, is shown to be due to the iron salts in solution. This difficulty is overcome by employing as a blank a solution containing the sample, and cooling both solutions to the same temperature. The recommended procedures differ according to the presence or absence of vanadium in the sample.

Reagents—Ammonium vanadate reagent—Dissolve 2·5 g. of ammonium vanadate in 500 ml. of water, add 30 ml. of concentrated nitric acid, and dilute to 1 litre. *Sodium vanadate reagent*—Dissolve 2·5 g. of sodium vanadate in 50 ml. of water, add 20 ml. of concentrated nitric acid, and dilute to 100 ml.

Procedure—(A) *Vanadium present*—Dissolve two 1-g. samples in 30 ml. of diluted nitric acid (1 + 2) and boil to expel nitrous fumes. Add 10 ml. of freshly prepared, 15 per cent. ammonium persulphate solution, and boil for 5 min. to destroy the excess of persulphate. Remove from the hot-plate, and cool to room temperature the solution that is to be the blank. To the sample solution add 10 ml. of ammonium vanadate reagent, mix, add 40 ml. of 5 per cent. ammonium molybdate solution, shake to dissolve any precipitate, and cool to room temperature. Dilute both solutions to 100 ml. and measure the absorption of the coloured solution against the blank at 465 mμ. in a 4- or 5-cm. cell. Calculate the phosphorus content from a calibration curve obtained with standards prepared from a standard phosphate solution and a steel of known, low-phosphorus content.

(B) *Vanadium absent*—Dissolve a 1-g. sample in 25 ml. of diluted nitric acid (1 + 2) and boil to expel nitrous fumes. Add 1 ml. of 2·5 per cent. potassium permanganate solution, and boil for 2 min. Add 1 ml. of 10 per cent. potassium nitrite solution to reduce manganese dioxide, and boil for 1 min. to expel fumes. To the hot solution add 10 ml. of 10 per cent. sodium molybdate solution and, while still warm, dilute to 50 ml. Pipette two 10-ml. aliquots into 1·5-cm. cells and add to one solution 0·3 ml. of sodium vanadate reagent. Mix well and cool both cells to room temperature in running water. (If it is desired to work with cold solutions allow 5 min., after adding reagents, for full colour development.) Measure the absorption of the colour solution against the blank at 465 mμ. Calculate the phosphorus content from a calibration curve obtained with standards prepared from a standard phosphate solution and a steel of known, low-phosphorus content.

In procedure (B), ammonium salts are excluded to avoid precipitation of ammonium phosphomolybdate in the blank. H. J. CLULEY

Determination of Nitrogen in Ferro-Alloys and Other Materials by Nesslerisation without Distillation. W. C. Newell (*J. Iron and Steel Inst.*, 1945, **152**, 333–337P)—*Method*—Dissolve 1 g. of the sample in 5 ml. of sulphuric acid and 20 ml. of water and add a few drops of 30 per cent. hydrogen peroxide to attack carbides. Evaporate and fume for 30 min., first adding 2 g. of potassium sulphate if the sample contains titanium. Cool the solution, dilute it, and transfer it to a 250-ml. graduated flask. Add 40 ml. of 20 per cent. sodium hydroxide solution, dilute to the mark, and allow the precipitate to settle. Decant some of the liquid through a filter paper that has been washed with dilute sodium hydroxide solution to remove ammonia. Take 100 ml. of the filtrate in a Nessler cylinder and add 5 ml. of 5 per cent. gum arabic solution and 2 ml. of Nessler reagent. Compare in the usual way with a blank solution to which a standard solution of ammonium chloride is added.

Remove ammonia from the gum solution before use by passing it through a column of base-exchange zeolite, adding one-tenth of its volume of Nessler solution and filtering. Insufficient hydrogen peroxide is added to the sample to oxidise all the iron. The hydroxide precipitate does not absorb

ammonia from the solution, unless it is allowed to dry before the filtration is completed. The ammonia blank is kept as small as possible by using re-distilled acid, distilled water boiled with a little sodium hydroxide, boiled sodium hydroxide solution, and hydrogen peroxide distilled from sulphuric acid. (0·1 g. of sodium peroxide may be used instead of hydrogen peroxide.) Results obtained with the method agree well with those obtained by vacuum fusion or by a distillation method. No evidence has been found for the suggestion that steels contain some nitrides that are hydrolysed to ammonia only during an alkaline distillation process.

The method, with slight modification, can be used for organic materials. L. A. DAUNCEY

Rapid Methods of Determining Copper in Ferro-Titanium. B. J. Barkov (*Zavod. Lab.*, 1946, **12**, 546–549)—Three variants of an internal electrolysis method are described.

