THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATHS

WE record with deep regret the deaths of

The Rt. Hon. Lord Clinton (Honorary Member)
Gerald Roche Lynch.

NORTH OF ENGLAND SECTION

THE Twentieth Summer Meeting of the Section was held at Llandudno from Friday, May 17th, to Monday, May 20th, 1957.

The Chairman of the Section, Mr. A. N. Leather, B.Sc., F.R.I.C., presided over an Ordinary Meeting at 10.15 a.m. on May 18th, 1957, at which V. L. S. Charley, B.Sc., Ph.D., gave a talk entitled "Some Chemical Features of the Composition of Fruit Juices of Interest to Analysts and their Ladies." This provoked an animated and prolonged discussion.

On the Sunday afternoon the party was taken on a coach tour of the coast and hinterland between Llandudno and Caernarvon, and at the latter place tea was taken and the Castle was visited.

MIDLANDS SECTION AND MICROCHEMISTRY GROUP

A JOINT Meeting of the Midlands Section and the Microchemistry Group was held at 7 p.m. on Friday, May 10th, 1957, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Midlands Section, Dr. R. Belcher, F.Inst.F., F.R.I.C., who introduced the Chairman of the Microchemistry Group, Mr. D. F. Phillips, F.R.I.C.

A discussion on "The Micro-determination of Functional Groups" was opened as follows: "Some Developments in the Analysis of Functional Groups," by W. I. Stephen, Ph.D., A.R.I.C.; "The Determination of N-Methyl Groups," by M. K. Bhatty, M.Sc., A.R.I.C.; "The Determination of Equivalents," by T. S. West, Ph.D., A.R.I.C.; "Titrations in Non-aqueous Media on the Sub-micro Scale," by T. S. West, Ph.D., A.R.I.C. (see summaries below).

Before the meeting, at 2.30 p.m., a visit was made to Little Bromwich Hospital, Yardley Green Road, Birmingham, 9, to see some of the laboratory work being carried out in the Metabolic Ward, the Regional Virus Laboratory and the Pathological Laboratory.

SOME DEVELOPMENTS IN THE ANALYSIS OF FUNCTIONAL GROUPS

DR. W. I. STEPHEN gave a survey of recent literature on the determination of organic functional groups. A great deal of work had been reported in this branch of quantitative organic analysis, but only those developments that had improved the methods for the more important organic groups were discussed.

One of the most important determinations was that of the alkoxyl group, and recent work had been aimed at improving the apparatus, increasing the accuracy and extending the scope of the method. The main difficulty in the past had been the wash liquid used to free the alkyl iodide liberated by the classical Zeisel procedure from iodine, hydriodic acid and possibly hydrogen sulphide. Cadmium sulphate or antimony potassium tartrate solutions appeared to be the best wash liquids. Numerous modifications to apparatus and procedure had been described. Noteworthy were the contributions

of Kirsten and Erlich-Rogozinsky (*Mikrochim. Acta*, 1955, 786) and of Gran (*Svensk Papperstidning*, 1952, **55**, 255, 287; 1953, **56**, 179, 202; 1954, **57**, 702). The latter had perfected a suitable procedure for the simultaneous determination of methoxyl and ethoxyl groups.

Closely related to the alkoxyl group was the alkylimino group. Franzen and co-workers had made an extensive study of the various factors involved in this determination and recommended simplified apparatus (*Mikrochim. Acta*, 1955, 845).

Acetyl and C-methyl groups were determined by hydrolysis and oxidation, respectively, by using Wiesenberger's apparatus, recently standardised in B.S. 1428 : Part C1 : 1954. Wiesenberger had further modified his apparatus to deal with volatile acidic substances formed during the hydrolysis of certain acetylated carbohydrates of high molecular weight.

The Zerewitinoff method as used by Soltys was widely applied in the determination of active hydrogen. Recent work had tended to favour the use of lithium aluminium hydride in place of the conventional Grignard reagent. Recently, Stevens (*Anal. Chem.*, 1956, **28**, 1184) had shown that a solution of methyl magnesium chloride in tetraethyleneglycol dimethyl ether functioned satisfactorily as a general reagent for active-hydrogen determinations.

The work of Kainz (*Mikrochim. Acta*, 1953, 349) on the determination of the primary amino group had resulted in a great simplification of the established van Slyke procedure. The new apparatus was simple to use and gave accurate results.

Finally, the methoxymercuric acetate methods of Marquardt and Luce and of Martin for the determination of unsaturation in organic compounds had been improved by Das (*Anal. Chem.*, 1954, **26**, 1086). Excess of mercuric acetate was determined by non-aqueous titration. The method was by no means universally applicable, but a number of important ethylenic substances, including esters such as vinyl and allyl acetates, could be determined with accuracy.

The Determination of N-Methyl Groups

Mr. M. K. Bhatty said that the Herzig - Meyer method for methylimino and methylimino and methoxyl groups had been adapted to the 50-μg scale of analysis. An apparatus consisting of a double-distillation arrangement for hydriodic acid had been modified for the ultra-micro determinations. The substance was allowed to react with hydriodic acid, the excess of which was then distilled over and kept boiling in a second flask under a reflux condenser. The residual quaternary ammonium iodide was decomposed at 300° to 360° C in presence of ammonium iodide and gold chloride. The decomposition of the salt was best achieved by filling the flask initially with glass beads. The resulting methyl iodide was absorbed in a solution of bromine in sodium acetate - glacial acetic acid mixture. The iodine in the solution was determined by titration with 0·01 *N* sodium thiosulphate.

Three distillations and decompositions for the substance under test and two for a blank gave a net titre representing the amount of N-methyl group.

When methylimino and methoxyl groups were present together, the methoxyl was determined first by digestion with hydriodic acid for 1½ hours. The methylimino group was then determined, as outlined above, after the distillation of the acid and decomposition of the salt.

Emphasis had been laid on precautions for minimising the blanks. High blanks due to aerosol formation had been eliminated by passing the hot gas stream through the boiling acid in the second flask. Methyl iodide vapours were purified in an efficient condensation - scrubbing system. Heating and sweeping of the apparatus had been regulated.

Determination of tertiary nitrogen—An ion-exchange method had been devised on the ultra-micro scale for the determination of tertiary nitrogen. About 50 μg of the substance were treated with methyl iodide, the excess of which was afterwards removed by evaporation. The methiodide salt was passed down an anion-exchange column of Amberlite IRA-400(OH) resin, which produced the corresponding quaternary ammonium hydroxide in the effluent. The base was titrated acidmetrically, 0·01 per cent. alcoholic solutions of methyl red and methylene blue being used as indicators. The limitations of the method were enumerated.

The Determination of Equivalents

Dr. T. S. West said that ultra-micro samples were weighed by difference on an ultra-micro balance and dissolved in hemispherical-ended borosilicate-glass tubes of 1 cm diameter. Each sample was dissolved in 200 μl of ethanol and treated with an excess of standard aqueous sodium hydroxide. The latter was titrated with standard aqueous benzoic acid to a phenolphthalein end-point by using a micrometer-syringe burette. Standard illumination from a "daylight" lamp was used throughout the titrations. Identical tubes were placed on either side of the titration tube and were filled with the same volume of indicator, alcohol and water to act as comparison standards for the detection of the end-point. The titrations were carried out with protection from atmospheric carbon dioxide. Results by this method were as accurate as those obtained on the micro scale, provided the volume of ethanol present was controlled within certain limits.

Titrations in Non-aqueous Media on the Sub-micro Scale

Dr. T. S. West said that many substances that were too weakly acidic or basic to be titrated in aqueous solution could be satisfactorily determined in anhydrous organic solvents such as benzene, butylamine and glacial acetic acid. Generally speaking, protogenic solvents enhanced basicity and protophyllic solvents encouraged acidity. Ionisation was not, however, a necessary process, and many aprotic solvents had much to offer because of their excellent solvent powers on organic molecules and because they minimised the solvolysis of reaction products and so on.

Ultra-micro titrations of many substances could be carried out in non-aqueous solvents. For example, the alkali-metal salts of carboxylic acids could be titrated as strong bases in acetic acid by dissolving the sample in an excess of standard perchloric acid in glacial acetic acid and titrating the excess with standard sodium acetate in glacial acetic acid, with crystal violet as indicator and with a comparison system (± 1 μl of titrant contained in each tube) as described previously. Quaternary ammonium halides could be determined similarly by application of the mercuric acetate reaction. Visual end-points in non-aqueous solvents were somewhat unsatisfactory and more precise results could be obtained by application of potentiometric or radio-frequency end-point techniques.

BIOLOGICAL METHODS GROUP

The Summer Meeting of the Group was held on Thursday, May 23rd, 1957, when, by kind permission of the Director, 30 members paid a visit to the Wellcome Research Laboratories, Langley Court, Beckenham, Kent.

In the morning, visits were made to the Pharmacological and Chemical Research Laboratories, after which the Group was entertained to lunch at the Eden Park Hotel. In the afternoon demonstrations were given by the Bacteriological and Immunological Departments and visits were made to the Stables and to the Serum Concentration Laboratory.

At the close of the meeting the Chairman of the Group, Dr. S. K. Kon, F.R.I.C., expressed thanks on behalf of the visitors.

WESTERN SECTION

An Ordinary Meeting of the Section was held on Saturday, November 10th, 1956, taking the form of a visit to the factory of Messrs. H. W. Carter and Co. Ltd., Coleford, Glos. During the morning, the party was conducted round the factory and laboratories, and in the afternoon the following papers were presented and discussed: "The Application of the Polarised Platinum Electrode to the Determination of Ascorbic Acid in Fruit Products," by R. C. Curtis, B.Sc.; "The Effect of Ripening on Certain Constituents of the Blackcurrant," by Miss A. D. Ayres, B.Sc., R. C. Curtis, B.Sc., M. J. Egerton, B.Sc., A.R.I.C., and H. Fore, B.Sc., Ph.D., F.R.I.C.

Obituary

LESLIE HERBERT LAMPITT

LESLIE HERBERT LAMPITT died on June 3rd at the age of 69; by his death chemistry, particularly that branch dealing with food, lost one of its outstanding men and personalities. This was clearly demonstrated by the large number of people who attended the funeral at the church of St. Mary, Harrow-on-the-Hill. There were representatives from all the main chemical societies in this country, and Professor Stoll, President of the International Union of Pure and Applied Chemistry, came to England especially for the service, as did others, including Professor R. Delaby. All wished to pay their last respects to an outstanding man.

Lampitt was educated at the old Birmingham Technical School, and then entered Birmingham University as a Priestley Scholar in 1906. He had a brilliant career as a student and obtained first-class honours in chemistry and biochemistry. One of his first positions (1911) was that of chemist to the large Belgian flour mill, the Meunerie Bruxelloise. He fought in the 1914–18 war, during which time he first met Mr. S. M. Gluckstein of J. Lyons & Co. Ltd.

His life's work proper began when he started and organised, after the 1914–18 war, the now world-famous laboratories of the firm of J. Lyons & Co. Ltd. There are many first-class chemists, but alas, only few with the vision of Lampitt and with the enthusiasm and the organising and business ability to make the vision come true. The best monument to him must still remain the magnificent, well equipped and efficiently run laboratories at Cadby Hall. It was no simple task to do this, but Lampitt was as able a business man as he was a chemist. He had uncanny judgment and collected around him able lieutenants whom he could inspire. It is not surprising, therefore, that before long he was elevated to the Board.

Lampitt realised the importance of organised chemistry and, as far as this country was concerned, although he helped all and sundry, his main devotion was to the Society of Chemical Industry. He was President in 1946–48, Honorary Foreign Secretary in 1935–36 and 1948–57, Honorary Treasurer in 1936–46, Chairman (and Founder) of the Food Group, 1931–36, and Jubilee Memorial Lecturer for 1933–34. He became the Society's Medalist in 1943. However, he was active in many other chemical and scientific organisations. He was a Member of Council of the Royal Institute of Chemistry in 1928–31, 1935–38, 1939–42, 1949–50 and a Vice-President in 1942–45. In our own Society he was a Member of Council in 1925–26, 1933–34 and 1939–40, as well as being a Vice-President in 1935–36 and 1941–42.

He turned his attention to international chemistry and was one of the leading lights and a driving force of the International Union of Pure and Applied Chemistry, of which he was the Honorary Treasurer. He was often the Chairman of Executive Committees for organising International Congresses, and this was a job he could do so much better than anyone else. He watched carefully to see that the interests, dignity and importance of the United Kingdom were looked after.

It is difficult to write of him briefly. He never lost close contact with his main interest, the chemistry of food, and did as much as any man to ensure high standards, with particular attention to hygiene. He was able to impress upon food manufacturers that they undertook great responsibilities in providing food and had to maintain high standards, only possible by proper attention to the use of scientific methods. In his own laboratory he was an absolute autocrat, but a benevolent one.

The time he gave to outside activities, only some of which have been mentioned, was enormous. Many came to him for advice and he had time for all, and great understanding. Like all great personalities he had his critics, but all were agreed as to his ability and honesty of purpose. He knew good food and wine as do few men, and indeed was a great diner-out. One side of him not always realised was that he was a devout Christian, worked for the Church and was always doing quietly some kind and thoughtful act that might have surprised those who only knew him as a public man. He lived at Harrow-on-the-Hill, and his hobby was his wonderful garden. He was married in 1915 to Edith Potter Potts, who survives him, and who is so well known and respected in all chemical circles, while his son is also a frequenter of chemical social functions.

We shall all miss Leslie Herbert Lampitt and it is hard to see how he can be replaced—certainly no one man can do it.

D. W. KENT-JONES

The Determination of Atmospheric Mercury Trapped in Permanganate Solutions: A Modified Method

By R. G. DREW and E. KING

The use of hydrogen peroxide as a reducing agent for acid potassium permanganate solutions before the determination of trapped mercury is described. The degree of accuracy of a modified extractive titration method under various interfering conditions is indicated.

THE problems involved in measuring atmospheric mercury fall into two main categories, associated with the method of sampling and the method of analysing the samples so obtained. The principle of sampling suggested by Kuziatina,[1] involving the use of bubblers containing acid potassium permanganate as the air-scrubbing solution, has been adopted. Since dithizone, the reagent used in this work for the determination of the mercury, is sensitive to oxidation, it is necessary to reduce the permanganate before using this reagent. This paper reports the use of hydrogen peroxide as a reducing agent and the suitability of the chemical determination under conditions of contamination and interference likely to be encountered under actual sampling conditions.

PRINCIPLE OF THE METHOD—

As in the method described by Kuziatina, a known volume of the atmosphere to be tested is drawn through a bubbler containing acid permanganate solution. The permanganate is decolorised by reduction with aqueous hydrogen peroxide. The mercury in the sample is then determined by extractive titration with dithizone solution.

In the method described below, the chief innovation is the use of hydrogen peroxide as a reducing agent to prepare the contents of the bubbler for determination. This reagent gives more reproducible results under varying conditions than either oxalic acid or hydroxylamine, which have been used hitherto. Our whole experience during this work was that dithizone is remarkably stable towards hydrogen peroxide at the dilutions used and under the conditions of the method.

Extractive-titration methods have been employed in many instances for the separation and determination of heavy metals with dithizone solution. Eckert[2] and Maren[3] used the method for the determination of mercury. Strafford, Wyatt and Kershaw[4] recommended the extractive titration of zinc when present in large amounts. Milton and Hoskins[5] revived the method for the preferential extraction of mercury in the presence of copper, and Buckell[6] adopted the method for the determination of mercury in the atmosphere.

