The ANALYST

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

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

Volume 84

No. 1004, Pages 621-676

November 1959

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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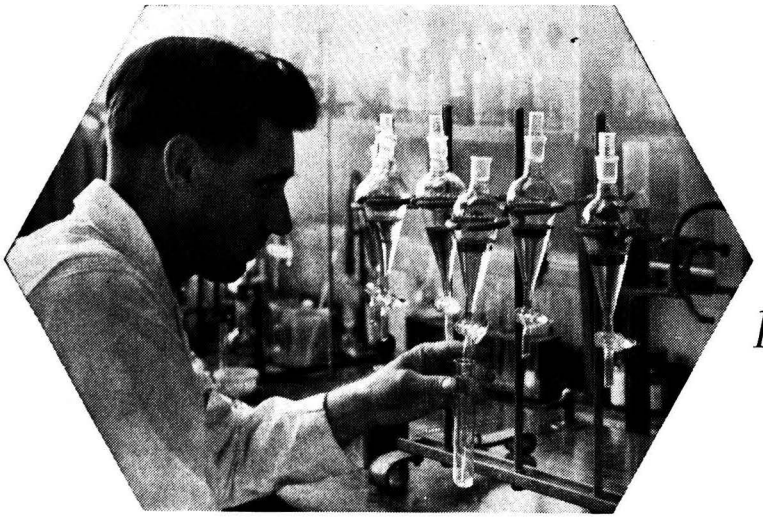
Volume 84, No. 1004 November 1959

Incidental information

No. 13



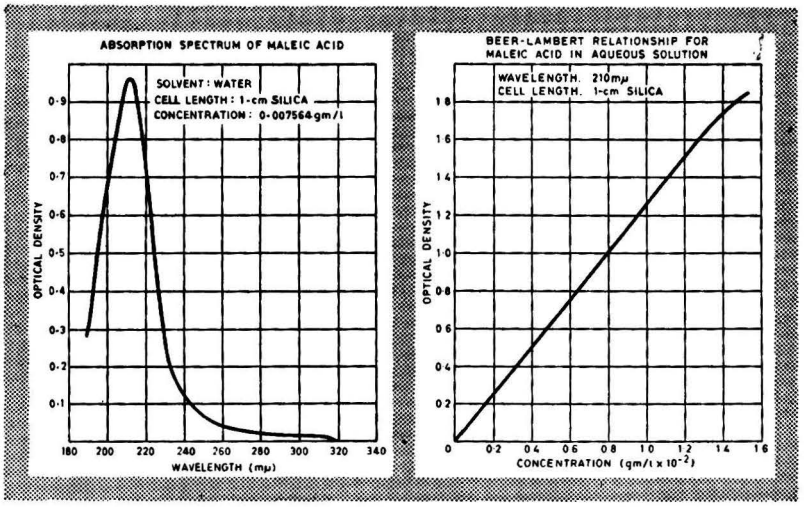
Items of interest from our laboratory notebooks

▶ Most analysts know about 1:10-phenanthroline and many use it for iron determinations. Not so many people seem to know that **4:7-diphenyl-1:10-phenanthroline** is twice as sensitive as 1:10-phenanthroline in the colorimetric determination of iron. There are several papers on the subject but the latest is *Analyst*, 1958, **83**, 80. The reagent is also called **Bathophenanthroline**, and we make it.

▶ Then, again the substitution of methyl groups in the 2:9 positions has the interesting effect of making the reagent insensitive to iron and we then have a selective and sensitive reagent for copper (see *Anal. Chem.*, 1956, **28**, 1158). Hopkin & Williams make **2:9-dimethyl-1:10-phenanthroline** (sometimes called **Neocuproin**).

▶ One does not think of sulphate as a radical one can determine absorptiometrically, but this is now possible for low concentrations. **Barium chloranilate** is the reagent and there are two papers on the subject—*Anal. Chem.*, 1957, **29**, 281 and *Anal. Chem.*, 1958, **30**, 202. Hopkin & Williams make it.

▶ Hopkin & Williams Ltd. were also early off the mark with supplies of the remarkable new colour-producing reagent for fluoride ions, **3-aminomethylalizarin-NN-diacetic acid**, described by Belcher, Leonard and West (*Talanta*, 1959, **2**, 92.) This important reagent is already available from stock.



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THE SOCIETY FOR ANALYTICAL CHEMISTRY

FORMERLY THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to all branches of analytical chemistry; to study questions relating to the analysis, nature and composition of natural and manufactured materials generally; and to promote, or assist to promote, the efficiency and the proper administration of the laws relating to the control and composition of such materials.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society who shall provide written testimony of their personal knowledge as to his scientific and professional fitness. If the Council in their discretion think fit, such testimony may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £3 3s. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No Entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The Entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st of each year.

Scientific Meetings of the Society are usually held on the first Wednesday in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. The Annual General Meeting is usually held in London on the first Friday in March. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

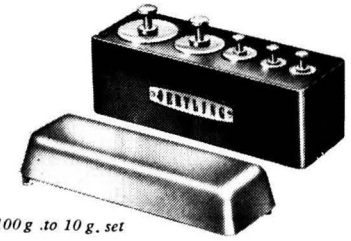
The Analyst, the official organ of the Society, is issued monthly, to all Ordinary and Junior Members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas. The Microchemistry, Physical Methods and Biological Methods Groups have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.



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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, November 4th, 1959, in the meeting room of the Chemical Society, Burlington House, London. W.1. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The following papers were presented and discussed: "A Spectrographic Method for the Analysis of High-purity Acids," by J. H. Oldfield, F.R.I.C., and E. P. Bridge, B.Sc.; "The Colorimetric Microdetermination of Copper in Water: A Survey of Available Methods," by B. Tuck, B.Sc., A.R.I.C., and E. M. Osborn; "Spectrofluorimetry of Lubricating Oils—Determination of Oil Mist in Air," by C. A. Parker, B.Sc., Ph.D., F.R.I.C., and W. J. Barnes.

NORTH OF ENGLAND AND SCOTTISH SECTIONS

A JOINT Meeting of the North of England and Scottish Sections was held at 7.15 p.m. on Friday, September 25th, 1959, in the Central Hotel, Victoria Viaduct, Carlisle. The Chair was taken by the Chairman of the North of England Section, Dr. J. R. Edisbury.

The subject of the meeting was "Water: Determination and Examination," and the following papers were presented and discussed: "Determination of Water," by J. H. Thompson, B.Sc., Ph.D., A.R.I.C.; "Water Analysis as a Guide to Potability," by J. G. Sherratt, B.Sc., F.R.I.C.; "Determination of Radioactive Contaminants in Water," by G. E. Eden, B.Sc., A.R.I.C.

The meeting was preceded at 2.15 p.m. by a visit to Carr's Biscuit Works, Carlisle.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Thursday, September 17th, 1959, in the Mason Theatre, The University, Edmund Street, Birmingham 3. The Chair was taken by the Vice-Chairman of the Section, Dr. H. C. Smith, F.R.I.C.

A discussion on "Some Applications of EDTA" was opened by T. S. West, B.Sc., Ph.D., A.R.I.C., J. Blenkin, B.Sc., and C. A. Johnson, B.Pharm., B.Sc., F.P.S., A.R.I.C. (see summaries below).

SOME APPLICATIONS OF EDTA

DR. T. S. WEST discussed recent advances that had been made in the technique of complexometric analysis. The subject matter ranged over new complexones, indicators, methods of physico-chemical end-point detection, masking procedures and other topics. Recent trends were examined and he made some comments on possible developments in the near future.

MR. J. BLENKIN gave a brief description of the development of some methods incorporating EDTA titrimetry for the analysis of the ash from compounded rubber.

These methods had particular reference to:

(*a*) The determination of barium sulphate by solution in ammonium EDTA after pre-treatment of the sulphate.

(*b*) The determination of strontium in the presence of zinc, using differential pH techniques and ion-exchange resins.

(*c*) The determination of iron, aluminium and magnesium in silicates after fusion in sodium hydroxide.

MR. C. A. JOHNSON said that during the past few years EDTA had been used increasingly for routine analytical control in the pharmaceutical industry. He reviewed some of the types of problem occurring in this field and gave a somewhat more detailed account of the analysis for aluminium. The use of complexometric methods for the determination of alkaloids and of sulphate were considered. Finally he made some attempt to assess the contribution that EDTA had so far made to routine control analysis.

AN Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, October 14th, 1959, in the Technical College, The Butts, Coventry. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "The Analytical Chemistry of Tantalum and Niobium," by A. R. Powell, F.I.M., F.R.I.C., F.R.S.

BIOLOGICAL METHODS GROUP

A DISCUSSION Meeting of the Group was held at 6.30 p.m. on Thursday, October 15th, 1959, in "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. J. S. Simpson, F.I.M.L.T.

A discussion on "Routine Toxicity Tests in the Control of Pharmaceuticals" was opened by P. Andrews, B.Sc.

The Determination of Mercury by Distillation from its Compounds and Preparations*

BY H. E. BROOKES AND L. E. SOLOMON

(*Boots Pure Drug Co. Ltd., Pharmaceutical Division, Standards Department, Nottingham*)

The use of mercury and its derivatives is extensive, and a survey of the literature shows that there are many analytical methods described for its determination in the widely different materials employed. No one method has been available that would deal with them all.

The advantage of the proposed method is that it has been found to be applicable to a wide range of mercurial compounds and preparations. The mercury is isolated, free from interfering substances, by a distillation procedure in which a fused-silica distillation flask is used. The metal is then dissolved in nitric acid and titrated with ammonium thiocyanate solution.

The application of the method to seed dressings and pharmaceutical preparations is described.

DISTILLATION has long been used for isolating mercury quantitatively from compounds and impurities. In 1844, Erdmann and Marchand[1] used distillation with lime to determine the atomic weight of mercury. Although the method is capable of giving a high degree of accuracy,[2,3,4,5] it has not generally been used, presumably owing to difficulty and inconvenience in manipulation.

All the modifications of the distillation method have been similar in principle, the material being heated in a horizontal tube in the presence of lime and the distilled mercury being amalgamated or condensed and weighed.

We saw the advantages of distillation for determining mercury when Rose's method,[6] as described by Lunge and Keane,[7] was studied by a Joint Committee[8] of members of the Ministry of Agriculture, Fisheries and Food and the Association of British Insecticide Manufacturers. A Pyrex-glass tube, closed at one end, was filled successively with magnesite,

* Presented at the Meeting of the Society on Wednesday, October 2nd, 1957.

mercurial mixed with lime, cupric oxide and lime, and was closed with an asbestos plug. The open end of the tube was drawn out and bent at right angles immediately after the plug. The tube was then strongly heated in an iron guttering; its open end dipped into a beaker containing water, in which the mercury was collected and finally weighed.

It occurred to us that a vertical retort consisting of a distillation flask would be superior to a horizontal tube for the following reasons—

(*i*) The distillate would rise by convection, as in conventional liquid distillation, and would not require the constant passage of an inert gas to assist it.

(*ii*) When the organic matter was destroyed by heat, the contents of the bulb would pack down under gravity, whereas, in a horizontal tube, channels are formed, along which the inert gas will pass instead of diffusing through the whole.

(*iii*) Heating would be simplified, as most of the heat could be concentrated on the bulb of the flask.

Previously the mercury had been collected and weighed. Under the conditions of the experiment, mercury is often finely divided and contaminated by distillation products that are difficult to remove by washing without loss of mercury, particularly when present in small amounts. We therefore decided to collect the mercury on zinc wool, dissolve it in nitric acid and titrate with ammonium thiocyanate solution. The conditions of this titration were established by Kolthoff and Stenger[9] and by other workers,[10,11,12] and the procedure was shown to be accurate; Sudo, Shimoe and Miyahara[5] found the method to be accurate at semi-micro levels.

Experimental

Preliminary experiments—

A Pyrex-glass flask having a 40-ml bulb and a neck 11 cm in length and 1·2 cm in diameter fitted with a B14 standard cone was attached by means of standard joints to a small delivery tube and a U-tube receiver packed with zinc wool. The bulb was filled with a mixture of iron filings, lime and the mercurial, and the neck with a mixture of iron filings and lime. The delivery tube and receiver were fitted, the latter being cooled by dipping under water in a beaker. The neck and then the flask were heated strongly. When evolution of gas had subsided, the receiver was detached and placed in a titration flask. The amalgam was dissolved in nitric acid and the mercury was titrated with 0·1 *N* ammonium thiocyanate. The results were promising, although difficulty was experienced owing to softening of the glass. This difficulty was overcome by using a fused-silica flask. Further, since it was noticed that small amounts of mercury adhered to the delivery tube, it was found to be more convenient to make the delivery tube and receiver in one piece, the whole of which could be placed in the titration flask. Several determinations were made on seed dressings, which happened to be available to us from collaborative work previously carried out in connection with the Joint Committee; results were reproducible and recoveries were at least as good as and often superior to those obtained by a variety of methods in the collaborative study.[8] The seed dressings had been compounded according to results obtained for the constituent mercurials by other methods. It was therefore considered necessary to test the method with an inorganic mercury salt. Mercuric chloride was chosen because of its volatility and as a test of complete reduction. The mixture of lime and iron filings was found to be inadequate for reducing the mercuric chloride completely, and recoveries were only about 95 per cent. Various reducing agents were tried unsuccessfully, but 2 g of sucrose provided the required atmosphere and sufficient gases to drive all the mercury into the receiver within 15 minutes.

Several mercury compounds and preparations were examined by this procedure; the results are shown in Tables I and II.

Loss of mercury—

After several determinations on the same sample of analytical-reagent grade mercurous chloride by different workers, it became clear that there was a fairly constant loss of mercury when the standard receiver and procedure were used.

TABLE I

RECOVERY OF MERCURY FROM COMPOUNDS BY DISTILLATION

Compound	Recovery, %	Compound	Recovery, %
Mercuric chloride.. ..	99·1	Mercuric sulphide ..	99·5
Mercurous chloride ..	99·3	Mercuric iodide	99·1
Mercuric oxide	99·2	Mercuric oxycyanide ..	99·2

TABLE II

RECOVERY OF MERCURY FROM VARIOUS PREPARATIONS

Preparation	Mercury content by distillation, %	Mercury content by other methods, %	Recovery (if result by distillation is correct), %
Mercury ammoniated	77·7	78·4*	99·1
Mercurochrome	25·6	25·2†	—
Mersalyl	38·6	38·1‡, 38·6§	100·0
Methylmercury chloride	79·1	76·0*	98·6 (calculated)
Phenylmercury *p*-hydroxybenzoate	47·6	47·65*	99·8
Ethylmercury phosphate	71·5	71·6*	99·8
Phenylmercury nitrate	63·0	63·1*	99·8

* By the method described by Gjalddalk *et al.*[13]
† By the method described in the British Pharmaceutical Codex.
‡ By the method described in the British Pharmacopoeia.
§ By the method described by Theimer *et al.*[14]

To minimise this loss, the receiver of the standard apparatus was modified; an inverted small bulbous funnel packed with glass wool was fitted by means of a standard joint. When the standard procedure was carried out with the modified receiver on the sample of mercurous chloride used in the previous experiment, the loss was reduced to less than 1 mg of mercury, which is sufficiently small to be insignificant for macro determinations. The results of both series of experiments are shown in Table III.

TABLE III

LOSS OF MERCURY FROM MERCUROUS CHLORIDE DURING DISTILLATION

Amount of mercury taken, mg	Amount of mercury found, mg	Recovery, %	Amount of mercury lost, mg
When standard receiver was used—			
357·5	356·0	99·5	1·5
282·2	280·4	99·3	1·8
273·5	271·6	99·2	1·9
264·5	262·8	99·3	1·7
250·5	248·6	99·2	1·9
239·1	237·6	99·3	1·5
208·0	206·6	99·4	1·4
190·2	189·1	99·3	1·1
137·9	135·9	99·0	1·3
4·13	2·48	60·0	1·65
When modified receiver with glass-wool trap was used—			
215·4	215·0	99·8	0·4
194·2	193·7	99·7	0·5
171·7	171·4	99·8	0·3
163·0	162·4	99·5	0·6
18·97	18·47	97·2	0·5
6·2	5·95	95·9	0·25
0·76	0·54	71	0·22
0·76	0·28	37	0·48

INTERFERING SUBSTANCES—

The promise shown by the method with mercury compounds led us to test it on a range of mercury preparations, which for assay purposes can be classified in three main groups—

(*a*) Tablets and pills.

(*b*) Seed dressings, dusting and other mercury powders.

(*c*) Ointments.

The substances likely to be associated with the mercurial in such preparations are chlorine, bromine and iodine, sulphur, benzene hexachloride, dieldrin, boric acid, bismuth subgallate and lead arsenate. Distillation experiments were carried out on mercurous chloride mixed successively with 1-g amounts of chlorine, bromine and iodine, sulphur, boric acid and bismuth subgallate, 2-g amounts of benzene hexachloride and dieldrin and 5 g of lead arsenate. These amounts had no measurable effect on the recovery of mercury, except in presence of iodine and bromine, when it was necessary to introduce sufficient copper filings above the iron filings - lime mixture to occupy about 1 inch of the neck of the flask. Large amounts of chlorides and gas were produced when benzene hexachloride was present, and it was found to be advisable to limit the amount of this material in the flask to 2 g.

When sulphur was present it was noticed that the mercury distilled free from tarry matter produced by the decomposition of organic material. Sulphur was therefore used later to cleanse the distillate, particularly from ointments.

EXAMINATION OF OINTMENTS—

The nature of ointments makes it inconvenient to use a large amount of sample. It is difficult to pass much more than 2 g of an ointment through the neck of the flask, and, further, a large proportion of the ointment-base distils with the mercury. This presents no difficulty for ointments containing 5 per cent. or more of mercury, as the loss, approximately 0·5 mg, when the glass-wool trap is used, is insignificant. However, at lower concentrations of mercury this loss becomes appreciable.

To reduce the loss further, a more efficient receiver was used; it consisted of a vertical 75-mm Liebig condenser, internal diameter 6 mm, sealed to the open end of the existing receiver. In an earlier experiment, aluminium had been used to trap the mercury as an amalgam, since it was thought that the mercury was present as vapour, which might be lost. After several materials had been tried, we found that well packed glass-wool was equally effective, which suggests that mercury was probably lost as a spray rather than a vapour. When this apparatus was used, less than 0·1 mg of mercury was lost. This loss is again not significant for ointments containing 1 to 5 per cent. of mercury if a 1-g sample is taken for the assay.

We have found that results are erratic when the amount of mercury distilled is greater than 50 mg, which appears to be the capacity of the receiver. No attempt has been made to modify the apparatus for macro determinations, for which a satisfactory receiver has been described.

Owing to the difficulty of preparing small samples of ointment accurately in the laboratory, we examined several factory batch samples by distillation and compared the results with those by the official or other well known methods previously found to be reliable. The results are shown in Table IV.

The seed dressings examined at the start of the investigation were then assayed by the distillation procedure in its final form, receiver *b* and a glass-wool trap being used in all the determinations; the results are shown in Table V and the constituents of the seed dressings in Table VI.

METHOD

APPARATUS—

The complete apparatus is shown in Fig. 1.

Fused-silica flask—The capacity of the bulb is approximately 25 ml; the neck is 1·2 cm in diameter and 11 cm long (including the B14 cone, which is 2·8 cm long).

Pyrex-glass receivers—The dimensions of the receivers are shown in Fig. 2.

TABLE IV

MERCURY CONTENTS OF OINTMENTS BY DISTILLATION AND OTHER METHODS

Sample		Mercury content by distillation, %	Mercury content by other methods, %	Calculated mercury content, %	Weight of sample, g	Receiver used (see Fig. 2)
Mercury oleated B.P. ..		18·15	17·86	18·50	1	*b*
Ointment mercurous chloride B.P. 1948		18·46	18·7, 18·26	16·98	1	*b*
Ointment mercuric nitrate strong B.P.	(1)	7·20	7·14	6·99	2	*b*
	(2)	6·72, 6·63	7·15			
	(3)	6·99	6·24			
	(4)*	6·92	6·48			
	(5)	7·06, 6·96	6·0, 6·30			
Ointment mercury oleated B.P.	(1)	4·95	4·68	4·62	1	*c*
	(2)	4·46	4·43, 4·50			
Ointment mercury, lead and zinc B.P. 1949	(1)	2·30	2·04	2·33	2	*c*
	(2)	2·12	1·90, 1·93			
Ointment mercury ammoniated B.P.		2·01	1·85	1·97	2	*c*
Ointment ammoniated mercury and coal tar	(1)	1·95	1·91	1·97	2	*c*
	(2)	2·04	1·98			
Ointment mercuric nitrate dilute B.P.	(1)	1·50	1·39	1·40	2	*c*
	(2)	1·34	0·93, 0·86, 1·10, 1·16			
	(3)	1·48	1·39			
	(4)	1·33	1·22			
	(5)	1·48	1·28			

* Sample No. 3 re-mixed.

TABLE V

MERCURY CONTENTS OF SEED DRESSINGS

Each sample was mixed with 1 g of sucrose before distillation

Sample No.	Weight of sample, g	Mercury content found, %	Calculated mercury content, %
1	8	2·46	2·50
2	15	1·46	1·50
3	5	2·94	2·96
4	20	1·01	1·01
5	10	1·00	1·06
	5	1·01	
6	10	1·13	1·14
7	20	1·15	1·15

TABLE VI

CONSTITUENTS OF SEED DRESSINGS

Sample No.	Substances present
1	Phenylmercuric acetate, soluble filler, oil and wetting agent
2	Phenylmercuric acetate, ethoxyethylmercury silicate, insoluble filler, dye and oil
3	As in sample No. 2 *plus* 40 per cent. of added benzene hexachloride
4	Phenylmercuric acetate, ethylmercury chloride, insoluble filler, dye and oil
5	As in sample No. 4 *plus* 40 per cent. of added benzene hexachloride
6, 7	Phenylmercuric acetate, lead arsenate and dye

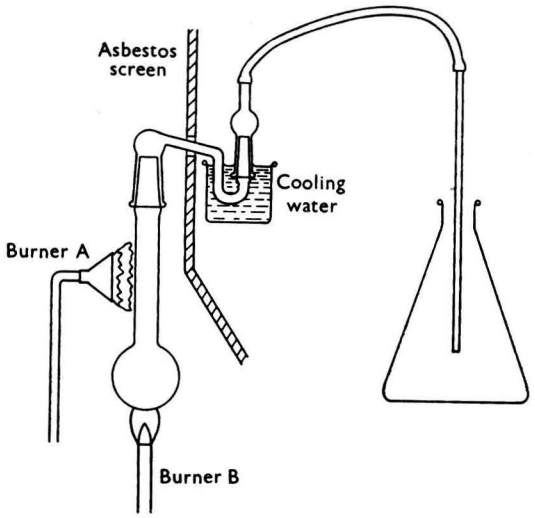

Fig. 1. Distillation apparatus

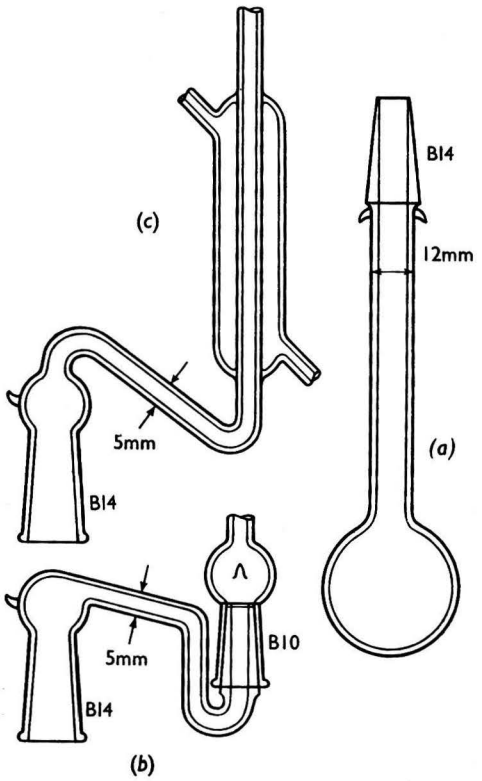

Fig. 2. (*a*) Fused-silica flask; (*b*) Pyrex-glass receiver; (*c*) Pyrex-glass semi-micro receiver. Scale: half size

Bunsen burner A, *4-inch* × *½-inch brass tube*—Over this, in order conveniently to heat the neck of the flask, is fitted a piece of ½-inch diameter copper tubing, bent to a right angle, having a 4·5-cm flame-spreader attached to its horizontal end by means of Emerto gas-pipe fittings.

Bunsen burner B, *3½-inch* × *$\frac{7}{16}$-inch brass tube.*

Asbestos screen—A suitably shaped piece of asbestos to shield the receiver and beaker of water from the bunsen burners.

REAGENTS—

Sucrose.
Calcium oxide, granular, B.P.C. 1934—Particle size 20- to 40-mesh.
Iron filings—Fairly coarse.
Zinc wool—Analytical-reagent grade.
Ammonium thiocyanate, 0·1 N.
Ammonium ferric sulphate solution, 10 *per cent. w/v.*
Potassium permanganate solution, 5 *per cent. w/v.*
Ferrous sulphate.