Internal electrolysis without preliminary separation of iron and titanium—Dissolve 0·25 g. of ferro-titanium in 20 ml. of diluted sulphuric acid (1 + 3), oxidise the carbides with 2 to 3 ml. of diluted nitric acid (sp.gr. 1·20), and evaporate to fuming. Cool the solution, add water to dissolve the salts, filter off the silica, and wash with hot water. Heat the filtrate (volume about 120 to 150 ml.) to 75° C., add 1 ml. of a 0·5 per cent. solution of hydrazine sulphate or hydrochloride to reduce iron, and insert the electrodes. The cathode is a platinum gauze electrode, and the anode a smooth aluminium plate that has been rubbed with emery paper, then immersed for 1 to 2 min. in 30 per cent. sodium hydroxide solution, and finally washed with water; they are joined together outside the solution by means of a copper clamp, which must make good contact with each electrode. The upper 3 to 4 mm. of the gauze cathode projects out of the solution. Allow electrolysis to proceed for 20 min. at 75° to 80° C., then cover the gauze by adding hot water, and electrolyse for a further 6 to 7 min. Absence of copper on the upper part of the gauze shows that deposition was complete. Quickly transfer the electrodes to a beaker containing distilled water, remove and transfer them to another beaker containing distilled water, then disconnect them, wash the cathode with alcohol, place it in an oven at 100° to 105° C. for 2 or 3 min., and weigh.

Internal electrolysis of the solution of the residue insoluble after dissolution of the alloy in sulphuric acid—Tzinberg (1931) showed that copper-containing steels, when dissolved in sulphuric acid in absence of air, yield the whole of the copper as an insoluble, spongy, black residue. The method is applied to the determination of copper in ferro-titanium. Place 0·5 g. of ferro-titanium in a 250-ml. conical flask, add 80 ml. of diluted sulphuric acid (1 + 10), immerse a small spiral of aluminium as a precaution against dissolution of some of the copper, close the flask by means of a Bunsen valve, and heat the contents of the flask until the ferro-titanium is dissolved. Remove the flask from the source of heat, take out and wash the aluminium spiral, filter the solution quickly through a folded paper, wash the flask twice with hot water, collecting the insoluble matter on the filter, and give the filter two further washes with hot water. Then place the paper and contents in the original flask and add 10 ml. of diluted nitric acid (sp.gr. 1·20). After dissolution of the black residue, transfer the solution to a 200-ml. graduated flask, add an excess of diluted ammonia solution (1 + 1) (about 25 ml.), cool, and make up to the mark with cold water. Take 100 ml. of the filtered solution, add diluted sulphuric acid (1 + 1) until the odour of ammonia disappears and then 4 ml. more, heat to 75° C., and then electrolyse as described above.

Internal electrolysis after complete dissolution of the alloy and removal of iron and titanium by means of ammonia—Dissolve 0·5 g. of ferro-titanium in 25 ml. of diluted sulphuric acid (1 + 4), oxidise with 2 to 3 ml. of diluted nitric acid (sp.gr. 1·20), transfer to a 200-ml. graduated flask, add about 50 ml. of diluted ammonia solution (1 + 1), cool, make up to the mark, mix, and filter off 100 ml., add diluted sulphuric acid (1 + 1) as described in the second variant, and electrolyse.

Results are compared with those obtained gravimetrically and by photo-colorimetric determination of the copper - ammonia complex in the ammoniacal solutions obtained in the second and third variants given above. With 1 to 3 per cent. of copper, agreement to 1 part in 100 is obtained. [*Note*—The author does not refer to the possibility of loss of copper by adsorption in the Group-III hydroxide precipitate—*Abstractor*.] G. S. SMITH

Colorimetric Determination of Antimony in Copper-Base Alloys. A. C. Holler (*Anal. Chem.*, 1947, **19**, 353–355)—The method described is suitable for the determination of up to 1 per cent. of antimony in copper-base alloys. The antimony is co-precipitated with stannic oxide by nitric acid treatment of the sample, the oxides dissolved in sulphuric and hydrochloric acids and the antimony converted to the yellow iodoantimonite complex. Iodine, liberated by traces of copper occluded in the stannic oxide precipitate, is removed by addition of sodium thiosulphate. The only element likely to cause interference is bismuth, which forms a yellow iodide complex analogous to that of antimony.

Reagents—Standard antimony solution—Dissolve 0·125 g. of antimony metal in 25 ml. of hot sulphuric acid and dilute to 250 ml.; 1 ml. ≡ 0·5 mg. antimony.

Potassium iodide - sodium hypophosphite reagent—Dissolve 100 g. of potassium iodide and 20 g. of sodium hypophosphite in 100 ml. of water and filter.

Preparation of calibration curve—To volumes from 5 to 30 ml. of the standard antimony solution add sufficient sulphuric acid to bring the total amount of acid in each to 10 ml., and dilute to about 100 ml. Add 10 ml. of hydrochloric acid, cool to 20° C., and dilute to 250 ml. Take a 25-ml. aliquot and add 10 ml. of the iodide - hypophosphite reagent and 1 or 2 drops of 0·5 per cent. starch solution. If a starch-iodine colour forms, add 5 per cent. sodium thiosulphate solution dropwise until the

colour is discharged, adding 1 drop in excess. Make up to 50 ml., adjusting the temperature to 20° ± 1° C. Measure the absorption of the solution at 450 mμ. in a 2-cm. cell, using water as a reference solution.