METHOD

APPARATUS—

The bubblers used in this work are of the glass-bead type, as described by Milton and Duffield,[7] but without the attached filter-paper head. Before use, soak each bubbler in chromic acid cleaning solution. Rinse the bubbler, first with tap water and then with water redistilled in all-glass apparatus. Carry out a blank by filling the bubbler completely with 0·05 *N* potassium permanganate solution and setting it aside for 1 hour, after which the contents should be titrated for mercury as described below. Not more than 0·5 ml of dithizone solution should be required.

In this work some 15 to 20 ml of potassium permanganate solution are necessary to fill the bubblers adequately and allow a reasonable sampling rate without undue splashing. In view of this, a fixed amount (20 ml) of potassium permanganate solution was taken for most of the interference experiments.

All glass apparatus, including the bubblers, should be made from Pyrex glass. All apparatus should be thoroughly cleaned in chromic acid solution.

REAGENTS—

All reagents should be of recognised analytical grade. After normal distillation in a metal still, all water should be redistilled in Pyrex-glass apparatus.

Potassium permanganate solution, 0·05 N—Prepare this solution in 5 per cent. v/v sulphuric acid (1 litre contains 1·58 g of potassium permanganate). It should be discarded if drastic ageing reduces the permanganate concentration owing to decomposition.

Hydrogen peroxide, 20-volume—This should be stored in the dark at about 4° C.

Chloroform, redistilled—Redistil analytical-reagent grade chloroform in all-Pyrex-glass apparatus.

Dithizone stock solution—Dissolve 0·05 g of diphenylthiocarbazone (dithizone) in 100 ml of redistilled chloroform. This solution should be stored in the dark at about 4° C, and it should not be used if it shows any signs of deterioration, *i.e.*, an increase in the volume of the solution equivalent to a known amount of mercury. The solid dithizone used should be up to a reliable standard of purity and, once decided upon, the source of supply should not be changed.

Dithizone extraction solution—Dilute the stock solution 1 in 100 with redistilled chloroform. Prepare this solution freshly each day and, when it is not in use, keep it in the dark.

Standard mercury solution A—Dissolve 0·06767 g of pure mercuric chloride in water and dilute to 100 ml.

Standard mercury solution B—Dilute standard mercury solution A to 1 in 100 with water. This solution should be freshly prepared as required.

$$1 \text{ ml} \equiv 5 \ \mu\text{g of mercury.}$$

PROCEDURE—

The method is essentially an extractive titration in which the technique has been rigorously standardised.

Wash the contents of the bubbler into a beaker with redistilled water until the washings are only faintly pink, and then rinse the bubbler with 10 ml of hydrogen peroxide and finally with redistilled water. The hydrogen peroxide removes any brown deposit in the bubbler that has not been affected by washing with water. If much dilution with water has occurred, complete reduction of the permanganate may be slow, but the reaction can be accelerated by adding a few millilitres of 50 per cent. v/v sulphuric acid.

After complete reduction, quantitatively transfer the solution or an aliquot to a separating funnel and dilute to a standard volume with distilled water. As the manner and time of shaking and settling out in the separating funnel must be carefully standardised, the contents of the separating funnel must be diluted to an approximately constant volume before the titration; we dilute to about 250 ml in a 500-ml separating funnel. At this point check the acidity of the diluted sample to ensure that its pH is 1 or less, adding more 50 per cent. v/v sulphuric acid if necessary. The use of universal indicator paper is sufficiently accurate to check the acidity. In practice, further addition of acid is rarely necessary. Saturate the aqueous layer with chloroform by vigorously shaking it with about 5 ml of redistilled chloroform. After settling out, run off the lower chloroform layer and discard it. Then add measured portions of the dithizone extraction solution from a burette. After each addition, mix the two phases intimately by shaking the separating funnel 2 or 3 times per second for 20 seconds. After each shaking, set the funnel aside for 30 seconds to allow settling out of the lower chloroform layer. In the presence of mercury this layer will be orange, owing to the conversion of the blue-green dithizone to the orange-red mercury dithizonate, which is also soluble in chloroform. Run off the bulk of the chloroform and discard it, and swirl and shake the contents of the funnel to dislodge drops of chloroform suspended from the top aqueous layer. Run off this residue also. Add the next portion of dithizone solution and repeat the shaking procedure as before. By this means mercury is gradually extracted from the aqueous layer.

The end-point of the titration is reached when a final addition of 0·5 ml of dithizone solution, after shaking, does not turn orange-red, but has a grey-green appearance. The judgment and selection of this end-point is a matter of experience, but it has been found that it is better, in the presence of other metals, not to titrate to a full green, but to a green-tinted grey colour. The end-point shade of green decided on by the operator must be adhered to rigorously.

In order to make the procedure quantitative, it is necessary to reduce the volumes of dithizone solution added as the end-point is approached. To do this, an approximate estimate of the mercury content of the sample is first made by titrating a small aliquot

(say one-tenth) of the sample, 2 to 3-ml portions of the dithizone solution being added. From this preliminary titration, an approximate expected titration value for a larger aliquot can be determined. This must be done by multiplying the approximate mercury value determined from the calibration graph by the factor for the larger aliquot to be taken. The expected mercury content for the larger aliquot is then converted, from the graph, to an expected approximate titration volume. In carrying out the accurate titration, single additions of dithizone solution should not exceed 5 ml, and a few millilitres before the expected end-point the additions should be in 1-ml or 0·5-ml amounts, according to the degree of accuracy required.

PROCEDURE FOR PREPARING A CALIBRATION GRAPH—

Construct the calibration graph by making up a series of samples, each containing approximately 20 ml of permanganate solution plus various amounts of mercury, added as standard mercury solution B, through a range of 0 to 60 μg of mercury. Decolorise the samples by adding 10 ml of hydrogen peroxide and titrate. Plot the volume of dithizone solution in millilitres against the amount of mercury in micrograms. The graph is slightly curved, as shown by Figs. 1 and 2. Each time the calibration curve is used it should be checked at one or two points, as any variation in conditions, technique or solutions is likely to be shown up by such a procedure.

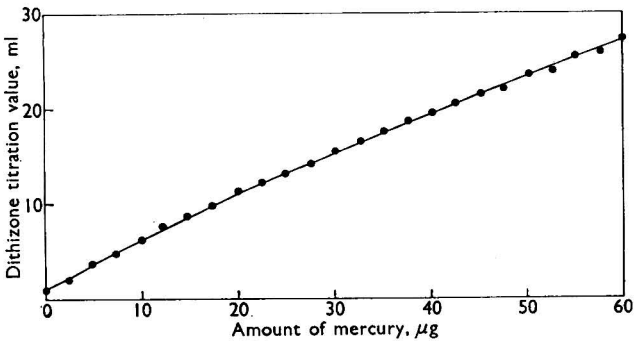

Fig. 1. Calibration graph prepared by using mercuric chloride solution

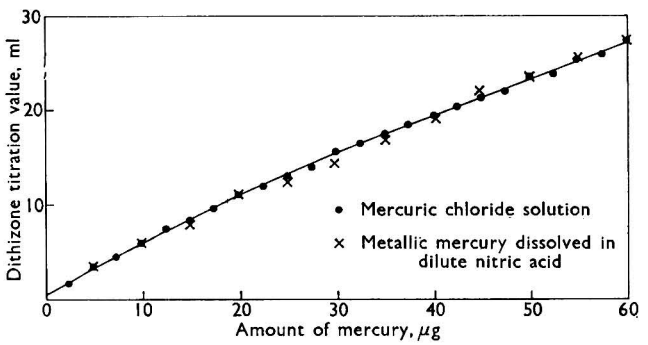

Fig. 2. Comparison of standard solutions for the preparation of a calibration graph

NOTES ON THE METHOD—

We have found that mercury dithizonate is sensitive to natural light, especially when in contact with reduced permanganate solutions (see Barnes[8] and Irving, Risdon and Andrew[9]), and variations in the intensity of natural light have caused difficulties in this laboratory. We therefore consider it essential to carry out the titrations in a "dark-room"

illuminated by electric-light bulbs. Fluorescent-tube lighting is not so satisfactory for assessing the end-point colours.

In view of evidence of losses of mercury from reduced permanganate solutions on standing, it is recommended that all samples should be stored in the permanganate form and not reduced until immediately before titration (see p. 466). The temperature at which samples are stored and analysed should be as close as possible to 20° C, as higher temperatures sometimes cause loss of mercury.

Checks on the method

Owing to the coarseness of the titration technique, the accuracy of the method has a definite limit. However, results were obtained on a simple solution of mercuric chloride in reduced potassium permanganate solution to a precision of $\pm$ 0·5 ml of dithizone solution, which is approximately equivalent to 1 μg of mercury. In order to apply this analysis technique to samples taken from the atmosphere in factories, etc., it was necessary to find out how the chemical accuracy varied with other factors. The main procedure was always as follows. Twenty millilitres of permanganate solution were taken and a known amount of standard mercury solution was added. Contaminating material was then added or a possibly interfering factor was allowed to operate, and then the hydrogen peroxide was added. The sample was titrated as described under "Procedure." Small variations in this procedure are mentioned as they occur in the separate sections that follow. These checks were carried out by five different workers, two of whom had not participated in the original development work on the method.

Interference from other metals—

Sandell[10] has stated that dithizone reacts preferentially with copper, mercury, silver and palladium when these metals are in aqueous solution at a pH of 1 or less, *i.e.*, in 0·1 to 0·5 *N* acid. Milton and Hoskins[5] have shown that, if the pH of the solution is kept within the limits 0·075 to 0·15 *N* with respect to hydrochloric acid, then mercury will be extracted by a chloroform solution of dithizone in preference to copper. In general, all other heavy metals do not normally react with dithizone under these conditions. In view of these facts, it has been specified in this method that the sample shall have a pH of 1 or less, to reduce interference from other metals. Variations of acidity towards the more acid side in practice did not cause great interference.

Table I

Effect of contaminating metals

Range of amounts of contaminant	Number of recovery experiments	Range of absolute errors for mercury contents of 5 to 60 μg, μg	Recovery for 5 μg of mercury (single value or average), %	Recovery for contents within the range 10 to 60 μg of mercury (average), %
20 to 35 mg of Cu	7	0 to + 2	140	103
16 to 30 mg of Fe^{II} and Fe^{III}	16	0 to + 7	130	113
32 to 200 mg of Zn	13	0 to + 3	140	103
20 to 50 mg of Pb	7	0 to + 2	130	101
8 to 64 mg of Cr^{VI}	4	0 to + 1 (20 μg of Hg only)	—	101
8 to 64 mg of Al	4	0 (20 μg of Hg only)	—	100
8 to 64 mg of Cd	7	0 to + 1	120	101
50 mg of Mg	3	0 to + 2	140	102
50 mg of Ca	3	0 to + 1	120	100

In spite of the precautions described above, it was nevertheless thought desirable for two reasons to check the effect of other metals. The chemical system involves the use of dithizone in a solution containing an excess of hydrogen peroxide. This chemical environment might have altered the reactivity of other metals towards dithizone. Also, the amount of contaminating metals such as copper and iron encountered in some factory atmospheres

is likely to be very large, *e.g.*, of the order of milligrams in the presence of micrograms of mercury.

Standard solutions of various metal salts were made up in water or acid and known amounts were added to samples containing known amounts of mercury. The results are shown in Table I, and they indicate that when a small amount of mercury, *i.e.*, 5 μg or below, is being determined, there is considerable interference from other metals in terms of percentage recoveries, although the absolute deviations are rarely more than a few micrograms. When mercury is being determined in the 10 to 60-μg range, the percentage recoveries are considered acceptable, except when iron is present. The iron probably causes gradual oxidation of the dithizone, as it is well known that in basic solutions this oxidation easily occurs.[11]

INTERFERENCE FROM MIXTURES OF METALS—

Mixtures of standard solutions were made up in a series of increasing complexity. The first series had known mercury contents and the results were as follows—

Number of determinations	= 9
Range of amounts of metal added	= 10 to 20 mg of Fe; 5 to 50 mg of Cu; 30 to 50 mg of Zn; 30 to 50 mg of Pb; 50 mg of Cd; 50 mg of Ca; 25 mg of Cr^{VI}
Range of mercury recoveries as a percentage of the mercury added	= 100 to 115 per cent.
Average mercury recovery	= 111 per cent.
Range of absolute errors	= 0 to +3 μg of mercury

The second series again contained mixtures of metals, but the mercury content was unknown to the operator. Consequently, preliminary aliquots had to be taken. The results were as follows—

Number of determinations	= 9
Contaminating materials in each sample	= 20 mg of Fe; 30 mg of Cu; 50 mg of each Al, Zn, Mg, Cd, Cr^{VI} and Ca
Range of mercury content	= 7 to 57 μg
Range of recoveries as a percentage of mercury added	= 96 to 127 per cent.
Average mercury recovery	= 110 per cent.
Range of absolute errors	= − 1 μg to + 4 μg of mercury

There were three anomalous values not included in the above results and these gave recoveries of 178, 172 and 136 per cent. They were found to be due to a worker titrating past the end-point. It is essential, in the presence of much contaminating material, to titrate to the first perceptible shade of green that appears. This was done with a second group of unknown samples by the same operator, with the following results—

Number of determinations	= 3
Contaminating materials as in the previous group of experiments	
Range of mercury content	= 22 to 48 μg
Range of recoveries as a percentage of the mercury added	= 103 to 118 per cent.
Average mercury recovery	= 108 per cent.
Range of absolute errors	= + 1 μg to + 4 μg of mercury

It is realised that some of the metals tested here do not combine with dithizone. Nevertheless, in view of their possible occurrence in samples from factory atmospheres, it did not seem advisable to assume that they could not interfere in any way and so this was checked.

INTERFERENCE FROM SOLID OXIDES—

A mixture containing the following solid oxides in unknown proportions was prepared: ferric oxide, lead dioxide, lead monoxide, stannic oxide, manganese dioxide, chromic oxide and cupric oxide. Approximately 0·5 g of this mixture was added to each sample of mercury in permanganate and the determination procedure was carried out normally, except that each sample, after reduction, was extracted several times with pure chloroform, in order to clear away (at the chloroform - water interface) most of the undissolved solid. The results were as follows—

Number of determinations	= 6
Range of mercury content	= 5 to 60 μg
Range of recoveries as a percentage of the mercury added	= 95 to 120 per cent.
Average mercury recovery	= 106 per cent.
Range of absolute errors	= − 1 μg to + 2 μg of mercury

INTERFERENCE FROM MANGANESE DIOXIDE—

If the atmosphere being sampled contains small amounts of reducing substances, a partial decomposition of the permanganate trapping solution sometimes occurs to give a deposit of brown solid on the inside of the bubbler. This deposit probably contains manganese dioxide and therefore the effect of adding solid manganese dioxide to samples was determined.