PROCEDURE FOR DECOMPOSING POWDERS—

Weigh a suitable amount of the sample, mix with 2 g of powdered sucrose, if necessary (see Note), and transfer to the flask by means of a funnel. (A small amount of calcium oxide mixed with the powder helps it to flow more freely.) Fill the flask to the neck with iron filings, and fill the neck to the bottom of the cone with a mixture of equal parts by volume of iron filings and calcium oxide. Fit the cone with a tight plug of steel wool (grade O Supreme, obtained from the Brillo Manufacturing Co. Ltd., was used). Plug the end of receiver *b*

with zinc wool. Prepare the glass-wool trap by plugging the bulbous portion with glass-wool that has been washed with acid and then dried; keep in place by means of zinc wool. Attach the trap to the receiver, and fix by a spring.

Lubricate the B14 joint with silicone grease, attach the receiver to the flask, and secure by tying iron wire round the lugs provided. Clamp the flask vertically with the U-bend of the receiver immersed in water in a 100-ml beaker.

If the semi-micro receiver, *c*, is used, charge the flask in the way just described, but use a weight of sample equivalent to less than 50 mg of mercury.

Heat the neck of the flask to a dull red for 5 minutes with burner A. (A piece of wire gauze over the standard joint protects it from the direct heat of the flame.) Heat the bulb of the flask with burner B; use an extremely small flame, so that evolution of gas is not too rapid (5 to 10 minutes). Adjust burner B so that a steady stream of gas is evolved for a total time of about 15 minutes. When evolution of gas has considerably reduced, heat for another 2 minutes with the full heat of burner B. (Owing to great dissimilarity in mercury-containing preparations, it is not possible to state exact heating periods for the whole range of preparations, but a total heating period of 15 to 20 minutes was generally ample.) Pass the evolved gases into a flask containing carbon tetrachloride by means of a length of narrow rubber tubing attached to the outlet of the receiver. A good indication of the rate of evolution of gas can be obtained from the position of the "ring" of condensing mercury globules in the receiver, which should pass about 1 inch beyond the upper bend of the receiver.

At the end of the heating period, turn off burner A, cut the iron wire, and detach the receiver. Turn off burner B, and allow the receiver to cool (about 5 minutes).

NOTE—Organic mercurials, except the most volatile (such as methylmercuric chloride), decompose when heated, and sucrose need not be added to preparations containing these mercurials, *e.g.*, seed dressings, or to those containing a large proportion of vegetable matter. Sucrose must be added to preparations that are more difficult to decompose and to inorganic mercury compounds. When ignited with mercurous chloride, a 2-g portion of sucrose produces enough gas to clear the flask of mercury in about 20 minutes, a fairly steady flow of gas being evolved.

PROCEDURE FOR DECOMPOSING TABLETS—

Crush twenty tablets to a powder, transfer a suitable amount to the flask, and add powdered sucrose until the total weight of sample is about 2 g. Charge the flask with iron filings and calcium oxide, and then continue in the way described for decomposing powders.

PROCEDURE FOR DECOMPOSING OINTMENTS—

Accurately weigh 1 or 2 g of ointment on a piece of grease-proof paper. With the ointment spread fairly evenly, roll the paper into a 2½- to 3-inch roll. Place the roll in the flask, and press it against the bottom of the bulb with a glass tube. Add 1 g of powdered sulphur, charge the flask with iron filings and calcium oxide, and then continue in the way described for decomposing powders. (In our experience, the periods for which the bulb of the flask is heated are approximately the same for most ointments—6 minutes with an extremely low flame, 6 minutes with a slightly higher flame and 5 minutes at full heat.)

PROCEDURE FOR TITRATING MERCURY—

Amounts of mercury greater than 50 *mg*—Wash the receiver free from tarry matter by adding acetone through the B14 socket, and then wash several times with water to remove acetone. Detach the glass-wool trap, and transfer the receiver to a wide-necked 500-ml conical flask. Add 15 ml of water and then 15 ml of concentrated nitric acid, slowly at first, until most of the zinc dissolves. Warm on a steam-bath to dissolve the mercury, and then boil to remove nitrous fumes. Add 5 per cent. w/v potassium permanganate solution dropwise until a permanent pink colour is obtained, cool, and decolorise by adding ferrous sulphate. Dilute with water to about 120 ml, cool to below 15° C, and titrate against 0·1 *N* ammonium thiocyanate with 10 per cent. w/v ammonium ferric sulphate solution as indicator.

Amounts of mercury less than 50 *mg*—Invert the receiver, and fix it in the neck of a flask by means of a cork that has a second hole through which a glass tube passes so that suction may be applied. Wash with acetone until free from tarry matter. (Slight suction is necessary to draw liquid through at first; it then percolates through by siphon action.) Wash the receiver thoroughly with water to remove acetone.

Transfer the receiver and cork attachment to the neck of a 100-ml conical flask. Dissolve the mercury by allowing three 2-ml portions of concentrated nitric acid to percolate through, wash with six 3-ml portions of water, and remove the receiver. Place the flask on a steam-bath for a few minutes, and add 5 per cent. w/v potassium permanganate solution until a permanent pink colour is obtained. Cool, and decolorise by adding a freshly prepared 2 per cent. w/v solution of ferrous sulphate. Cool to below 15° C, add 0·5 ml of 10 per cent. w/v ammonium ferric sulphate solution, and titrate against 0·01, 0·02 or 0·05 *N* ammonium thiocyanate until the solution has a distinct brown tinge (the titre should be between 5 and 10 ml).

Conclusions

The proposed distillation procedure for isolating mercury from its compounds and preparations can be applied to a wide range of preparations with a high level of accuracy. It can be used for small amounts of mercury with a small, constant and determinable error. The procedure is simple and rapid; any type of preparation can be examined in 1 to 1¼ hours. The apparatus is inexpensive and virtually indestructible in use. Because of the small space required, several determinations can conveniently be made concurrently.

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Received *April 27th*, 1959

A Modification of Thorn and Shu's Method for Organic Carbon in Soils

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A modification of Thorn and Shu's method for determining organic carbon in soils is described. Results are presented for the determination of carbon in a number of pure compounds. The values for the carbon contents of eight soils have an average coefficient of variation of 0·9 per cent. and agree favourably with those found by a dry-combustion method.

For the determination of organic carbon in soils, several rapid methods are available,[1,2] all of which depend on oxidation. However, it is well known that the extent of oxidation can vary considerably and is complete for only a few soils. For example, during 1949, six New Zealand soils containing from 2 to 30 per cent. of carbon were analysed by either Allison's[1] or Schollenberger's[2] method in five laboratories, and the results were compared with those obtained by dry combustion.[3] As the carbon contents found by dry combustion were from 1·13 (brown granular clay) to 0·94 (skeletal soil) times those found by the rapid methods, Davies and his co-workers concluded that, although the rapid methods gave reproducible results, there was no correction factor of general application.

Most of the wet-combustion methods in which more drastic oxidation is used are less useful, as they are slower and do not achieve complete oxidation, or even a constant fraction of it. However, van Slyke and Folch[4] found that a mixture of sulphuric, orthophosphoric,

chromic and iodic acids completely oxidised all the substances analysed, including cholesterol and stearic acid. Bremner[5] successfully applied this method to soils and soil extracts. Thorn and Shu[6] also used van Slyke and Folch's mixture to oxidise natural compounds, but instead of measuring manometrically the carbon dioxide evolved, they determined it by absorption in standard alkali under reduced pressure. Zinc turnings were used to remove acid vapours from the carbon dioxide, and iodine was removed by solid potassium iodide.

Experimental

When soils were analysed by Thorn and Shu's method, results were 5 to 10 per cent. low in the range 10 to 20 mg of carbon. This was attributed to slow diffusion of the carbon dioxide through the oxygen produced by the large excess of oxidant. The oxygen increased the pressure by more than 5 cm of mercury, but, when oxygen was removed by passing the gases through a roll of copper gauze heated in a silica tube, the initial pressure increased, after absorption of carbon dioxide was complete, by less than 1 cm of mercury. Replicate results were nearer to the correct values (as determined by dry combustion for soils and by calculation for pure compounds), but were extremely variable owing to uncontrollable amounts of acid vapours passing through the zinc trap. Attempts to prevent the acid vapours from passing into the standard alkali by lengthening the zinc trap and by inserting a trap containing sulphuric acid were unsuccessful. Little improvement was effected by passing the gases through a trap containing alkali and subsequently liberating carbon dioxide with lactic acid. The amount of alkali used had been calculated to react with the interfering gases and only a small proportion of the carbon dioxide, on the assumption that the stronger acids would be preferentially retained. However, the slightly longer procedure of quantitatively absorbing the carbon dioxide in an excess of sodium hydroxide also resulted in the quantitative absorption of the acid vapours. The traps containing zinc and potassium iodide were therefore discarded. Additional interference from halogens was then encountered and was not avoided when the trap containing potassium iodide was re-inserted or when hydrazine was added to the sodium hydroxide,[4] but was overcome by placing a spiral of silver wire immediately after the roll of copper gauze in the silica tube.

Method

Apparatus—

The apparatus used is shown in Fig. 1 and is based on that described by Thorn and Shu.[6] The roll of copper gauze is wound on stiff resistance wire for ease of manipulation. Copper oxide is periodically removed by heating the roll of gauze in a bunsen flame, immersing it in the vapour above warmed methanol in a flask and leaving it there to cool. A clamp is inserted to control the gas flow from the combustion to the absorption flasks. This limits foaming of the reacting mixture and so permits more rapid heating than previously.

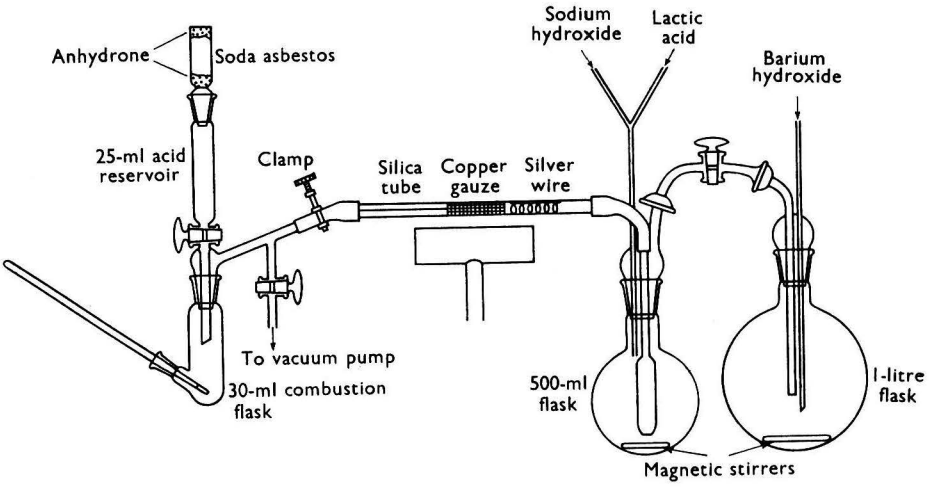

Fig. 1. Apparatus for determining carbon in soils by wet combustion

REAGENTS—

Iodate - dichromate mixture[7]—Grind together two parts by weight of potassium iodate and one of potassium dichromate.

Acid mixture[7]—Heat 67 ml of fuming sulphuric acid, 33 ml of syrupy phosphoric acid and 1 g of potassium iodate at 160° to 190° C until the iodate dissolves.

Sodium hydroxide, 0·5 N—Prepare free from carbonate.

Barium hydroxide, 0·2 N.

Lactic acid, 0·5 N.

Hydrochloric acid, 0·1 N.

Thymolphthalein indicator solution—Prepare a 0·1 per cent. w/v solution of thymolphthalein in 96 per cent. ethanol.

PROCEDURE—

Add 2 g of iodate - dichromate mixture to the finely ground sample, containing about 15 mg of carbon, in the combustion flask. Lubricate the ground-glass joints of the combustion flask and the stopcock from the acid reservoir with phosphoric acid. Evacuate the apparatus; during evacuation, allow 25 ml of 0·2 *N* barium hydroxide and 25 ml of 0·5 *N* carbonate-free sodium hydroxide to run into the 1-litre and 500-ml flasks, respectively, and heat the copper gauze in the silica tube to a dull red.

Close the stopcocks to the vacuum pump and to the second absorption flask, and allow 5 ml of the acid mixture in the acid reservoir to flow on to the sample. Heat the mixture carefully by means of a micro burner, and check any tendency to violent foaming by holding the flame at the base of the foam collar and by tightening the clamp between the combustion flask and the silica tube. When the temperature reaches about 150° C, close the clamp, heat to between 200° and 210° C, and then cautiously release the gases into the sodium hydroxide solution. After 5 minutes, remove the flame from beneath the copper gauze, close the clamp, run into the sodium hydroxide solution an amount of 0·5 *N* lactic acid calculated to give a final pH of 3 to 4, and open the tap leading to the flask containing the 0·2 *N* barium hydroxide. After a further 5 minutes, warm the first flask by means of a small flame until its contents boil. Absorption of the released carbon dioxide by the barium hydroxide is complete within 10 minutes. Restore the pressure in the apparatus to atmospheric by introducing air (free from carbon dioxide) through the pump line, and titrate the excess of 0·2 *N* barium hydroxide against 0·1 *N* hydrochloric acid to a faint blue end-point with thymolphthalein indicator solution. Note that the blank value can be taken as the trace amount of carbonate in the sodium hydroxide solution, but is best checked by oxidising an analytical standard, *e.g.*, hippuric acid.

TABLE I

CARBON CONTENTS OF VARIOUS PURE COMPOUNDS

Compound	Weight of sample, mg	Carbon content found, %	Theoretical carbon content, %
Hippuric acid	31·9	60·5	60·3
	35·3	60·0	
	23·5	60·0	
	26·6	60·2	
Potassium hydrogen tartrate	66·1	25·5	25·5
	54·9	25·5	
Sucrose	40·0	42·1	42·1
	36·2	42·0	
Glucose	39·3	39·9	40·0
	47·6	40·0	
Stearic acid	19·6	75·6	76·0
	26·2	76·0	
p-Chlorobenzoic acid	32·4	53·4	53·7
	39·4	53·7	
Cystine	52·9	30·0	30·0
	60·9	29·9	

Results

Some selected compounds were analysed, including some containing nitrogen, an alkali metal, sulphur or a halogen; the results are shown in Table I. The oxidation of sucrose produced far more iodine than did that of other compounds and, in absence of silver wire, caused results to be up to 2 per cent. higher than the theoretical value; results for chlorobenzoic acid were over 6 per cent. higher when the silver wire was omitted.

In Table II the results of analyses of eight soils by the proposed method are compared with those obtained by a micro dry-combustion method. For only one soil, a yellow-brown loam, did the carbon contents found by the two methods differ, the dry-combustion figure being, however, only 2·6 per cent. higher.

Table II

Carbon contents of various soils by the proposed and dry-combustion methods

Sample	Proposed method				Dry-combustion method	
	Number of determinations	Mean carbon content, %	Standard deviation, %	Coefficient of variation, %	Carbon content found, %	Mean carbon content, %
Brown granular clay loam	5	5·24	0·034	0·7	5·21, 5·27	5·24
Yellow-brown pumice soil	5	7·56	0·031	0·4	7·65, 7·57	7·61
Recent soil from alluvium	8	2·96	0·035	1·2	2·85, 2·96	2·91
Yellow-brown earth ..	5	3·45	0·037	1·1	3·40, 3·45	3·43
Yellow-brown loam {	15	15·0	0·14	0·9	15·4, 15·3	15·4
	5	8·31	0·027	0·3	8·30, 8·27	8·29
Gley soil	5	4·82	0·043	0·9	4·72, 4·91	4·82
Well decomposed peat ..	6	16·3	0·24	1·5	16·2, 16·4	16·3

Conclusion

Although titrimetric methods for determining carbon dioxide are inherently less precise than the gravimetric method,[8] the proposed method for determining carbon in soils is much more rapid than the dry-combustion - gravimetric procedure and is sufficiently accurate for soils when uniformity of sample is likely to be a limiting factor.

I thank Dr. R. H. Jackman for supplying the standard soils analysed by dry combustion and Dr. K. Taraniv for carrying out some of the soil analyses by the proposed method.

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Received *May 4th*, 1959

A Volumetric Micro Determination of Organically Bound Sulphur and Organic and Inorganic Sulphates

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A volumetric procedure for the micro determination of sulphur is described. It is based on precipitation of barium sulphate by a measured excess of barium chloride solution and potentiometric titration of the excess of barium with a solution of the disodium salt of ethylenediaminetetra-acetic acid. A mercury reference electrode is used.

THE many descriptions of volumetric procedures for determining sulphur recently published demonstrate the great interest of analysts in developing a rapid and accurate method[1 to 10] for this purpose. In my opinion, however, these methods are either too time-consuming or, when indicators are used, too subjective for many analysts to locate precisely the end-point of the titration.

The procedure described by Schöniger (combustion of small samples in an oxygen-filled flask) provides an excellent micro method for the rapid combustion of organic compounds and the ultimate determination of chlorine, bromine, iodine, fluorine, phosphorus and sulphur.[8]

FitzGerald[2] proposed a method for determining concentrations of sulphate ion by using a measured excess of barium chloride solution and titrating the excess of barium with a solution of disodium dihydrogen ethylenediaminetetra-acetate (EDTA), Solochrome black WDFA 150 being used as indicator. He reported excellent results, but mentioned that, in test solutions containing 5 to 10 mg of sulphur per 100 ml, the comparatively large amounts of barium sulphate present caused some difficulty in precise observation of the end-point. However, after the barium sulphate had been removed by filtration, the filtrate could be satisfactorily titrated. The proposed procedure does not include such a filtration, thereby saving time and preventing any loss of material during transfer.

Schmid and Reilley,[11] in determining the stability constants of metal chelates, used a mercury electrode as an indicator electrode for the various metal ions that in the presence of a chelating agent formed a 1 to 1 complex with bivalent mercury as well as with the metal ion being studied. They also showed that the potential of the mercury electrode was linearly related to log(metal concentration) and that consequently the electrode could serve as a pM electrode (analogous to a pH indicator electrode) in the presence of fixed concentrations of the metal and mercuric chelates.[12]

A combination of the combustion technique described by Schöniger, FitzGerald's titration with EDTA solution and Schmid and Reilley's mercury indicator electrode offered the possibility of a rapid, accurate and precise volumetric determination of sulphur in organic compounds.

Method

Apparatus—

Schöniger's apparatus[8] was used for the combustion of the sample.

The titration apparatus consisted of a 30-ml beaker fitted with a No. 7 rubber stopper. A mercury electrode, a calomel electrode and a nitrogen-inlet tube were fitted into three holes in the stopper; a fourth hole permitted the insertion of a 1-ml microburette. The mercury electrode used was J-shaped and prepared from Pyrex-glass tubing having an external diameter of 6 mm.

A Leeds and Northrup potentiometer, model 7663, or a Photovolt electronic pH meter, model 115, was used in the potentiometric titrations.

Reagents—

EDTA solution, 0·1 M—Dissolve 33·62 g of disodium ethylenediaminetetra-acetate in water, and dilute to 1 litre.

Mercuric EDTA solution—Mix 10 ml each of 0·1 *M* mercuric acetate and 0·1 *M* EDTA solution.

Barium chloride, 0·005 M—Dissolve 1·22 g of barium chloride, $BaCl_2.2H_2O$, in water, and dilute to 1 litre.

Standard zinc solution, 0·01 M—Dissolve 326·9 mg of analytical-reagent grade zinc in 10 ml of 3 *N* nitric acid, and evaporate to dryness on a steam-bath. Dissolve the residue in 1 ml of glacial acetic acid, and dilute with water to 500 ml in a calibrated flask.

Ammonium acetate solution, aqueous, 1 M.

Ammonia solution, sp.gr. 0·880—Analytical-reagent grade.

Hydrogen peroxide solution—Dilute 5 ml of Superoxol (Merck & Co. Inc.) to 25 ml with water.

STANDARDISATION OF EDTA SOLUTION—

Place 5 ml of 0·01 *M* zinc solution in a 30-ml beaker, and add 2 ml of 1 *M* ammonium acetate, 0·1 ml of mercuric EDTA solution and 2 ml of ammonia solution. Dilute with water to 20 ml, and insert the stopper containing the electrodes and the nitrogen-inlet tube. De-gas the solution with nitrogen for 2 minutes, and then, with nitrogen flowing to agitate the solution, titrate with EDTA solution potentiometrically in the usual manner.

STANDARDISATION OF BARIUM CHLORIDE SOLUTION—

Place 10 ml of barium chloride solution in a 30-ml beaker, and titrate in the way just described for standardising the EDTA solution.

PROCEDURE FOR DETERMINING SULPHUR—

Place an accurately weighed sample (see Note) on a 1-inch × 1¼-inch strip of Whatman No. 1 filter-paper, fold the strip, insert a 1-inch × ¼-inch strip of filter-paper as a wick, and place in the platinum basket of a Schöniger apparatus.[8] Place 3 ml of hydrogen peroxide solution in the Erlenmeyer flask of the apparatus, and displace the air in the flask with oxygen. Ignite the wick, immediately insert the basket into the flask, and make sure that the ground-glass stopper is tight. When, after a few seconds, the combustion is complete, shake the flask for 2 minutes to complete the conversion to sulphate.

Rinse the contents of the flask into a 50-ml glass evaporating dish with water, add exactly 10 ml of 0·005 *M* barium chloride, and evaporate to dryness to remove hydrogen peroxide.

Stir the residue into a slurry with water, add 2 ml of 1 *M* ammonium acetate, 0·1 ml of mercuric EDTA solution and 2·0 ml of ammonia solution, sp.gr. 0·880, and transfer the mixture to a 30-ml beaker. Determine the excess of barium chloride present by potentiometric titration with 0·1 *M* EDTA solution.

The potential equilibrates quickly after each addition of titrant, so that the titration can be carried out rapidly. The sharpness of the titration is indicated by the fact that the value of $\Delta E/\Delta V$ at the end-point was 3600. It was necessary to remove all the hydrogen peroxide so that the characteristic change in potential would occur.

NOTE—Use a weight of sample less than 3 mg if the sulphur content is 50 per cent., less than 5 mg if the sulphur content is 30 per cent., less than 7 mg if the sulphur content is 20 per cent. and less than 10 mg if the sulphur content is 15 per cent.

DISCUSSION OF RESULTS

The proposed procedure has been used in this laboratory for almost 1 year and has given excellent results (see Table I). Each of the compounds listed was considered to be analytically pure, since acceptable results were obtained for the other elements present.

A few liquid organic samples have been analysed by using the same titration procedure after the sample had been decomposed in a Carius tube. In these determinations the solution was rinsed from the Carius tube with water, 0·005 *M* barium chloride was added, and the titration was carried out after the mixture had been evaporated to dryness.

Water-soluble sulphates of organic bases have also been analysed. The compound was dissolved in water, an excess of 0·005 *M* barium chloride was added, and the excess was titrated with 0·1 *M* EDTA solution after 1 *M* ammonium acetate, mercuric EDTA solution and ammonia solution had been added.

The proposed titrimetric procedure could be used for determining inorganic sulphates. However, before the barium chloride solution was added, any metal ions forming chelates with EDTA would have to be removed by passage through an ion-exchange resin.

TABLE I

COMPARISON OF RESULTS BY THE PROPOSED METHOD AND THE CALCULATED SULPHUR CONTENTS

Compound	Sulphur content found, %	Calculated sulphur content, %
Dithioline sulphate pentahydrate	10·78	10·94
β-Mercaptoethylamine salicylate	15·18	14·90
2-Methylmercapto-4 : 5-imidazole diethylcarboxylate	12·66	12·42
A tetrasulphone (research compound)	28·35	28·34
Galactose diethylmercaptal	22·80	22·39
Anisaldehyde thiosemicarbazone (batch No. 1)	15·40	15·33
Anisaldehyde thiosemicarbazone (batch No. 2)	15·45	
Anisaldehyde thiosemicarbazone (batch No. 3)	15·43	
Anisaldehyde thiosemicarbazone (batch No. 4)	15·22	
2 : 5-Dibromo-3 : 4-dinitrothiophen	9·58	9·63
p-Acetylaminobenzaldehyde thiosemicarbazone	13·68	13·55
6-Mercapto-9-β-D-ribofuranosylpurine	11·10	11·24

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Received *May 18th*, 1959

The Determination of Soil Nitrates with a Brucine Reagent

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A rapid modified procedure has been developed for the quantitative determination of soil nitrates with a brucine reagent after the destruction of nitrites present in the extract. It is not possible to determine nitrate-N alone in the presence of nitrite-N with this reagent simply by varying the ratio of water to concentrated sulphuric acid during colour development. However, the addition of a small amount of aminosulphonic acid selectively destroys nitrite-N and permits the determination of from 1 to 10 p.p.m. of nitrate-N by this method with a standard deviation of 0·4 p.p.m. The method can be used for calcium sulphate soil extracts, but is not satisfactory for potassium chloride extracts.

DURING a study of mineral nitrogen in red lateritic loam coffee soils, it became desirable to find a rapid quantitative method for determining nitrate-N in samples of fresh soil. The phenoldisulphonic acid method described by Roller and McKaig[1] was used satisfactorily, but this method requires the evaporation of an aliquot of the soil extract to dryness (usually overnight) before reagent additions for colour development are made.

The determination of nitrate-N by means of the colour produced with brucine in the presence of sulphuric acid[2] was investigated in an empirical manner (*a*) to ascertain whether or not the method, which is more rapid than the phenoldisulphonic acid method, was sufficiently accurate quantitatively, bearing in mind the uncontrolled variation involved with field samples,[3] (*b*) to compare it with the phenoldisulphonic acid method, which was then the standard technique used in this laboratory, (*c*) to determine whether or not nitrite-N extracted simultaneously from the soil samples with nitrate-N was included in the determination and, if so, to what extent it affected the final result and (*d*) to find whether or not nitrate-N could be determined by this method in a potassium chloride extract of the soil sample made for the determination of available ammoniacal nitrogen.[4] (Nitrate-N is extracted with a reagent containing 25 ml of a saturated solution of calcium sulphate in 200 ml of water.)