Procedure—Dissolve 1 to 5 g. of sample, depending on the antimony content, in 25 ml. of nitric acid, and heat until evolution of nitrous fumes ceases. Add 100 ml. of hot water and digest at just below boiling temperature for 1 hr. Filter off the precipitate through a double, No. 42 Whatman paper, wash thoroughly with hot water, and transfer the precipitate and paper to a conical flask. Add 25 ml. of nitric acid and 10 ml. of sulphuric acid and evaporate to fuming, repeating the addition of nitric acid and the evaporation to fuming if necessary. Cool, wash down the sides of the flask and again evaporate to fuming. Cool, dilute to about 150 ml., add 10 ml. of hydrochloric acid, and boil until the solution becomes clear. Cool to 20° C. and dilute to 250 ml. Take a 25-ml. aliquot and develop the colour and measure the absorption as directed above.

To ensure complete co-precipitation of the antimony with stannic oxide, the ratio of tin to antimony should be at least 10 to 1. If necessary, tin should be added at the start of the analysis.

H. J. CLULEY

Detection of Scandium, Rare Earths, Zirconium, and Thorium with Murexide. G. Beck (*Analyt. Chim. Acta*, 1947, **1**, 69–71)—The purple colour of murexide (ammonium purpurate) is changed to yellowish-orange by rare earths and scandium when they are added as sulphate solutions, and by zirconium and thorium when added as chloride or nitrate solution. The lowest concentrations of these elements that can be detected by this test are: cerium earths, 20 μg. per ml.; thorium, 30 μg. per ml.; and zirconium, 75 μg. per ml. Of the purpurates formed, only scandium purpurate is decomposed by the addition of α,α′,α″-trimethylamine-tricarboxylic acid (nitrilotriacetic acid), the purple murexide being reformed. The yellow zirconium and thorium purpurate solutions become violet on adding sodium iodate, and scandium purpurate solutions become red on adding sodium acetate in the presence of sodium iodate. An advantage of the murexide test is that aluminium does not interfere. The group of ions considered in this paper can easily be separated from the other ions, zinc, cadmium, ferrous iron, nickel, cobalt, manganous, and uranyl, that give orange colours with murexide.

The possibility of using the murexide reaction for a volumetric determination of scandium has been examined; the end-point is not sharp.

B. ATKINSON

Volumetric Determination of Rhodium. V. S. Syrokomsky and N. N. Proshenkova (*J. Anal. Chem. Russ.*, 1947, **2**, 247–252)—It is shown for the first time that rhodium can form quinquevalent salts by the action of certain oxidising agents; thus, solutions of tervalent rhodium sulphate containing not more than 10 per cent. by volume of concentrated sulphuric acid are oxidised by sodium bismuthate in the cold to give an intense bluish-violet, stable colour, sensitive to the presence of 0·01 mg. of rhodium in 1 ml. The oxidised solution can be titrated with ferrous ammonium sulphate solution in presence of phenylanthranilic acid as indicator. The determination is accurate to 1 part in 20 or more over the range of 1 to 12 mg. of rhodium.

Iridium salts are oxidised similarly to the quinquevalent state, but not quite quantitatively; palladium salts are oxidised to an unstable quadrivalent state, but platinum salts are unaffected; hence rhodium may be determined in presence of platinum.

Oxidation of tervalent rhodium does not occur with ceric sulphate, potassium permanganate, or boiling perchloric acid mono-hydrate. Ammonium persulphate and silver nitrate together, like sodium bismuthate in the cold, oxidise hot solutions, but some reduction occurs during the subsequent heating required for decomposition of the persulphate excess, making an accurate determination of rhodium impossible. With sodium bismuthate, oxidation is quantitative after 1·5 hr., and the quinquevalent rhodium salt is stable in presence of the excess of bismuthate for several hours. Chlorides must be absent since the ion $[RhCl_6]'''$ is stable towards these oxidising agents.

Iridium salts are rapidly oxidised by hot perchloric acid, and by sodium bismuthate in hot solutions, but bismuthate reacts very slowly in cold solutions, and ammonium persulphate with silver nitrate has no effect. Palladium salts are oxidised by perchloric acid, sodium bismuthate, and by ammonium persulphate in the presence of silver nitrate, but reduction of the quadrivalent salt formed and evolution of oxygen occur almost at once.

The colour intensity of quinquevalent rhodium solutions follows the Lambert - Beer law and can be used for colorimetric determination of rhodium. The oxidised solution gives no precipitate with potassium, ammonium, iron, silver, platinum, iridium, and palladium salts, but aqueous ammonia and sodium hydroxide solutions yield black precipitates of variable composition. Phenylanthranilic acid is instantaneously oxidised, giving an intense cherry-red coloration. This reaction is sensitive to 0·17 μg. of rhodium in 1 ml., and provides a very sensitive test for rhodium.