Each sample was made by adding about 1·5 g of commercial manganese dioxide to 20 ml of permanganate solution, known amounts of mercury then being added. The samples were decolorised in the cold, either with hydrogen peroxide, as in the standard method, or with hydroxylamine or oxalic acid, as used in earlier methods. With hydroxylamine sulphate 10 ml of a 20 per cent. w/v solution (stripped of interfering metals by extraction with dithizone at about pH 1) were used per sample, and with oxalic acid, 14 ml of a 10 per cent. w/v solution were used per sample. The results were as follows—

Reducing agent	Hydrogen peroxide		Hydroxylamine		Oxalic acid	
Mercury added, μg	20	35	20	35	20	35
Difference in dithizone titration value from standard sample, ml	−0·5	0	−3	−5	−3·5	−4·5

Although these experiments were carried out with more manganese dioxide than can be produced from one charge of trapping solution, it is apparent that the use of hydrogen peroxide removes any significant variation in the results.

EFFECT OF ADDITIONAL TRAPPING SOLUTION AND HYDROGEN PEROXIDE—

When bubblers are used for an extended period in dry atmospheric conditions, evaporation sometimes necessitates addition of a further charge of trapping solution. Although, therefore, the normal volume of solution used is about 20 ml, checks were carried out for possible interference from larger volumes. The results were as follows—

Number of determinations .. = 6
Amount of mercury added .. = 20 μg in all tests
Range of volumes of permanganate solutions added .. = 20 to 200 ml
Mercury recovered .. = 20 μg in all tests

In a single experiment in which 100 ml of 20-volume hydrogen peroxide were added to a mercury sample the recovery was 100 per cent.

EFFECT OF STORING SAMPLES—

In view of the fact that atmospheric sampling is sometimes carried out in places distant from the analytical laboratory, when it is impossible immediately to analyse the samples, it was considered advisable to check the keeping qualities of a typical sample.

There are two possible situations, one when the samples are kept in the original form in the bubbler, and the other when the samples are reduced and kept in the colourless form.

With samples kept as the permanganate form—

Range of added mercury contents .. = 5 to 60 μg
Range of times left standing .. = 50 minutes to 24 hours
Air temperature .. = 20° to 22° C
Range of mercury recoveries .. = 100 to 103 per cent.
(there was also one value of 80 per cent., representing a loss of about 0·5 μg out of 2·5 μg of mercury)
Range of absolute errors .. = $-\frac{1}{2}$ μg to +1 μg of mercury

With samples kept in the reduced form—

Range of added mercury contents .. = 20 to 60 μg
Range of times left standing .. = 60 to 85 minutes
Air temperature .. = 18° to 21° C
Range of mercury recoveries .. = 90 to 98 per cent.
Range of absolute errors .. = −2 μg to −2·5 μg of mercury

The losses occurring on setting aside a sample with additional hydrogen peroxide (60 ml of 20-volume solution) are higher.

Recoveries dropped to a minimum of 75 per cent. after the sample had been set aside for 2 hours.

SAMPLING CHECK UNDER WORKSHOP CONDITIONS—

In order to check the performance of this method under actual sampling conditions a series of atmosphere samples was taken in an M.R.C. workshop (metal work and wood

work). With most samples a low artificial mercury content was introduced into the atmosphere by exposing dishes of liquid mercury to the air. An aliquot from each sample was analysed for trapped mercury and then a known amount of mercury (standard solution) was added to an identically sized aliquot and the determination was repeated. Hence recoveries under practical conditions could be assessed.

Of the first group of twelve determinations carried out, there were three anomalous recoveries of 280, 220 and 150 per cent. The nine remaining results may be summarised as follows—

Range of recoveries	= 90 to 130 per cent.
Average recovery	= 120 per cent.
Range of absolute errors	= −1 to +3 μg of mercury

Two of the anomalous values mentioned above were associated with the operation of "melting down" lead piping. Further atmosphere samples were taken during a similar operation and this time recoveries were 100 to 110 per cent. It is thought that the first anomalies may have been due to silver derived from laboratory plumbing, although this could not be verified.

We record here our appreciation of the technical assistance received in the above work from Miss A. Murdon, Mr. B. Biles, Mr. T. R. Emerson and Mr. E. Palmer. Acknowledgment is also made to Mr. H. Hardwick (Engineer—Maintenance) for co-operation in carrying out the sampling check in the M.R.C. Workshop. We finally thank Dr. P. L. Bidstrup for her continuing interest and assistance in the preparation of this paper.

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Department for Research in Industrial Medicine
M.R.C. Laboratories, Holly Hill
Hampstead, London, N.W.3

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Comparison of Flame-photometric and Chemical Methods for Determining Sodium and Potassium in Soil, Plant Material, Water and Serum

By M. Puffeles and N. E. Nessim

Flame-photometric and chemical methods of determining sodium and potassium in soil, plant material, water and serum are compared. The paper shows how the correction of the results by the chemical methods for the recovery error and of the results by flame-photometric methods for interference by calcium and phosphate considerably reduces the otherwise large discrepancies.

Time-consuming and laborious determinations of sodium and potassium by chemical methods are rapidly being replaced by more convenient and economical flame-photometric methods. Procedures have been described for such determinations in industrial, agricultural and biological material.[1,2,3,4,5,6,7,8,9] Whereas the sources of error and their elimination have been investigated by many workers who used American flame photometers based on internal-standard techniques,[10,11] relatively little attention has been devoted to the simpler, single-cell

flame photometers, such as the British-made E.E.L. instrument (Evans Electroselenium Ltd.), which has lately come into use in many laboratories here and elsewhere.[12]

The work described is a study of sodium and potassium determinations in soil, plant extracts, natural waters and blood serum carried out with the E.E.L. flame photometer and by chemical methods, with a view to preparing flame-photometric correction curves for the two main interfering ions, calcium and phosphate, and thereby to achieve better agreement of results.

Physical sources of error such as (*a*) fluctuation of the spraying rate, (*b*) changes of air and gas pressures, (*c*) variation of flame temperature, (*d*) impurities affecting the viscosity and surface tension of the test solution, and (*e*) insufficiency of the filters, were not taken into consideration. These errors are discussed, among others, by Fox[13] and Robinson and Ovenston.[14]

Halstead and Chaiken[15] state that calcium interferes with the flame-photometric determination of sodium and potassium. Spector[16] reports a positive error due to calcium for 30 p.p.m. of sodium that increases as the concentration of calcium ions increases and amounts to 40 per cent. for 500 p.p.m. of calcium. The corresponding error effect on 50 p.p.m. of potassium is about 12 per cent. Both errors are appreciably high compared with the interference of potassium in the determination of 10 p.p.m. of sodium (3 per cent. for 1000 p.p.m. of potassium) and of sodium on the determination of 10 p.p.m. of potassium (0 per cent. for 1000 p.p.m. of sodium). Of all the cations dealt with, calcium seems to be the main source of interference in the flame-photometric determination of both sodium and potassium.

Collins and Polkinhorne,[12] however, call attention to the large differences in interference caused by *N* solutions of various anions. Phosphate causes a 34 per cent. depression in the reading for 10 p.p.m. of sodium and 78 per cent. for 10 p.p.m. of potassium, these figures representing the greatest effect caused by any anion investigated.

Experimental

The E.E.L. flame photometer used in this work has been described in detail by Collins and Polkinhorne.[12] The main parts were illustrated and its operation was explained. Essentially it is a single-cell direct-reading instrument. A butane - air flame is used, primarily because it is expected to cause lower mutual interference effects between sodium and potassium themselves than would be experienced with, for example, an oxygen - acetylene flame.

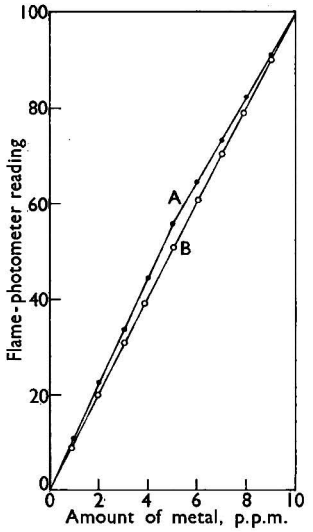

Fig. 1. Calibration curves: curve A, sodium; curve B, potassium

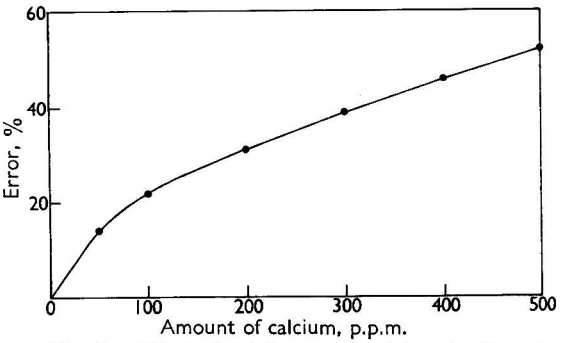

Fig. 2. Effect of calcium on the determination of 10 p.p.m. of sodium

AnalaR reagents and demineralised water (containing not more than 0·5 p.p.m. of total salts expressed as sodium chloride) were used throughout. All determinations were carried

out in duplicate. To ascertain the reproducibility of the results, flame-photometric readings were first made serially and then repeated, the scale adjustment being re-checked against standard solutions during the repeat tests.

Fig. 1 shows the calibration curves for sodium and potassium prepared by spraying standard solutions of their chlorides.

EFFECT OF CALCIUM ON SODIUM—

TABLE I

ERROR IN DETERMINING 10 p.p.m. OF SODIUM IN THE PRESENCE OF CALCIUM BY FLAME PHOTOMETER

Calcium present, p.p.m.	Sodium found, p.p.m.	Error, %	Apparent sodium exhibited by sprayed solution of sodium-free calcium, p.p.m.
50	11·4	+14	0·6
100	12·2	+22	1·3
200	13·1	+31	2·6
300	13·9	+39	3·6
400	14·6	+46	4·3
500	15·2	+52	4·9

The E.E.L. flame photometer is adjusted to produce a scale reading of 0 for demineralised water and 100 (full-scale deflection) for 20 p.p.m. of sodium. A series of solutions containing 10 p.p.m. of sodium and various amounts of calcium as calcium chloride are then sprayed and the reading for sodium is recorded. Solutions of calcium chloride containing the same amounts of calcium as before but free from sodium are then sprayed and the apparent sodium reading is noted. It can be seen from Table I that the error caused by the insufficiency of the sodium filter to eliminate the transmittance of the neighbouring calcium bands does not appear to be quite additive. The presence of calcium together with sodium seems to enhance the emission of the latter.

Brealey and Ross[17] reported a constant negative error of 2·8 per cent. caused by 100 and 1000 p.p.m. of calcium when working with the first of their two flame photometers on 30 p.p.m. of sodium. This effect of calcium on sodium was never observed with our E.E.L. instrument, nor with the Lange[16] or Beckman model DU[18] instruments.

EFFECT OF CALCIUM ON POTASSIUM—

This was found to be negligibly small below 300 p.p.m. of calcium (see Table II). With 400 p.p.m. a positive interference error of only 0·4 per cent. was found for 10 p.p.m. of potassium. This is significantly lower than that reported by Spector,[16] who used the Lange single-cell flame photometer. This improved reduction of the calcium effect may be due partly to the higher selectivity of the E.E.L. potassium filter and to the lower temperature butane - air flame used, as acetylene, chosen by Spector, burns at a much higher temperature, thereby increasing the mutual interference effect.

TABLE II

ERROR IN DETERMINING 10 p.p.m. OF POTASSIUM IN THE PRESENCE OF CALCIUM BY FLAME PHOTOMETER

Calcium present, p.p.m.	Error, %	Calcium present, p.p.m.	Error, %
100	+0·11	700	+0·65
200	+0·21	1000	+0·90
300	+0·31	2000	+1·65
400	+0·40	4000	+2·75
500	+0·49	7000	+3·55
600	+0·57	10,000	+4·20

EFFECTS OF PHOSPHATE ON SODIUM AND POTASSIUM—

The effects were investigated by preparing two series of standard solutions containing 10 p.p.m. of sodium and 10 p.p.m. of potassium, respectively, and various amounts of AnalaR ammonium dihydrogen phosphate. The solutions were sprayed into the E.E.L. flame photometer, the instrument having been adjusted to give full-scale deflections with both 10 p.p.m.

of phosphate-free sodium and with potassium. The results are shown in Fig. 3 and confirm Collins and Polkinhorne's report[12] that phosphate ions cause negative interference errors in the determination of both sodium and potassium, the effect on potassium being by far the greater.

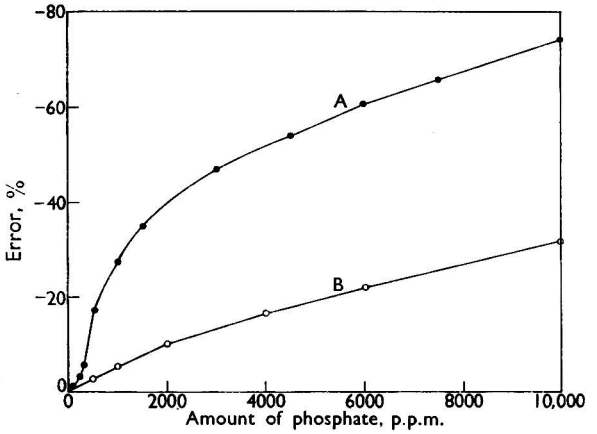

Fig. 3. Effect of phosphate ions on the determination of sodium and potassium: curve A, 10 p.p.m. of potassium; curve B, 10 p.p.m. of sodium

DETERMINATION OF SODIUM AND POTASSIUM IN SOIL EXTRACTS—

Six samples of typical local soils were extracted with neutralised *N* ammonium acetate solution. Sodium and potassium were then determined in the extracts (*a*) with the E.E.L. flame photometer and (*b*) by chemical methods, *i.e.*, sodium gravimetrically as sodium magnesium uranyl acetate[19] and potassium volumetrically as cobaltinitrite.[20] The results are given in Tables III and IV.

TABLE III

COMPARISON OF THE RESULTS OF THE DETERMINATION OF SODIUM IN SOIL SAMPLES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Type of soil	Calcium in sprayed solution, p.p.m.	Correction for calcium, %	Phosphate in sprayed solution,* p.p.m.	Sodium found by chemical method— uncorrected, p.p.m.	Sodium found by chemical method— corrected, p.p.m.	Sodium found by flame-photometric method— uncorrected, p.p.m.	Sodium found by flame-photometric method— corrected, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Light ..	41	−11·0	30·2	2·46	2·52	2·80	2·49	+17·5	−1·2
Medium ..	155	−27·5	17·6	7·03	7·20	9·70	7·02	+37·8	−2·5
Medium ..	56	−15·0	15·4	4·59	4·70	5·50	4·67	+19·8	−1·5
Heavy ..	57	−15·5	11·1	17·36	17·74	21·98	18·60	+26·6	+3·7
Heavy ..	150	−27·0	8·5	11·98	12·25	18·80	12·72	+17·2	+3·8
Loess ..	50	−14·0	2·0	28·80	29·45	33·63	28·95	+12·9	−1·7

* No correction for phosphate was applied, as any interference caused by the concentrations found was negligible.

DETERMINATION OF SODIUM AND POTASSIUM IN PLANT-ASH EXTRACTS—

For the purpose of this investigation, ten samples of leaves from four types of local plants were chosen. About 5 g of each dried sample were ashed in silica crucibles at 550° to 600° C, and the ash was extracted with 2·5 ml of concentrated hydrochloric acid and hot water. The extracts were then filtered, cooled and made up to 250 ml with demineralised water.[21] Sodium and potassium were determined in the extracts both chemically and flame photometrically. The results are expressed as parts per million in dry matter and are given in Tables V and VI.