Experimental

Modification of brucine method—

The technique for the brucine method described by Snell and Snell[2] was modified successfully by reducing the amounts of reagents used. A 2-ml portion of clear calcium sulphate[1] soil extract was placed, by pipette, in a 50-ml beaker and 0·5 ml of the 5 per cent. w/v brucine in chloroform reagent was added. With constant agitation, a 4-ml portion of analytical-reagent grade concentrated sulphuric acid was added down the side of the beaker. The beaker was then placed on an asbestos mat, covered with a watch-glass and allowed to cool for 15 to 20 minutes, after which the colour developed was measured in a 2·5-cm cell with an E.E.L. long-cell absorptiometer (Evans Electroselenium Ltd.), a blue filter (No. 601) being used. The colour was stable for up to 24 hours.

This technique was compared with the phenoldisulphonic acid method[1] on a calcium sulphate extract of a single air-dried sample of top soil that had been sub-sampled ten times. No measurable amount of nitrite-N was found in the extract by a modification (described in a personal communication from K. Shaw, Rothamsted Experimental Station) of Shinn's method.[5]

The comparison did not reveal any significant difference between the mean values found by the two methods, although the accuracy and reproducibility between duplicate determinations were better when the phenoldisulphonic acid method was used. The results are shown in Table I.

Table I

Nitrate contents of top soil extracts

The soil used was a lateritic red loam derived from recent trachytic tuff. Each result, which is the mean of ten replicate determinations, is expressed as parts per million of nitrate-N in the air-dried soil

Method	Mean nitrate content, p.p.m.	Standard deviation, p.p.m.	Standard error of mean, p.p.m.	Average difference between duplicate results, p.p.m.
Brucine	8·2	0·9	±0·3	1·0
Phenoldisulphonic acid..	8·1	0·7	±0·2	0·8

Effect of nitrite—

The influence of nitrite-N on the nitrate-N colour developed with the brucine reagent was initially determined by comparing the colours produced in two series of standard solutions; the results are shown in Table II.

The presence of nitrite-N had no significant influence on results by the phenoldisulphonic acid method, but an appreciable and approximately constant effect was observed when the brucine method was used.

A further series of standard solutions was prepared in which the concentration of nitrite-N was varied over a wide range in the presence of 5 p.p.m. of nitrate-N. The optical-density values for this series, which suggested that the brucine reagent is sensitive to increasing concentrations of nitrite-N, were as follows—

Nitrite-N present, p.p.m. ..	0·0	2	4	6	8
Optical density	34·2	37·4	42·3	49·5	54·0

TABLE II

EFFECT OF NITRITE ON NITRATE COLOUR

Concentration of nitrite-N present, p.p.m.	Concentration of nitrate-N present, p.p.m.	Optical density of colour produced by— phenoldisulphonic acid method	Optical density of colour produced by— brucine method
	0	Blank setting	Blank setting
	2	8·5	13·8
Nil	5	20·9	33·2
	10	36·5	49·6
	15	49·5	63·8
	0	0·0	10·6
	2	7·5	23·9
4	5	19·4	43·8
	10	35·0	59·5
	15	49·8	70·0

EFFECT OF RATIO OF EXTRACT TO ACID—

Snell and Snell[2] state that "if nitrites are not to appear in the final results, two parts of sulphuric acid must be present for every part of water. To determine nitrites as well as nitrates, lessen the amount of sulphuric acid so that the ratio of water to sulphuric acid is 2 to 1."

Three series of solutions were prepared in the calcium sulphate extracting solution. The series contained 0, 1, 2, 4 and 8 p.p.m. of (*i*) nitrate-N, (*ii*) nitrite-N and (*iii*) the same concentrations of nitrate-N *plus* nitrite-N, *i.e.*, the third series contained 0, (1 + 1), (2 + 2), (4 + 4) and (8 + 8) p.p.m. of each. Colour was developed in 6-ml portions of the solutions with the brucine in chloroform reagent, and the ratio of solution to sulphuric acid was varied as 1 to 2, 2 to 1, 1 to 3, 3 to 1 and 1 to 1. Some of the results are summarised in Table III.

At a ratio of 3 to 1, no measurable colour was produced by the solutions containing nitrate-N alone and values were not closely reproducible for the other two series of solutions. At a ratio of 1 to 1, no values were reproducible, but at ratios of 1 to 2, 2 to 1 and 1 to 3, reproducible values were obtained.

TABLE III

EFFECT OF RATIO OF SOLUTION TO SULPHURIC ACID ON INCLUSION OF NITRITE WITH NITRATE

In these determinations, the calcium sulphate extracting reagent was used as blank

Ratio of solution to sulphuric acid	Concentration of solution, p.p.m.	Optical density of solution containing— nitrate-N	nitrite-N	nitrate-N *plus* nitrite-N
	0	4·5	3·3	8·6
	1	12·7	6·5	19·8
1 to 2	2	18·5	11·7	26·3
	4	30·4	17·6	50·5
	8	48·5	28·2	61·0
	0	2·1	1·1	1·1
	1	2·2	6·8	7·7
2 to 1	2	2·5	12·5	11·2
	4	2·7	21·5	24·3
	8	3·3	34·6	36·5
	0	4·0	2·6	3·0
	1	4·8	3·8	7·6
1 to 3	2	9·6	5·2	13·6
	4	19·8	7·3	21·4
	8	32·2	14·8	43·5

The ratio 1 to 2 gave strictly additive results for nitrate-N *plus* nitrite-N at the lower concentrations only. The concentration of the solution containing 8 p.p.m. each of nitrate-N and nitrite-N was too high for complete reaction with the volume of reagent added, and the highest practical concentration of reacting nitrogen, *i.e.*, nitrate-N, nitrite-N or nitrate-N *plus* nitrite-N, was found to be 10 p.p.m.

Table III shows that, at the ratio 2 to 1, colour is apparently developed for nitrite-N alone, and at the ratio 3 to 1, the effect of nitrite-N is almost additive, although the sums of

the optical-density readings for nitrate-N and nitrite-N are generally greater than the readings for the solutions containing nitrate-N *plus* nitrite-N.

The results demonstrated that, under these conditions, it was not possible to exclude the measurement of nitrite-N by varying the ratio of solution to sulphuric acid. It therefore became necessary to find a means by which any nitrite-N present in the soil extract could be removed without interfering with the quantitative measurement of nitrate-N.

REPLACEMENT OF CHLOROFORM IN THE REAGENT BY GLACIAL ACETIC ACID—

It was apparent that a more stable solvent than chloroform for the brucine reagent was desirable for routine analysis. At an altitude of over 5000 feet above sea level, the loss of chloroform was such that the concentration of brucine increased appreciably in 1 to 2 weeks, thereby making it necessary to renew this reagent at frequent intervals. Further, the final addition of concentrated sulphuric acid removed chloroform by boiling and consequently diminished the final volume of the mixture.

Horne and Denmead[6] developed a modified brucine reagent for determining soil nitrate under field conditions, chloroform being replaced by glacial acetic acid. This reagent was found to overcome the two objections previously noted in preliminary tests, and the use of a reagent containing 5 per cent. w/v of brucine in glacial acetic acid was adopted at this stage.

Before proceeding further, however, the previous experiments with brucine in chloroform were repeated with brucine in glacial acetic acid, except that the ratios of solution to sulphuric acid were restricted to 1 to 2 and 2 to 1. Although the optical densities were not identical, the same conclusions were drawn.

SELECTIVE DESTRUCTION OF NITRITES—

Preliminary results by using a technique noted by Feigl,[7] who quoted Baumgarten and Marggraff,[8] in which a 2 per cent. w/v solution of aminosulphonic acid in distilled water was used for the selective destruction of nitrite-N in the presence of nitrate-N were sufficiently encouraging for further experiments to be undertaken.

METHOD FOR DETERMINING 1 TO 10 p.p.m. OF NITRATE-N

PROCEDURE—

By pipette, place a 2-ml portion of clear calcium sulphate soil extract or standard solution in calcium sulphate extractant in a 50-ml beaker, add 1 drop of a 2 per cent. w/v solution of aminosulphonic acid in distilled water, and swirl to mix. (The reaction is almost instantaneous for concentrations of nitrite-N up to 4 p.p.m.) Add 0·5 ml of a 5 per cent. w/v solution of brucine in glacial acetic acid, swirl again, and then add, with continuous agitation, 4 ml of concentrated sulphuric acid. (During the addition of sulphuric acid, the initially colourless mixture becomes orange-yellow and then yellow.) Cover the beaker with a watch-glass, place on a sheet of asbestos, and allow to cool for 15 to 20 minutes. Measure the optical-density in a 2·5-cm cell with an E.E.L. long-cell absorptiometer fitted with a blue No. 601 filter. (Optical densities were also measured in 0·5-cm cells at 454 mμ with a Unicam SP600 spectrophotometer, but it is not necessary to use an instrument having the accuracy of this spectrophotometer.)

Note that, when possible, the reagents used should be of recognised analytical grade.

RESULTS

Nine sub-samples from a bulk sample of top soil were extracted both with the calcium sulphate and the 2 *N* potassium chloride[4] reagents. Both extracts were analysed by the proposed method, and, for comparison, the calcium sulphate extract was analysed by the phenoldisulphonic acid method. The original extracts were then analysed after the addition of 3 p.p.m. of nitrate-N; the results are summarised in Table IV.

These results illustrate a fact that has consistently shown up in this work, namely that both the brucine reagent method[2] and the proposed method give slightly higher results for nitrate-N in extracts of this type of soil than does the phenoldisulphonic acid method. There is no significant difference in the results found with the two extracting reagents, and all three methods have detected the added nitrate-N reasonably well.

A second bulk sample of top soil was sub-sampled seven times and extracted with the calcium sulphate and potassium chloride reagents. Aliquots of the extracts were analysed by the proposed method, both with and without the addition of aminosulphonic acid; further

aliquots, to which 2 p.p.m. of nitrite-N had been added, were similarly analysed. The results are summarised in Table V; the mean values found for the untreated extracts in either extracting reagent are not shown, although they differed significantly. Calibration graphs were prepared to cover a range of nitrate-N concentrations in the extracting reagents, both in the presence and absence of aminosulphonic acid.

TABLE IV

NITRATE-N FOUND IN A SOIL EXTRACT BY PROPOSED AND PHENOLDISULPHONIC ACID METHODS

The addition of aminosulphonic acid was omitted. Each result is expressed as parts per million of nitrate-N in the oven-dried soil

Extracting reagent ..	Calcium sulphate		Calcium sulphate		Potassium chloride		
Method	Modified brucine		Phenoldisulphonic acid		Modified brucine		
	No added nitrate-N	3 p.p.m. of added nitrate-N	No added nitrate-N	3 p.p.m. of added nitrate-N	No added nitrate-N	3 p.p.m. of added nitrate-N	Least significant difference
Nitrate-N found—							
Means of 9 results, p.p.m.†	5·8	9·1	5·2	8·1	6·3	9·2	N.S.
Means of 18 results, p.p.m.	7·5		6·6		7·7		0·40*, 0·76***
Means of 27 results, p.p.m.	5·8				8·8		0·35***
	(No added nitrate-N)			(3 p.p.m. of added nitrate-N)			

† Method ± means of nitrate-N interaction.
* Statistically significant at the 5 per cent. level of significance.
*** Statistically significant at the 0·1 per cent. level of significance.

TABLE V

AMOUNTS OF NITRATE-N FOUND IN SOIL EXTRACTS BY PROPOSED METHOD IN PRESENCE AND ABSENCE OF ADDED NITRITE-N

Each result is expressed as parts per million of nitrate-N in the oven-dried soil

Extracting reagent	Calcium sulphate				Potassium chloride				
Method of treating extract† ..	A	B	C	D	A	B	C	D	Least significant difference
Nitrate-N found—									
Means of 7 results, p.p.m. ..	5·8	5·6	6·8	5·7	6·3	8·3	4·7	8·4	0·5*, 0·7**
Means of 28 results, p.p.m. ..		6·0				6·9			0·4*, 0·6**

† Treatment of extracts—
A. No aminosulphonic acid added during colour development.
B. Aminosulphonic acid added during colour development.
C. Nitrite-N (2 p.p.m.) added before colour development.
D. Nitrite-N (2 p.p.m.) and aminosulphonic acid added before colour development.
* Statistically significant at the 5 per cent. level of significance.
** Statistically significant at the 1 per cent. level of significance.

The addition of aminosulphonic acid to destroy nitrite-N was satisfactory when the soil had been extracted with the calcium sulphate reagent. Aminosulphonic acid added to the potassium chloride soil extracts resulted in significantly higher values for apparent nitrate-N, both in the presence and absence of added nitrite-N. This factor largely accounts for the significantly higher mean (apparent) value for nitrate-N in the potassium chloride soil extract.

Finally, several samples of red lateritic loam top soil were collected at random from coffee fields and extracted in duplicate with calcium sulphate and potassium chloride reagents. Nitrate-N was determined in the calcium sulphate extracts by both the proposed and, as a control, the phenoldisulphonic acid method. The potassium chloride extracts were analysed by the proposed method only. The results are shown in Table VI.

The use of aminosulphonic acid in the proposed method leads to higher values for nitrate-N when potassium chloride is used as extracting reagent than when soil nitrates are extracted with calcium sulphate. This would not seem to be the result of greater nitrate extraction by the potassium chloride, but of some reaction occurring after the addition of the aminosulphonic acid reagent.

The results in Table V suggest that the presence of an appreciable amount of nitrite, with which the aminosulphonic acid reacts, does not diminish this effect. The use of the

potassium chloride extractant also gives more variable results and, in particular, a greater difference between duplicate determinations.

TABLE VI

NITRATE-N FOUND IN TOP SOILS

Each result is the mean of duplicate determinations and is expressed as parts per million of nitrate-N in the oven-dried soil

Sample No.	Nitrate-N found in calcium sulphate extract by— phenoldisulphonic acid method, p.p.m.	Nitrate-N found in calcium sulphate extract by— modified brucine method, p.p.m.	Nitrate-N found in potassium chloride extract by modified brucine method, p.p.m.
1	10·5	12·3	14·7
2	5·7	8·3	9·5
3	7·8	8·7	11·9
4	0·9	0·0	4·1
5	26·3	23·6	20·2
6	9·8	9·9	9·8
7	25·0	26·0	25·2
8	1·8	3·0	4·9
9	2·4	3·1	3·3
Standard deviation	—	1·2	1·5
Average difference between duplicate results	—	1·4	1·7
Means and 95 per cent. confidence limits	10·0	10·5 ± 0·6	11·5 ± 0·7

Extraction with calcium sulphate and determination of soil nitrates by the proposed method has a satisfactory accuracy when fresh field samples are analysed. In comparison with the phenoldisulphonic acid method, it has the advantages of requiring less time for manipulation and the use of less laboratory equipment; it is more rapid when large numbers of samples have to analysed quickly. On the average, for a red lateritic loam, it has given slightly higher values for nitrate-N than has the more accurate phenoldisulphonic acid method, and the results are less reproducible.

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Received *March 23rd*, 1959

An Automatic Titrimeter for Plotting True-scale Titration Curves

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Modifications to a Honeywell - Brown recorder are described; these modifications permit the chart and a micro syringe delivering 0·005 to 0·02 ml of titrant per minute to be driven by the same motor. When used in conjunction with suitable electrodes and an electronic pH meter, the device permits titration curves (10 inches × 18 inches) to be plotted automatically. The ordinate scale of these curves remains directly proportional to the volume of titrant used irrespective of its rate of admission, which can be varied as appropriate during the titration.

When used in conjunction with a Vibron electrometer and associated pH meter, the apparatus can be made to discriminate to better than 0·003 unit of pH and the pH scale, 2 to 10 units, can be extended to a length of 64 inches.

The use of the apparatus is outlined for certain organic preparations requiring control of pH, for studies of kinetics at constant pH and for automatic titrimetry of various kinds.

Dissociation constants of weak acids and the stability constants of the complexes they form with metals can be evaluated from pH measurements made during the titration with alkali of solutions of the acidic chelating agent in absence and in presence of metal ions.[1,2]

Attempts have been made to facilitate and to increase the accuracy of such potentiometric titrations by using some mechanical means of delivering the alkali accurately and at a controlled rate.[3,4] If the addition has to be stopped to take a pH reading, some mechanism must be used to lift the tip of the burette or syringe from the titration mixture to prevent slow effusion of titrant and a consequent drift of potential. If, on the other hand, the pH is plotted continuously by some recording device, both the speed of the recorder chart and the rate at which titrant is added must be constant throughout if a certain length of chart is to correspond exactly to a known volume of added titrant.

For some time past we have been using a hypodermic syringe driven by a constant-speed motor in conjunction with a model 23 E.I.L. electronic pH meter (Electronic Instruments Ltd.) and a Honeywell - Brown recorder to plot titration curves automatically. Unknown to us, essentially the same combination had been successfully operated by Haslam and Squirrell, who have recently published a full account of their apparatus and its application to a wide variety of potentiometric titrations.[5]

When the potential increases rapidly during a titration (for example, in an acid - alkali titration, the value of $\delta(\mathrm{pH})/\delta V$ increases as the end-point region is approached), more time is needed for equilibrium between the solution and the electrodes to be established and there is a real danger that the almost instantaneous record on the chart will give an incorrect picture of the true titration curve. This can be avoided in two ways. The first is to increase the over-all time taken for the titration by introducing the titrant at a much slower rate; this is certainly the simplest expedient and probably the most effective. The second is to simulate the normal practice of manual titration and add aliquot portions of titrant, ΔV, which are successively diminished between successive observations of the potential, E, in relation to the extent that $\delta(\Delta V)/\delta E$ increases. In an automatic titration, this can be achieved by decreasing the rate at which titrant is added. However, if this rate is altered without simultaneously altering the chart speed in an exactly equivalent manner, the ordinate scale of the graph drawn by the recorder will no longer be linearly proportional to the volume of titrant added and a distorted titration curve will result.

All these disadvantages have been overcome in the titration apparatus described here. The recorder chart and the micrometer syringe used to add titrant are both driven by the same motor through non-slip gears. The speed of this motor can be varied by a Variac transformer so that the rate at which titrant is added does not exceed the rate at which equilibrium is attained. Such changes in the speed of the motor, which are under immediate

manual control and can be applied as soon as the gradient on the chart is seen to increase or decrease, will, however, affect the rate at which titrant is added and the chart speed equally. The ordinate axis of the graph plotted automatically on the chart will therefore be linear with respect to the volume of titrant added, and the resulting plot will be a true-scale titration curve.

The titrant is added by means of an Agla micrometer syringe, and the pH of the solution is measured with a glass electrode - calomel reference electrode assembly, a direct-reading E.I.L. electronic pH meter, model 23, being used. The pH readings are recorded by a chart recorder having a pH scale from 0 to 14 (Honeywell - Brown, model Y153X17/V/X-6R). With this apparatus up to 0·5 ml of titrant can be added (with a precision of $\pm 0{\cdot}0002$ ml) over periods of time ranging from 100 down to 25 minutes and pH values can be read to approximately 0·02 unit of pH.

TITRATION APPARATUS AND MOTOR-DRIVEN SYRINGE—

The titration apparatus consists of a double-walled glass cell through which water from a thermostat is circulated. The vessel is closed by a rubber stopper bored with holes for a glass electrode, the reference electrode, the tip of the syringe (or solenoid-operated burette), a glass stirrer and an inlet tube for the nitrogen used for stirring or maintaining an inert atmosphere. This nitrogen is pre-saturated by passage through a solution similar to that being investigated. Titrant from the syringe is added through a fine capillary jet, which is at right angles to the syringe and dips under the surface of the solution being titrated. The syringe is driven by a 1/25 horse-power series-wound electric motor, the speed of which is reduced by means of suitable gearing and can be varied by a Variac transformer. This permits fine adjustment to the rate at which titrant is added.

The drive is transferred from the gears to the micrometer through a pinion, E (see Fig. 1), of length greater than that of the maximum traverse of the micrometer screw on the Agla syringe, *i.e.*, greater than 2·5 cm. A suitable gear-wheel, F, is attached to the end of this micrometer screw on a shaft screwed into the thread normally used to secure the ratchet stop. A sensitive micro switch is situated in a position such that it is actuated by gear-wheel F when the zero mark is reached; this switches off the drive motor and so prevents damage should the apparatus be left unattended for too long. A further switch is inserted to cut out this safety device when the micrometer drive is reversed in order to refill the syringe.

In addition to the slow forward drive, a fast reverse drive and neutral gear are included to facilitate the operation of refilling the syringe. These gears are engaged by moving the lay shaft, G, to engage either gear-wheel K with idling-pinion L (fast reverse) or gear-wheel H with pinion J (slow forward). At the half-way position neither H nor K is engaged; a neutral position is thus provided. An arm, M, free to revolve on G, is incorporated to engage with one of three slots fixed rigidly to the chassis of the titration apparatus and thus to lock shaft G in any of the three positions.

MODIFICATION TO RECORDER—

As supplied by the makers, the chart of a Honeywell - Brown recorder is driven by a synchronous motor through a gear-box. The four leads to this motor were cut and a ganged 4-pole 2-way switch, S, was inserted (see Fig. 2). It was then possible, at will, to operate the chart by an external motor or to use it in the manner for which it was originally designed.

With the chart-drive motor isolated electrically by opening switch S, movement of the chart is controlled by a flexible drive, N, direct from a shaft in the gear-box of the motor that operates the syringe. This flexible drive is connected to pinion A on the shaft of the chart-drive motor by means of shaft D having internal spline ends. One end of shaft D meshes with the teeth of pinion A and the other has a standard 0·125-inch key-way to accommodate the end of the flexible drive. A locking washer, held firmly to the casing by nut B, locks into a groove cut in a rigid brass plate, C. This plate is bolted rigidly to the chassis of the instrument and, when the chassis is in the closed position, protrudes through a hole cut in the case of the recorder. The chassis cannot now swing from the recorder case while the drive is connected and accidental damage to it is prevented.

An E.I.L. pH meter, model 23, provides an output of 20 μA per unit of pH (*i.e.*, 280 μA at full-scale deflection) for a recorder having an input impedance less than 1500 ohms. The Honeywell - Brown recorder, which had an impedance of 150 ohms and was calibrated

from pH 0 to 14 at a full-scale deflection of 280 μA, could be used directly with it. However, when the recorder was used in conjunction with an E.I.L. Vibron electrometer, model 33B, and the associated pH-measuring attachment, model C33B, slight modification was necessary. Since this unit provided full-scale deflection at 1 mA and required an impedance of about 1500 ohms when coupled to a recorder, a suitable high-stability resistor was connected in series with the output of the pH meter and the recorder was shunted with a 100-ohm wire-wound resistor, R, as shown in Fig. 3.

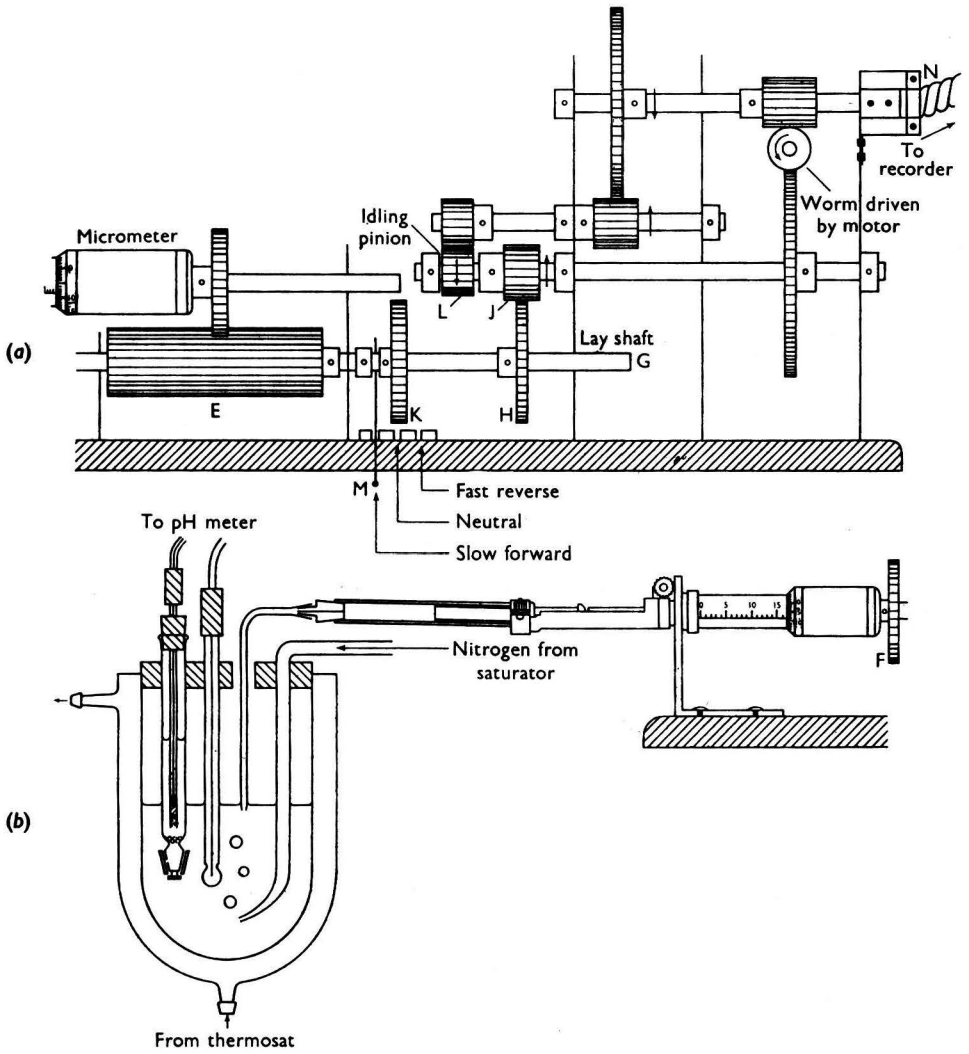

Fig. 1. Potentiometric titration apparatus (not to scale and semi-diagramatic): (*a*) micrometer-syringe drive and gear-box; (*b*) titration vessel

By appropriate adjustment of the range-switches on the Vibron assembly and of resistor R, it was possible to arrange for a reading of 10 units on the recorder (about an 8-inch width of chart) to correspond to 1 unit of pH. This permitted direct readings to be made from the chart graduations to 0·01 unit of pH and an estimate to be made to 0·003 unit of pH or better. If a particular titration covers a range of several units of pH, the pen of the recorder can be re-started at zero when the next higher integral pH value is reached by adjusting the pH UNITS switch on the pH-measuring attachment and making the corresponding adjustment to

the Vibron unit. The pH scale for a titration covering a range of 8 units of pH then extends for about 64 inches, which makes it possible to exploit the full sensitivity and high stability of the Vibron electrometer.