Volumetric determination—Procedure—Obtain the rhodium in solution as chloride, *e.g.*, by fusion with barium peroxide, dissolution of the melt in *aqua regia*, and removal of barium as sulphate. Treat a suitable volume [quantity not stated] with 50 ml. of water and 15 ml. of concentrated sulphuric acid, evaporate the solution to fuming, and maintain copious fuming for 20 to 30 min. When the pink colour of the complex chloride is replaced by the yellow colour of the sulphate the conversion is complete. Allow the solution to cool, dilute with water to 100 to 120 ml., and cool to 15° to 20° C. Introduce about 1 g. of sodium bismuthate, and leave the solution for 2 to 3 hr. Filter, and wash the residual bismuthate with diluted sulphuric acid (1 + 10) until a drop of the filtrate on a

porcelain plate no longer gives a coloration with phenylanthranilic acid solution. Titrate the filtrate with 0·01 *N* ferrous ammonium sulphate until the colour of the solution becomes paler, add 2 or 3 drops of 0·04 per cent. phenylanthranilic acid in 0·1 per cent. sodium carbonate solution, wait for 1 to 2 min., and complete the titration slowly adding the last drops of the titrant at 15- to 20-sec. intervals. The end-point is shown by a sharp colour change from an intense cherry-violet to pale yellow-green. One ml. of 0·01 *N* ferrous solution is equivalent to 0·51 mg. of rhodium.

Visual observation of the end-point is satisfactory if the rhodium content does not exceed 10 to 12 mg. Platinum may be present to twenty times the rhodium content provided its weight does not exceed 0·03 g., for otherwise a difficultly soluble complex sulphate of platinum containing rhodium may be formed during the evaporation with sulphuric acid. With greater amounts of platinum a preliminary separation of rhodium is necessary (*cf.* Wichers, *J. Amer. Chem. Soc.*, 1924, **46**, 1882). The method is satisfactory for the analysis of alloys of platinum with 7 to 10 per cent. of rhodium. The degree of accuracy noted above was obtained with synthetic mixtures. It is stated that no other method exists for this purpose; the thionalide method (Kienitz and Rombock, *Z. anal. Chem.*, **1939, 117**, 241) is inapplicable in presence of other platinum metals. A method based on oxidation of the complex $K_3[Rh(NO_2)_6]$ with ceric sulphate (*cf.* similar method with $K_3[Co(NO_2)_6]$, Bennett and Harwood, ANALYST, 1935, **60**, 677) gave variable results. G. S. SMITH

Use of Background Intensity instead of a Comparison Line for the Spectroscopic Determination of Cobalt and Nickel in Ores. L. N. Ovchinnikov (*J. Anal. Chem. Russ.*, 1947, **2**, 225–228)—The introduction of an element to serve as an internal standard complicates the spectrographic determination of ores, etc. It is now shown that in the determination of cobalt and nickel in copper sulphide ores the background intensity may be used in place of the intensity of a comparison line. With a Q-24 Zeiss quartz spectrograph, a Zeiss semi-automatic microphotometer, and orthochromatic plates, H and D 200, the lines Co 3405·12 A. and Ni 3414·77 A. and the nearby background were measured on the microphotometer. Standard samples were used for comparative purposes. From the results of several hundred determinations on different samples the probable error (standard deviation × 0·67), calculated from duplicate results, for the background method was ±8 per cent. of the content with cobalt, and ±9 per cent. with nickel, at concentrations between 0·001 and 1 per cent. G. S. SMITH

Physical Methods, Apparatus, etc.

Application of Emission Spectrum Analysis to Molecular Vapours. M. Handelsmann (*Helv. Chim. Acta*, 1946, **29**, 881–888)—The lower limit of detectability of the emission band spectra of certain molecules has been investigated. As the bands of OH, CO, CN, etc., were nearly always detected in the discharge, halides of zinc, cadmium, and mercury were used, as their vapour pressures are negligible at room temperature. The spectra were excited in a discharge tube, fitted with plane quartz windows, by an external high frequency source to obviate the use of internal electrodes and so prevent adsorption of gases on the electrodes. The tube was situated in an electric oven of controlled variable temperature.

Weighed amounts of salts were introduced into the discharge tube and photographs obtained at various temperatures to determine the minimum temperature or corresponding saturated vapour pressure where the characteristic band spectra of the salt vapours first began to show. With mercuric chloride, the bands were prominent when a pressure of 0·2 mm. reduced to 0° C. had been reached.

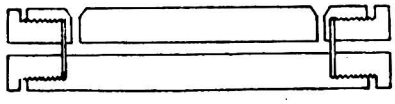
The excitation of molecular spectra was then considered in relation to the existing saturation vapour pressure and the corresponding saturation temperature conditions. Results obtained with the nine halides examined are tabulated below, the spectra occurring only faintly at the lower temperatures (given in brackets), and clearly at the higher temperatures.