TABLE IV

COMPARISON OF THE RESULTS OF THE DETERMINATION OF POTASSIUM IN SOIL SAMPLES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Type of soil	Calcium in sprayed solution,* p.p.m.	Phosphate in sprayed solution, p.p.m.	Correction for phosphate %	Potassium found by chemical method— uncorrected, p.p.m.	Potassium found by chemical method— corrected, p.p.m.	Potassium found by flame-photometric method— uncorrected, p.p.m.	Potassium found by flame-photometric method— corrected, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Light ..	41	30·2	+0·36	8·22	8·47	8·52	8·55	+3·6	+0·9
Medium ..	138	17·6	+0·21	12·50	12·89	12·99	13·02	+3·9	+1·0
Medium ..	56	15·4	+0·18	18·40	18·95	18·73	18·76	+1·8	−1·0
Heavy ..	57	11·1	+0·13	40·61	41·90	41·25	41·30	+2·1	−1·4
Heavy ..	135	8·5	+0·10	35·05	36·15	35·44	35·80	+1·0	−1·8
Loess ..	50	2·0	+0·00	14·22	14·64	14·52	14·52	+2·1	−0·8

* No correction for calcium was applied, as any interference caused by the concentrations found was negligible.

TABLE V

COMPARISON OF THE RESULTS OF THE DETERMINATION OF SODIUM IN PLANT LEAVES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Type of leaves	Calcium in sprayed solution, p.p.m.	Correction for calcium, %	Phosphate in sprayed solution,* p.p.m.	Sodium found by chemical method— uncorrected, p.p.m.	Sodium found by chemical method— corrected, p.p.m.	Sodium found by flame-photometric method— uncorrected, p.p.m.	Sodium found by flame-photometric method— corrected, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Green eucalyptus	50	−14·0	17·7	2900	2970	3500	3010	+20·6	+1·3
Yellow eucalyptus	16	−4·5	5·2	9020	9240	9460	9050	+4·9	−2·1
Dried tobacco	330	−41·0	56·6	607	621	1020	602	+68·1	−3·1
Dried tobacco	325	−40·5	52·4	735	752	1110	660	+51·0	+1·2
Dried tobacco	451	−49·0	41·3	581	595	1140	582	+91·2	−2·1
Desert weed	3	−0·8	1·6	43,500	44,500	33,700	33,400	−0·2	−2·0
Green citrus	135	−26·0	74·4	659	675	940	695	+58·0	+3·0
Green citrus	135	−26·0	74·4	631	646	860	636	+36·2	−1·5
Yellow citrus	143	−26·5	13·6	2062	2118	2905	2142	+40·8	+1·0
Yellow citrus	150	−27·0	13·6	2050	2100	2835	2070	+27·7	−1·4

* No correction for phosphate was applied, as any interference caused by the concentrations found was negligible.

TABLE VI

COMPARISON OF THE RESULTS OF THE DETERMINATION OF POTASSIUM IN PLANT LEAVES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Types of leaves	Calcium in sprayed solution,* p.p.m.	Phosphate in sprayed solution, p.p.m.	Correction for phosphate, %	Potassium found by chemical method— uncorrected, p.p.m.	Potassium found by chemical method— corrected, p.p.m.	Potassium found by flame-photometric method— uncorrected, p.p.m.	Potassium found by flame-photometric method— corrected, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Green eucalyptus	50	17·7	+0·21	5820	6001	6020	6033	+3·5	+0·5
Yellow eucalyptus	125	41·3	+0·50	2220	2320	2280	2291	+2·6	−0·1
Dried tobacco	42	7·1	+0·09	20,420	21,080	20,900	20,919	+2·3	−0·8
Dried tobacco	108	17·5	+0·21	10,010	10,334	10,300	10,321	+2·8	−0·1
Dried tobacco	150	13·8	+0·17	6650	6850	6940	6952	+4·4	+1·5
Desert weed	7	3·3	+0·04	22,740	23,444	23,200	23,209	+2·1	−1·0
Green citrus	14	7·4	+0·09	13,850	14,290	14,200	14,213	+2·5	−0·5
Green citrus	20	10·6	+0·13	8630	8900	9040	9052	+5·0	+1·7
Yellow citrus	143	13·6	+0·17	6797	7048	7000	7012	+2·9	−0·6
Yellow citrus	150	13·6	+0·17	7150	7372	7430	7443	+3·9	+0·4

* No correction for calcium was applied, as any interference caused by the concentrations found was negligible.

DETERMINATION OF SODIUM AND POTASSIUM IN NATURAL WATERS—

Six samples of deep-well and river waters were concentrated by evaporation. As before, sodium and potassium were determined in aliquots of the concentrated samples both by chemical methods and with the E.E.L. flame photometer. The results are shown in Tables VII and VIII.

TABLE VII

COMPARISON OF THE RESULTS OF THE DETERMINATION OF SODIUM IN NATURAL-WATER SAMPLES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Source of water	Calcium in sprayed water, p.p.m.	Correction for calcium, %	Phosphate in sprayed water,* p.p.m.	Sodium found by chemical method— uncorrected, p.p.m.	Sodium found by chemical method— corrected, p.p.m.	Sodium found by flame-photometric method— uncorrected, p.p.m.	Sodium found by flame-photometric method— corrected, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Bat-Yam ..	19·7	−4·3	0·11	21·1	22·3	23·1	22·1	+6·0	−1·1
Holon ..	13·7	−3·0	0·10	50·0	51·2	54·4	52·8	+8·8	+1·3
Yarkon ..	8·9	−2·0	0·14	123·8	126·8	128·8	126·2	+4·1	−0·5
Kfar-Ono ..	11·3	−2·5	0·13	26·2	26·8	27·6	26·9	+5·3	+0·4
Tel-Aviv ..	6·1	−1·3	0·02	230·0	235·5	236·0	232·9	+2·6	−1·1
Tel-Gibborim	15·5	−3·4	0·10	46·0	47·1	48·0	46·4	+4·3	−1·5

* No correction for phosphate was applied, as any interference caused by the concentrations found was negligible.

TABLE VIII

COMPARISON OF THE RESULTS OF THE DETERMINATION OF POTASSIUM IN NATURAL-WATER SAMPLES BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Source of water	Calcium in sprayed water,* p.p.m.	Phosphate in sprayed water,* p.p.m.	Potassium found by chemical method— uncorrected, p.p.m.	Potassium found by chemical method— corrected, p.p.m.	Potassium found by flame-photometric method, p.p.m.	Deviation of uncorrected results, %	Deviation of corrected results, %
Bat-Yam ..	59	0·33	1·50	1·55	1·62	+8·0	+4·5
Holon ..	55	0·40	2·58	2·66	2·62	+2·7	−1·5
Yarkon ..	62	0·80	6·80	7·02	7·12	+4·7	+1·4
Kfar-Ono ..	34	0·40	1·17	1·21	1·20	+1·8	−0·8
Tel-Aviv ..	122	0·50	6·15	6·35	6·27	+2·0	−1·3
Tel-Gibborim	62	0·40	0·92	0·95	0·95	+3·3	0·0

* No correction has been applied to the flame-photometric results, as the interference due to calcium and phosphate was negligible.

DETERMINATION OF SODIUM AND POTASSIUM IN SERUM—

Sodium was determined in six fresh samples of human blood sera both chemically by the potassium pyroantimonate method[22] and flame photometrically by preparing and spraying a (1 + 499) dilution in demineralised water.

Potassium was determined in the same hemolysis-free sera both chemically by the cobaltinitrite method[22] and flame photometrically by preparing a (1 + 49) dilution in demineralised water. The results are shown in Table IX.

TABLE IX

COMPARISON OF THE RESULTS OF THE DETERMINATION OF SODIUM AND POTASSIUM IN SAMPLES OF BLOOD SERA BY CHEMICAL AND FLAME-PHOTOMETRIC METHODS

Calcium in sprayed diluted (1+499) serum,* p.p.m.	Phosphate in sprayed diluted (1+49) serum,* p.p.m.	Sodium found by chemical method,† p.p.m.	Sodium found by flame-photometric method, p.p.m.	Deviation, %	Potassium found by chemical method,† p.p.m.	Potassium found by flame-photometric method, p.p.m.	Deviation, %
0·22	0·25	3160	3250	+2·8	193	200	+3·6
0·19	0·26	3120	3200	+2·6	220	225	+2·3
0·21	0·22	3200	3170	−0·9	202	195	−3·5
0·24	0·20	3400	3350	−1·5	220	210	−4·5
0·17	0·23	3350	3450	+3·0	170	165	−2·9
0·20	0·28	3280	3220	−1·9	172	180	+4·6

* No interference caused by the concentrations present. † Corrected for error of recovery.

Discussion of results

Very large discrepancies are observed when the results of chemical determinations are compared with those of flame-photometric determinations, especially for sodium, amounting in one extreme case to 91·2 per cent. (see Table V). The flame-photometric results are almost always higher than those by chemical methods. For sodium this seems to be due chiefly to two factors (*a*) the low recovery by chemical determinations [a series of ten recovery tests on standard solutions containing 2 to 5 mg of sodium (the usually encountered range) was carried out by the same chemical method and under similar conditions; the average for the well agreed results obtained was 97·6 ± 0·3 per cent.] and (*b*) a relatively large positive interference effect due to calcium, which appears to be caused by the insufficiency of the sodium filter to prevent completely the transmission of calcium bands (6030, 6240 and 6480 A), which are quite close to the sodium doublet (5890 and 5896 A), and the enhancement of the sodium emission in presence of calcium. The negative error effect of phosphate ions is too weak to counteract the calcium effect, as the concentration of phosphate ions in all the soil, plant-material, water and serum samples investigated was found to be either below the limit required to cause interference or, at the most, negligibly small.

The relatively high flame-photometric results for potassium are mainly due to the low recoveries in the chemical determinations. The average obtained for a series of ten recovery tests on standard solutions containing 1 to 5 mg of potassium was 96·9 ± 0·4 per cent. The calcium effect on 10 p.p.m. of potassium is negligible up to 300 p.p.m., as their bands are widely separated, the potassium doublet being at 7660 and 7690 A. However, none of the samples analysed contained such a high concentration of calcium. The negative error effect of phosphate ions, although stronger for potassium than sodium, was negligibly small with the concentrations of phosphate ions encountered.

To obtain better agreement between the results of the chemical and flame-photometric determinations, it was found necessary to apply a constant positive correction for the percentage error of the recovery of sodium and potassium by the chemical methods, and, a negative correction for calcium and a positive correction for phosphate ion-concentrations of the sprayed solutions on which sodium and potassium were determined flame photometrically. For this purpose calcium and phosphate concentrations were determined and the corrections applied were based on Figs. 2 and 3. The phosphate-ion concentrations of the samples of soil, plant material, water and serum were below the level required to affect the determination of sodium. This is also true of the calcium effect on the determination of potassium in these samples and in all such cases no correction was applied. With blood serum neither the calcium nor phosphate-ion content warranted any significant interference correction for either sodium or potassium.

The success of the applied corrections can be seen from the percentage deviation of the flame-photometric results from the chemical ones (see Tables III to IX). Most of the large discrepancies have been considerably reduced and the deviations kept within acceptable limits, considering the small quantities measured.

It was found that, in general, the corrected results of the determinations of sodium and potassium were lower by the flame-photometric method than by the chemical methods. This is probably because of a negative error effect due to the presence of anions other than phosphate. The exceptions may indicate the presence of some interfering agents that have a positive error effect and that have not been considered. Small corrections for reagent blanks were applied in the calculation of the results of the chemical determinations.

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LABORATORY FOR CHEMICAL ANALYSIS
MINISTRY OF AGRICULTURE
TEL-AVIV, ISRAEL

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The Determination of Excess of Zinc in Zinc Oxide

BY H. J. ALLSOPP

This paper describes a hydrogen-evolution technique whereby excess of zinc in zinc oxide is determined. Under vacuum, the sample to be analysed is dissolved in previously distilled hydrochloric acid. After solution, the acid is evaporated to ensure that all the evolved gas is released. The gases are then analysed for hydrogen content, from the amount of which the excess of metal is calculated.

The method was standardised radiochemically and, although the values obtained showed only about a 70 per cent. recovery, the method is believed to be absolute. The influence of grinding on the specimen has been studied.

It is estimated that the method is sensitive to a 0·1 p.p.m. excess of zinc in zinc oxide by weight. The zinc oxide specimens analysed so far have been found to contain an excess of zinc within the range 0·2 to 18 p.p.m. by weight. The reproducibility of the method is good.

A KNOWLEDGE of the amount of excess of zinc contained in zinc oxide was desired during diffusion and sintering investigations.[1,2,3,4]

In the first investigation, the diffusion of zinc in zinc oxide was being determined in atmospheres of differing oxygen content and it was important to find out whether the diffusion rates observed could be correlated with departure of the oxide from stoicheiometry. In the second investigation, the sintering behaviour of zinc oxide powder had been found to depend on the amount of oxygen in the surrounding atmosphere, and here again it was important to try to establish whether the sintering could be correlated directly with changes in the composition of the powder.

It was clear at the start that the excess of zinc to be determined was very small.[5] Little attention has been paid so far to the measurement of small departures from stoicheiometry, so that, apart from the above-mentioned immediate requirement, it appeared that any attempt to determine non-stoicheiometry in samples of zinc oxide would be worth while.

The greatest merit of the determination of the extent of non-stoicheiometry by chemical means is the directness of the approach. Measurements on the loss of weight of zinc oxide after being heated are not helpful, since there is appreciable volatility, *i.e.*, loss of both zinc and oxygen, under the conditions in which the presence of excess of zinc becomes evident, *e.g.*, in terms of colour change or semi-conductivity. Optical and electrical methods of estimating non-stoicheiometry involve assumptions about the relation between the relevant defect centres and the departure from stoicheiometry.

It must be emphasised that the chemical analysis of a material, the over-all composition of which is non-stoicheiometric, cannot in principle give any information about the way in which the non-stoicheiometry is present in the material. For example, when excess of zinc is determined in zinc oxide, the method of analysis cannot itself distinguish between zinc oxide crystals incorporating an excess of zinc in their lattice and a mechanical mixture of stoicheiometric zinc oxide crystals with free zinc metal. However, it is reasonable to believe

that any excess of zinc found in zinc oxide that has been subjected to conditions of temperature and atmosphere in which no zinc metal would be expected to remain in the free state can be ascribed to a non-stoicheiometric lattice.

Previous work on chemical determination of excess of zinc in zinc oxide has been carried out by Ehret and Greenstone,[6] and by von Wartenburg (reported by Mollwo and Stöckmann[7]). Ehret and Greenstone studied the red variety of zinc oxide first prepared by Kutzelnigg[8,9] in which the excess of metal was found to be approximately 0·02 per cent. by weight by (*i*) vacuum distillation of metal from the oxide at 335° C, (*ii*) reduction of potassium dichromate, (*iii*) reduction of potassium permanganate and (*iv*) reaction with bromobenzene.

Von Wartenburg made use of the reduction of potassium permanganate, with precautions to prevent spurious reduction of the reagent. This method was applied to determining the excess of zinc in specimens of zinc oxide that had been deposited by Mollwo and Stöckmann on glass plates inserted in a coal-gas flame containing zinc vapour. By varying the amount of oxygen in the burning gas, the colour of the zinc oxide deposit could be varied from white, through yellow to black. Von Wartenburg found a 0·5 to 1 per cent. excess of zinc by weight in the yellow deposits.