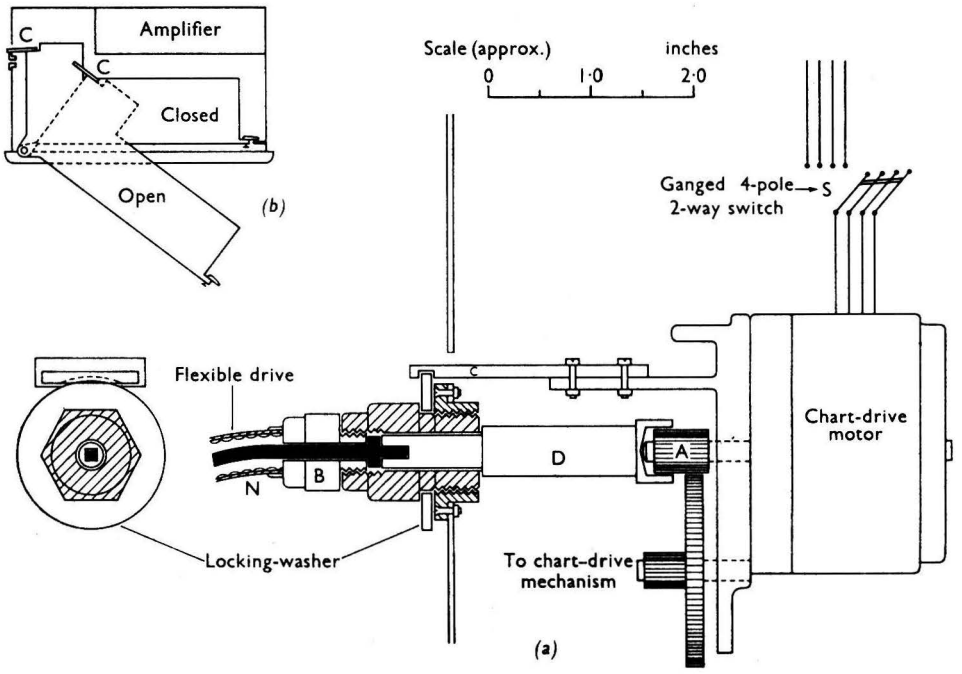

Fig. 2. (*a*) Modification to chart-drive mechanism of Honeywell - Brown recorder; (*b*) plan view of Honeywell - Brown recorder

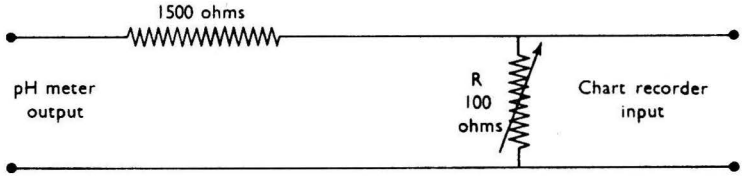

Fig. 3. Modification to Honeywell - Brown recorder when used in conjunction with Vibron electrometer

When used at this sensitivity, it was essential to shield all leads to the electrodes. For most commercial shielded glass electrodes, the shielding stops at the composition collar joining the glass to the screened leads. In our experience, the unshielded section is sufficient to introduce random fluctuations in pH owing to capacity effects. These fluctuations could be greatly reduced by fitting small additional earthed shields over the unscreened sections and could be completely removed by enclosing the whole titration vessel in an earthed shield made conveniently from perforated zinc gauze. To ensure long-term stability and to exploit instrumental sensitivity to the full, a good low-resistance earth is essential. This should be independent of the earthed connection of the a.c. mains supply, connection to which invariably introduces a high noise level and may make it impossible to use the recording titrimeter in a building in which other electrical apparatus is being switched in and out of circuit.

In practice it was found that a convenient maximum rate of adding titrant was about 0·02 ml per minute (corresponding to about 0·75 inch of chart travel). When this rate was decreased by means of the Variac transformer, a convenient minimum rate was 0·005 ml

per minute, *i.e.*, about 0·2 inch of chart travel. That equilibrium had been reached in any particular titration could be checked by stopping the motor drive for a time and withdrawing the tip of the syringe, or by repeating the titration at a slower speed. In this connection, we found that certain glass electrodes did not reach equilibrium with sufficient rapidity in the more alkaline regions. Of several examined, the GHS23 electrodes marketed by Electronic Instruments Ltd. were found to have an entirely satisfactory and rapid response and were accurate over the whole range up to pH 13. The shorter-range electrode, GH23, is even more rapid over the pH range 0 to 10.

PRECISION—

To illustrate the precision attainable with the titrimeter, a true-scale titration curve for the neutralisation of 50 ml of 0·001 *M* ethylenediaminetetra-acetic acid (H_4Y) in a medium of constant ionic strength (0·1 *M* potassium chloride) at 20° C was automatically plotted during 40 minutes. Values of pH corresponding to known volumes of added alkali (of exactly known concentration) were read at many points along the entire length of the graph. From the latter figures were calculated the corresponding values of R, in accordance with the equation—

$$R = \frac{a\,C_S + [H^+] - [OH^-]}{C_S}$$

where C_S is the total concentration of acid taken and a is the degree of neutralisation, *i.e.*, the number of equivalents of alkali added per molecule of acid taken. From considerations of electroneutrality it can be easily shown that, if the successive dissociation constants of the acid are defined by the equation—

$$K_n = \frac{[H^+]\,[H_{4-n}Y^{-n}]}{[H_{5-n}\,Y^{1-n}]}$$

then—

$$R\,[H^+]^4 + (R-1)K_1[H^+]^3 + (R-2)K_1K_2[H^+]^2 + (R-3)K_1K_2K_3[H^+] + (R-4)K_1K_2K_3K_4 = 0$$

When this equation was solved for all the pairs of values for $[H^+]$ and R obtainable from the experimental results (successive approximations were used), the values obtained for the dissociation constants were as follows—

$$pK_2 = 2{\cdot}61 \pm 0{\cdot}04$$
$$pK_3 = 6{\cdot}18 \pm 0{\cdot}04$$
$$pK_4 = 10{\cdot}29 \pm 0{\cdot}04$$

The values found by Schwarzenbach and Ackermann[6] for the same medium and temperature were, respectively, 2·67, 6·161 and 10·26.

It need hardly be emphasised how valuable such a titrimeter can be for determining the equivalent weight of an acid or base, for following changes in the composition of a mixture of acids or bases and in similar studies of purity. With different electrodes, *e.g.*, a glass reference electrode and a silver-wire indicator electrode for titrations of mixtures of halides, the apparatus can be used for all the titrations described by Haslam and Squirrell,[5] with the added advantage that the rate of titration can be decreased at strategic points without departing from the ideal condition that the ordinate scale should remain an exact measure of the volume of titrant added at any stage.

An obvious development of this type of apparatus, which would incorporate the advantages of true-scale recording and variable rate of addition of titrant with fully automatic operation, would be to control the speed of the motor providing the common drive to the syringe and recorder chart by a signal proportional to the first derivative of the d.c. input to the recorder (see Note).

NOTE—Since the work described was carried out, our attention has been drawn to a fully automatic titrimeter, the Titrigraph, and pH-Stat embodying these principles, which is marketed by Messrs. Radiometer, Emdrupvej 72, Copenhagen NV, Denmark. For workers unlikely to make extensive use of automatic titrimetry, the apparatus described in this paper has the merits of low cost and simplicity and the special advantage that the small modification to the recorder does not in any way affect its use for other laboratory purposes.

FURTHER APPLICATIONS—

For analytical purposes, in which greater interest may attach to the end-point of the reaction or to some intermediate state at which $\delta(pH)/\delta v$ is a maximum or minimum, it may be more convenient to plot values of this derivative on the recorder chart. This can easily be achieved by interposing an electronic differentiating device between the output of the pH meter and the input of the recorder. A small additional refinement is to include a cam-operated micro switch actuated by the micrometer screw of the syringe, by means of which a second "operations" pen on the recorder can be made to inscribe a scale of volumes on the right-hand side of the chart.

When the Honeywell - Brown recorder was equipped with two adjustable micro switches, which could be set to open or close external circuits at one or both of two pre-determined pH values, the titration assembly could be used in several other useful ways. In one application, the attainment of a pre-determined pH before the true end-point can be used to close the first micro switch and reduce the speed of the drive motor and the rate at which titrant is added. The operation of the second micro switch then stops the titration. Should it be more convenient, the Agla micrometer syringe can be replaced by a burette having FAST and SLOW delivery (*e.g.*, the Delivery Unit, catalogue No. 11610, marketed by W. G. Pye and Co. Ltd., Cambridge), the changes from FAST to SLOW and from SLOW to STOP being controlled by the two micro switches on the recorder. With either arrangement, the apparatus is convenient for the automatic addition of alkali or acid to maintain a constant pH, *e.g.*, as specified by Haslam and his co-workers in their elegant procedure for the complexometric titration of metals with ethylenediaminetetra-acetic acid[7,8] or that needed in kinetic studies of hydrolysis, enzyme action or other processes at a pre-determined pH.[9] It is also useful in the preparation of certain chelating agents (complexones) from amines and sodium chloroacetate when it is necessary to add concentrated alkali at a rate such that the pH is maintained between the change points of phenolphthalein and thymolphthalein, *i.e.*, between 8·5 and 9·5.[6]

We thank Electronic Instruments Ltd. for the extended loan of a Vibron electrometer and associated pH-measuring equipment, and Imperial Chemical Industries Ltd. for the loan of a Honeywell - Brown chart recorder. One of us (L.D.P.) wishes to thank the Department of Scientific and Industrial Research for a maintenance grant.

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Received *March 17th*, 1959

An Inexpensive High-frequency Titration Apparatus for General Laboratory Use

Application to some EDTA and Precipitation Titrations

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The construction of a stable high-frequency titrimeter is described; it will respond to both effective over-all capacitance and over-all conductance changes in the titration cell. The instrument incorporates a simple 2 megacycles per second crystal-controlled oscillator circuit and a valve voltmeter, and operates from a 220-volt 50-cycles mains supply. It can easily be constructed, is inexpensive and is simple to operate. Examples of its use are given; these include some EDTA and precipitation titrations.

The circuit used for the instrument is a modification of that described by Hall.[1] It is basically a 2 megacycles per second crystal-controlled oscillator combined with a valve voltmeter. The glass titration cell (equipped with two external aluminium or copper electrodes) and two calibrated variable capacitors form part of the resonant circuit; the unit is designed to provide a means of measuring or cancelling any change in cell capacitance consequent upon the addition of a reagent. The principle of operation is simple. If the tuning capacitor of a crystal oscillator is set at a value too great for oscillation to occur and then the capacitance is gradually increased, a point is reached at which oscillation starts abruptly; this point can be reproduced accurately. This feature permits the over-all capacitance changes of a high-frequency titration cell to be followed. If the tuning capacitance of the crystal oscillator is adjusted until maximum resonant voltage is attained,* the grid-bias voltage is at a maximum. The maximum grid-bias voltage at each point of a titration provides an indication of the effective conductance change of the cell. The grid-bias voltage is measured by the valve voltmeter and is assumed to be proportional to the conductance changes in the vicinity of the equivalence point.

It is also possible to use the change in capacitance of the system during the titration to evaluate the end-point; one condenser is set at a suitable value and the other standard condenser is adjusted to a maximum reading on the microammeter. The end-point is deduced graphically from a plot of the condenser reading against the volume of titrant. Owing to the small changes in capacitance, the procedure is less satisfactory than that dependent upon measurement of changes of the maximum grid-bias voltage.

Description of titrimeter

The circuit diagram is shown in Fig. 1, and the assembled titrimeter (including the titration cell) is shown in Fig. 2. A list of components required for the construction of the instrument is given in an Appendix (see p. 653); the total cost is about £25. Most of the parts are standard radio components; the manufacturers of the more important components are named.

The titrimeter can easily be modified for use at other pre-determined frequencies up to about 15 megacycles per second. This merely involves introducing plug-in quartz crystals oscillating at the desired frequency and the appropriate plug-in type of coil.

The titration cell, shown in Fig. 2 (compare Hall and Gibson[2]), consists of a Pyrex-glass tube, 150 mm × 25 mm, provided with two 25-mm wide bands of aluminium sheet (1·5 mm thick) fitted tightly around the tube and held in position (about 2·5 cm apart) by a Bakelite strip. The cell is mounted inside a glass vessel, which may be filled, if desired, with paraffin wax. Connection to the titrimeter is made with a screened cable. The level of the liquid in the cell should be about 1 cm above the top of the upper metal strip before measurements are made; the initial volume is about 35 ml.

* In many titrations adjustment to the minimum grid voltage is somewhat more sensitive; the end-point thus obtained is identical with that derived from the maximum grid-voltage readings.

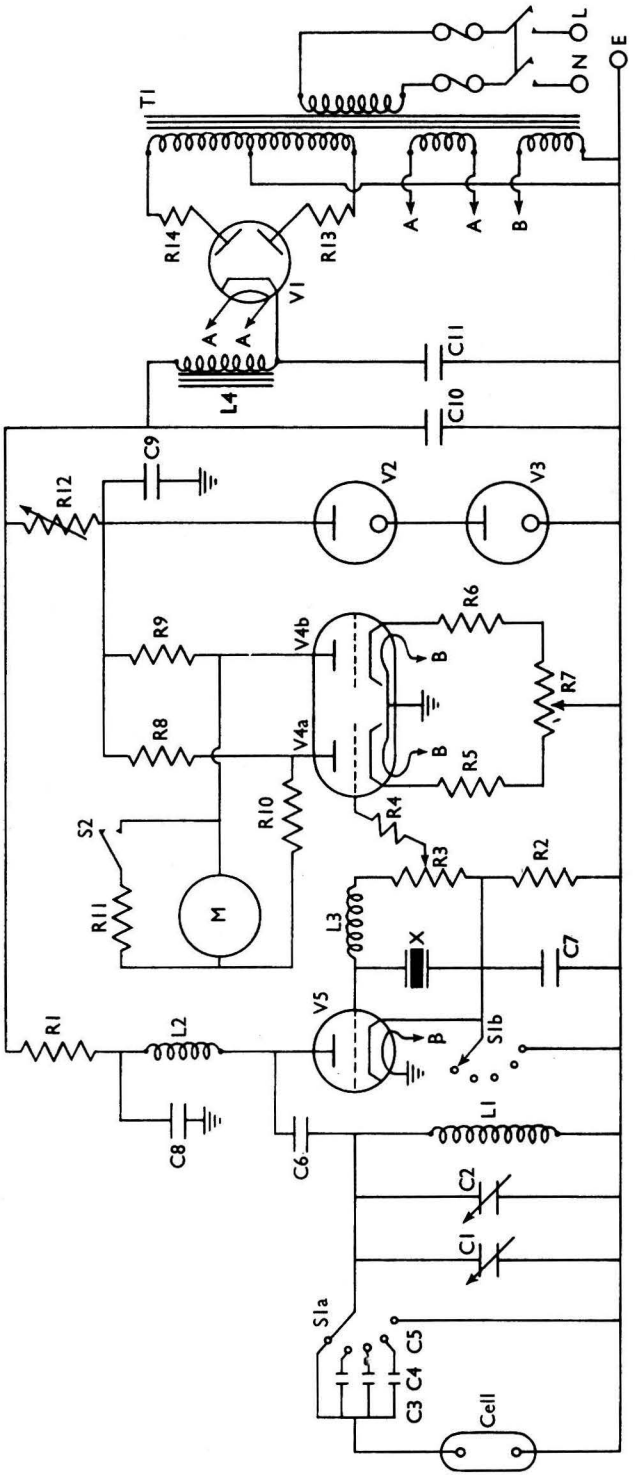

Fig. 1. Circuit diagram of high-frequency titrimeter (for values of components, see Appendix, p. 653)

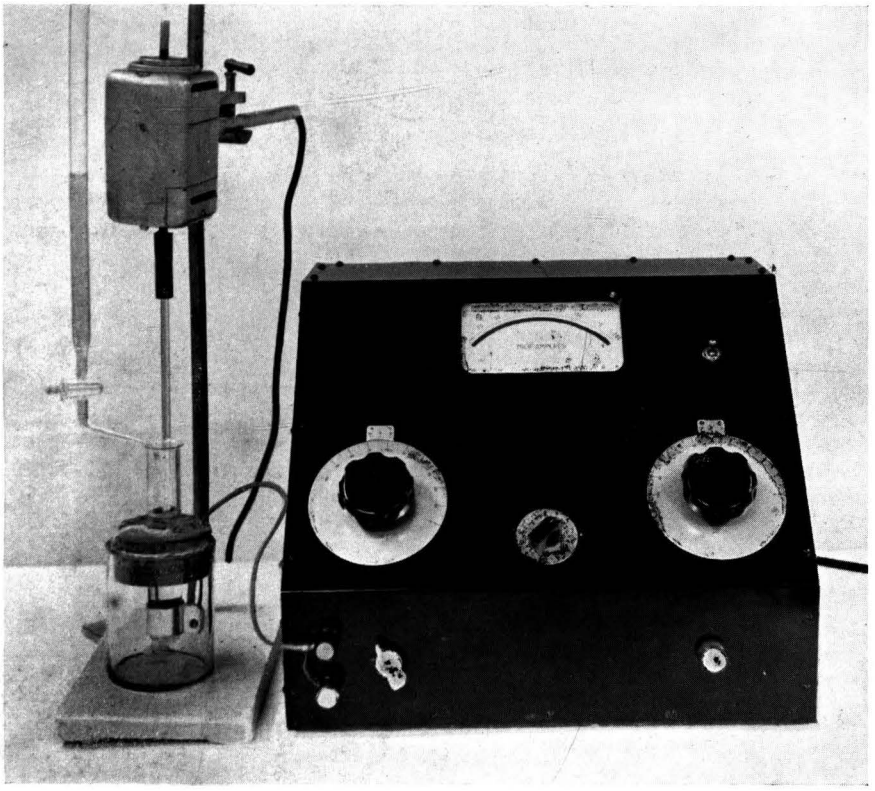

Fig. 2. High-frequency titrimeter and titration cell

[To face p. 648

Fig. 3 shows the response curves for the instrument as the concentration of an electrolyte solution (hydrochloric acid, sodium hydroxide or sodium chloride) is increased from zero to about 0·22 *M* (compare Hall[3] and Blaedel, Malmstadt, Petitjean and Anderson[4]).

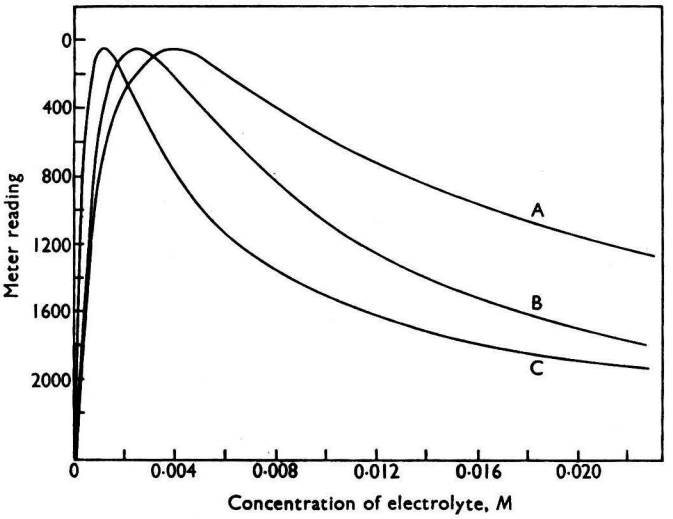

Fig. 3. Response curves of instrument for different electrolytes : curve A, sodium chloride; curve B, sodium hydroxide; curve C, hydrocloric acid

Method of use

The solution under test is placed in the titration cell and diluted to bring the liquid level at least 1 cm above the upper metal band (about 35 ml). If measurements are to be made only near the end-point, the total volume of the test solution *plus* the added titrant should be about 35 ml before these measurements are made. The solution is stirred mechanically with a glass stirrer and the titrant is added from a burette (usually of the semi-micro type) provided with a bent delivery tip. The sensitivity of the meter, M, is reduced by closing switch S_2. The instrument should be switched on at least 15 minutes before use in order to attain temperature equilibrium. The cell is connected in circuit, and the variable condensers C_1 and/or C_2 are rotated to give the maximum deflection on the meter. The reading on meter M is adjusted by means of the sensitivity control, R_3, so that it does not exceed one-fifth of the full-scale deflection. The meter is then restored to full sensitivity by opening switch S_2, and condenser C_2 is rotated to produce maximum deflection. For each addition of titrant, the condenser C_2 is slowly turned until the reading is a maximum; condenser C_1 is left in its original position during the whole of the titration. As the end-point is approached a marked change of meter reading per unit volume of titrant generally occurs. The end-point is evaluated from a plot of meter readings against volume of the reagent.

Typical determinations

To save space, the titration curves, in most cases in the vicinity of the end-points, of the various determinations are assembled in groups.

The apparatus was first used for three simple titrations—

(*i*) silver with 0·1 *M* sodium chloride (concentration of sodium chloride solution, 0·1014 *M*; volume of sodium chloride solution at end-point, 10·10 ml; silver found, 108·3 mg; silver present, 108·3 mg);

(*ii*) standardisation of about 0·1 *N* hydrochloric acid with 0·10035 *M* sodium carbonate (volume of sodium carbonate solution at second end-point, 9·99 ml; normality of hydrochloric acid found, 0·1005 *N*; true normality, 0·1005 *N*);

(*iii*) standardisation of about 0·1 *N* sodium hydroxide with 0·1005 *N* hydrochloric acid (volume of hydrochloric acid at end-point, 9·95 ml; normality of sodium hydroxide found, 0·1000 *N*; true normality, 0·09996 *N*).

The titration curves are shown in Figs. 4, 5 and 6.

TITRATIONS WITH EDTA—

Satisfactory results were obtained for the titrations of Zn^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Th^{4+} and La^{3+} with about 0·01 *M* EDTA (compare Hara and West[5] and Blaedel and Knight[6]). The metal ion contents of the various solutions were determined by standard (usually gravimetric) methods.

The 0·01 *M* EDTA was prepared from a weighed amount of dried analytical-reagent grade disodium ethylenediaminetetra-acetate; it was further standardised by titration with about 0·01 *M* zinc chloride solution prepared by dissolving about 0·7 g (accurately weighed) of analytical-reagent grade zinc pellets in diluted hydrochloric acid (1 + 1), boiling off the excess of acid on a water bath and diluting to 1 litre. Some typical results were: molarity of EDTA solution—from weight of salt, 0·009990 *M*; from Zn^{2+} titration, 0·009985 *M*. In all subsequent titrations, the EDTA concentration used for calculation was that derived from the standardisation with zinc.

The various metallic ion solutions used in the titrations were about 0·01 *M* and were prepared from the following salts: analytical-reagent grade $CuSO_4.5H_2O$; analytical-reagent grade $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$; analytical-reagent grade $Pb(NO_3)_2$; analytical-reagent grade $3CdSO_4.8H_2O$; pure $CoSO_4.(NH_4)_2SO_4.6H_2O$ and, preferably, pure cobalt sponge (Johnson and Matthey)—a known weight of the latter was dissolved in diluted nitric acid (1 + 1), the excess of acid boiled off on a water bath and the resulting solution diluted to 1 litre; pure $NiSO_4.(NH_4)_2SO_4.6H_2O$; analytical-reagent grade $Th(NO_3)_4.xH_2O$; pure $La(C_2H_3O_2)_3$. The results are collected in Table I and the titration curves are shown in Figs. 7 to 15.