Salt	Saturation temperature ° C.	Saturation pressure mm.	Zero pressure mm.
$HgCl_2$	(70) 80	(0·011) 0·023	(0·008) 0·015
$HgBr_2$	(75) 85	(0·013) 0·031	(0·009) 0·021
HgI_2	(90) 100	(0·011) 0·024	(0·008) 0·016
$CdCl_2$	(420) 440	(0·005) 0·011	(0·002) 0·004
$CdBr_2$	(390) 410	(0·009) 0·023	(0·004) 0·008
CdI_2	(300) 320	(0·005) 0·012	(0·002) 0·005
$ZnCl_2$	(280) 300	(0·0015) 0·004	(0·0009) 0·002
$ZnBr_2$	(290) 310	(0·008) 0·002	(0·004) 0·008
ZnI_2	(240) 260	(0·002) 0·006	(0·001) 0·003

The zero pressure is the saturation pressure reduced to 0° C. by considering an average temperature difference of 30° C. between the salt and the middle of the discharge tube. The average value of the minimum saturation pressure appears to be about 0·01 mm. For mercuric chloride, the lower limit of detectability is approximately 3 μg. D. A. POYNTER

Capillary Absorption Cells in Spectrophotometry. P. L. Kirk, R. S. Rosenfels, and D. J. Hanahan (*Anal. Chem.*, 1947, **19**, 355–357)—The sensitivity of spectrophotometers may be increased by developing the colour in a volume merely sufficient to fill the absorption cell, and also by reducing cell-size to the diameter of the

light beam. With instruments such as the Beckman spectrophotometer containing photo-emission elements, the current of which is amplified, further advantage may be obtained by reduction of the cell-diameter to less than that of the light beam and corresponding increase of the cell-length, increased amplification being used to increase the sensitivity. Capillary absorption cells designed for this purpose are described.



The cells, as shown in cross section in the figure, are made from Teflon, a fluorinated hydrocarbon, which is inert to most aqueous reagents and organic solvents and, being opaque, renders unnecessary the use of masks for reducing the diameter of the light beam. The end windows, of glass or clear plastic, are held in by plugs screwed into the main cell. Ports for filling are provided at both ends inside the windows. The solution is introduced by means of a pipette, care being necessary to avoid trapping of air bubbles. A special cell-holder is also described.

The two sizes of cell used for routine work have diameters of 2 mm. and 4 mm., cell-length being 5 cm. in each case, and the final volumes of solution 0·2 ml. and 0·4 ml., respectively. The use of these cells permit the determination of a few millimicrograms of a constituent. The following table indicates the sensitivities obtainable.

CAPILLARY ABSORPTION CELL SENSITIVITIES

Constituent	Method	Amount in μg. giving 90 per cent. transmission in: 10 ml.	1·0 ml.	0·2 ml.
Iron	*o*-Phenanthroline ..	0·44	0·044	0·0087
Chromium ..	Diphenylcarbazide ..	0·14	0·014	0·0028
Manganese ..	Permanganate ..	3·5	0·350	
Phosphate ..	Molybdenum blue ..	0·38	0·038	
Silicon	Molybdenum blue ..	0·11	0·011	

H. J. CLULEY

Tempometric (Drop-Time) Method of Potentiometric Titration. I. A. K. Kalje (*Zavod. Lab.*, 1946, **12**, 773–776)—*General principle*—The reagent is added dropwise at a constant rate of flow (normally, 1 drop per sec.) to about 40 ml. of test solution contained in a glass funnel of 9 cm. diameter, with a fused-in porous glass diaphragm in its conical portion. The funnel below the diaphragm contains a suitable electrolyte and a rod electrode which passes through a short length of rubber tubing, attached to the tube of the funnel, and dips into mercury. The funnel, etc., is made to rotate by means of an electric motor, and contact between the rod electrode and a millivoltmeter or a galvanometer is made through the mercury and the motor. An electrode, immersed in the test solution, is in the form of a plate, 3 cm. × 3 cm × 1 cm. It is stationary and serves also as a stirrer. This electrode, of platinum, gold, palladium, tungsten, silver, or other suitable metal, is called the positive, although in the course of a titration it may become negative. It is connected with the positive terminal of the millivoltmeter, and the lower electrode, usually of cadmium in saturated cadmium nitrate, is connected with the negative terminal. In view of the high ohmic resistance of the porous glass diaphragm the current is very small, and polarisation of the electrodes is practically absent. Thus, it is possible to carry out a variety of titrations, including certain neutralisation, precipitation, and oxidation - reduction titrations, that are difficult or impossible by normal means.

During a titration the reagent solution is added until a characteristic swing of the needle of the millivoltmeter or galvanometer is observed. The result is given in seconds and calculated to millilitres from the known rate of flow of the reagent. The characteristic swing that determines the end-point differs in type according to the kind of titration, and, before any new application of the method may be made, it is necessary first to discover, by work with standard solutions, if a characteristic swing does in fact occur, and if the time at which it occurs with solutions of different concentrations is strictly proportional to these concentrations. Serviceable end-points are obtainable often where there is apparently no connection between them and the stoicheiometric end-points. The characteristic swing of the needle in one type of titration might correspond to its passage through the zero, and in another to a swing that takes it nearly off the scale.