The above-mentioned varieties of zinc oxide may indeed consist of crystals containing all the measured excess of metal in a non-stoicheiometric lattice, although it is possible to argue against this. However, the excess of zinc in the specimens of zinc oxide used in the diffusion and sintering work already mentioned (prepared in oxygen or argon at temperatures up to 1450° C) and believed to be contained in the crystal lattice was, on the whole, certainly much less than the excess of zinc reported by Ehret and Greenstone and by von Wartenburg. Many of the present specimens are typical of those used in studies made of the semi-conduction of zinc oxide.

Experimental

Early work and considerations—

At first a method similar to that of von Wartenburg was tried. Samples of zinc oxide that had been sintered in argon at 1300° C and ground in an alumina mortar were dissolved in an excess of 0·01 *N* potassium permanganate containing 30 per cent. w/v of sulphuric acid. The residual permanganate was then measured by adding an excess of ferrous ammonium sulphate and titrating with further permanganate. Dissolution and titration were carried out under oxygen-free nitrogen and all solutions were prepared with doubly distilled water. The results obtained were erratic and too close to the estimated limit of detection of the technique (0·002 per cent. excess of zinc by weight) to be acceptable. An attempt to improve this approach by measuring the residual permanganate spectrophotometrically failed for the same reason. These reactions could also be criticised on the grounds that it was conceivable that some hydrogen from the reaction of the excess of metal with the acid could escape before reacting with the permanganate or other reagent used. Another method was therefore sought.

Attack of the oxide by acid, followed by measurement by a gas-analysis technique of the hydrogen produced by the excess of metal was an attractive proposition. In principle, this idea was similar to the method used with success to determine excess of barium in barium oxide,[10,11,12,13] although with this oxide the excess of metal was greater and water or water-vapour could be used as reactant.

By solution in acid, the solid zinc oxide could be completely broken down, giving the excess of metal the best possible opportunity to react. Further, if the solution could be distilled *in vacuo* in the apparatus, it would be reasonably certain that all the hydrogen was liberated.

In the first experiments by the hydrogen-evolution technique, sulphuric acid was used as the reactant. Under a vacuum of 10^{-4} mm of mercury or better, the specimen to be analysed was dropped into 90 per cent. w/v sulphuric acid at room temperature (the acid having been previously degassed by being boiled under the same vacuum) and the gas evolved during the reaction was analysed for hydrogen in the way to be described later. Unfortunately sulphuric acid was found to be objectionable (*i*) because of too slow dissolution of the zinc oxide and (*ii*) because it attacked the wax used on some of the joints of the apparatus. However, a few experiments in which pieces of zinc metal, weighed on a microbalance, were used as specimens gave encouraging results, which are shown in Table I. (The rapid solution in this case obviated the troubles named in (*i*) and (*ii*).)

TABLE I

RECOVERY OF METALLIC ZINC, WITH SOLUTION IN SULPHURIC ACID: ANALYTICAL VOLUME, 685 ml; TEMPERATURE, 20° C

Metallic zinc (by microbalance), μg	Hydrogen pressure, μ of Hg	Zinc equivalent, μg
89 ± 3	31·75	83
50 ± 3	21·55	56
30 ± 5	8·46	22

THE HYDROCHLORIC ACID METHOD—

From the experience gained with sulphuric acid, it was decided to investigate the possibility of using hydrochloric acid instead. The ease of solution of zinc oxide in this acid would be helpful, and experiments soon showed that its vapours could be condensed in liquid-nitrogen traps and that dry hydrogen chloride was absorbed by soda-asbestos. Hence the wax that it was desired to use in joints in much of the apparatus could be protected and also it would be possible to use a distillation process to ensure release of all hydrogen produced.

APPARATUS—

The apparatus consists essentially of two parts, a reaction system and an analytical system. A schematic diagram of the whole apparatus is shown in Fig. 1.

The reaction system consists of four bulbs, which may be cooled with liquid nitrogen. Bulb 1 is provided with a side-arm to house the sample and a sealed glass tube filled with iron powder to act as a "pusher." Bulb 2 is provided with a side-arm through which 50 per cent. v/v hydrochloric acid may be added.

The reaction vessels are connected to the main apparatus by a cone and socket joint, "A," and then via a mercury-vapour cold-trap, 1, and tap T_1 to a three-stage mercury-diffusion pump, P_1. This pump transfers the gases evolved from the reaction vessels to a small analytical system containing a palladium tube, platinum filament, Pirani gauge, soda-asbestos bulb, McLeod gauge and a cold-trap, 2. The palladium tube, soda-asbestos bulb, McLeod gauge and cold-trap can each be isolated from the analytical system by taps. A second mercury-diffusion pump, P_2, is used to evacuate the whole system and is backed by a conventional rotary oil pump. The reaction vessels can, when necessary, be directly evacuated by this backing pump via tap T_7.

ANALYTICAL PROCEDURE—

With the portion of the apparatus up to tap T_1 and the reaction vessels filled with oxygen-free nitrogen, bulbs 2 and 4 are cooled with liquid nitrogen. Fifteen millilitres of 50 per cent. v/v hydrochloric are placed in bulb 2 and the arm is sealed. The 0·5 g of crushed sample in a glass boat together with the "pusher" are placed in the side-arm of bulb 1 and this arm is sealed, and the system is then evacuated. Bulb 1 is heated with a hand torch, after which liquid nitrogen is placed around bulbs 1 and 3 and trap 1.

The liquid nitrogen at bulb 2 is removed, and the bulb is allowed to warm and the acid to distil into bulbs 1 and 3 (the liquid nitrogen at bulb 4 preventing any acid vapours that may escape from bulb 3 from reaching the waxed joints). Bulb 2 is again cooled.

Tap T_1 is closed, the sample and pusher are transferred magnetically to bulb 1 and the liquid nitrogen is removed so that the acid thaws to react with the oxide and distils back into bulb 2. A powdery deposit of zinc chloride is left in bulb 1. Solution is assisted by magnetic stirring.

The analytical system is prepared by heating the platinum filament at 600° C and the palladium tube at 350° C for 2 minutes and then cooling. Liquid nitrogen is placed at trap 2 and the soda-asbestos is exposed to the system as a precaution in the event of traces of dry hydrogen chloride findings its way past bulb 4 and trap 1. Tap T_3 is closed.

The gases are then transferred from the reaction vessels to the analytical system over a period of 5 minutes and the pressure is measured by the Pirani gauge. Any oxygen present is allowed to react with the hydrogen on the platinum filament at 600° C, and the pressure drop is measured. The remaining hydrogen is diffused to the atmosphere through the palladium tube at 350° C and the pressure drop is again measured. This procedure was recommended by Ransley.[14] The total hydrogen present is found from these two pressure changes and the zinc equivalent is then calculated.

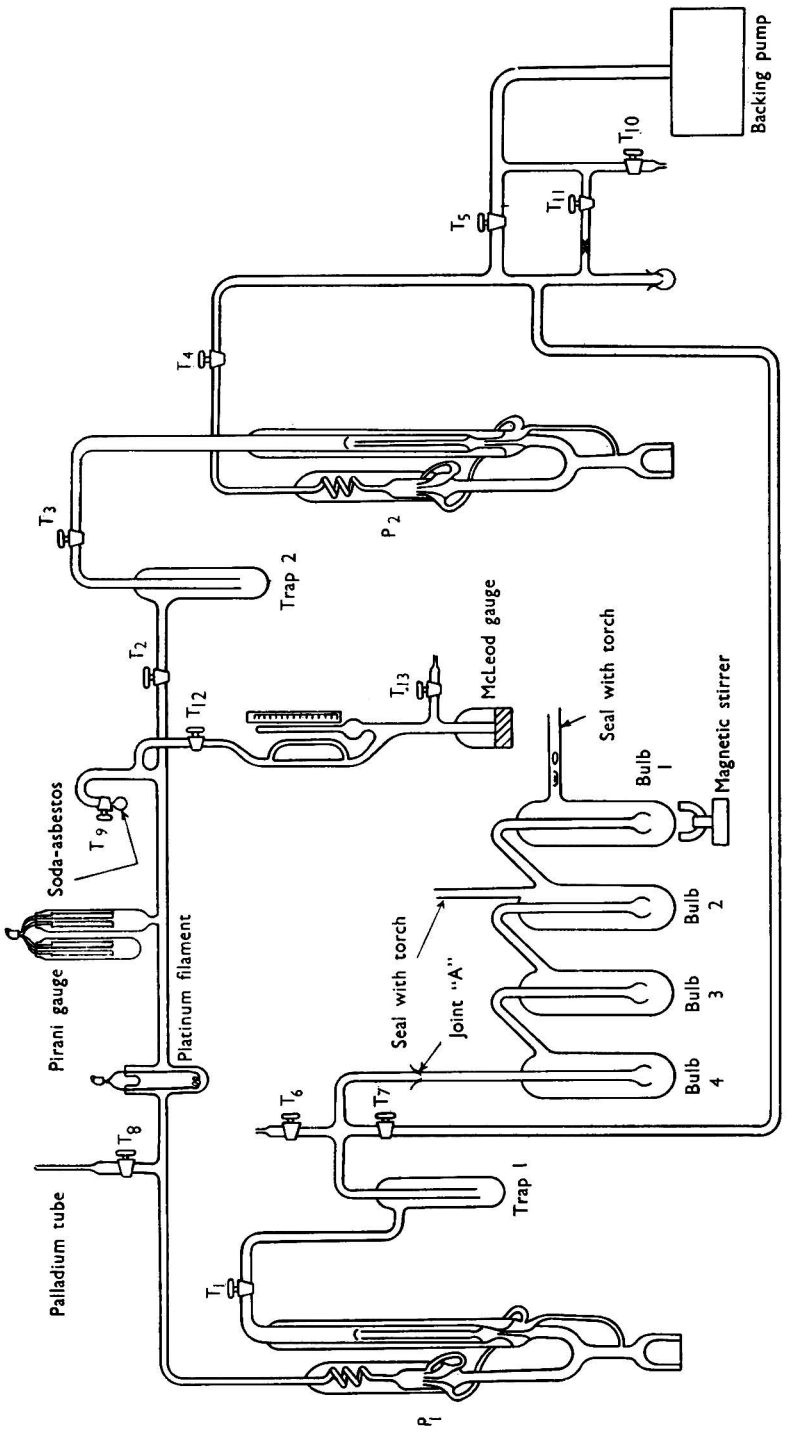

Fig. 1. Diagram of the apparatus

STANDARDISATION OF THE METHOD—

In order to standardise the method it was desired to test known amounts of pure zinc and to compare the weights with those obtained from their hydrogen equivalents. It was decided to work with evaporated layers of radioactive zinc. The evaporation technique afforded easy deposition of microgram amounts of metal, the mass of which could be determined radiochemically. This was less tedious and more accurate than the microbalance approach used in the early work with sulphuric acid.

It was found to be essential to evaporate the zinc *in vacuo* straight into the reaction system. Preliminary experiments in which evaporated deposits were first made in a separate apparatus showed that the rate of oxidation of the zinc deposit when exposed to air at room temperature was sufficiently great as to render the experiments valueless, since the reaction with hydrochloric acid would give the zinc metal equivalent, whereas the radiochemical determination would give the total zinc, including any that had become oxidised.

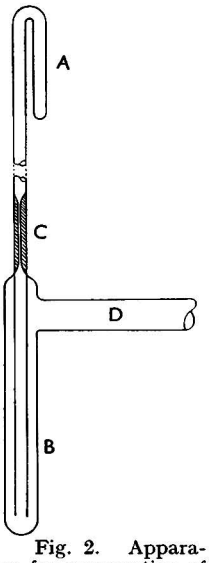

Fig. 2. Apparatus for evaporation of zinc

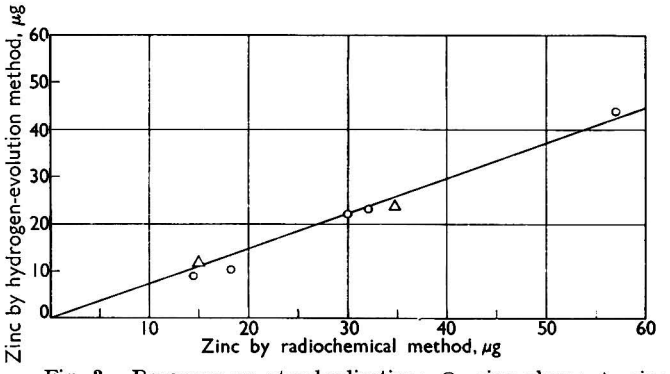

Fig. 3. Recovery on standardisation: ○, zinc alone; △, zinc + 0·5 g of zinc oxide

The evaporation apparatus constructed is shown in Fig. 2; it consists of an inverted U-tube, A, closed at one end and joined to the centre tube of a bulb, B, by means of a short length of 1-mm capillary tubing, C; the envelope of bulb B was provided with a side-arm, D. Milligram portions of the radioactive zinc (zinc-65 with a specific activity of 50 mC per g approximately) were placed in the closed end of the U-tube before assembly and the side-arm, D, of the bulb, B, was joined to the arm of bulb 1 of the reaction system (see Fig. 1). Then 50 per cent. v/v hydrochloric acid was added (under oxygen-free nitrogen) to bulb 2, the apparatus was sealed and evacuated and a portion of the acid was distilled from bulb 2 to bulb 1, which, together with bulbs 3 and 4, was surrounded with liquid nitrogen. The bulb, B, was cooled with liquid nitrogen and the U-tube was heated to 450° C. Most of the zinc condensed in the U-tube above the capillary, but microgram portions condensed at the bottom of bulb B (see Fig. 2). At this stage the capillary was sealed with a torch, so that the small portion of zinc that had reached bulb B was incorporated in the apparatus for analysis.

The reaction system, still evacuated, was then isolated from the main apparatus and a portion of the acid in bulb 1 was distilled into bulb B. The liquid nitrogen was removed from bulb B, and the acid was allowed to thaw and react with the zinc and distil back into bulb 1. The evolved gases were pumped into the analytical system and analysed for hydrogen content as already described. The zinc equivalent was then calculated.

The bulb, B, was then disconnected from the reaction system and the residue of zinc chloride was dissolved in 20 ml of 30 per cent. v/v hydrochloric acid. The gamma activity

of 10 ml of this solution was measured by means of a scintillation counter. The activity per dissolved microgram of the radioactive zinc metal used in these standardisation experiments had been determined previously by dissolving weighed samples (10 mg approximately) and diluting until the solution had a convenient count-rate (the same counting geometry was maintained throughout). Hence, after correction had been made for decay (the half-life of zinc-65 is 250 days), the mass of zinc deposited in bulb B in each evaporation was easily calculable. After allowance had been made for errors in the counting, the decay constant and the measurement of volumes, and errors in the calibration, the accuracy of the radiochemical determination is estimated to be within ± 3 per cent.

The results from the hydrogen evolution were plotted against those determined by the radiochemical method and are shown in Fig. 3. It will be seen that the relationship was nearly linear, but the values obtained from the hydrogen evolution were only about 70 per cent. of those obtained radiochemically.