TABLE I

TITRATIONS OF SOME METALLIC IONS WITH EDTA

Concentration of EDTA solution, *M*	Volume of EDTA solution used, ml	Metallic ion	Amount of ion found, mg	Amount of ion present, mg
0·009985	9·85	Cu^{2+}	6·26	6·28
0·009985	9·90	Fe^{3+}	5·53	5·52
0·009985	10·11	Pb^{2+}	20·92	20·85
0·009985	10·04	Cd^{2+}	11·27	11·25
0·010000	9·44	Co^{2+}	5·56	5·56
0·009891	10·06	Ni^{2+}	5·84	5·84
0·009891	9·23	Th^{4+}	21·19	21·10
0·009891	9·25	La^{3+}	12·71	12·75

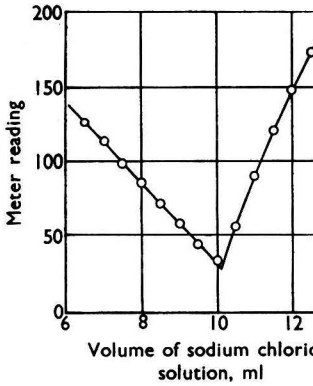

Fig. 4. Titration of silver nitrate with sodium chloride solution

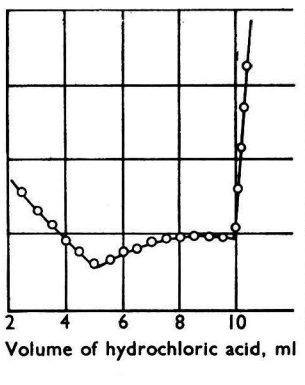

Fig. 5. Titration of sodium carbonate with hydrochloric acid

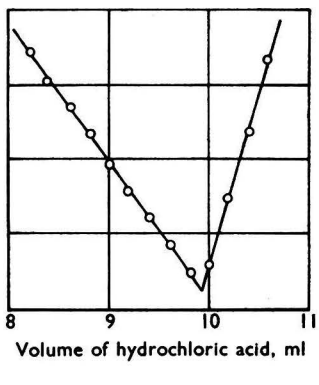

Fig. 6. Titration of sodium hydroxide with hydrochloric acid

Precipitation titrations

Sulphate with barium chloride solution—

The sulphate ion solution (about 0·01 *M*), prepared from analytical-reagent grade potassium sulphate, was titrated slowly with 0·01 *M* barium chloride (also prepared from the analytical-reagent grade salt). It may be necessary to wait for 3 to 5 minutes before meter readings are steady, particularly within 3 to 4 ml of the equivalence point. The titration curve is shown in Fig. 16. Some typical results were: concentration of barium chloride solution, 0·009918 *M*; volume of barium chloride solution, 10·03 ml; SO_4^{2-} found, 9·56 mg; SO_4^{2-} present, 9·58 mg. Milner[7] determined sulphate ion, largely at concentrations below 1 mg, by titration at a frequency of about 18·5 megacycles per second; the shape of his titration curves differed somewhat from our own.

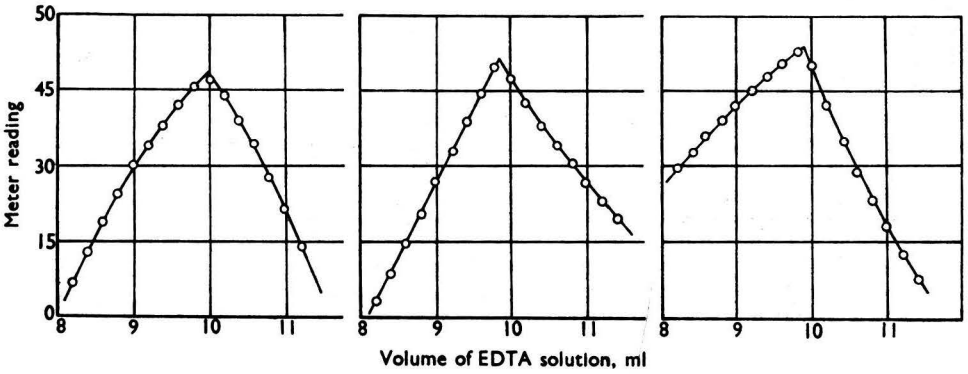

Fig. 7. Titration of Zn^{2+} ions with EDTA

Fig. 8. Titration of Cu^{2+} ions with EDTA

Fig. 9. Titration of Fe^{3+} ions with EDTA

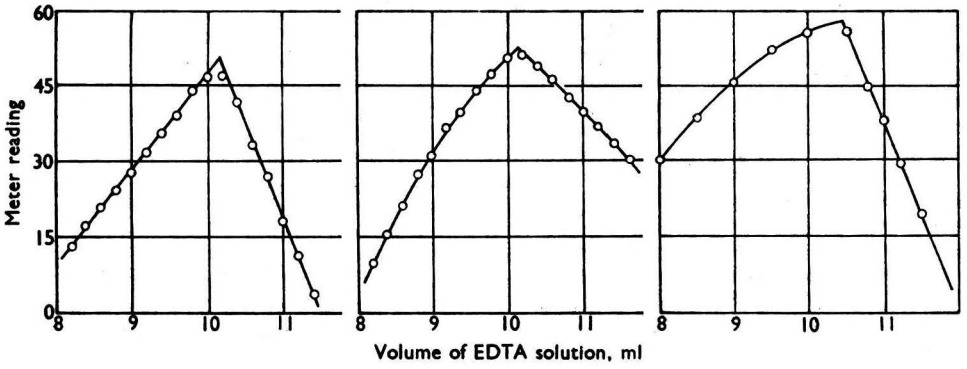

Fig. 10. Titration of Pb^{2+} ions with EDTA

Fig. 11. Titration of Cd^{2+} ions with EDTA

Fig. 12. Titration of Co^{2+} ions with EDTA

Jamieson[8] determined sulphate by titration, *inter alia*, with aqueous barium acetate; he used an apparatus operating at 30 megacycles per second (see Dowdall, Sinkinson and Stretch[9]) with the titration cell fitted into the grid coil. Results were satisfactory in an ethanol - water mixture (1 + 4 v/v), but not in water, for amounts of sulphate between 1·5 and 21 mg in 40 ml of solution. It is stated that equilibrium was established in 30 seconds. In our experiments at 2 megacycles per second, 2 to 3 minutes were required for attainment of equilibrium near the equivalence point in aqueous solution, and accurate results were obtained for 5 to 20 mg of sulphate in 35 to 40 ml of solution.

Thorium with sodium oxalate—

A 0·01 *M* thorium nitrate solution, prepared from analytical-reagent grade $Th(NO_3)_4.xH_2O$, was titrated with standard 0·02000 *M* sodium oxalate. The latter was

prepared by dissolving the appropriate weight of analytical-reagent grade sodium oxalate in 1 litre of 0·01 *M* nitric acid. The titration curve is shown in Fig. 17. Typical results were: concentration of sodium oxalate solution, 0·02000 *M*; volume of sodium oxalate solution, 11·00 ml; Th^{4+} found, 21·11 mg; Th^{4+} present, 21·19 mg. Similar results were obtained by titration with standard 0·02 *M* oxalic acid. Blaedel and Malmstadt[10] determined thorium by high-frequency titrimetry by a similar method; the titration cell was placed in the field of a high-frequency oscillator operating at 30 megacycles per second and the changes in frequency of the oscillator were measured during the titration.

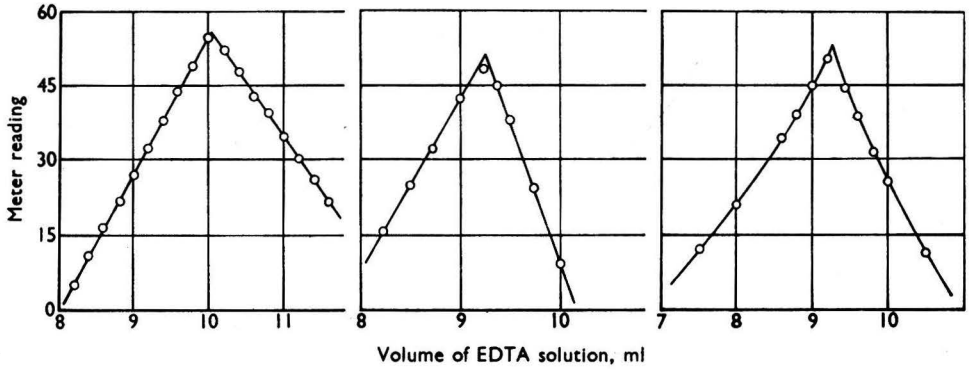

Fig. 13. Titration of Ni^{2+} ions with EDTA

Fig. 14. Titration of Th^{4+} ions with EDTA

Fig. 15. Titration of La^{3+} ions with EDTA

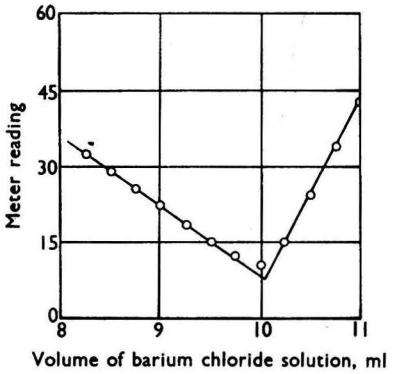

Fig. 16. Titration of SO_4^{2-} ions with barium chloride solution

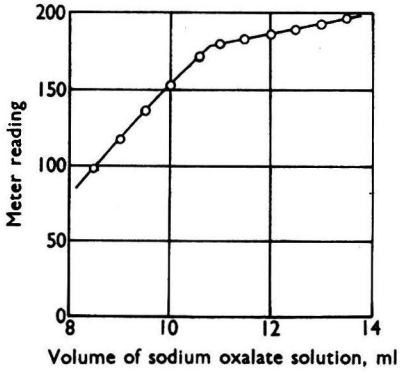

Fig. 17. Titration of Th^{4+} ions with sodium oxalate solution

FLUORIDE WITH LANTHANUM ACETATE—

This titration was first carried out satisfactorily by Dowdall, Sinkinson and Stretch[9]; they used a high-frequency titrimeter (15 to 20 megacycles per second) with the titration cell located within the anode coil. In our experiments about 0·01 *M* sodium fluoride, prepared from a known weight of the dry analytical-reagent grade salt, was titrated with about 0·01 *M* lanthanum acetate. The lanthanum acetate solution was prepared by dissolving 3·16 g of pure lanthanum acetate in water containing 0·4 to 0·5 ml of glacial acetic acid and diluting to 1 litre; its concentration was determined by titration with standard 0·01 *M* EDTA and also by precipitation as the oxinate. Ten millilitres of the fluoride solution were treated with 5 ml of 0·1 *M* acetic acid, diluted to about 40 ml and titrated with the standard lanthanum solution. A further check of the fluoride concentration was obtained by passage through an ion-exchange column and titration of the hydrofluoric acid effluent with standard sodium hydroxide solution and methyl red as indicator. The titration curve is shown in Fig. 18. Some typical results were: concentration of lanthanum acetate solution, 0·009149 *M*; volume of lanthanum acetate solution, 3·520 ml; F^- found, 1·836 mg; F^- present, 1·842 mg.

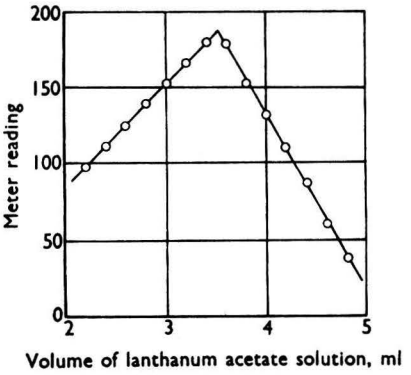

Fig. 18. Titration of F^- ions with lanthanum acetate solution

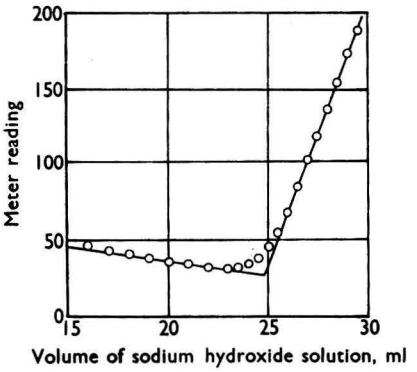

Fig. 19. Titration of Be^{2+} ions with sodium hydroxide solution

BERYLLIUM WITH SODIUM HYDROXIDE—

This titration, for 12·7 to 37·1 mg of beryllium, was first performed by Anderson and Revinson,[11] who used an apparatus operating at 22 megacycles per second, and 0·4320 *N* sodium hydroxide. Our titrations were carried out with a beryllium sulphate solution containing about 2 mg of beryllium (prepared from an analytical-reagent grade salt) and about 0·02 *N* sodium hydroxide. Some typical results were: concentration of sodium hydroxide solution, 0·02020 *N*; volume of sodium hydroxide solution, 24·70 ml; beryllium found, 2·250 mg; beryllium present, 2·260 mg. The titration curve is shown in Fig. 19.

We thank Imperial Chemical Industries Ltd. for a grant.

APPENDIX

LIST OF COMPONENTS USED IN THE CONSTRUCTION OF THE HIGH-FREQUENCY TITRIMETER (Fig. 1.)

C_1	=	100-$\mu\mu$F high-stability standard variable condenser (Eddystone,* catalogue No. 738) fitted with vernier slow-motion dial (Eddystone,* catalogue No. 843).
C_2	=	27·5-$\mu\mu$F high-stability standard variable condenser (Eddystone,* catalogue No. 588) fitted with vernier slow-motion dial (Eddystone,* catalogue No. 843).
C_3	=	500-$\mu\mu$F silver - mica fixed condenser.
C_4	=	250-$\mu\mu$F silver - mica fixed condenser.
C_5	=	100-$\mu\mu$F silver - mica fixed condenser.
C_6	=	100-$\mu\mu$F condenser.
C_7, C_8, C_9	=	0·10-μF condenser.
C_{10}, C_{11}	=	8-μF electrolytic condenser, 450-volt d.c. working.
R_1	=	33,000-ohm resistance.
R_2	=	1500-ohm high-stability resistance, 1 per cent. tolerance.
R_3	=	33,000-ohm variable resistance.
R_4	=	1-megohm resistance.
R_5, R_6	=	2200-ohm high-stability resistance, 1 per cent. tolerance.
R_7	=	1000-ohm potentiometer.
R_8, R_9	=	30,000-ohm high-stability resistance, 1 per cent. tolerance.
R_{10}	=	820-ohm high-stability resistance, 1 per cent. tolerance.
R_{11}	=	47-ohm high-stability resistance, 1 per cent. tolerance.
R_{12}	=	20,000-ohm variable resistance.
R_{13}, R_{14}	=	100-ohm resistance.
L_1	=	Tuning coil consisting of 50 turns of 26-gauge enamelled copper wire on a 1-inch paxolin former.
L_2	=	2·5-millihenry radio-frequency choke (Eddystone,* catalogue No. 737).
L_3	=	13-millihenry radio-frequency choke (Eddystone,* catalogue No. 1066).
L_4	=	10-henry 60-mA choke.
T_1	=	Mains transformer: primary windings, 10–0–200–240 volts; secondary windings, 350–0–350 volts at 100 mA; additional windings, 5 volts at 2 amps and 6·3 volts at 3 amps.
S_1	=	5-way 2-pole switch (Yaxley).
S_2	=	Single-pole toggle switch (Bulgin).
V_1	=	5V4 rectifier.
V_2, V_3	=	85A2 neon regulator.

V_4 = 12AT7.
V_5 = 12AT7 (only one half used).
X = 2-megacycles per second crystal, 0·01 per cent. (type 10X†).
M = Moving-coil d.c. microammeter, 0–250 μA with mirror scale (model 500‡).

* Eddystone products are manufactured by Stratton & Co. Ltd., Alvechurch Road, West Heath, Birmingham 31.
† Obtained from Quartz Crystal Co. Ltd., 71, Kingston Road, New Malden, Surrey.
‡ Manufactured by Taylor Electrical Industries Ltd., Montrose Avenue, Slough, Bucks.

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Received *April 14th*, 1959

Notes

A RAPID METHOD FOR DETERMINING THE CONSTITUENT COMPONENTS IN CAROTENOID EXTRACTS FROM LEAF TISSUE

THE methods described for the rapid assay of β-carotene and total xanthophylls in plant tissues[1,2] are unsuitable for the determination of the individual substances. It is now recognised[3] that the major xanthophylls in most green leaves are lutein (3:3′-dihydroxy-α-carotene), violaxanthin (3:3′-dihydroxy-5:6:5′:6′-diepoxy-β-carotene) and neoxanthin (structure unknown). Neoxanthin can be easily separated chromatographically from lutein and violaxanthin, but it is difficult to separate the two last-named substances from each other without prolonged chromatography; the problem is further complicated because the relative positions of these two compounds on chromatographic columns can be altered by different combinations of adsorbent and developer. Further, the absorption spectra of solutions of the two compounds in light petroleum are almost identical, that of lutein having maxima at 422, 443 and 473 mμ and that of violaxanthin having maxima at 420, 440 and 470 mμ, so that it is not possible to determine the individual components in a mixture of the two by spectroscopy alone. However, the addition of 1 drop of concentrated hydrochloric acid to such a mixture converts violaxanthin almost completely to auroxanthin,[4] the absorption spectrum of which has maxima at 378, 400 and 420 mμ. If, therefore, the optical density of the mixture is measured at 400, 442 and 472 mμ both before and after treatment with concentrated hydrochloric acid, the amounts of lutein and violaxanthin originally present can be calculated.

Method

REAGENTS—

Acetone—Analytical-reagent grade.

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

Adsorbent mixture—Mix 2 parts by weight of chromatographic-grade magnesium oxide and 1 part of Celite 545.

PREPARATION OF CHROMATOGRAPHIC COLUMN—

Pour dry adsorbent mixture into a 15-cm × 1·8-cm glass chromatographic tube, and pack down under suction to give a column 6 cm high. Pack a 1-cm layer of anhydrous sodium sulphate on top of the column of adsorbent mixture.

PROCEDURE FOR DETERMINING CAROTENOIDS—

Completely extract the pigments from up to 1 g of leaf tissue or the equivalent amount of homogenates or chloroplasts with 40 to 60 ml of cold acetone, and transfer them to 20 ml of light petroleum by adding a half-saturated aqueous solution of ammonium sulphate.[5] Moisten the column with light petroleum, and pour the entire solution of pigments on to it.

Develop the chromatogram with portions of light petroleum that contain increasing amounts of acetone, and collect the fractions of eluate in calibrated flasks. Elute α-carotene, if present, with light petroleum containing 2 per cent. v/v of acetone, β-carotene with light petroleum containing 5 per cent. v/v of acetone, violaxanthin *plus* lutein (one fraction) with light petroleum containing 20 per cent. v/v of acetone and neoxanthin with light petroleum containing 40 to 50 per cent. v/v of acetone. Take care to avoid contamination of the neoxanthin fraction by chlorophyll, which, if present, can be removed by cold saponification.[6]

Determine α-carotene, β-carotene and neoxanthin in their eluted fractions by spectrophotometry; take the values of $E^{1\%}_{1cm}$ as 2700 at 443 mμ, 2500 at 448 mμ and 2270 at 437 mμ, respectively.

Measure the optical density of the violaxanthin - lutein mixture at 400, 442 and 472 mμ. From the end of a stirring rod, add 1 drop of concentrated hydrochloric acid to the solution in the cuvette, and, after 10 minutes, again make optical-density measurements at the same wavelengths. (Although only the second set of readings at 400 and 472 mμ is required to determine the individual compounds, the first set can be used to calculate the approximate total of the two, if the value of $E^{1\%}_{1cm}$ for the mixture is assumed to be 2500 at 442 mμ.)

NOTE—The carotenoids in amounts of leaf tissue greater than 1 g can be determined by using a larger column. The separation of the pigments on a small or large column can be hastened by applying pressure to the top of the column; care should be taken, however, as the bands tend to run into one another if too much pressure is applied.

CALCULATION OF RESULTS—

By using values for $E^{1\%}_{1cm}$ of 2500 at 443 mμ and 2550 at 440 mμ for lutein and violaxanthin, respectively, in light petroleum containing 20 per cent. v/v of acetone, we have calculated values of $E^{1\%}_{1cm}$ from the optical densities of purified lutein and purified violaxanthin after treatment with concentrated hydrochloric acid. (Violaxanthin does not yield pure auroxanthin; the product contains traces of other pigments.[4]) The values for acidified violaxanthin (mainly auroxanthin) are 190 at 472 mμ and 2200 at 400 mμ; for lutein, the values are 2280 at 472 mμ and 1020 at 400 mμ.

The concentrations of the two carotenoids in the eluted fraction can be calculated from the expressions—

$$\text{Violaxanthin present, } \mu g \text{ per ml} = \frac{E_{400} - 0{\cdot}448\, E_{472}}{0{\cdot}211}$$

$$\text{Lutein present, } \mu g \text{ per ml} = \frac{E_{472} - 0{\cdot}086\, E_{400}}{0{\cdot}219}$$

in which E_{400} and E_{472} are the optical densities of the mixture at 400 and 472 mμ, respectively, measured in 1-cm cells after treatment with concentrated hydrochloric acid.

The standard error of the mean of twenty-one replicate determinations by the proposed method was ±2 per cent.

One of us (T.O.M.N.) was supported by a Fellowship of the National Science Foundation.

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Received *May 20th*, 1959

THE IDENTIFICATION OF NYLON AND RELATED POLYMERS BY RING PAPER CHROMATOGRAPHY

CLASPER, Haslam and Mooney's method for identifying nylon and related polymers by paper chromatography[1] has proved to be generally useful, but chromatographic examination of the hydrolysis products of the polymer takes a considerable time—about 7 hours. It seemed to us that, if ring paper chromatography could be applied, a considerable saving in time would be effected. We decided, therefore, to examine this procedure and in particular that form of chromatographic test proposed by Miss and Segal,[2] who used the method for the separation of amino acids, antibiotics, mono- and polycarboxylic acids, higher fatty acids, polyalcohols, etc. The method is essentially that described by Rutter,[3] but the eluting liquid is fed to the chromatographic paper by means of a thread having suitable dimensions. Miss and Segal claim that their procedure gives much more reproducible separation times than does Rutter's method and provide evidence for this claim.

After threads of all descriptions had been tested, it was decided that Miladi No. 16 thread (a cotton mending thread manufactured by Miladi Needlework Products, The British Thread Mills Ltd., East Park Road, Leicester) provided a useful wick and permitted us to separate the various hydrolysis products in about 2 hours. The method described below has been used in the examination of nylon and related polymers.

METHOD

PROCEDURE—

A 25-mg portion of the polymer is first hydrolysed as described previously,[1] *i.e.*, overnight at 120° C with diluted hydrochloric acid (1 + 1). The hydrolysis product is then washed out of the tube with water, and the mixture is evaporated to dryness before extraction with 1 ml of hot ethanol.

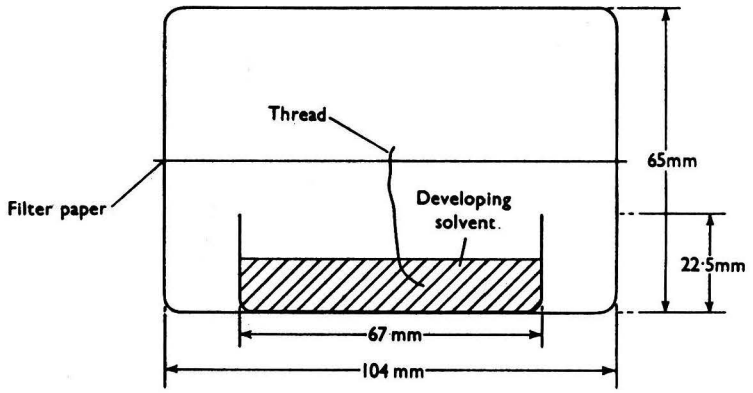

Fig. 1. Apparatus for ring paper chromatography

The apparatus is then assembled as shown in Fig. 1, the inner compartment, a small Petri dish, containing approximately 20 ml of the developing solvent (a mixture of 6 volumes of *n*-propanol, 3 volumes of ammonia solution, sp.gr. 0·880, and 1 volume of water). The mixture is allowed to equilibrate for a few minutes. In the test for bases and ϵ-aminocaproic acid a 5-μl portion of the ethanolic solution of the hydrolysis products is applied to the centre of an 11-cm Whatman No. 1 filter-paper by means of a micropipette and is allowed to dry. A piece of Miladi No. 16 thread (see Note) is then threaded through the centre of the filter-paper, the edges of the hole being made quite smooth with the thick end of the needle, and the wick is cut so that it projects about 0·5 cm above and approximately 3 cm below the filter-paper. The paper is then transferred to the apparatus, the wick is immersed in the solvent mixture, and the whole apparatus is closed. It is important that the upper and lower Petri dishes are well ground, so that a good fit is obtained and solvent vapours cannot escape. Particular attention should be paid to the distance between the surface of the solvent and the filter-paper; in our experience 2¼ cm are satisfactory.

The elution is allowed to proceed at room temperature for 1½ to 1¾ hours, although care should be taken to ensure that the developing solvent does not reach the edges of the Petri dishes.

The filter-paper is then removed and dried, first in air and then at 105° C for 20 minutes, after which it is sprayed with a solution containing 0·3 g of ninhydrin in a mixture of 95 volumes of *n*-butanol and 5 volumes of 2 *N* acetic acid. The paper is then dried in air and finally at 105° C for 5 minutes, after which it is ready for examination.

The test for acids, such as adipic and sebacic acids, is carried out in the same way, except that three separate 5-μl portions of the ethanolic solution of the hydrolysis products are applied to the centre of the filter-paper before chromatography. In this test, spraying is carried out lightly with methyl red - borate buffer reagent[1] (a solution containing 0·03 g of methyl red in 100 ml of a buffer solution prepared by dissolving 12·368 g of boric acid and 14·912 g of potassium chloride in water, adding 35 ml of *N* sodium hydroxide and diluting to 1 litre; the pH of the buffer solution should be 8·0 $\pm$ 0·1). The sprayed paper is dried between pieces of clean filter-paper. It is advisable to carry out controlled tests on known copolymers under the same conditions.