Examples—Neutralisation—With palladium as positive electrode, and cadmium, in saturated cadmium nitrate solution, as negative electrode, 0·1 *N* sulphuric acid was titrated with 0·090 *N* barium hydroxide, delivered dropwise through a capillary tube from a Mariotte bottle with a constant head of liquid. The galvanometer reading, originally 9 divisions, dropped very slowly at first, then passed rapidly through zero, and started to move back slowly. The zero reading was close to the equivalence point. The titration of varying amounts of acid in 40 ml. of water showed that if the first passage through zero was taken as the characteristic swing, the time was proportional to the volume of acid present (here, 36 sec. for 1 ml. of 0·1 *N* sulphuric acid) and that with 2 to 4 ml. of acid the accuracy was better than 1 part in 100 Similar results were obtained in the titration of hydrochloric, succinic, and other acids.

Precipitation—In the normal potentiometric titration of bromide with silver nitrate the very slow attainment by the silver electrode of constant potential after each addition of reagent makes the operation tedious and prolonged. The new method, used on a semimicro scale, enables a result to be

obtained in a few minutes. With a positive silver electrode, and the negative cadmium electrode as before, 0·1 *N* sodium bromide was titrated with 0·1 *N* silver nitrate. Several noticeable displacements of the needle were observed, but one was much greater than all the rest and took the needle nearly off the scale. This was taken as the characteristic swing. It corresponded, however, to nearly double the stoicheiometric amount of silver nitrate. Thus, for each ml. of 0·1 *N* bromide, the time to attain this point with 0·1 *N* silver nitrate flowing at the rate of 1 ml. per 32·5 sec. was 61·3 sec.

Ferrocyanides, which give no clear indications of an end-point in the ordinary potentiometric titration, yielded a well-defined characteristic displacement by the new method of titration with silver nitrate. This occurred at 35 sec. per ml. of 0·1 *N* potassium ferrocyanide, instead of the stoicheiometric 32·5 sec., under the conditions used for titrating bromide.

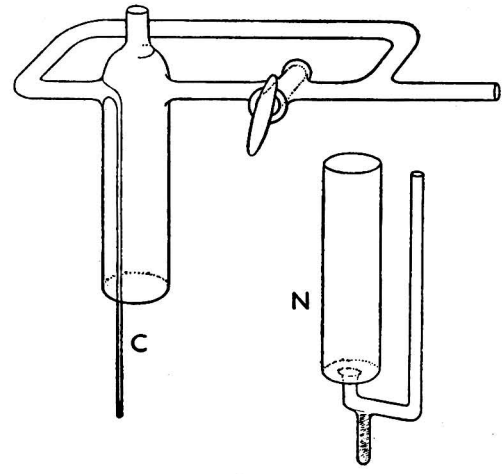
Oxidation - reduction—With a positive gold electrode, and the negative cadmium electrode as before, 0·096 *N* stannous chloride was titrated with 0·1 *N* potassium dichromate in presence of about 0·1 *N* hydrochloric acid. The rate of addition of dichromate was 1 ml. per 32·2 sec. The characteristic displacement occurred at 33·0 sec. per ml. of stannous solution, instead of at the stoicheiometric 31·2 sec.

In general the results, referred to those of standards under identical conditions, are accurate to 1 part in 100. The method should prove most useful in cases where the normal potentiometric method is inapplicable. G. S. SMITH

Use of Multi-Tip Electrodes in Polarographic Work. J. McGilvery, R. C. Hawkings, and H. G. Thode (*Canad. J. Res.*, 1947, **25**, **B**, 132–134)—The sensitivity of the polarograph can be increased if the dropping mercury electrode consisting of a single capillary is replaced by one consisting of two or more capillaries connected in series. The total diffusion current given by an electrode of five capillaries has been shown to agree with the value obtained by substituting in the Ilkovic equation a value for $m^{2/3}t^{1/6}$ that is equal to the sum of the $m^{2/3}t^{1/6}$ factors of the individual capillaries, where m is the mass of mercury flowing per second, and t is the drop-time.