Having established a recovery for zinc metal, it was thought desirable to do experiments in which zinc metal was deposited on zinc oxide in order to check whether the large residue of zinc chloride had any catalytic or similar effect on the hydrogen produced. This was done by using the same apparatus as before with 0·5 g of zinc oxide (previously found to possess a 0·25 p.p.m. excess of zinc in zinc oxide by weight by the method described in this paper) present in bulb B. The radioactive zinc was then evaporated on to the zinc oxide powder and processed as before. The results showed a recovery of the same order as that for zinc alone (see △, Fig. 3).

Possible change of composition during grinding—

It was considered essential to check whether the initial grinding of the specimen, which was necessary to obtain dissolution in a reasonable time, could alter the composition of the oxide. To investigate this, a block of zinc oxide (sintered at 1300° C in argon for 20 hours) was progressively ground and samples were taken for determination of the excess of zinc.

First a portion of this block was crushed in an alumina mortar, and the particles were shaken on to a piece of millimetre graph paper and particles of about 1 mm and 0·5 mm diameter were hand-picked from there. The remaining particles were then graded into those less than 0·25 mm, much less than 0·25 mm and very much less than 0·25 mm by "tabling" on an inclined paper. Another portion of the block was ground in a mechanical agate mortar and samples were taken after grinding for 5, 15, 20, 25, 30, 45, 60 and 120 minutes. A third portion was ground by hand in an alumina mortar for less than 1 minute in exactly the same way as in the recommended procedure and a fourth portion was ground in an alumina mortar for 120 minutes.

All these samples were then analysed.

Results and discussion

The fact that the gas evolved, on dissolution of the oxide, was hydrogen was confirmed by the Pirani - McLeod factor.

The results of the grinding experiment described in the previous section are presented first (see Fig. 4). All of the figures obtained up to 15 minutes' grinding time are in agreement and then the figures increase sharply up to 30 minutes' grinding time, after which, as far as can be seen, they remain roughly constant. It would seem, therefore, that particles of the oxide lose oxygen when heated during the short intervals of time during which they are under the pestle and that cooling is too rapid for the replacement of all the oxygen lost. However, the important practical point is that the usual grinding procedure does not appear to alter the composition of the oxide (see □, Fig. 4).

The result from the long grinding in an alumina mortar (see X in Fig. 4) agrees with the result of long grinding in agate, suggesting that contamination from mortar materials had not occurred in either case, since it would have been a remarkable coincidence if contamination had occurred to the same extent in both mortars.

Table II contains the results of determination of the excess of zinc in various zinc oxide preparations. It will be seen that the excess of zinc in the specimens was about 0·2 to 18 p.p.m. by weight, which is considerably less than that in the specimens studied by other workers.[6,7]

The reproducibility of the method was good and the sensitivity was estimated to be a 0·1 p.p.m. excess of zinc in zinc oxide by weight from figures obtained from blanks with

15 ml of 50 per cent. v/v hydrochloric acid alone. The radiochemical determinations suggested that the results were certainly accurate within 30 per cent. However, it is believed that the 30 per cent. discrepancy in the standardisation experiments arose for the following reasons (*i*) that hydrochloric acid does not react with all of the evaporated zinc that is deposited in the upper part of the inner sleeve of bulb B and (*ii*) that oxidation of zinc occurs during the evaporation. It is therefore thought that the accuracy of the determinations of excess of zinc in zinc oxide is considerably better than 70 per cent.

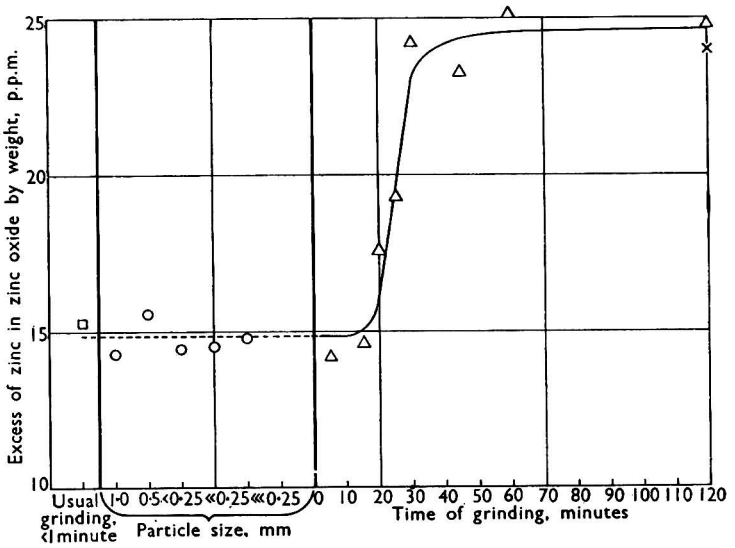

Fig. 4. Change of composition of zinc oxide during grinding: △, ground in an agate mortar; X, ground in an alumina mortar

Work is in progress on the further standardisation of the method by means of a molecular-beam technique in order to concentrate the zinc molecules on to a desired spot in bulb B. The effective volume of the distillation apparatus will be much smaller, so minimising the danger of oxidation.

It is not proposed to enter here into a full discussion of the interpretation of the various results given in Table II, since the purpose of this paper is to describe the method of analysis, but the following comments must be made. It seems reasonable to suppose that the results on sintered material correspond to the excess of zinc present in non-stoicheiometric zinc oxide crystals, because any free zinc present in the starting powder might have been expected to oxidise or vaporise during sintering. Nevertheless, it should be emphasised that (*i*) it is not yet clear how uniform is the distribution of the excess of metal in the specimens analysed and (*ii*) the first results suggest a variation of non-stoicheiometry among specimens of different raw materials treated similarly.

It is considered that (*ii*) is probably due to the influence of impurities on the non-stoicheiometric composition attained at high temperatures. However, it must not be overlooked that substituted impurity cations of valency higher than zinc could also cause evolution of hydrogen during solution of the oxide in hydrochloric acid, but the small amount of hydrogen produced by some of the preparations investigated gives an upper limit to this effect of impurity and suggests that the effect is probably small with the raw materials from which the present specimens were made.

Turning to the results for powders, the relatively high content of excess of zinc in powder heated in air at 400° C (compared with that in the powder before being heated and also after being heated in air at 700° C) will be investigated further, as will powder heated under other conditions in this temperature range, which is of particular importance to studies of the sintering, catalytic and other properties of zinc oxide. The powders were made by the French process (burning zinc vapour), so that it is possible that the excess of zinc in the powder as received could be due to free metal, but this is uncertain. Similarly, the state of the excess of zinc in powder heated to 400° C is uncertain.

Finally, previous estimates of the concentration of excess of zinc in non-stoicheiometric zinc oxide by non-chemical methods should be given. From measurement of the temperature dependance of the Hall coefficient, Hahn[15] calculated that the donor concentration in powder pressings sintered in air at temperatures between 895° and 1400° C (with various cooling schedules) was in the range $1{\cdot}9 \times 10^{16}$ to $9{\cdot}0 \times 10^{17}$ per cubic centimetre; if it is assumed that the numbers of atoms with excess of zinc equals the number of donors, the excess of zinc content of the specimens of zinc oxide would have been in the range 0·4 to 18 p.p.m. by weight. In their work on the electrical conductivity of zinc oxide with additions of aluminium oxide, Hauffe and Vierk[16] estimated that there is a fractional concentration of interstitial zinc in pure zinc oxide at 400° C of the order of 4×10^{-5}. Scharowsky[17] estimated by dispersion theory the concentration of absorption centres in needle crystals of zinc oxide that had been heated in zinc vapour at temperatures between 920° and 1220° C (the concentration of zinc atoms in the vapour ranging from $7{\cdot}2 \times 10^{18}$ to $6{\cdot}1 \times 10^{19}$). The concentration of centres varied from $6{\cdot}6 \times 10^{17}$ to $6{\cdot}8 \times 10^{18}$ per cubic centimetre. If it be assumed that the number of atoms in the excess of zinc equals the number of absorption centres, the non-stoicheiometry of the crystals would range from a 13 to 140 p.p.m. excess of zinc in zinc oxide by weight. The order of magnitude of the non-stoicheiometry estimated from these researches seems to be in rough accord with the present results of direct chemical analysis for excess of zinc.

TABLE II

TYPICAL RESULTS BY THE PROPOSED METHOD: ANALYTICAL VOLUME, 405 ml; TEMPERATURE 20° C

Specimen	Conditions of sintering: Atmosphere (pressure of 1 atmosphere)	Conditions of sintering: Temperature, °C	Conditions of sintering: Time, hours	Mass of sample, g	Hydrogen evolved, μ of Hg	Excess of zinc in zinc oxide by weight, p.p.m.
Sintered bar, approximately 1·2 cm × 1·2 cm × 10 cm (material BD)	argon	1300	20	0·506	5·68	17·5
				0·502	5·69	17·6
				0·505	5·80	17·9
				0·507	5·86	18·0
	air	1080 to 1150	26	0·504	0·92	2·8
				0·500	0·88	2·7
				0·508	0·61	1·9
				0·504	0·83	2·6
Sintered plate, approximately 1·2 cm × 1·2 cm × 0·2 cm	Bar: oxygen	1200 and then	22	0·345	0·77	3·5
Cut from bar, approximately 1·2 cm × 1·2 cm × 10 cm (material DA)	Plate: argon	1300	229	0·373	0·77	3·2
Sintered plate (as above)	oxygen	1200 and then	24	0·286	1·58	8·6
Cut from bar (as above) (material AF)	argon	1300	229	0·321	1·90	9·1
Powder, particle size originally 0·1 to 1 μ (material BH)	argon	1300	7	0·503	5·39	16·5
				0·504	5·49	17·0
Powder, particle size originally 0·1 to 1 μ (material AF)		As received		0·496	0·16	0·5
	air	400	7	0·497	1·61	5·0
	air	700	2	0·488	0·07	0·2
Powder, particle size originally 0·1 to 1 μ (material BC)		As received		0·503	0·82	2·5
	air	400	7	0·506	1·61	5·0
				0·507	1·68	5·2
	air	700	2	0·505	0·09	0·3

METHOD

PROCEDURE—

Store the specimen when received in a clean glass tube and, when required, crush it to a powder in a clean alumina mortar. Weigh 0·5 g into a small glass boat.

With all taps closed, switch on all three pumps and when pumps P_1 and P_2 are working open taps T_1, T_2, T_3, T_4 and T_5. When a suitable vacuum is reached, as indicated by the Pirani gauge, *i.e.*, less than 0·2 μ of mercury, close T_1, bleed in oxygen-free nitrogen through T_6 and when the pressures are equalised remove the cap from joint "A."

Flush the reaction system with oxygen-free nitrogen through the side-arm of bulb 1 and connect to the main apparatus with the nitrogen still flowing and leaving the vessels from the side-arm of bulb 2. Place liquid nitrogen at bulbs 2 and 4, add 15 ml of 50 per cent. v/v hydrochloric acid into bulb 2, stop the flow of nitrogen gas to bulb 1 and seal off the side-arm at bulb 2. Place the boat containing the specimen to be analysed together with a sealed glass tube containing iron powder as a pusher into the side-arm of bulb 1, and draw the side-arm down to a fine capillary with the aid of a hand-torch; stop the flow of nitrogen by closing T_6 and seal.

Close T_4 and evacuate the reaction system roughly through T_7 and close T_7. Open T_4 and then evacuate the vessels through the mercury-diffusion pumps by opening T_1. Heat bulb 1 carefully to remove any adherent oxygen and, when a suitable vacuum is reached, again less than 0·2 μ of mercury, place liquid nitrogen at bulbs 1 and 3 and trap 1. Remove the liquid nitrogen from bulb 2 and allow the acid to distil into bulbs 1 and 3, assisting distillation with gentle heat. When the distillation is complete, close T_1, and transfer the boat containing the oxide and the pusher to bulb 1, using a magnet for the purpose; replace the liquid nitrogen at bulb 2 and remove that at bulb 1 to allow the acid to thaw and react with the oxide and to distil back into bulb 2. Solution is assisted by mechanical magnetic stirring. Allow the solution and distillation to proceed without heat, topping up the cold-traps with liquid nitrogen meanwhile.

When solution and distillation are complete, run the platinum filament at dull-red heat (600° C) and the palladium tube at 350° C for 2 minutes with T_8 open, and then cool, and close T_8, isolating the palladium tube. Open T_9 to expose the soda-asbestos, place liquid nitrogen at trap 2 and close T_3. Open T_1 and pump the gases from the reaction vessels to the analytical system over a period of 5 minutes. Close T_1 and take the pressure reading on the Pirani gauge. Run the platinum filament at about 600° C for 2 minutes and record the reading on the Pirani gauge. Open T_8, place the small furnace, running at 350° C, over the palladium tube and diffuse out the hydrogen until the pressure is constant. Note the reading on the Pirani gauge and remove the small furnace from the palladium tube. Open T_3, evacuate the analytical system and close T_8 and T_9.

Remove the liquid nitrogen from bulbs 2 and 3 and trap 1, and open T_6 to the atmosphere to let in air to the reaction system. When the system is full of air, remove the vessels from the apparatus, replace the cap at joint "A," close taps T_4 and T_6 and open tap T_7 to roughly evacuate the system to T_1. When trap 1 has thawed completely, close T_7 and open T_4 and then T_1. Pump for about 1 hour and during this period remove the liquid nitrogen from trap 2. Close all taps, switch off the mercury-diffusion pumps, open T_{10} and switch off the backing pump.

Tap T_{11} is used only for slow rough evacuation of the system and T_{12} and T_{13} when the McLeod gauge is being used.

CALCULATION—

The pressure drops during catalysis (if any) and during the diffusion through palladium are converted to microns of mercury on an air-calibration graph and then to the pressure of hydrogen as microns of mercury, 1·60 being taken as the Pirani factor for hydrogen (determined experimentally), as follows—

$$\frac{\text{Pressure drop on catalysis on platinum, microns of mercury}}{2{\cdot}10} = \text{Hydrogen pressure, microns of mercury.}$$

$$\frac{\text{Pressure drop on diffusion through palladium, microns of mercury}}{1{\cdot}60} = \text{Hydrogen pressure, microns of mercury.}$$

$$\frac{\text{Total hydrogen, microns of mercury} \times 65{\cdot}38 \times \text{analytical volume, ml}}{22{\cdot}4 \times 760 \times \text{weight of sample, g}} = \text{Excess of zinc, p.p.m.}$$

I express my gratitude to Mr. J. P. Roberts for unstinted help and advice and for the radiochemical determination of zinc, to Mr. H. W. Sumner for glassblowing, to Messrs. H. C. Davis, W. N. Mair and W. Watt for advice, particularly during the early stages of this

work and to Mr. T. R. F. W. Fennell for weighing microgram amounts of zinc metal. Reproduced by permission of the Controller, H.M. Stationery Office.

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Determination of Gold in Sea Water by Radioactivation Analysis

BY R. W. HUMMEL

Small portions of sea water were irradiated with slow neutrons in a Harwell Pile and the radioactivity from the gold-198 produced was compared with that from a standard gold solution. The gold contents found depended on the distance from the shore that the samples were taken, and varied from about 400 μg per cubic metre for English coastal water to about 15 μg per cubic metre for water from the north-western limit of the Bay of Biscay.