NOTE—It is possible that a commercial thread might not always contain the same proportion of natural or other lubricant and for this reason may not always behave in a standard way. We suggest that this difficulty may be avoided by using a scoured yarn of almost pure cotton cellulose, as prepared by Shirley Developments Ltd., 40 King Street West, Manchester 3.

RESULTS

The various bases and acids formed by hydrolysis of the polymers tested produced characteristic uniform rings, although the R_F values for individual constituents were not the same as those found originally by ascending-solvent paper chromatography.[1] Different polymers examined by us have exhibited the behaviour described below.

Test for bases—*pp'*-Diaminodi*cyclo*hexylmethane dihydrochloride produced a blue ring (R_F 1), and the approximate R_F values for hexamethylenediamine dihydrochloride, ω-aminoheptanoic acid hydrochloride and ϵ-aminocaproic acid hydrochloride were 0·90, 0·78 and 0·73, respectively.

Test for acids—Sebacic acid produced a pink ring (R_F approximately 0·82), and the R_F value for adipic acid was approximately 0·62. In addition, a pink ring (R_F approximately 0·72) due to ammonium chloride is invariably obtained in this test.

Sometimes the ring produced by ammonium chloride is not well separated from that produced by adipic acid, but in this event the combined ring is broad and, when the behaviour in this test is taken in conjunction with the test for bases, little difficulty in identification is experienced.

From results obtained in this way, valuable information may readily be obtained about nylon polymers and copolymers.

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Received *May 28th*, 1959

IMPROVEMENTS IN THE AGAR-PLATE TEST FOR THIAMINE FACTORS WITH *LACTOBACILLUS FERMENTI* 36

DURING a study of the nutritional aspects of *Lactobacillus fermenti* 36 (ATCC 9338), I found that nutritional conditions rather than inherent biological inconsistency of the strain were responsible for incidental discrepancies in the assay of thiamine in fluid media.[1] Tryptophan was found to be an essential nutrient for *L. fermenti*, and the presence of a reducing agent, such as cysteine or ascorbic acid, was confirmed to be critical when experiments were performed on semi-synthetic media based on Sarett and Cheldelin's original medium,[2] from which the alkali-treated peptone supplement was omitted because of its interference with a systematic nutritional investigation.

When these studies were repeated by auxanographic tests on agar plates, the growth of *L. fermenti* with thiamine in the media recommended by different workers for thiamine assay[2,3,4,5,6] was rather faint and ill-defined, an effect previously observed by Bacharach and Cuthbertson.[3]

Urine, extracts of yeast and liver or peptone gave double zones, the inner zone showing abundant bacterial growth. Some additional factor or factors not encountered in these media was therefore indispensable to a successful agar-plate test. Supplements of thiamine-depleted yeast extract[3] or alkali-treated peptone[2] in the basal medium did not adequately replace the missing factor, but the addition of Tween 80 (polyoxyethylene sorbitane mono-oleate) overcame the insufficiency. The growth zones obtained in this way were superior in density and visibility to those obtained by many of the other agar-plate methods for different vitamins of the B group. In tube tests for thiamine with *L. fermenti*, oleic acid and Tween 80 are not necessary nutrients,[1,2,6] although MaciasR[7] has recently reported that thiazole utilisation is enhanced by the presence of these supplements.

For the basal medium, I recommend the composition previously described,[1] *viz.*, a semi-synthetic medium based on Sarett and Cheldelin's medium[2] without alkali-treated peptone, but with tryptophan, cysteine, ascorbic acid, pantethine, Tween 80 and agar. When the peptone supplement is omitted, the vitamin-free casein component should be increased from 5 to 12 g per litre of double-strength medium.

In contrast to former agar-plate methods,[2,4] the size of the inoculum is not important in this test. Adjustment of the twice-washed cell suspension to a light-transmission value between 30 and 40 per cent. at 6000 A will, however, ensure greater uniformity between runs (1 ml per 25 ml of basal medium).

The incorporation of any of the thiamine-depleted natural supplements previously recommended (peptone, yeast extract, etc.), although not essential, will exert a beneficial effect by counteracting the slight background growth and will to some extent improve the visibility of the zones of exhibition when incubation is prolonged or when too dense an inoculum is used.

Batches of the basal medium, without agar, can be stored in double-strength form below −10° C for as long as 6 months. Seeded plates were successfully stored at 4° to 8° C for up to 2 weeks before use. The special treatments used by Jones and Morris,[4] *i.e.*, allowing the medium to mature at room temperature or pre-incubating the plates before the standard or test solutions were poured into the cups, are superfluous under the proposed conditions. However, if the plates with the poured cups are pre-cooled at 4° to 8° C for 1 to 4 hours before incubation, the size of the zones is increased; in this way, the sensitivity range of the assay can be increased without serious effect on the visibility of the growth zones. The normal incubation period varies between 8 and 10 hours, although growth is usually detectable after 5 or 6 hours of incubation at 35° C. The optimum range of assay depends on the type of cups or paper discs used and, in my experience, is between about 10 and 100 mμg of thiamine per cup.

Since thiamine occurs in different phosphorylated and disulphide forms in natural extracts and body fluids, several such substances were tested under the proposed conditions; the responses agreed fairly well with the quantitative results obtained when thiamine was used. One of the main advantages of the proposed conditions is that cocarboxylase and thiamine have equal potencies on a molar basis; this is in contrast to earlier tube techniques, for which a 30 to 40 per cent. increase in thiamine activity was claimed after pyrophosphorylation.[3,8]

Discussion of results

Table I shows the results found for different derivatives of thiamine under the proposed conditions.

The appearance of the growth zones is shown in Fig. 1, in which inner zones of inhibition can be observed in cups Nos. 6 to 10 and 46 to 50, as well as in cups Nos. 21 to 25 and 61 to 65, to which sulphite-treated yeast-extract solution had been added with the thiamine or cocarboxylase standards, as recommended for the improvement of bacterial growth in a cup-plate test for thiamine.[3] However, it can be seen from Fig. 1 that such an addition is unnecessary when the proposed conditions are used, as no further improvement resulted.

Recovery experiments with thiamine standards or dilutions of the different derivatives of thiamine tested together with crude natural extracts, *e.g.*, yeast autolysate, produced uniform growth zones and results in good agreement with the theoretical values, except for thiamine disulphide[11]; this compound exhibited anomalous response in some batches of media. Thiamine disulphide could be used to test the suitability of the mixture in the basal medium. When low values were obtained with this compound, the addition of yeast autolysate significantly increased the growth zones (compare cups Nos. 31 to 35 and 71 to 75 with cups Nos. 36 to 40 and 76 to 80 in Fig. 1). This activating effect of yeast extract on thiamine disulphide is at present being investigated in more detail.

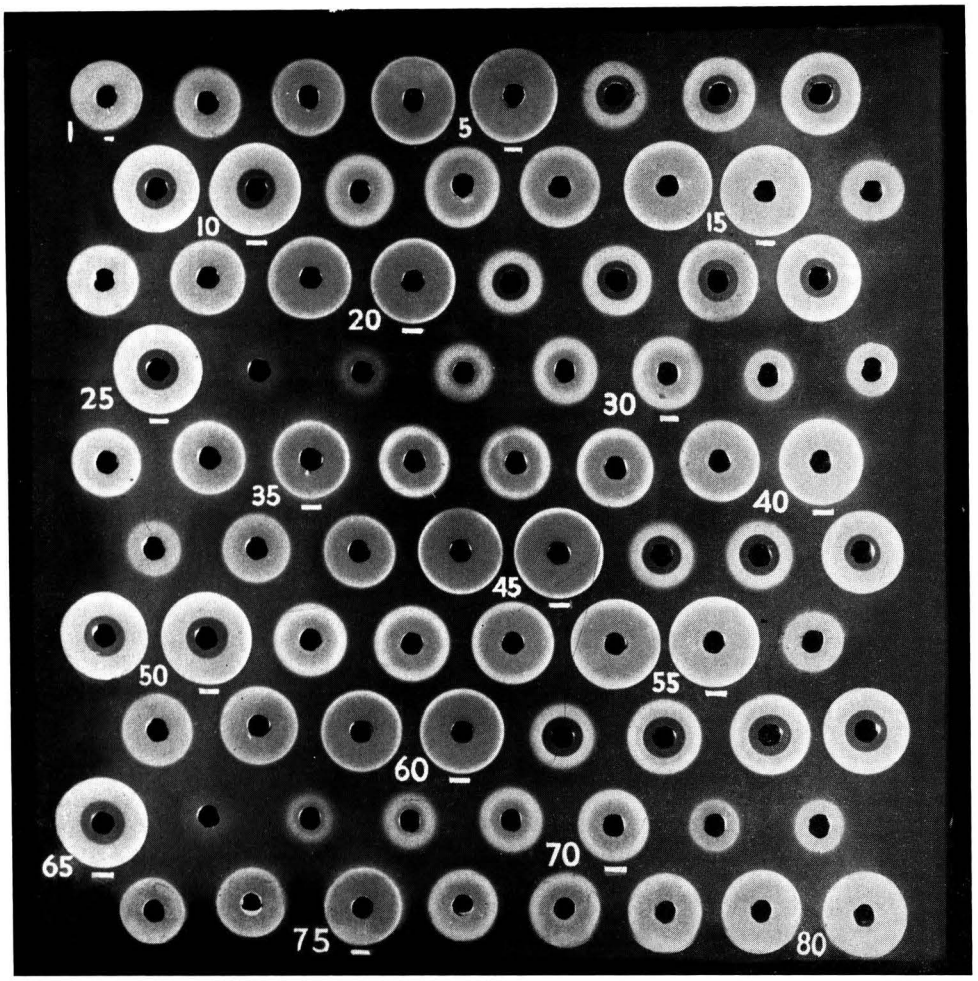

Fig. 1. Large-plate test of thiamine and its derivatives with *L. fermenti* on a semi-synthetic agar medium containing Tween 80 : cups Nos. 1 to 5 and 41 to 45, thiamine standards; cups Nos. 6 to 10 and 46 to 50, thiamine *plus* constant levels of sulphite-treated Bacto yeast extract; cups Nos. 11 to 15 and 51 to 55, thiamine *plus* constant levels of Bacto yeast-extract percolate; cups Nos. 16 to 20 and 56 to 60, cocarboxylase; cups Nos. 21 to 25 and 61 to 65, cocarboxylase *plus* sulphite-treated Bacto yeast extract; cups Nos. 26 to 30 and 66 to 70, Bacto yeast extract in increasing concentrations; cups Nos. 31 to 35 and 71 to 75, thiamine disulphide; cups Nos. 36 to 40 and 76 to 80, thiamine disulphide *plus* constant levels of Bacto yeast extract

[To face p. 658

Although the response with thiamine disulphide is a criterion for the quality of the basal medium, in microbiological thiamine assays it represents a problem similar to that in the thiochrome method, in which this substance is inactive unless reduced by an excess of cysteine or glutathione. According to my preliminary results, the treatment used in the thiochrome test will restore the activity of the disulphide form of thiamine in the agar-plate test.

TABLE I

GROWTH ZONES OBTAINED WITH VARIOUS THIAMINE COMPOUNDS

Compound tested	Number of cups at each concentration	Average diameter of growth zone produced by— $4{\cdot}62 \times 10^{-9}$ g of thiamine per ml of test solution, mm	$9{\cdot}25 \times 10^{-9}$ g of thiamine per ml of test solution, mm	$18{\cdot}5 \times 10^{-9}$ g of thiamine per ml of test solution, mm
Thiamine hydrochloride	6	24·9	27·4	30·1
Thiamine orthophosphate hydrochloride	3	25·2	27·1	29·9
Thiamine pyrophosphate hydrochloride (cocarboxylase)	3	25·7	27·2	29·2
Thiamine - cysteine complex (crude)*	3	24·7	27·0	29·7
Allithiamine (crude)†	3	24·3	27·5	30·3

* Prepared as described by Matsukawa *et al.*[9]
† Prepared as described by Matsukawa *et al.*[10]

It has been reported that, among the precursors of thiamine, the thiazole moiety of the thiamine molecule can replace thiamine in rather high concentrations under conditions favourable to *L. fermenti*.[7] In the agar-plate test, 0·2 to 5 mg per ml of the thiazole (4-methyl-5-oxyethylthiazole) will promote growth and give rise to somewhat diffuse zones. The appearance of the growth zones with thiazole improved and was similar to that of the thiamine zones when low concentrations of the pyrimidine portion (2-methyl-4-aminomethylpyrimidine hydrochloride) of the thiamine molecule were added to the same or adjacent cups.

Interference from thiazole can be easily removed by paper chromatography of these precursors. In several solvent systems, *e.g.*, *n*-butyl alcohol - water - acetic acid (25 + 25 + 6) or *n*-butyl alcohol - ethanol - acetic acid - water (4 + 1 + 1 + 10), thiazole exhibits a high mobility and moves with the solvent front, but thiamine and its derivatives have R_F values between 0·0 and 0·6, thus permitting easy separation from thiazole.

The work described was supported financially by the Swedish Natural Science Research Council, and I thank Mrs. Eva Börje-Lööv for valuable assistance.

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First received *November 10th*, 1958
Amended, *April 13th*, 1959

SOME FACTORS IN THE DETERMINATION OF CARBONATE IN SOILS

DURING analysis of soil samples from liming trials for residual limestone, some refinements were made to Schollenberger's method.[1] Splashes from the reaction mixture were prevented from entering the absorption flask by using a Davies instead of a Liebig condenser. All reagents were stored in aspirators and passed into the apparatus through a system of tubing isolated from the

atmosphere. In this way, contamination was avoided and the additions were made more rapidly. The ferrous chloride and hydrochloric acid were made up in one solution.

CARBON DIOXIDE FROM HYDROLYSIS AT DIFFERENT TEMPERATURES—

It is well known that, unless precautions are taken in the acid decomposition of carbonate in soils, carbon dioxide can also be produced from the oxidation and hydrolysis of organic material.[1] In Schollenberger's method, oxidation is inhibited by adding ferrous chloride, and hydrolysis is minimised by keeping the reaction temperature between about 20° and 30° C. As the reaction temperature may sometimes be above this range, *e.g.*, in the analysis of dolomite, which is relatively unreactive at room temperature, or as a result of an accidental increase in pressure, the effect of temperature on the carbon dioxide evolved from hydrolysis was studied. For a yellow-brown loam containing 7 per cent. of carbon, the results were as follows—

Carbon dioxide evolved, milli-equivalent %	0·18	0·27	0·49	0·93	1·49	1·97
Reaction temperature, °C	22·5	29·5	47	58·5	69	74·5

These results show that the amount of carbon dioxide evolved from hydrolysis of organic compounds during 20 minutes at 75° C is about ten times that evolved at 25° C. It begins to increase rapidly above about 45° C, so that a vacuum pump should be chosen to permit boiling at least below this temperature.

DECOMPOSITION OF CARBONATE IN AIR-DRIED SOIL SAMPLES—

When air-dried soil samples from a liming trial, ground to pass through a 60-mesh sieve, were re-analysed after storage in screw-topped bottles at room temperature for 3½ years, all except one had a lower carbonate content. The original values for the pH and carbonate content and the loss of carbonate after 3½ years are shown in Table I. Samples Nos. 1, 2, 3 and 4 contained a less-reactive limestone than did samples Nos. 5, 6, 7 and 8. To samples Nos. 1, 2, 5 and 6 half as much limestone had been applied in the field as to samples Nos. 3, 4, 7 and 8, which were saturated to 60 per cent. of the cation-exchange capacity. The moisture content of the samples, determined by drying at 105° C, varied from 5 to 6 per cent.

The control sample had a pH of 5·1, contained 5·3 per cent. of moisture and was saturated to 25 per cent. of its cation-exchange capacity; when mixed with a few hundred milligrams of analytical-reagent grade calcium carbonate, 10 g of this finely ground sample yielded 0·02 milli-equivalent of carbon dioxide after 16 hours. When water was added to give a soil to water ratio of 1 to 5, 0·5 milli-equivalent of carbon dioxide was evolved after only 30 minutes. When calcium carbonate was mixed with another soil sample (moisture content made to 20 per cent., pH 4·9, saturated to 10 per cent. of the cation-exchange capacity), 0·05 milli-equivalent of carbon dioxide was evolved within 30 minutes.

TABLE I

DECOMPOSITION OF CARBONATE IN AIR-DRIED SAMPLES OF SOIL

Sample No.	Original calcium carbonate content, milli-equivalent %	Decrease in calcium carbonate content over 3½ years, milli-equivalent %	Loss of calcium carbonate on storage, %	Original pH
1	6·0	0·0	0	5·8
2	1·7	0·6	35	6·0
3	17·2	2·4	14	6·2
4	8·0	0·6	8	6·5
5	2·6	0·8	31	5·9
6	1·2	0·4	33	6·0
7	11·6	2·0	17	6·5
8	8·4	1·6	19	6·6

For samples Nos. 2, 3, 4, 5, 6, 7 and 8 in Table I, the loss of calcium carbonate is related to the pH of the soil and to the amount originally present. Other factors involved are the time in contact with the soil (by comparison of the losses over 3½ years and over 16 hours or less) and the moisture content of the soil. The reactivity of the limestone may also play a role. Loss is suffered even from air-dried samples.

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Received *May 4th*, 1959

A RAPID VACUUM-DISTILLATION METHOD FOR DETERMINING CARBONATE IN SOILS WITH ETHYLENEDIAMINETETRA-ACETIC ACID

THE method described by Schollenberger[1] for determining carbonate in soils gives precise results, but the time taken for a determination, especially with a resistant sample of limestone, is rather long. Methods involving acid at a higher reaction temperature, although rapid, have the unavoidable disadvantage of producing carbon dioxide by hydrolysis of organic material.[1] As shown in this Note, replacement of the acid by a solution of the disodium salt of ethylenediaminetetra-acetic acid (EDTA), which has a pH of 4·5, removes this disadvantage, but maintains the rapid reaction rate of the high-temperature methods.

METHOD

APPARATUS—

The apparatus is shown in Fig. 1. The barium hydroxide solution is stirred continuously by a magnetic stirrer during absorption of carbon dioxide. The condenser maintains a vacuum in the absorption flask by condensing the steam, passing in at 90° to 95° C, from the first section of the

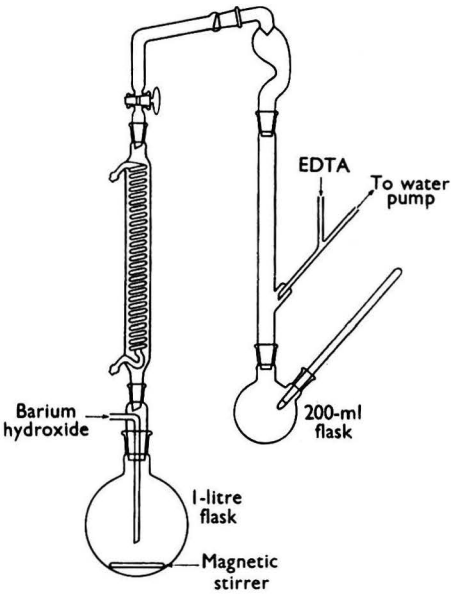

Fig. 1. Apparatus for determining carbonate

apparatus. The splash-head and the tubing leading to it from the reaction flask prevent the soil suspension from "bumping" over into the absorption flask. The tube from the EDTA solution and the water pump is sealed into a bulb on the side of the tube connecting the reaction flask and the splash head in order to avoid direct contamination from "bumping" splashes.

REAGENTS—

EDTA solution, 0·5 *per cent.*, *w/v*.
Barium hydroxide, 0·04 N.
Hydrochloric acid, 0·02 N.
Thymolphthalein indicator solution—Prepare a 0·1 per cent. w/v solution of thymolphthalein in 96 per cent. ethanol.

PROCEDURE—

Place the finely ground soil sample, containing sufficient limestone to neutralise about half of the barium hydroxide in the 200-ml flask. Evacuate the apparatus, and run in 25 ml of 0·04 *N* barium hydroxide and 150 ml of 0·5 per cent. w/v EDTA solution. Partly close the tap, and heat the soil suspension rapidly, by means of a gas burner, until its temperature reaches 90° C.

Maintain the temperature at 90° to 95° C, and adjust the tap so that the distillation rate does not cause the temperature in the absorption section to exceed about 40° C. After 5 minutes (or less, according to the resistance of the limestone), remove the flame, and carefully open the tap fully. After a further 5 minutes to complete the absorption of carbon dioxide, restore atmospheric pressure by introducing carbon dioxide-free air. With thymolphthalein as indicator, titrate the excess of barium hydroxide against 0·02 *N* hydrochloric acid to a faint blue end-point.

Results

In Table I four results by the proposed method are compared with those obtained by Schollenberger's method.

Table I

Comparison of results for carbon dioxide

Sample	Type of limestone	Carbon dioxide found by— proposed method, milli-equivalent %	Carbon dioxide found by— Schollenberger's method, milli-equivalent %
Reclaimed marine soil ..	Shell	31·3	31·0
Yellow-brown loam ..	Marble	15·4	15·2
	None	0·2	0·2
Rendzina	None	2·8	0·2

The results for limestone are in good agreement. The sample of yellow-brown loam containing no limestone yielded, in a reaction time of 20 minutes, 0·3 milli-equivalent per cent. of carbon dioxide at 30° C and 2 milli-equivalent per cent. at 75° C by Schollenberger's method. Hydrolysis by EDTA solution, which produces 0·2 milli-equivalent per cent. of carbon dioxide, is slight, even at 95° C, being less than in Schollenberger's method at 30° C. However, there is a large discrepancy between the results for rendzina. Even 2 *N* hydrochloric acid in 20 minutes yielded, at 74° C, only 0·8 milli-equivalent per cent. of carbon dioxide. The additional carbon dioxide produced by the proposed method is, therefore, probably formed not by decomposition of organic material but from carbonates less reactive than calcium carbonate.

Reference

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Received *May 4th*, 1959

THE USE OF ANTAZOLINE FOR IDENTIFYING NITRATE AND NITRITE

A disadvantage of most reagents described for detecting nitrate and nitrite[1] is that they either give the same reaction with both radicles or serve to identify only one of them. The antihistamine drug 2-(N-benzylanilinomethyl)iminazoline (antazoline) forms distinct colours with nitric and nitrous acids[2] and, under suitable conditions, can be used to identify either in the presence of the other. The test has the additional advantage that antazoline is cheap and readily available.

In its simplest form, the test is carried out by placing 1 drop of the test solution on a spotting-tile and adding 1 drop of a 1 per cent. aqueous solution of antazoline hydrochloride and then 1 drop of concentrated sulphuric acid. Nitrates produce a deep red colour; nitrites a bright yellow, which may appear initially as orange if nitrite is present in excess.

Test for nitrate in presence of nitrite

To test for nitrate in the presence of nitrite, use is made of the fact that the latter is rapidly and completely destroyed by sulphamic acid.[3] To 1 drop of the test solution are added 1 drop of a 5 per cent. solution of sulphamic acid and then 1 drop of a 5 per cent. solution of antazoline hydrochloride in concentrated sulphuric acid. The sulphamic acid used must be recrystallised, as the commercial product usually contains nitrate.

When the test is carried out in this way, one part of nitrate can be detected in the presence of a 20-fold excess of nitrite, provided that the latter is not present in a concentration higher than 1 per cent. Acetate, arsenate, bicarbonate, bismuthate, borate, bromide, carbonate, chloride, cyanide, molybdate, oxalate, perchlorate, periodate, phosphate, picrate, sulphate, sulphite and tungstate form no colour with the reagent and do not interfere, even when present in 50-fold excess. Oxidising agents, such as chlorate, bromate, iodate, chromate, hypochlorite and vanadate, form red colours with the reagent, and iodine is liberated from iodides. The presence of any of these ions therefore masks the colour formed by nitrate, a disadvantage common to most tests of this type. Persulphate, arsenite, thiosulphate and thiocyanate form no colour with the reagent, but prevent formation of the red colour. Interfering substances can be selectively destroyed,[4] but this adds to the complexity of the test and detracts considerably from its sensitivity.

Test for nitrite in presence of nitrate

If hydrochloric acid is used instead of sulphuric, the yellow colour with nitrite develops, but the red colour with nitrate does not. To test for nitrite in the presence of nitrate, 1 drop of a 1 per cent. solution of antazoline hydrochloride in 5 *N* hydrochloric acid is added to 1 drop of the test solution. Acetate, arsenate, arsenite, bicarbonate, bismuthate, borate, bromide, carbonate, cyanide, iodate, molybdate, nitrate, oxalate, perchlorate, periodate, persulphate, phosphate, sulphate, thiocyanate and tungstate form no colour and do not interfere, even when present in 500-fold excess. Iodide and chlorate interfere only when present in large excess. Bromate, chromate, hypochlorite, permanganate and vanadate form different shades of red or orange and thus mask the colour formed by nitrite. Selenium dioxide inhibits the test, as do compounds that destroy nitrites, *e.g.*, sulphite and thiosulphate.

Discussion of the tests

Organic matter, unless highly coloured, does not interfere with either test, and both nitrate and nitrite can be detected in the presence of extremely large amounts of protein or carbohydrate.