Since the drops falling from the different capillaries are not synchronised, galvanometer oscillations are considerably reduced without the use of artificial damping devices. The sensitivity of a polarograph using multi-tip electrodes is limited by the high residual current obtained. J. G. WALLER

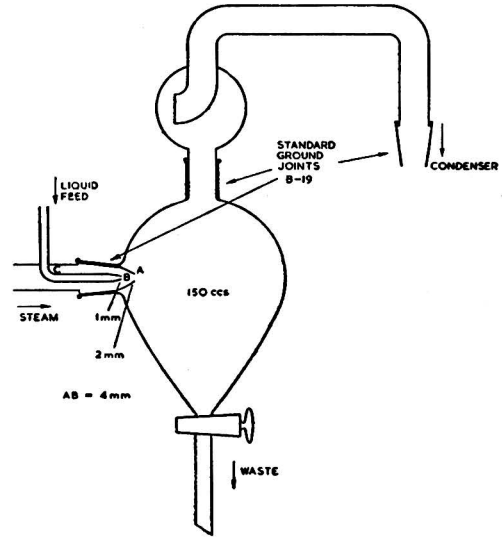
New Electrolyte Vessels for Polarographic Analysis. J. V. A Novak (*Coll. Czech. Chem. Comm.*, 1947, **12**, 237–244)—A vessel has been devised to reduce the number of manipulations required in routine and research work. It consists of an outer cylindrical jacket, *C*, and a close-fitting inner vessel, *N*, to hold the electrolyte. A capillary tube is sealed into the outer jacket and serves to pass an inert gas through the solution in order to remove dissolved oxygen. By opening a stop-cock the inert gas can be passed over the solution while the polarogram is recorded. The inner vessel has a slightly smaller diameter than the outer jacket.



Electrical contact is normally made to the mercury pool, but if a separate unpolarisable electrode is required, a modified electrolyte vessel can be used.

J. G. WALLER

Continuous Steam Distillation Apparatus. W. A. Immerwahr (*Chem. and Ind.*, 1947, **34**, 520)—The Pyrex-glass apparatus shown must be well lagged, and it is sometimes necessary to have several splash traps. Once the rates of flow of filtered liquid and steam are adjusted, the distillation may be allowed to proceed. Pre-heating of high-



boiling liquids is effected by lengthening BC, and low-melting solids may be distilled if the supply vessel is enclosed in a steam-jacket.

M. E. DALZIEL

Reviews

THE DEHYDRATION OF FOOD. By T. N. MORRIS, M.A. Pp. 174. London: Chapman & Hall, Ltd. 1947. Price 15s.

This book, which has special reference to war-time developments in the United Kingdom, describes not only the scientific principles underlying the dehydration of foods, but also the practical and technological developments that have taken place partly under the stimulus of war-time conditions and necessities. It is little but good—very good. That is not to say that no points of detail, no references or descriptions could be criticised; but in general the fundamentals of the subject are well and truly described, and commercial processes are adequately outlined. So great has been the success achieved in practice that, as the author remarks, any faulty specimens are apt to be regarded as representative of dehydrated foods, while the normal product is erroneously thought to be cooked fresh food.

The subjects particularly dealt with include, after a very interesting historical survey, data on nutritive values, a comparison of dehydration with canning or refrigeration, and the general characteristics of biological materials in relation to the problems of dehydration. Then follow the application of these principles to vegetables, to meat, fish, milk, eggs and other foods. Storage and packaging are dealt with, also methods for the practical examination, including the bacteriology, of dehydrated foods and the control of infestation.

Altogether this is a book much to be commended to all who are interested in food, whether they be analysts, dietitians or technologists. It is well written and contains a remarkable lot of information and data in a comparatively small compass. The author's position as a member of the Low Temperature Research Station and his work under the Food Investigation Board are a guarantee of competence and knowledge of the subject. He, with the publishers, has produced a very useful, very readable and informative book. H. E. Cox

FORENSIC MEDICINE. By KEITH SIMPSON, M.D. Pp. 322. London: Edward Arnold. 1947. Price 16s.

Included in the training of students of medicine is a short course on Forensic Medicine and Toxicology and it is usually something of a problem to decide how much the average medical student should be required to know, for a medical man may at any time be called upon to deal in the first place with cases presenting medico-legal problems.

Certain well-known textbooks on forensic medicine deal very fully with the subject and are of considerable value to those having a flair for it, but for the average medical student they may be dull and confusing.

The book under review fills a gap and presents an account of the subject in a very attractive manner, sufficiently in detail to meet the needs of the average medical student. The first thirteen chapters deal with essentially medical problems, the next four mainly with medico-legal aspects and the remaining twelve with toxicology. In a space of 322 pages the author has presented the fundamentals of the subject in a very readable manner and included many instructive illustrations.

Here and there the author's classical background is indicated. In general, there is a suggestion that the book has, in parts at any rate, been somewhat hastily written, with the result that a few vague and, in some instances, inaccurate statements remain. For example, on p. 127, in a reference to the Rouse case (1930) it is stated that the body in the burnt car "was that of a woman, and the post-mortem examination showed that she had been murdered by some blunt instrument such as a hammer"; the body was, in fact, that of a man, and it appeared to be the intention of Rouse to give the impression that he himself was the man. Again, on p. 57, in a description of the disposal of a body in another murder, we find the following: "In that case a woman had been struck on the side of the head with a stool (subsequently burned) and, stripped of all clothing, had been toppled from a bicycle into a stream sewn up inside four potato sacks." On p. 257, under "Tests for the detection of strong mineral acids"—"in solution" and "solution" should read "insoluble" and "soluble" respectively; also "HN_4OH" should read "NH_4OH."