OF the many investigations of the gold content of sea waters, the first reported apparently was that of Sonstadt.[1] Many more followed until after the First World War, when Fritz Haber embarked on a seven-year study at first intended to relieve the burden of Germany's war debts, but which culminated in the desire to add to scientific knowledge by determining as closely as possible the actual gold content of sea water. This change in emphasis occurred when he discovered that the gold content was much lower than earlier studies had led him to believe.[2,3,4]

In their very careful studies involving thousands of analysed samples from the European and North American coasts and from both the tropical and arctic Atlantic, Haber and his co-workers strove to eliminate the disturbing effects of gold introduced with the reagents and by laboratory dust, as well as gold leached out of containers by the corrosive waters and gold adsorbed on the vessel walls. Bauer has stated[5] that Haber disregarded both the possibility of adsorption and the likelihood of the oxidation of colloidal gold to ionic forms by dissolved oxygen in the sea, but he was at fault in each case, as can be seen by a perusal of Haber's papers.[2,3]

The analytical method finally developed by Haber was, in brief, to precipitate lead sulphide in the sea-water sample within a few minutes of its collection by adding to it solutions of lead acetate and ammonium sulphide, all the gold being occluded and errors caused by adsorption on the walls or by leaching being minimised. After several minutes the precipitate was treated with lead formate and boric acid and the whole was converted by heating to a lead regulus embedded in a lead borate slag. Finally, by carefully controlled heating the lead nucleus was melted and scorified, leaving a gold - silver granule measurable without removing it from the slag in which it was embedded. Further careful heating near the melting-point of gold caused the silver to go into the slag. The gold granule embedded

in the slag was measured microscopically after placing it in 1-bromonaphthalene, the refractive index of which equalled that of the slag. The mean of all the gold determinations made on samples collected during trips across the South Atlantic by the German research ship "Meteor" (1635 samples were taken for gold analysis, at 186 stations) was 4×10^{-9} g of gold per kg of sea water.

Samples from North Atlantic and Arctic waters led Haber to believe that the South Atlantic was a somewhat gold-poor area, and he stated[3] that the sea near Iceland and east coast of Greenland exhibited a mean gold content about ten times greater than the average of the "Meteor" samples. Haber found not only that large sea areas appeared to differ in their gold contents, but also that individual samples taken almost simultaneously at the same location gave widely variable results, and he concluded, after many experiments, that the local variations were due to the likelihood of gold occurring not only as chloroauric ions but also as colloidal particles or adsorbed on or included in suspended inorganic and organic matter. This refutes the comment of Putnam[6] that Haber and others "have not appreciated the extreme variability of sea water with respect to biologically active trace elements."

Among the more recent investigations have been those of Caldwell,[7] Stark[8] and Putnam.[6] These re-emphasised the variability to be expected, the values found covering the range from 20 to 2000 μg per cubic metre. In general the highest values were found in coastal waters. Putnam, using a modification of Haber's method (but with ferric hydroxide to carry down the gold, a procedure considered unsuitable by Haber not only because small amounts of the precipitate do not occlude all the gold, but also because of its awkward filtering properties) and finally determining the gold colorimetrically by an improvement of the stannous chloride test used by Sonstadt in 1872, found no detectable gold in samples taken from both the eastern and western coastal areas of the United States, from the Gulf Stream near Florida or from a point 100 miles offshore from New York. He concluded that sea water ordinarily contains less than 0·5 μg of gold per cubic metre, since the analytical method was sufficiently sensitive to detect gold added to sea water at a concentration of 0·5 μg per ton.

In view of the wide variations and uncertainties in much previous work, owing almost certainly to the difficulties inherent in the classical methods when extended to their lower detection limits, it seemed desirable to apply the radioactivation method, which has a lower detection limit for gold, at a flux of 10^{12} neutrons per sq. cm per second and a minimum detectable radioactivity of 20 disintegrations per minute, of $2{\cdot}5 \times 10^{-11}$ g (the amount present in 2·5 ml of sea water containing 10 μg per cubic metre).

Experimental

The samples of sea water analysed for their gold contents were as follows—

(*a*) surface samples collected by the author at various distances offshore from Portland Bill,

(*b*) surface and sub-surface samples obtained at International Hydrographic Station E1, 25 miles offshore from Plymouth, through the kind co-operation of L. H. N. Cooper and F. A. J. Armstrong of the Marine Biological Association of the United Kingdom, and

(*c*) surface and sub-surface samples from the north-western limits of the Bay of Biscay at 46° 30′N, 08° 00′W, again collected by F. A. J. Armstrong.

The results of a number of analyses of deep-sea waters stored for several years in a glass carboy or in a polythene bottle will not be discussed, since it is suspected that adsorption of gold on the vessel walls has rendered them valueless. The amount of gold found in the polythene-stored water was so low compared with that stored in the carboy that adsorption was suspected. Experiments in which gold-198 was added to sea water in polythene bottles has confirmed this view, as has also an experiment in which surface water from 1 mile off Portland Bill was collected almost simultaneously in a polythene bottle and in six silica tubes in which the water was to be irradiated. Subsequent radioactivation analyses of these samples showed that over a 3-week interval the gold content of the sea water in the polythene bottle decreased to less than a quarter of that collected directly in the irradiation tubes.

New silica irradiation tubes were made for all samples collected from Portland, Plymouth and the Bay of Biscay. The silica was first rinsed with hot aqua regia, then with demineralised

water and finally flame-dried. The tubes were made with a long narrow neck and held from 8 to 10 ml. After fabrication they were flame-dried, partly evacuated on the inter-laboratory vacuum line and sealed off at the narrow end. In each experiment, four tubes contained only sea water, two contained standard gold solution (71·7 mg of gold per litre) either with or without sea water and two tubes were left empty. Both the sea-water tubes and blank tubes were made from the same two pieces of 12-mm and 3-mm bore silica tubing. The narrow ends of the standard tubes came from a second piece of 3-mm bore silica, but in this case any possible difference in the amount of gold leached from these tubes, compared with the other six, would be insignificant owing to the overwhelming preponderance of gold added in the standard solution.

The surface samples from Portland were collected simply by dipping the tubes into the water and breaking off their tips. Surface samples from Plymouth and the Bay of Biscay were taken in a polythene bucket and the irradiation tubes were filled from it within a minute of collection. Sub-surface samples were taken with a Perspex-lined Nansen - Petterssen water bottle from which portions of the water were run into a 200-ml polythene beaker in which the tips of the tubes were broken off. The beaker had been previously washed with aqua regia and kept free from dust until used. The water was in contact with the Perspex for 3 or 4 minutes and with the polythene for less than 1 minute. After collection, the open ends of the tubes were closed with plastic caps tightly fitted to keep an air gap between the caps and the sea water, so minimising the possibility of adsorption of gold by the caps.

On arrival at the laboratory the caps were removed and any excess of water was shaken out to give a remaining volume of from 4 to 6 ml. The outer surfaces of the tubes were then carefully rinsed with demineralised water. The tubes were weighed on a balance not used for any weighings in which milligram amounts of gold were involved.

The corresponding blank tubes then had their tips removed and were washed and weighed in the same manner as the sea-water tubes.

Then about 0·4 g of standard gold solution, containing 71·7 mg of gold per litre, was added to the remaining two tubes of the set. These may or may not have been filled with sea water. Control experiments revealed no appreciable difference in the results whether the gold solutions were irradiated with or without admixed sea water. The amounts of solution added were determined by weight difference. The gold used to make up the standard solution was precipitated with hydroquinone from a 2 per cent. w/v gold chloride solution. Subsequent irradiations of the standard solution, followed by chemical treatment as described below, revealed no detectable active impurity on following the decay through six to seven half-lives.

For the neutron irradiations of these samples in the Pile, the apparatus designed by Pate[9] was used, in which the eight open narrow-necked silica tubes were held vertically in a graphite housing placed inside an aluminium can. The samples were irradiated for 6 days in a thermal column of the Harwell BEPO Pile and then left for 2 or 3 days to allow the activity to fall to a convenient working level.

ANALYTICAL PROCEDURE—

The analytical procedure described below was evolved with tubes containing no added gold and is based on a method described by Goldberg and Brown.[10] The outside of the tube was rinsed with hot aqua regia containing a trace of inactive gold in order to remove possible active-gold contamination. Then about 2 ml of 10 per cent. hydrochloric acid were introduced and the tube was heated gently until, in those instances where the tube had gone dry during irradiation, the solids had dissolved. The tube was then inverted over a tall 250-ml Pyrex-glass beaker that had previously been cleaned with hot aqua regia containing inactive gold and the washing solution expelled by heating the closed end. (All beakers were numbered and reserved for use only with similar samples. For example, beakers 1 and 2 were used for the blanks and never contained gold activities greater than several hundred disintegrations per minute per ml. Other glassware was similarly reserved. Between experiments all were rinsed two or three times with hot aqua regia containing added inactive gold.) The tube was washed once more with 10 per cent. hydrochloric acid, twice with hot aqua regia containing a trace of inactive gold and finally twice more with 10 per cent. hydrochloric acid. Five millilitres of 2 per cent. w/v gold chloride solution were added and the solution was evaporated nearly to dryness. A few drops of aqua regia were added to ensure complete chemical exchange between active and inactive gold, and the solution was again evaporated

almost to dryness. It was then transferred to a separating funnel by using 25 ml of 10 per cent. hydrochloric acid and then an equal volume of ethyl acetate. After extraction, the acetate layer was washed twice with equal volumes of the 10 per cent. acid and then transferred back to the original beaker. This in the meantime had been thoroughly rinsed to ensure that any minute particles of silica originating from the breaking off of the tip of the tube were not carried through to contaminate the gold finally precipitated and prepared for counting. After evaporation of the ethyl acetate solution to dryness, the gold was dissolved in several drops of aqua regia. This was heated nearly to dryness to remove most of the nitric acid and then diluted to give about 25 ml of 25 per cent. hydrochloric acid solution. The solution was heated to boiling, 10 ml of 5 per cent. hydroquinone solution were added and the mixture was boiled gently for several minutes or until the gold had coagulated to leave a clear solution. The gold was washed twice with hot water and then dissolved in a few drops of aqua regia. The entire procedure from extraction with ethyl acetate to precipitation with hydroquinone was repeated. The final gold precipitate was washed three times with hot water (cold water was added and brought to the boil, so as to break up the larger particles and make possible a more even deposit on the counting tray) and twice with acetone. After being dried, the coarse gold powder was transferred directly to a weighed aluminium counting tray and flattened down with a numbered spatula. A measured amount of a dilute acetone solution of collodion was added to affix the gold to the tray. A few minutes' drying under an infra-red lamp was followed by weighing and counting.

The contents of the tubes containing the standard solution were extracted in the same way, but were expelled into a 1-litre flask containing 50 ml of concentrated hydrochloric acid and 5 ml of 2 per cent. w/v gold chloride solution. After dilution up to the mark, 10 ml were put by pipette into a 100-ml flask containing acid and gold in the same proportions as in the 1-litre flask. One millilitre of this was transferred to the appropriate 250-ml beaker for treatment as described above.

The empty irradiation tubes were finally dried and weighed to determine the weights of sea water irradiated.

An end-window Geiger tube was used for counting. The usual corrections were made for coincidence, background and chemical yield. The last was generally between 60 and 80 per cent. Decays of blank, sea water and standard samples were followed on several occasions over 6 to 7 half-lives and throughout that period exhibited a half-life of 2·69 days, within the statistical counting errors.

The mean activity of the two blank tubes was subtracted from the activities obtained from the sea waters. The blank activities averaged slightly more than 7 per cent. of the sea-water activities for all the experiments reported in Table I. The ratio increased to 20 per cent. for the last five experiments, the blanks averaging 57 counts per minute and the sea water samples 290 counts per minute.

TABLE I

GOLD CONTENTS OF SEA WATERS BY RADIOACTIVATION

Source of water	Date of collection	Depth	Number of samples	Gold content, μg per cubic metre
Portland, 1 mile..	Dec. 7, 1955	Surface	2	320 ± 150
			2	506 ± 14
			2	411 ± 10
			6	63*
Portland, breakwater	Jan. 19, 1956	Surface	3	15 ± 9
Portland, 5 miles	Jan. 19, 1956	Surface	4	185 ± 115
Plymouth, 50° 02′ N, 04° 22′ W	Feb. 21, 1956	Surface	4	96 ± 30
		20 metres	2	19 ± 13
		50 metres	2	89 ± 58
	Mar. 26, 1956	Surface	4	14 ± 9
		20 metres	4	29 ± 12
46° 30′ N, 08° 00′ W	June 22, 1956	Surface	4	13 ± 2
		45 metres	4	21 ± 6
		~600 metres	3	10 ± 3

* Mean of three experiments with water collected in a polythene bottle.

Irradiated gold from both standard solution and sea water were assumed to have the same specific activity, so that—

$$\frac{\text{Weight of gold in standard}}{\text{Corrected count rate of standard}} = \frac{\text{Weight of gold in sea water}}{\text{Corrected count rate of sea water}},$$

and hence the weight of gold per unit weight of sea water could be calculated. However, the results of this investigation are arbitrarily expressed in terms of the weight of gold per unit volume of sea water. The resulting error is considered insignificant.

The results are given in Table I. The values of the gold contents given are the means of the several values obtained by comparing each sea-water sample (the numbers of which are given in the Table) with each of the two standard samples. The standard deviation of each mean is given also.

Conclusions

The results are in general agreement both with those workers who have only been able to obtain quantitative results with coastal samples containing relatively large amounts of gold and with Haber and his co-workers, who, by extremely careful application of refined techniques, were able to determine the gold contents of deep-sea samples as well as of coastal samples.

Somewhat smaller amounts of gold were found when samples were obtained progressively further out to sea. The means of the samples collected at Portland (1 mile), Portland (5 miles), Plymouth and Bay of Biscay are, respectively, 409, 185, 49 and 15 μg per cubic metre.

The lowest values found were still well within the sensitivity of the method, taking as the criterion the relative activities of blanks and sea-water samples. As little as 3 to 5 μg per cubic metre should be distinguishable by using the method described here. If many samples were taken, to allow confident statistical treatment of the results, the lower limit would be extended still further. Reduction of the blank values seems to offer the widest scope for improvement of the method. This might best be accomplished by the use of a less corrosive reagent to remove the irradiated samples from the tubes, so reducing the amount of gold leached from the tubes themselves.

The deviations given in Table I tend to confirm Haber's opinion that much of the gold content of sea water is associated with suspended particles of organic or inorganic materials. This hypothesis is supported by the decrease in gold contents as samples are obtained progressively further from shore.

The results of some analyses of sea-water samples collected at the same time as those examined for their gold contents are given in Table II. These are reproduced with the permission of the Marine Biological Association and the Government Chemist. A decrease of about 15 per cent. in the dissolved silicate contents between February 21st and March 26th, possibly associated with the spring outburst of phytoplankton, is accompanied by a decrease in the gold contents of about 60 per cent. Many more experiments would be required to establish if there is a reliable correlation.