The test for nitrite is considerably more delicate than that for nitrate. A 0·001 *M* solution of the former gives a positive result, whereas a 0·01 *M* solution of the latter is required. The sensitivity of the test naturally depends on the size of drop used. If microdrops (0·1 μl), as described[5] for the detection of alkaloids, are used, the sensitivity is 0·05 μg for nitrate and 0·005 μg for nitrite. If 0·5-μl drops are used, as recommended by Feigl,[1] the dilution limits are 1 in 10,000 for nitrate and 1 in 100,000 for nitrite, the corresponding limits of identification being 5 μg for nitrate and 0·5 μg for nitrite.

Aqueous solutions of antazoline hydrochloride are colourless and, when protected from light, stable almost indefinitely; solutions in 5 *N* hydrochloric acid are stable for at least 3 months. The sensitivity of a solution in concentrated sulphuric acid deteriorates rapidly and has disappeared after 24 hours; this reagent solution should be freshly prepared as required and is satisfactory for 3 or 4 hours.

I thank Ciba Laboratories Ltd. for a generous supply of antazoline.

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Received *March 2nd*, 1959

THE PRECIPITATION OF MANGANESE IN SILICATE-ROCK ANALYSIS

The distribution of manganese during a silicate analysis by established methods is uncertain, and unless special techniques are used it is distributed between the ammonia, the oxalate and the phosphate precipitates.[1,2] Probably the best approach is to precipitate the manganese with the members of the iron group by means of an oxidising agent. Hillebrand and Lundell[1] recommend

the use of ammonium persulphate for this purpose and accept the findings of Epperson,[3] who applied the method to hydrated lime and Portland cement and found no significant retention of calcium in the iron-group precipitate. Holt and Harwood[2] were of the opinion that some calcium would be precipitated, as manganite, with that precipitate and preferred to use bromine in place of ammonium persulphate.

During the analysis of two rocks, we have used both methods to make a comparative study of the distribution of manganese; our results are summarised in Table I.

TABLE I

DISTRIBUTION OF MANGANESE DURING ANALYSIS OF ROCKS

Sample No. 1718, an aegirine granite from Rockall, North Atlantic, contained 0·23 per cent. of calcium oxide and 0·04 per cent. of magnesium oxide. Sample No. 1014, a hornfels from Bannerdale, Cumberland, contained 0·82 per cent. of calcium oxide and 2·06 per cent. of magnesium oxide

Manganese found, as MnO, in—					Total manganese found, as MnO, %	Method
iron-group precipitate, %	sulphide precipitate, %	oxalate precipitate, %	phosphate precipitate, %	filtrate from phosphate precipitation, %		
Sample No. 1718—						
0·007	—	n.d.*	0·036	0·054	0·097	Washington, H. S.[4]
0·011	0·077	n.d.*	0·001	0·007	0·096	Washington, H. S.[4]
0·076	—	—	0·013	0·012	0·101	Holt, E. V., *et al.*[2]
0·102	—	0·001†	—	—	0·103	Hillebrand, W. F., *et al.*[1]
Sample No. 1014—						
0·140	—	n.d.*	0·374	0·005	0·519	Washington, H. S.[4]
0·190	0·324	0·001	0·011	0·002	0·528	Washington, H. S.[4]
0·448	—	0·002	0·070	0·013	0·533	Holt, E. V., *et al.*[2]
0·525	—	0·003†	—	—	0·528	Hillebrand, W. F., *et al.*[1]

* Not detected.
† Filtrate from iron-group precipitation.

When Hillebrand and Lundell's ammonia - ammonium persulphate method was applied to a rock containing 15·12 per cent. of calcium oxide, 0·01 per cent. of strontium oxide, 0·04 per cent. of barium oxide and 0·16 per cent. of manganous oxide, and to a synthetic mixture containing the equivalent of 25 per cent. of calcium oxide, 0·6 per cent. of barium oxide, 0·4 per cent. of strontium oxide and 2·0 per cent. of manganous oxide, no calcium could be detected by oxalate precipitation in the iron-group precipitates. Spectrographic examination of the precipitates confirmed the virtual absence of these metals, only traces of calcium and strontium being recorded. In both experiments, barium was in part precipitated with iron and alumina, but most of it passed into the filtrate.

CONCLUSIONS

The results in Table I indicate that, if no special precautions are taken, manganese is distributed between the iron-group and the magnesium precipitates and that, for rocks low in magnesium, some is found in the final filtrate.

We found the precipitation of manganese as sulphide to be incomplete, and this, in the analysis of a silicate rock, would give rise to positive errors in the magnesium determination.

The ammonia - ammonium persulphate method, by ensuring the virtually complete precipitation of manganese with the metals of the iron group, has provided the best method of collecting this element in our analyses. The method has the further advantage that a sulphide precipitation is avoided and the magnesium is precipitated free from manganese. We found no evidence of significant retention of calcium or strontium in the iron-group precipitate, but partial precipitation of barium does occur. Precipitation of manganese by Holt and Harwood's ammonia - bromine method was significantly less complete.

We thank the Acting Government Chemist and the Director of the Geological Survey and Museum for permission to publish this Note.

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THE APPLICATION OF ULTRA-VIOLET LIGHT AND DIPHENYLAMINE TO SPOT TESTS FOR EXPLOSIVES

A RECENT communication[1] from this laboratory reported that, under the influence of short-wavelength ultra-violet radiation, diphenylamine and inorganic nitrate salts react to produce a yellow-coloured product. The reaction appears specific and relatively sensitive, being capable of detecting 1 μg of nitrate ion in 0·01 ml of solution. L. D. Hayward (personal communication) found, and we have subsequently confirmed, that organic nitrates behave in a similar fashion. Finnie and Yallop[2] found that sulphuric acid solutions of diphenylamine and related compounds, when used as spot-test reagents, detected both nitrate and nitramine explosives, but could not be used to distinguish between them nor to indicate individual compounds. This Note describes the results obtained when the ultra-violet light induced diphenylamine - nitrate reaction is used as a spot test for explosives.

METHOD

REAGENTS—

Diphenylamine solution, 1 per cent. w/v—Dissolve 1 g of reagent grade diphenylamine in 95 per cent. ethanol and dilute to 100 ml with 95 per cent. ethanol.

APPARATUS—

Ultra-violet light source—A Hanovia* analytical model quartz lamp with type "L" burner equipped with filters SC5028 and SC5025 (range 2300 to 4200 A, maximum at 3200 A) was used.

PROCEDURE—

Weigh out 0·05 g (see Note) of the substance to be examined, and dissolve it in about 5 ml of solvent, usually acetone or hot distilled water. Write the numbers 1 to 4 in pencil on an 11-cm Whatman No. 1 or similar grade of filter-paper. Under number 1 place one drop, under 2 two drops and under 3 three drops of test solution, allowing the paper to dry between successive additions. Observe the appearance of the stained areas in visible and ultra-violet light. Place 1 drop of diphenylamine solution on each of the stained areas and also under number 4 (blank); note any change in appearance. Replace the test paper in ultra-violet light for 3 minutes, and then remove and examine it.

NOTE—The intensity and tint of the stain may vary slightly with concentration; hence in comparative tests it is advisable to use the same amounts of unknown and control substances.

RESULTS

Table I summarises the results obtained on a number of explosives and related substances; the details given are reproducible. This procedure not only distinguishes between different kinds of explosives, *e.g.*, nitrates (PETN, dynamite, black powder) and nitramines (RDX, tetryl, picrite), but in many instances readily identifies the individual compounds. Thus, TNT, dynamite (acetone extract), tetryl, picrite, DNT and picric acid are easily distinguished from one another. RDX and PETN are less readily identified, but offer little difficulty to an experienced operator. Smokeless powders contain cellulose nitrate (nitrocellulose), which is insoluble in water, but dissolves readily in acetone to give a yellow or yellow-green colour when spot tested with diphenylamine as described above. Black powders, on the other hand, contain inorganic nitrates, which are

* Obtainable from Hanovia Chemical and Manufacturing Co., 100 Chestnut St., Newark 5, New Jersey, U.S.A.

TABLE I

APPEARANCE OF EXPLOSIVES ON TEST PAPER IN VISIBLE AND ULTRA-VIOLET LIGHT BEFORE AND AFTER REACTION WITH DIPHENYLAMINE

Explosives and related substance	Appearance of explosive on test paper		Appearance of explosive - diphenylamine on test paper	
	Visible	Ultra-violet	Visible	Post-ultra-violet
TNT (trinitrotoluene)	Colourless or slightly dark; white deposit at high concentration	Dark brown	Orange colour at periphery of diphenylamine drop, the whole area changing to light brown as solvent evaporates	Same as visible
Nitroglycerin and glycol dinitrate (acetone extract of dynamite)	Grease-like stain	Very pale yellowish white, lighter than surrounding paper	Same as explosive only	Yellow to blue-green
RDX (*cyclo*trimethyl-enetrinitramine)	Colourless	Darker than surrounding paper	Same as explosive only	Colourless to faint yellow; difficult to distinguish from diphenyl-amine alone
PETN (pentaerythritol tetranitrate)	Colourless; white deposit at high concentration	Yellowish white periphery with darker centre	Same as explosive only	Faint to definite yellow
Smokeless powder	Colourless to slightly dark or faintly yellow	Darker than surrounding paper	Same as explosive only	Yellow to yellow-green
Tetryl (2:4:6-trinitro-phenylmethyl-nitramine)	Colourless to yellow	Dark brown	Light reddish brown	Same as visible
Picrite (nitroguanidine)	Colourless	Slightly darker than surrounding paper	Same as explosive only	Faint reddish tint to definite red colour
DNT (dinitrotoluene)	Colourless; white deposit at high concentration	Dark brown	Yellow	Same as visible
Guanidine nitrate	Colourless	Bluish white periphery with darker centre	Same as substance only	Faint to strong yellow
Picric acid	Pale yellow	Greenish brown	Brown coloration on periphery of diphenylamine where it overlies the picric acid; slowly turns green	Same as visible
Ammonium nitrate	Colourless	Bluish white periphery with light brown centre	Same as explosive only	Bright yellow
Sodium nitrate	Colourless	As above	As above	As above
Potassium nitrate	Colourless	As above	As above	As above
Black powder	Colourless to slightly dark	Bluish white periphery with dark centre	Same as explosive only	Bright yellow
Ammonium picrate	Yellow	Greenish brown	Same as explosive only	Same as visible; light brown when water is the solvent

insoluble in acetone, but soluble in water; hence the aqueous extract of a black powder gives a bright yellow colour in this test. In general, the yellow colour obtained with inorganic nitrates develops more quickly and is more intense than the yellow colour produced with nitrate esters. Picrite is relatively insoluble in acetone but dissolves readily in hot water; with the latter solvent the red colour produced by reaction with diphenylamine in ultra-violet light is unmistakable.

I thank the Commissioner, Royal Canadian Mounted Police, for permission to publish this Note.

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THE FORMALDEHYDE METHOD FOR DETERMINING AMMONIA

Ronchese's formaldehyde method[1] for determining ammonia does not appear to be entirely satisfactory. It is based on the equation—

$$4NH_4Cl + 6H{\cdot}CHO \longrightarrow (CH_2)_6N_4 + 4HCl + 6H_2O.$$

Formaldehyde, usually in considerable excess, is added to the solution of the ammonium salt, and the liberated acid is titrated after a few minutes against standard sodium hydroxide solution with phenolphthalein as indicator.

Experiments have shown that the cause of the small inaccuracy is two-fold. The reaction is reversible and will not reach completion unless the pH is increased; it is also somewhat slow, so that even if enough time is allowed to reach equilibrium at low pH, titration at the usual rate with sodium hydroxide solution does not allow enough time at the higher pH for the destruction of the remaining ammonium salt. These two effects are well known, but do not seem to have been considered together.

The method described below is recommended for determining the ammonia in an ammonium salt.

Method

Procedure—

Dilute 40 per cent. w/v formaldehyde solution with an equal volume of water, and make the solution slightly alkaline to phenolphthalein by adding sodium hydroxide solution. To a volume of this solution sufficient to leave an excess of about 1 ml of the diluted formaldehyde per 10 ml of final solution add the solution of the ammonium salt, and titrate at once against sodium hydroxide solution. Use phenolphthalein as indicator, and add about 2 ml of sodium hydroxide solution in excess. Close the flask with a rubber stopper, and set aside for 15 minutes. Complete the titration with hydrochloric acid, or, better, make the solution slightly acid with standard hydrochloric acid, and complete the titration with sodium hydroxide solution.

Discussion of the method

For accurate results, carbon dioxide must be excluded and controls must be used. The necessity for a control arises in part because the Cannizzaro reaction takes place to a small extent under these conditions.

$$2H{\cdot}CHO + NaOH \longrightarrow CH_3{\cdot}OH + H{\cdot}COONa$$

In a set of titrations, the volume of approximately 0·1 *M* sodium hydroxide equivalent to 25 ml of a solution of pure ammonium chloride was calculated to be 23·02 ml; the volume found was 23·01 $\pm$ 0·03 ml. The control correction was about 0·2 ml, of which about 0·15 ml could be attributed to the Cannizzaro reaction.

In arriving at the correction, the same weights of formaldehyde at the same concentrations were used in control and assay. As the ammonium salt removed only a small proportion of the formaldehyde, the final concentrations of formaldehyde in both solutions were approximately the same, and an error in the correction was more likely to be caused not by different concentrations of aldehyde but by the use of an unsuitable concentration of sodium hydroxide in the control. The volume of sodium hydroxide solution used was about 2 ml, to balance the 2-ml excess used in the assay.

In a further series of experiments, the titrations were carried out by the proposed method, but without pause, each being completed in about 4 minutes. The volume of sodium hydroxide solution equivalent to 25 ml of ammonium chloride solution, after a control correction of 0·08 ml, was found to be 22·93 ml. The flasks were then set aside, and, after 1 hour, the colour of the phenolphthalein was restored by adding a further 1 or 2 drops of sodium hydroxide solution. After a total correction of 0·10 ml, 23·03 ml of sodium hydroxide solution were found to be equivalent to 25 ml of ammonium chloride solution.

An appreciable error, therefore, is introduced when the titrations are made too rapidly.

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THE INDEPENDENT TITRATION OF CYANIDE AND SULPHIDE

There is no suitable method for determining sulphide and cyanide in the same sample. Singly, sulphide can be determined with high accuracy by titration against *o*-hydroxymercuribenzoic acid[1,2] in presence of dithizone or thiofluorescein as indicator, and cyanide can be determined with sodium nitroprusside as indicator, although the end-point is not distinct.

It was found that the presence of formaldehyde and the products of reaction between formaldehyde and cyanide did not interfere with the titration of sulphide, which could thus be determined in presence of cyanide after formaldehyde had been added.

The accuracy of the titration of cyanide *plus* sulphide with a solution of *o*-hydroxymercuribenzoic acid in presence of thiofluorescein is low, but cyanide can be accurately titrated with nickel sulphate solution[3] after the sulphide - *o*-hydroxymercuribenzoic acid complex has been formed.

A method for titrating cyanide with silver nitrate solution is also described.

Method for determining sulphide and cyanide

Procedure for sulphide—

To a sample containing less than 25 mg of hydrogen cyanide and 0·5 to 10 mg of hydrogen sulphide add 2 ml of 37 per cent. w/v formaldehyde solution, 5 ml of *N* sodium hydroxide and 1 ml of 0·1 per cent. w/v dithizone solution, and dilute to 100 ml. Titrate the sulphide with 0·03 *N* *o*-hydroxymercuribenzoic acid until the colour changes sharply from yellow to purple.

Procedure for cyanide—

To a sample containing less than 50 mg of hydrogen cyanide and less than 20 mg of hydrogen sulphide add 10 ml of solution containing 20 g each of ammonium chloride and ammonia per litre. Add a volume of 0·03 *N* *o*-hydroxymercuribenzoic acid corresponding to the result obtained in the previous titration *plus* a 4 per cent. excess and 1 ml of 0·2 per cent. w/v murexide solution. Titrate with 0·1 *N* nickel sulphate until the colour changes sharply from red to yellow.

Note that the presence of SO_3^{2-}, $S_2O_3^{2-}$ and CNS^- ions does not interfere in either of these titrations.

Argentimetric determination of cyanide

Procedure—

To a sample containing less than 50 mg of hydrogen cyanide add 5 ml of *N* sodium hydroxide and 1 ml of a freshly prepared 0·02 per cent. w/v solution of thiofluorescein in diluted ammonia, and titrate with 0·1 *N* silver nitrate until the blue colour changes sharply to a slight green tinge. Identical results were obtained in presence of ammonia and ammonia - ammonium chloride mixture; the presence of halogens and Zn^{2+} does not interfere with the titration.

Note that the error in the determinations described above is less than that of the burette.

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SHORT CUTS TO "LINES OF BEST FIT"

MANY determinations in analytical chemistry involve the relationship between two variables—calibration curves, for example. When the graph of the experimental points indicates a linear relationship, it is often possible to draw the "best" line by eye, and, if the points lie closely about a straight line, the error involved by the visual approach may be small in relation to experimental errors. There must, however, be an element of uncertainty in the visual method, which can be minimised by calculating the equation of the "line of best fit" based on the method of least squares.

For microbiological assays involving a linear relationship between vitamin dose and response, I have for many years preferred to calculate the equation of the line rather than trust entirely to the visual method.

The line may be represented by the equation $T = a + bV$, where T is the titre, V is the dose of vitamin, a is the intercept on the vertical axis (the blank value) and b is the slope of the line.

The values of a and b can be computed by the method of least squares, according to the expressions—

$$a = \frac{(\Sigma V \times \Sigma VT) - (\Sigma V^2 \times \Sigma T)}{(\Sigma V)^2 - n\Sigma V^2}$$

$$b = \frac{(\Sigma V \times \Sigma T) - n(\Sigma VT)}{(\Sigma V)^2 - n\Sigma V^2}$$

where ΣV is the sum of the vitamin doses, ΣVT is the sum of the products of the vitamin doses and the titres, ΣV^2 is the sum of the squares of the vitamin doses, ΣT is the sum of the titres, $(\Sigma V)^2$ is the square of the sum of the vitamin doses and n is the number of dose levels.

This somewhat formidable array can be reduced to simple terms for the vitamin doses normally used in assays.

For example, taking a standard line at dosage levels of 0, 0·5, 1·0, 1·5 and 2·0 ml, $\Sigma V = 5$, $\Sigma V^2 = 7{\cdot}5$, $(\Sigma V)^2 = 25$ and $n = 5$.

Substitution in the expressions for a and b gives—

$$a = \frac{5\Sigma VT - 7{\cdot}5\Sigma T}{25 - 5(7{\cdot}5)} = \frac{5\Sigma VT - 7{\cdot}5\Sigma T}{-12{\cdot}5} = \frac{3\Sigma T - 2\Sigma VT}{5}$$

$$b = \frac{5\Sigma T - 5\Sigma VT}{-12{\cdot}5} = \frac{2\Sigma VT - 2\Sigma T}{5}$$

In a particular assay, the standard gave the following results—

Vitamin dose (V), ml	..	..	0	0·5	1·0	1·5	2·0
Mean titre (T), ml	..	..	0·93	2·17	3·40	4·63	6·0 ($\Sigma T = 17{\cdot}13$)
Value of VT	..	..	—	1·085	3·400	6·495	12·000 ($\Sigma VT = 23{\cdot}430$)

Substitution for a and b then gives—

$$a = \frac{(3 \times 17{\cdot}13) - (2 \times 23{\cdot}430)}{5} = \frac{51{\cdot}39 - 46{\cdot}86}{5} = 0{\cdot}906$$

$$b = \frac{(2 \times 23{\cdot}430) - (2 \times 17{\cdot}13)}{5} = \frac{46{\cdot}86 - 34{\cdot}26}{5} = 2{\cdot}52$$

The equation of the best line is therefore given by the expression $T = 0{\cdot}906 + 2{\cdot}52\,V$. When $V = 0$, $T = 0{\cdot}906$ and when $V = 2$, $T = 5{\cdot}946$; the best line for the standard is obtained by drawing between these points.

A further example is given for the test sample used in conjunction with the standard; the same blank titre is incorporated.

Test extract (V), ml	..	..	0	1	2	3	4
Mean titre (T), ml	..	..	0·93	1·90	2·93	3·95	5·07 ($\Sigma T = 14{\cdot}78$)
Value of VT	..	..	—	1·90	5·86	11·85	20·28 ($\Sigma VT = 39{\cdot}89$)

In this instance, $\Sigma V = 10$, $\Sigma V^2 = 30$, $(\Sigma V)^2 = 100$ and $n = 5$. The values of a and b are therefore given by the expressions—

$$a = \frac{10\Sigma VT - 30\Sigma T}{100 - (5 \times 30)} = \frac{3\Sigma T - \Sigma VT}{5}$$

$$b = \frac{10\Sigma T - 5\Sigma VT}{-50} = \frac{\Sigma VT - 2\Sigma T}{10}$$

As before, the calculation is reduced to simple arithmetic as follows—

$$a = \frac{(3 \times 14{\cdot}78) - 39{\cdot}89}{5} = \frac{4{\cdot}45}{5} = 0{\cdot}89$$

$$b = \frac{38{\cdot}89 - (2 \times 14{\cdot}78)}{10} = \frac{10{\cdot}33}{10} = 1{\cdot}033$$

The equation of the best line is therefore given by the expression $T = 0{\cdot}89 + 1{\cdot}033\ V$. When $V = 0$, $T = 0{\cdot}89$ and when $V = 4$, $T = 5{\cdot}022$; the best line for the sample is obtained by drawing between these points.

Before even these simplified calculations are used, it is essential to plot a graph of the appropriate points in order to ascertain whether or not the assay is likely to have been satisfactory. In this way, it can be seen whether the responses are within that part of the curve that is normally linear or whether there have been any inadvertent errors in dosage levels in the tubes, etc. Provided that this preliminary inspection is satisfactory, the lines should then be drawn on the basis described.

Two criteria may be applied to the results in order to show whether the assay is satisfactory: (*a*) all experimental points should lie fairly closely about their appropriate best lines and (*b*) the calculated blank values should differ only slightly from the experimental blank value. In the example described, both criteria are satisfied.

It must be emphasised that no statistical estimate of error or validity is possible on the basis of this procedure, its object being merely to provide a short and simple arithmetical method for deriving the most suitable lines for standard and sample.

Since the zero-dose point applies to both standard and sample, a strict statistical computation would derive the two best lines by pooling all the values for both standard and test titres. In fact, I always use the "5-point 20-tube common-zero" design for this type of assay; further information on statistical aspects can be found elsewhere.[1,2] However, many workers are not able to expend the considerable time entailed by the necessarily lengthy calculations involved.

Abbreviated formulae can be derived for other ranges of doses, and Table I shows a selection from a Table that I have used for many experiments.

TABLE I

FACTORS USED TO CALCULATE EQUATIONS OF BEST LINES

	Factors for calculating value of *a*			Factors for calculating value of *b*		
Value of V, ml	ΣT	ΣVT	Divisor	ΣVT	ΣT	Divisor
0, 0·5, 1·0, 1·5, 2·0	3	2	5	2	2	5
0, 1, 2	5	3	6	1	1	2
0, 1, 2, 3	7	3	10	2	3	10
0, 1, 2, 3, 4	3	1	5	1	2	10
1, 2, 3	7	3	3	1	2	2
1, 2, 4	3	1	2	3	7	14
1, 2, 3, 4	3	1	2	2	5	10

The method of using Table I should be obvious by reference to the examples described, and it should also be obvious that, by choosing simple integral values for the units of one variable, the shortened formulae can be applied to many other examples of linear relationships.

REFERENCES

1. Wood, E. C., *Analyst*, **1946**, **71**, 1.
2. Wood, E. C., and Finney, D. J., *Quart. J. Pharm. Pharmacol.*, **1946**, **19**, 112.

DEPARTMENT OF APPLIED BIOCHEMISTRY
UNIVERSITY OF BIRMINGHAM

F. W. NORRIS
Received *March 20th*, 1959

Apparatus

A SMOKE-ELIMINATING DEVICE FOR A VAPOUR-PHASE CHROMATOGRAPHIC-FRACTION COLLECTOR

THE use of gas chromatography has made possible the separation of components of mixtures almost inseparable by distillation. When large volumes of sample are placed on a column having a diameter wider than normal, the eluted fractions can be collected for either spectroscopic or chemical analysis. Positive identification by the trapping technique is essential when investigating isomers that are eluted so close to one another that internal standards cannot be used.

For materials having low boiling-points, the efficiency of collection can be substantially increased by cooling the collecting device in a bath of ice. However, we have observed that the opposite is true when materials whose boiling-points are above 175° C at atmospheric pressure are investigated. Sudden cooling is invariably accompanied by the formation of a smoke, which is really a cloud of charged particles. The smoke collects on the walls of the chilled container, but re-forms when the container is warmed. If the hot exit vapours are allowed to condense in a tube at room temperature, the amount of smoke formed is considerably reduced, provided that the tube has been connected to the heated exit of the detector for a sufficient time to allow a thermal gradient to form, *i.e.*, if the temperature is gradually decreased along the condensing device. This effect can be achieved by lagging the condensing device with electrically heated resistance wire, a procedure that reduces the amount of smoke formed, but does not completely prevent its formation.

We have solved this problem by applying the principle of the Cottrell precipitator to precipitate the smoke by means of a high-tension current. The collection of smoke-forming fractions by this method is substantially quantitative.