The book not only gives guidance to the general practitioner as to what he should do, but also what he should not do, as on p. 22, and, referring to blood grouping on p. 45, "where the results are likely to carry grave consequences the test should be left to the expert." As a suggestion for subsequent editions, some guidance on procedure in submitting organs and other materials for toxicological examination in cases of suspected poisoning would be of value; in some cases within the reviewer's experience these have been incompletely and badly presented.

The publishers are to be congratulated on the production of this volume; the printing is good and the illustrations clear—a very creditable production in these days of austerity. J. B. FIRTH

HUMOUR AND HUMANISM IN CHEMISTRY. By Professor JOHN READ, F.R.S. Pp. 388 + xxiii. London: G. Bell & Sons Ltd. 1947. Price 21s.

The modern chemist does not seem to concern himself overmuch with the history of his science and he is perhaps still less acquainted with the lives and characters of the men whose labours have brought it to its present state. Beyond the names of those after whom apparatus, theories or reactions have been called and a few stories, such as those told of Kekulé's bus-ride through London and Gay-Lussac's way of celebrating a discovery by "justifying his first name," as Professor Read puts it, we usually know but little of the lives and characters of the men who made chemical history; nor, for that matter, have we been in the past too

plentifully supplied with reasonably full and readable books for repairing our loss. This state of affairs has, during recent years, been taken in hand by Professor Read, who has already given us several examples of his skill as a writer and historian.

In this, his latest book, dedicated to his pupils, the professor continues and amplifies his "*Prelude to Chemistry*"; but from a rather different view-point, the emphasis, this time, being focussed upon the personalities of the alchemists, chymists and chemists with whose activities he is concerned, rather than upon their actual work and theories. The treatment is chronological and the style anecdotal, light and, in many parts, humorous. The period covered begins with the gropings of the alchemists in their "smowking smydys," continues with the chymists of the iatro period and ends with modern research laboratories and some of the author's personal experiences of student life in Zurich, as a research assistant to Professor Pope in Cambridge and of his own work in the investigation of the forest wealth of Australia.

To search for the beginnings of chemistry in the records of alchemy savours somewhat of taking the family skeleton from the chemical cupboard. Nevertheless, everything must have a beginning somewhere, and many of the alchemists were interesting if doubtful characters; moreover, the author treats them with humour and sympathy and makes of them and their cryptic records a very convincing starting point for his story. But let it be said at the outset that most of the humour and a great part of the humanism in this book is the professor's own.

Among the curiosities to be found in the book there are extracts from Jane Marcet's "*Conversations on Chemistry*," a work that so inspired a young book-binder's assistant that he became professor at the Royal Institution, and eventually gave us the fundamental laws of electricity, with all that they have led to. Some idea of the importance formerly attached to this book and the oblivion that it has now reached is given by a copy of the fifth edition, 1817, in the reviewer's collection. It bears the bookplate of the Royal Military Academy, the impress of His Majesty's Stationery Office and the second-hand bookseller's price mark—"3d."

Professor Read ends his book with a flight of imaginative fancy in the form of a chemic drama or laboratory pantomime, written for the students of St. Andrew's University. This is named after the Peace Prize that was founded, paradoxically enough, by the inventor of dynamite. It is written in a style that recalls the verbal quips and quiddities of Hood and Barham; and, despite a key and a glossary, requires a sound knowledge of general chemistry and broad Scots to interpret its hidden mysteries of diction and science. It should go far to answer Kekule's exhortation, "Lehrnen wir Träumen," and to remove Baeyer's reproach that "So viele Chemiker haben nicht genügend Phantasie."

The comparatively moderate price asked for a book containing 60 plates on art paper and 29 illustrations in the text, and one that is well-printed on paper good by present-day standards, has been made possible by generous financial help, for which those who find pleasure in its pages will wish to thank the Walker Trust of the University of St. Andrews and Imperial Chemical Industries.

A fitting motto for this intellectual treat would be—

"I can teach you with a quip, if I've a mind;
I can trick you into learning with a laugh."

For like the Merryman in the song, the professor has a pretty turn for anecdote; he knows all the jests—ancient and modern—past, present and to come.

F. L. OKELL

FIDUCIAL LIMITS OF VITAMIN D_2 ASSAYS

THE Biological Methods Group has agreed jointly with the Nutrition Panel of the Society of Chemical Industry to collect evidence about the limits of error that may reasonably be expected in biological assays of vitamin D_2 (using rats). All members of the Biological Methods Group who have experience of assaying vitamin D_2 are therefore asked if they would be good enough to obtain from the Honorary Secretary of the Group (Dr. E. C. Wood, Virol Ltd., Hanger Lane, Ealing, London, W.5) a copy of a brief questionnaire and return it to him as soon as possible. It is hoped that in the light of the replies received, it should be possible to prepare and publish a statement that would be of assistance to all interested persons.

Volume 73, No. 867, Pages 303—366 June, 1948

Contents

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

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