DESCRIPTION OF DEVICE

High-tension current from a Ford model T ignition coil (FTC-40 ignition coil, manufactured by F. & B. Mfg. Co., Chicago 51, Illinois, and obtainable from Ford dealers) powered by a 6-volt

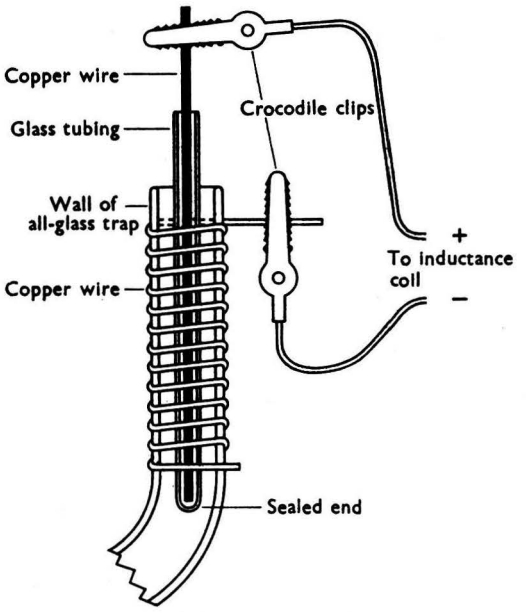

Fig. 1. Arrangement of electrodes at exit arm of trapping tube

battery is impressed across the flow of the exit gas. Fig. 1 shows a simple arrangement of electrodes on the exit arm of the trapping tube. The inner electrode is contained in an 8-cm × 3-mm piece of glass tubing. The tightly coiled spiral of copper wire encircling the trap can be easily placed in position and removed. The dimensions are not critical; satisfactory results have been obtained with tubes having internal diameters from 5 to 20 mm.

During operation, a silent discharge between the two electrodes produces a purplish glow. Tests with a power transformer operating on 115 volts a.c. (60 cycles) and connected through a rectifying tube to produce 4000 volts pulsating d.c. or 8000 volts a.c. showed that either a.c. or d.c. high-tension current will condense and precipitate the smoke. To avoid electric shocks, care should be exercised if the electrodes are handled during operation. The device should not be used when hydrogen is the carrier gas or under any other conditions when sparking might be dangerous.

Discussion of results

The proposed device has been used in a study of various types of quinolines and their isomers, which tend to form mists that can be condensed only by electrical discharge or solvation; any "freezing-out" procedure produces a solid that again forms a mist when warmed. Extensive experiments have been carried out on terpene alcohols, such as nerol, geraniol and citronellol, as well as on related compounds. Substituted *cyclo*hexyl-derivatives have also been successfully condensed, and the mists formed by both natural and synthetic high-boiling flavour components can be readily precipitated. So far, we have not encountered a compound forming a mist that cannot be precipitated.

Volumes of sample between 0·5 and 5·0 ml were injected on to the column, and the eluate, which contained the individual separated species, varied from 0·1 to 2 ml, depending upon the number of components in the sample.

When the proposed device was used, the efficiency of separation was increased from approximately 10 to above 90 per cent., the trap not being cooled in either instance. In our experience, compounds of the types just mentioned tend to form mists, and it is difficult to condense sufficient material for spectroscopic examination. However, by using our device, we have been able to collect sufficient material for spectroscopic as well as for standard chemical and physical tests.

Instrumental Analysis Laboratory
van Ameringen-Haebler Inc.
Rose Lane, Union Beach
New Jersey, U.S.A.

P. Kratz
M. Jacobs
B. M. Mitzner
Received *May 4th*, 1959

Silicate Analysis

The February number of the *Journal of the Society of Glass Technology*, 1959, volume 43, is devoted mainly to the papers read at a Symposium on Rapid Methods of Analysis for glass, held at Sheffield in November last. The introductory lecture was given by the President of the Society for Analytical Chemistry, Mr. R. C. Chirnside, who reviewed critically the present position of silicate analysis, especially as concerns what one might call modernised classical methods and new more rapid methods, both physical and chemical. This is followed by a paper in which the analytical uses of ethylenediaminetetra-acetic acid are neatly and clearly summarised by H. J. Cluley. Next comes a contribution by Miss Rosemary Sales, who gives details of methods for determining calcium oxide in limestone, calcium and magnesium oxides in soda - lime glasses and lead and aluminium oxides in lead-crystal glasses. As separations are eliminated, speed of determination is increased and an accuracy equal to that of classical methods is claimed. S. M. Budd, in the next contribution, deals with volumetric methods for determining the sodium carbonate and calcium carbonate contents of a soda - lime - silica batch and the application of the results to questions of sampling and batch inhomogeneity.

These papers are followed by a short one by H. Bennett on the gravimetric quinoline silicomolybdate method for silica, adapted to meet the routine control requirements of the ceramic industries and more fully described in the *Transactions of the British Ceramic Society*, volume 57. Then comes another communication from H. J. Cluley, in which the successful application of the gravimetric tetraphenylboron method to the determination of potassium in glass is described. A further saving of time has been recently found possible by using a volumetric finish, and details of this method are given.

The remaining papers deal with physical methods of analysis. R. J. Powell and Miss J. Todd describe their investigations into the determinations of the alkalis and calcium in glass by flame photometry and of calcium, aluminium and magnesium by spectrography; W. W. Fletcher gives

details for determining by means of an E.E.L. filter flame photometer the alkalis in three types of glass; and G. A. Hedgecock describes his experience with a Beckman flame photometer in analysing glasses and glass-making materials for the alkali metals.

This volume forms an interesting and valuable contribution to the literature on glass analysis and it is one that no silicate analyst can afford to ignore. The Chemical Analysis Committee of the Society of Glass Technology, who arranged the Symposium, and the authors who provided material for it are all to be congratulated on having rendered a real service to this subject.

L. S. THEOBALD

Book Reviews

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

In just over 100 pages, the authors have compressed a great deal of useful information about ethylenediaminetetra-acetic acid (EDTA) and its applications to the determination of various cations (17 pages), anions (3 pages), the hardness of water (5 pages) and the analysis of technical materials (7 pages). Physical methods of end-point detection in EDTA titrations are dealt with in 4 pages, but by far the largest section (22 pages) is devoted to metal indicators. Here, the formulae, with their dotted lines to indicate co-ordination, strike an old-fashioned note, and even three-membered chelate rings are depicted more than once. The formulae for the zirconium - SPADNS chelate is wrong, and it is surely misleading to show sulphonic acid groups as un-ionised under the conditions recommended for the titrations.

Some 10 pages are devoted to the use of masking and de-masking agents in EDTA titrations and the potentialities of the reagent itself as a masking agent. The book concludes with a short account (7 pages) of the analytical applications of other complexones. The authors have been at pains to avoid any theoretical treatment of complexometric titrations. Indeed, the only page of "theory" deals with the solubility of precipitates in EDTA, and the treatment is, unfortunately, quite unsound. It is certainly stated that the position of equilibrium in a cation - EDTA system will be dependent upon the pH of the solution, but nowhere are the acid dissociation constants of EDTA, or of other complexones, recorded.

The bibliography of 468 references is comprehensive and reliable (the only error detected being in reference No. 1!). It would have been more widely useful if it had been arranged alphabetically or chronologically. There is no index.

H. IRVING

PRINCIPLES OF OXIDATION AND REDUCTION. By A. G. SHARPE, M.A., Ph.D., F.R.I.C. Pp. vi + 30. London: The Royal Institute of Chemistry. 1959. Price 3s. 6d.

This monograph is designed primarily for teachers of chemistry at the Advanced Level of the G.C.E. and above, but will obviously appeal to a wider selection of readers.

After a preliminary introductory chapter, the electronic theory of oxidation and reduction is discussed in some detail. Consideration is given to the three different methods of balancing chemical reactions, and although analytical chemists tend to use the oxidation-state method they should be well aware of the implications of the oxygen-transfer and ion-electron methods of representation described in this part.

The most important chapter deals with the quantitative aspects of oxidation and reduction.

Sharpe makes some valuable observations on the sign conventions used in the expression of electrode potentials and on the measurement of these potentials. Further, he draws attention to the obvious limitations of Tables of redox potentials.

There is a final chapter on oxidation and reduction in gaseous and solid states and in non-aqueous media and some suggestions for further reading.

These suggestions might have been improved by more detailed reference to the text-books listed.

This booklet will be useful to analytical chemists who wish to get "nearer the bone" in understanding the volumetric titration procedures that they use.

J. HASLAM

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

Seven years ago, in reviewing the first edition of this book (*Analyst*, 1952, **77**, 165), I had the temerity to criticise it with all the weight of nearly three years' experience of editorial work behind me. The intervening years have taught me something of the size of the problem Dr. Dyson tackled in producing this guide for students and others to the ever-changing journals that make up chemical literature. Not that the treatment is exhaustive—it could never be that. But even in fulfilling his object of producing an introduction to the subject of literature searching, with a "selection of standard works . . . only given as *examples*," the author has had to sift through mountains of facts. One hesitates to point to faults that can only be seen by virtue of specialised knowledge, but analysts may find it useful to know that *The Analyst* continued to print abstracts during the years 1944–49 (despite what Dyson says), so providing an alternative source to *British Abstracts C* and *Chemical Abstracts* for those years. One criticism I made of the first edition I am glad to withhold from the second: the publisher's skill in operating a justifying typewriter has improved to such an extent that no fault can be found directly attributable to the production process. Any residual prejudice I have for printing from type-metal is solely due to the paucity of type-faces available on the typewriter.

The book contains valuable lists—for which the author specifically denies any claim to completeness—of dictionaries, encyclopaedias, chemical journals and periodicals, abstract journals, and text-books and special works of reference. Each entry is annotated as to contents, scope, dates of publication and—most important—the availability of collective indexes. There is also a short chapter on reference works on medicinal chemicals, selected as an example of a "borderland subject."

The heart of the book is chapter VI, a vivid description of "Making a Search of the Literature." The example has been selected with great care. Not only, as Dr. Dyson explains, are there not too many references, and not only do they occur in *Beilstein* and both supplements and in *Chemical Abstracts* and satisfy other criteria, including an odd reference in *Chemisches Zentralblatt*, but also (as it transpires) the Decennial Index to *Chemical Abstracts* has (just for once) failed to list the paper found in *Zentralblatt*, although it had in fact been duly abstracted. The trouble Dr. Dyson must have taken to find such a rare example! But it points the moral, which is that *everything* must be checked and cross-checked.

There are three valuable appendixes: Appendix II is a further example of a literature search and Appendix I lists some obsolete journals. Appendix III, as I wrote of the first edition, is the most useful: it is a chronological table of years and volume numbers for all the important chemical journals. Where these are irregular it is invaluable, and this includes particularly the years since the outbreak of the second World War. Since the 1951 edition, even, major changes have taken place, and users of the earlier edition must replace it, for out-of-date information is so often wrong information. This book, at its relatively low price among modern text-books, is a "must" for all research workers in all branches of chemistry. J. B. ATTRILL

METHODS OF BIOCHEMICAL ANALYSIS. Volume VII. Edited by DAVID GLICK. Pp. x + 353. New York and London: Interscience Publishers Inc. Price $9.50; 72s.

This volume maintains the high level set by its predecessors.

P. Grabar of the Pasteur Institute deals with immuno-electrophoretic analysis, a powerful technique first described in 1953. The mixture to be studied is subjected to electrophoresis in a gel (*e.g.*, agar); when sufficient separation is judged to have occurred, electrophoresis is stopped and a precipitating immune serum is allowed to diffuse perpendicularly to the axis of migration. The antibodies present in the serum and the antigenic constituents of the analysed liquid diffuse into the gel and arcs of specific precipitation become visible as insoluble antigen - antibody complexes are formed. Electrophoresis in a gel has distinct advantages over electrophoresis on paper, and the method permits definition or identification of the constituents of a liquid by (*a*) electrophoretic mobility, (*b*) immunological specificity and, often, (*c*) suitable staining methods. The disadvantage of the method is that the immune sera are difficult to standardise and the information gained is mainly qualitative.

A. S. Curry of the Home Office Forensic Science Laboratory reviews the analysis of basic nitrogenous compounds of toxicological importance. Modern methods of paper chromatography, electrophoresis and ultra-violet spectrophotometry have been applied to the detection of alkaloids.

Detailed Tables display the behaviour of alkaloids with spray reagents, and various commonly used alkaloids are considered from the point of view of detection or determination. This is an authoritative and well documented article.

K. Shibata of Tokyo deals with the spectrophotometry of translucent biological materials by the opal-glass transmission method. The directly observed absorption spectra of translucent biological preparations exhibit diffuse and intense absorption and poor definition. If this were the true absorption spectrum, the only remedy would be to reduce the turbidity, *e.g.*, by adding protein solution (Barer). It is not, however, possible to eliminate turbidity entirely by adjusting the refractive index of the medium, since the different structural parts of cells have different refractive indexes. Keilin and Hartree made diffuse bands sharper by freezing or by adding kieselguhr to translucent suspensions. Chance and others obtained sharp diffuse spectra by using two translucent materials instead of one with a transparent comparison medium.

Two new and successful techniques of using "opal glass" are based on a carefully worked out theory of light absorption and scattering; the first is a transmission method and the second a reflection method. Both give sharp absorption bands at low concentrations of, *e.g.*, cytochromes or chlorophylls. Opal glass has a uniform opalescence over the range 350 to 800 mμ and is used sandwiched between thick and very thin layers of transparent glass. An opalescent material suitable for the regions 220 to 400 and 700 to 1300 mμ is a paraffin-oil-impregnated filter-paper sandwiched between two quartz plates. The whole problem is discussed fully from the standpoint of both theory and practice.

J. M. McKibbin of Syracuse, New York, describes the determination of inositol, ethanolamine and serine in lipides. For inositol special attention is given to a microbiological procedure in which the assay organism is *Saccharomyces carlsbergensis*. A number of manometric chemical and colorimetric methods are given for determining ethanolamine and serine.

Lipoprotein lipase catalyses the hydrolysis of the triglyceride moiety of lipoproteins. Chylomicrons and some plasma lipoproteins act as substrates. The enzyme may be extracted from a variety of tissues by means of normal plasma or dilute aqueous ammonium hydroxide. After the injection of heparin the "post-heparin plasma" contains a "clearing factor" (lipoprotein lipase) not present in normal plasma. Thus lipaemic plasma is rapidly "cleared" as the enzyme hydrolyses the triglycerides in union with mainly α-lipoproteins. E. D. Kohn of the National Heart Institute at Bethesda, Maryland, deals with the assay of lipoprotein lipase *in vivo* and *in vitro*. This is an important topic having significant inter-relationships with atherosclerosis, blood clotting and hypocholesterolaemia. The analytical chemistry is described fully and the whole subject is ably placed in its proper setting.

J. F. Van Pilsum of Minneapolis gives a very clear account of the determination of creatine, creatinine, arginine, guanidinoacetic acid and related compounds.

The determination of ethanol in blood and tissues is discussed by F. Lundquist of Copenhagen. The practical importance of this is illustrated by the fact that "in the Scandinavian countries alone more than 25,000 cases are examined every year, each requiring three to six single analyses of blood or urine or both." The blood-alcohol concentrations that have any appreciable physiological effect are high compared with those of other possible volatile constituents. A concentration of 1·5 mg of ethanol per ml of blood represents a moderate degree of intoxication; this in molar terms is eight times that of normal blood sugar. There is at present no single analytical procedure completely specific for ethanol. Lundquist reviews the whole topic, but gives special and detailed attention to improved versions of the Widmark method whereby the volatile substances from blood diffuse from a small glass cup into a dichromate - sulphuric acid mixture. As now used the method requires 0·1 g of blood, so that sufficient material can be obtained from a finger tip or ear lobe for triplicate analyses. The enzymic method involving purified alcohol dehydrogenase from yeast is described fully and discussed critically.

The final chapter, by L. B. Jaques and Helen J. Bell of Saskatoon, Canada, is concerned with heparin. There is a fairly full account of its properties and of its extraction from tissues and body fluids. The determination of heparin is discussed under the headings of anticoagulant methods, coagulation test systems, chemical methods and tests for identity. Finally, methods for determining heparin in blood are described.

The Advisory Board and the Editor, Professor Glick, are to be congratulated on their continued success in selecting important topics and in finding contributors with both specialised experience and sound scholarship.

R. A. MORTON

Publications Received

THE ANALYSIS OF TITANIUM AND ITS ALLOYS. Third Edition. Pp. 119. London: Imperial Chemical Industries Limited. 1959. Price 21s.

ANNUAL REPORT 1958–9. Pp. 268. London: British Standards Institution. 1959. Price 7s. 6d.

STEROIDS. By LOUIS F. FIESER and MARY FIESER. Pp. xviii + 945. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1959. Price $18.00; 144s.

SOURCE BOOK OF INDUSTRIAL SOLVENTS. Volume III. MONOHYDRIC ALCOHOLS. By IBERT MELLAN. Pp. vi + 276. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1959. Price $10.00; 80s.

RADIOISOTOPES FOR INDUSTRY. By ROBERT S. ROCHLIN and WARNER W. SCHULTZ. Pp. x + 190. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1959. Price $4.75; 38s.

SOIL, GRASS AND CANCER. By ANDRÉ VOISIN. Pp. xviii + 302. Translated by CATHERINE T. M. HERRIOT and Dr. HENRY KENNEDY. London: Crosby Lockwood & Son Ltd. 1959. Price 30s.

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Septième Série. Pp. iv + 230. Paris: Masson et Cie. 1959. Price 4800fr.

AN INTRODUCTION TO CHEMICAL NOMENCLATURE. By R. S. CAHN, M.A., Dr. Phil. nat., F.R.I.C. Pp. viii + 96. London: Butterworths Scientific Publications. 1959. Price 10s. 6d.

Reprints of Review Papers

REPRINTS of the following Review Papers published in *The Analyst* are now available at the prices stated. Orders should be sent direct to the Assistant Secretary, The Society for Analytical Chemistry, 14, Belgrave Square, London, S.W.1 (not through Trade Agents), and MUST be accompanied by a remittance for the correct amount made out to The Society for Analytical Chemistry.

"X-Ray Fluorescence Analysis," by F. Brown (June, 1959). Price 2s. 6d.

"Ferrous Metallurgical Analysis," by B. Bagshawe (August, 1959). Price 5s.

Reprints of earlier Review Papers are still available as follows—

"The Analysis of Synthetic Detergents," by W. B. Smith (February, 1959). Price 2s. 6d.

"The Infra-red Analysis of Solid Substances," by G. Duyckaerts (April, 1959). Price 2s. 6d.

MAY & BAKER LTD., Dagenham, Essex, require ANALYSTS for either non-routine and development work or to take charge of sections engaged in the routine analysis of pharmaceutical and laboratory chemicals. Applicants should hold either a B.Sc. or A.R.I.C. and have had experience in one of the above fields. Apply initially in writing to the Personnel Officer, quoting Reference No. 131/59.

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CHEMIST, QUALIFIED, required for a branch factory in Suffolk. Bacteriological experience desirable particularly in meat products. The work will be in conjunction with and under the control of the Central Laboratory. Pension scheme in operation. A salary commensurate with qualifications and experience will be paid. Applications giving particulars of age, qualifications and experience to the Secretary, C. & T. Harris (Calne) Ltd., Calne, Wilts.

EAST MALLING RESEARCH STATION. CHEMIST, in the Plant Protective Chemistry Section, to assist in studies of insecticides and fungicides on fruit plants; degree in chemistry or equivalent qualification; experience in analysis an asset. Salary in Experimental Officer class. Particulars and application form from The Secretary, East Malling Research Station, Near Maidstone, Kent, to whom completed applications should be forwarded by 7th December, 1959.

THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION has a vacancy for a graduate analyst experienced in the examination of food products. The position is permanent and carries pension and life assurance rights. Applications, stating qualifications and experience and indicating the level of salary expected, should be marked "Private and Confidential" and addressed to the Director of Research, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

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HEALTH DEPARTMENT

APPOINTMENT OF SENIOR ASSISTANT ANALYST

Applications are invited for the above-mentioned appointment in the County Chemical Laboratory, Stafford. Applicants should have a University and/or a Royal Institute of Chemistry qualification, preferably an F.R.I.C. with Branch E Diploma. Previous experience in a Public Analyst's Laboratory will be an advantage.

The salary will be in accordance with the National Joint Council's A.P.T. IV scale (£1,065 × £55 (1) × £50(2) — £1,220).

Applications giving age, qualifications and experience, accompanied by copies of two recent testimonials, and stating whether or not the applicant is related to any member or senior official of the County Council, should be forwarded to the County Medical Officer of Health, County Buildings, Stafford, by not later than 22 November, 1959.

T. H. EVANS,
Clerk of the County Council.

Qualified Analytical Chemist, aged 25-30 years, required for Biscuit Factory Laboratory to develop and control system of raw materials and finished product analysis.

Experience of this type of work in either the food industry, or a laboratory, desirable.

Applications, stating age and giving particulars of qualifications and experience to The Secretary, F. E. Fox & Son Ltd., Biscuit Works, Batley, Yorkshire.

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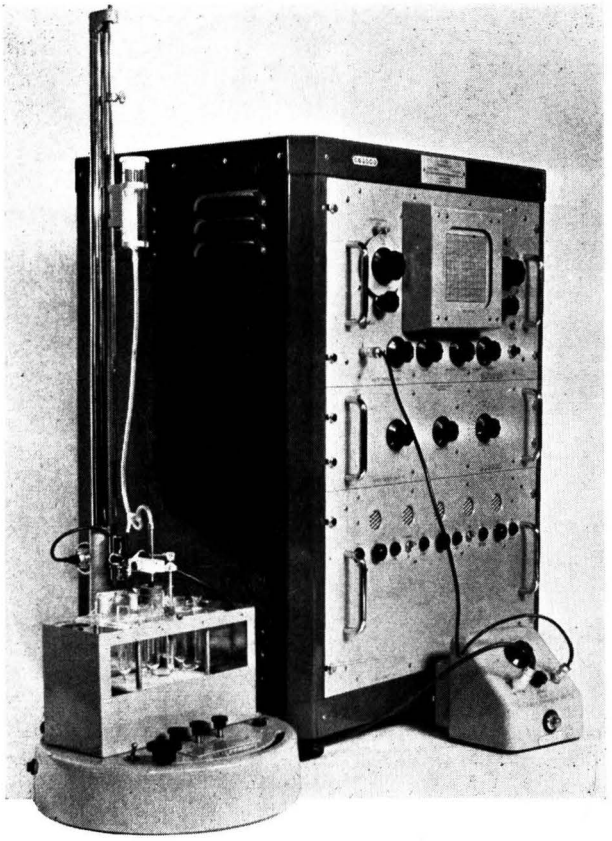

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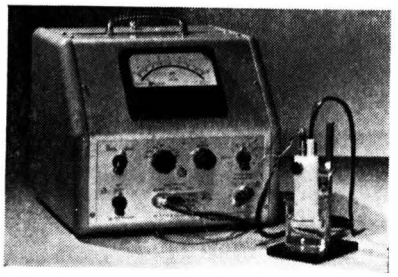


PYE UNIVERSAL pH METER CAT. No. 11067

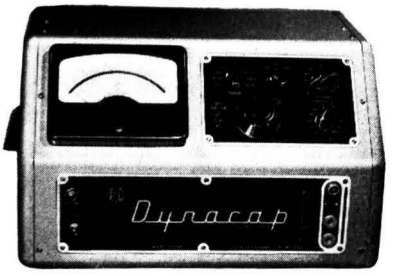
The Pye Universal *p*H Meter combines extreme accuracy, stability and simplicity of operation. The reading is given directly on a large meter of scale length 4½ inches. The range is 0-8 and 6-14*p*H units: each division is 0.1*p*H and readings can be estimated to 0.01*p*H. Millivolt ranges can also be selected: 0-800 and 600-1400 mV.

Normally there are only two controls to adjust each day and the readings are almost entirely unaffected by wide mains supply variations. Zero stability is ±0.02*p*H for mains voltage variations of ±15 per cent and frequency variations of ±3c/s. Circuit accuracy is such that the response is stable and linear to ±0.1 per cent under the above conditions of mains variation. Instrument sensitivity is 100 microamps/*p*H and external indicators and controllers can be plugged in without disturbing the panel meter. A total resistance up to 10 kilohms may be used. Both automatic and manual slope correction over the range 0-100°C. is provided.

A versatile electrode kit is supplied and a wide range of *p*H electrodes is also manufactured for special applications.

PYE for pH

DYNACAP pH METER CAT. No. 11072



Chemists have long awaited a pH Meter of this calibre: the use of a dynamic capacitor in the input stage provides exceptional zero stability, linearity and accuracy. Full advantage of this circuit is taken by the provision of expanded scales. The absence of grid current eliminates errors normally encountered when using robust (high resistance) electrodes. Absence of grid current is also important when operating over a wide range of temperature.

Basic scaling of the meter gives two main ranges of 0–10 and 4–14 pH units on a 5 inch scale. This can be expanded to give, in effect, a scale length of 35 inches, allowing excellent discrimination: a lower scale reads from 0–2.0 pH units each division being 0.02 pH: the range switch will select sub-ranges of 0–2 pH, 2–4 pH, 4–6 pH etc.

In addition to full automatic temperature compensation, manual temperature compensation is incorporated. Millivolt ranges 0–1000 mV; 400–1400 mV; expanded ranges of 200 mV throughout the full range of 0–1400 mV.

In all cases 1 mA output for full scale deflection is given, i.e. 100 μA/pH on main ranges, 500 μA/pH on expanded ranges. Recorders etc. to a total series resistance of 10 kilohms—(5 kilohms on expanded ranges) may be used.

SCIENTIFIC PYE INSTRUMENTS

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Volume 84. No. 1004, Pages 621 - 676 November, 1959

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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

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