Table II

Analyses of sea water from international hydrographic station E1, by the Marine Biological Association (salinities determined by the Government Chemist)

Date of collection	Depth	Temperature, °C	Salinity, ‰	Phosphate, μg-atom of P per litre	Silicate, μg-atom of Si per litre	Oxygen, ml per litre
Feb. 21, 1956	Surface	8·8	35·35	0·53	2·9	—
	20 metres	8·89	35·30	0·49	2·9	—
	45 metres	8·88	35·32	0·49	2·9	—
Mar. 26, 1956	Surface	8·7	35·43	0·54	2·6	—
	20 metres	8·63	35·39	0·46	2·5	—
At Station 46° 30′ N, 8° 00′ W *in Bay of Biscay*—						
June, 22, 1956	Surface	15·3	35·71	—	0·7	5·92
	45 metres	12·84	35·64	—	1·5	5·75
	600 metres	10·30	35·58	—	7·6	4·72

The very low value obtained with sea water from the breakwater at Portland Bill remains inexplicable.

It is a pleasure to acknowledge my indebtedness to Mr. A. A. Smales, Head of Analytical Chemistry Group, Chemistry Division, A.E.R.E., who proposed the problem and made several valuable suggestions during the course of the work.

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Formol Titration: An Evaluation of its Various Modifications

BY W. H. TAYLOR

The confusion of procedure and interpretation surrounding the formol-titration method of determining amino-acid nitrogen are reviewed, its origins are traced and the principles underlying the application of the method in various experimental circumstances are examined.

Under conditions that simulate protein hydrolysis and with a final formaldehyde concentration of 6 to 9 per cent., evidence from indicator and potentiometric titrations supports the conclusions that (*a*) in determining the degree of proteolytic hydrolysis a direct titration from the pH of hydrolysis to pH 8·5 in formaldehyde gives reasonably quantitative and reproducible results, (*b*) indirect procedures do not yield reproducible results, (*c*) with preliminary neutralisation to pH 9 amino acids are under-estimated slightly and peptides severely, and (*d*) with preliminary neutralisation to pH 7 peptides are under-estimated slightly, but amino acids are not.

ALTHOUGH formol titration has been widely used to determine the extent of proteolytic hydrolysis and to determine amino acids in biological fluids such as urine, published accounts of the method in original papers and in authoritative works of reference vary widely and may cause confusion to those who wish to use it for the first time. The various procedures that are in use are summarised in this paper and the principles underlying their application in various experimental circumstances are examined. A comparison of the accuracy of these procedures, when used for determining the degree of proteolysis, is attempted experimentally for the first time.

SUMMARY OF TECHNICAL PROCEDURES OF FORMOL TITRATION

There are two chief methods of carrying out formol titration, which may be called direct and indirect. Most works of reference mention only one of these, but both are described by Brown,[1] Jessen-Hansen,[2] Cole,[3] Van Slyke and Kirk[4] and Richardson.[5]

DIRECT METHOD—

Neutralised formaldehyde is added directly to the unneutralised solution under investigation, which is then titrated with alkali to its final end-point.[3,6,7,8,9,10,11,12] The procedure

may be illustrated by reference to the titration curves of single amino acids (see Figs. 1, 2 and 3, p. 490). Titration starts at the pH of solution in formaldehyde and follows curve A to the chosen end-point, which is usually near pH 9, with phenolphthalein as indicator. Cole[3] and Bodansky and Fay[11] prefer lower end-points of pH 8·3 and 8·5, respectively, but with use of the same indicator. The formaldehyde solution is neutralised to the pH of whichever end-point is selected. After subtraction of an appropriate blank, the direct titration is taken as a measure of the amino acids and peptides present. The composition of the blank or control solution varies. In determining the degree of proteolysis, when only the increase in amino groups is required, Bodansky and Fay[11] and Davis and Smith[12] used or recommend a sample of the digestion mixture at zero time. Sorensen and Jessen-Hansen[7] and Plimmer,[9] however, describe a technique for applying formol titration to coloured solutions, in which a blank of an equivalent volume of boiled distilled water and neutralised formaldehyde is used, the initial formol titration of the substrate thereby being ignored. For the determination of solutions containing only amino acids or peptides, a water - formaldehyde blank is used.

INDIRECT METHOD—

The solution under examination is first titrated with alkali to a given pH, formaldehyde (brought to the final pH) is added, and the resulting solution is titrated to an end-point at the same or a different pH. The value of the second titration, usually after subtraction of a blank, is taken as a measure of the amino acids and peptides present. The precise pH to which it is recommended that the preliminary titration should be taken varies. Dunn and Loshakoff[13] recommend pH 6. Neutralisation to pH 7, with litmus or neutral red as indicator, is recommended by Henriques,[14] Henriques and Sorensen,[15,16] Henriques and Gjaldbäk,[17] Luers,[18] Hoppe-Seyler and Thierfelder,[19] Northrop,[20] Van Slyke and Kirk,[4] Levy,[21] Borsook and Dubnoff,[22] Sisco, Cunningham and Kirk[23] and French and Edsall[24]; to pH 8 by Brown[1]; and to pH 9 by Kendall, Day and Walker,[25] Harding and McLean,[26] Berman and Rettger,[27] Cathcart, Paton and Pembrey,[28] Lloyd and Shore,[29] Hawk, Oser and Summerson,[30] Kirk,[31] Mills[32] and Mills, Munro and Leaf.[33]

The final pH to which the titration should be taken after the addition of formaldehyde also varies. Brown[1] recommends pH 8, with phenol red as indicator, and so do Borsook and Dubnoff[22] and Sisco, Cunningham and Kirk,[23] who used electrometric methods. All the other authors mentioned take the titration in formaldehyde to pH 9, with phenolphthalein as indicator. Hence in Figs. 1, 2 and 3 (see p. 490) preliminary titration follows curve B from the pH of solution to pH 6, 7 or 9. Formaldehyde is then added and thereafter the titration follows curve A.

When both preliminary and final titrations are taken to the same pH,[1] the same indicator serves for each and the procedure is referred to as the "one-indicator" technique.[5] When the pH values of the two end-points differ, separate indicators and a "two-indicator" technique are required. To prevent difficulties of colour matching when the second indicator is added, several different devices have been adopted, *viz.*, preliminary neutralisation to pH 7 is carried out with litmus paper,[17] a control colour standard is used,[20] or independent titrations with each of the two indicators are performed on aliquots.[5] Harris[34,35] overcame the difficulty by titrating first to an end-point in alcohol and formaldehyde at pH 9, with phenolphthalein as indicator, and then back-titrating to pH 5 (methyl red) with standard acid. At this latter pH the phenolphthalein used earlier is colourless and does not interfere. The back-titration is taken as a measure of amino acids and peptides.

As with the direct titration, the composition of the blank or control solutions varies. When the absolute amount of amino acids or peptides in a liquid such as urine is to be determined, a water - formaldehyde blank is used. When following the course of proteolysis, Henriques and Gjaldbäk[17] used a sample of the digestion mixture at zero time, but Northrop[20] recommends a water - formaldehyde blank for this purpose. Levy,[21] considering principally the determination of amino acids, found that the best quantitative results are obtained when a blank is omitted. In several of the papers that have been quoted no details of the composition of the blank are given.

PRINCIPLES UNDERLYING APPLICATION OF FORMOL TITRATION—

The reasons for these very great variations of procedure emerge, to some extent, from a combined study of the principles underlying the application of the formol titration and

the historical development of its use. Certain principles that have taken many years to become established, and which are still the source of confusion, may be stated as follows—

(*i*) *Groups determined by direct and indirect titration*—Direct titration measures the total carboxyl groups of amino acids and peptides; indirect titration measures the total amino groups of amino acids, peptides, primary and secondary amines and ammonia.[4,5] This occurs notwithstanding that in both procedures it is the basicity of the $-NH_2$ or $-NH-$ group that is altered by reaction with formaldehyde. This

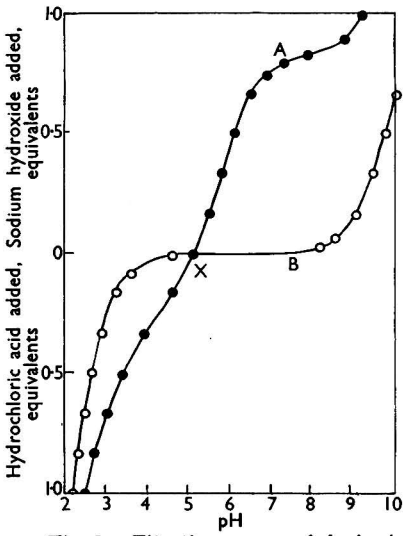

Fig. 1. Titration curves of glycine in 0·04 *M* aqueous solution at room temperature

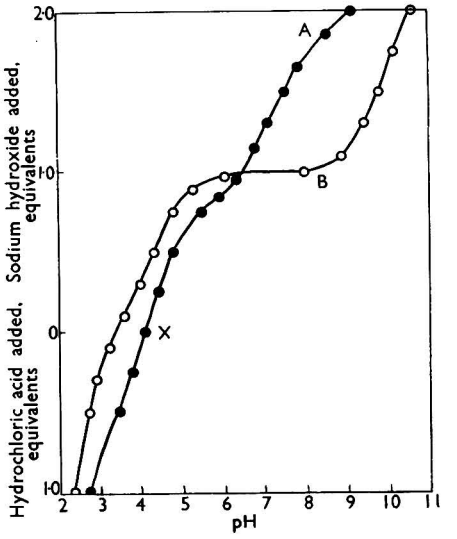

Fig. 2. Titration curves of glutamic acid in 0·04 *M* aqueous solution at room temperature

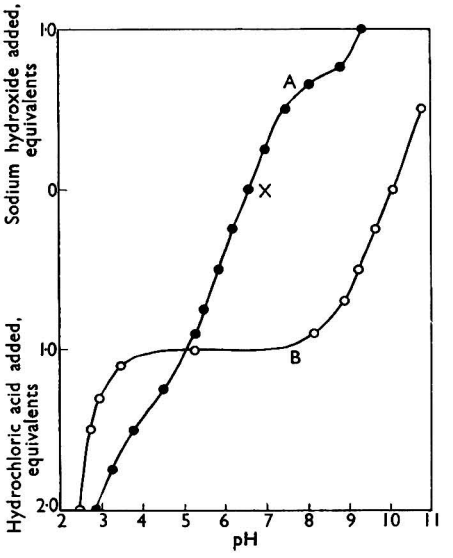

Fig. 3. Titration curves of lysine in 0·04 *M* aqueous solution at room temperature

In Figs. 1, 2 and 3, curve A was obtained with formaldehyde added. X marks the beginning of the direct titration

principle is illustrated by reference to Figs. 1, 2 and 3. Direct titration of a monoaminomonocarboxylic acid such as glycine from its pH of solution to pH 9 in formaldehyde requires one equivalent of base. Indirect titration, after neutralisation to, say, pH 7, similarly requires one equivalent of base. For a dicarboxylic acid such as glutamic acid, direct titration from the pH of solution requires however two equivalents of base, but indirect titration from pH 7 only one. For a diamino acid such as lysine the position is reversed; direct titration requires only one equivalent, indirect titration two.

(*ii*) *Accuracy of direct and indirect titrations*—With solutions containing only amino acids or peptides or both, the direct titration determines total carboxyl groups with almost theoretical accuracy; the indirect titration does not give theoretical accuracy unless the isoelectric point or points of the substance or substances being titrated and the pH of preliminary neutralisation are approximately the same.

The direct titration gives theoretical accuracy because each component amino acid or peptide is effectually titrated from its pH of solution to the chosen end-point in formaldehyde. This is what would be expected when any weak acid such as phosphoric acid or acetic acid or mixtures of both is titrated. With the indirect titration, it will be seen from Figs. 2 and 3 that titration from pH 7 in water to pH 9 in formaldehyde will require nearly the theoretical amounts of base, but that with a one-indicator technique, from, say, pH 8 or 9 in water to pH 8 or 9 in formaldehyde, less than the theoretical amount of base may be needed. Different amino acids may require differing end-points in order to be titrated with optimum accuracy, and this partly accounts for many of the modifications of the indirect procedure that have been devised.

(*iii*) *Presence of other titratable substances*—With solutions such as urine, which contain titratable substances other than amino acids and peptides, both procedures will fail to determine amino or carboxyl groups accurately, unless the interfering substances can be removed, *e.g.*, by filtration of phosphates and carbonates precipitated with barium chloride and hydroxide,[14] or unless allowance can be made for them in controls, or unless the indirect one-indicator technique be used, in which after adding formaldehyde only amino groups will be titrated. For urine and similar solutions the indirect procedure should be used and a choice made between two errors that cannot usually both be eradicated; with a one-indicator technique there is a risk of under-estimation of amino groups, with a two-indicator technique other groups titrating between the chosen pHs are included in the determination.

(*iv*) *Accuracy of direct titration in proteolytic digests*—With proteolytic digests, in which only the increase of amino groups is to be determined, quantitative accuracy may be obtained by the direct method despite the presence of other titratable substances.[2,5] This is because it is possible to use as a blank the digestion-mixture at zero time. Hence, even though digestion takes place at, say, pH 2, liberated amino acids and peptides are in effect titrated from their own pH of solution. On theoretical grounds the indirect titration could also be used to determine the degree of proteolysis, but it is shown experimentally later that, when this is attempted, considerable inaccuracies may arise. Should side-reactions during digestion cause liberation of other titratable groups, these would be determined by the direct method and an error introduced.

(*v*) *Blanks*—It follows that when the degree of proteolysis is being determined, only a digestion mixture at zero time should be used as blank. On other occasions a water - formaldehyde blank is suitable.

(*vi*) *Final concentration of formaldehyde*—The optimal concentration of formaldehyde in the reaction mixture at the end-point should be 6 to 9 per cent.[21]

It should be noted that these principles are derived in the main from theoretical considerations. Practical confirmation of the theoretical behaviour of the direct and indirect procedures has been obtained for single amino acids,[34,35,36] but not for mixtures of amino acids and proteolytic digests, and only occasionally for peptides.

The confusion that has surrounded the use of both procedures has arisen from lack of appreciation of these principles. Sorensen[6] originally used the direct procedure and applied

it first to pure solutions of amino acids, when its quantitative accuracy was established, and then to protein digests. The indirect procedure was devised by Henriques[14] for determination of amino acids in urine. After precipitation of phosphates and carbonates with barium chloride and barium hydroxide, the filtered urine was neutralised to litmus in order to prevent titration of the second carboxyl groups of the dicarboxylic amino acids. A footnote in Henriques' paper makes this clear—

> "Lackmuspapier wird zum Neutralisieren angewandt, weil Lösungen von Aminosäuren, die Monocarbonsäure sind, auf Lackmus neutral reagieren, dasselbe gilt von Aminosäuren die Dicarbonsäuren sind, wenn die eine Carboxylgruppe neutralisiert worden ist."

Henriques and Gjaldbäk[17] next applied the indirect method to the determination of proteolytic hydrolysis. As a result, subsequent workers and writers were justified in inferring the indirect method to be of general application to the determination of amino groups, both absolutely, in biological fluids such as urine, and relatively, as when following proteolysis. The method thus assumed greater importance than the direct procedure, and underwent the many modifications that have been described.

Several attempts to restore the balance between the direct and indirect procedures have been made. As early as 1923, Brown[1] found it necessary to quote extensively from Sorensen's original paper[6] to prove that the direct method was first used—

> ". . . because the description of Sorensen's method in certain text books of physiological chemistry would lead one to believe that the solution to be analysed should be titrated to the colour of phenolphthalein produced in the second stage of the control before the formalin is added."

Jessen-Hansen,[2] in a particularly authoritative account of the formol titration, stressed the importance of the blank and emphasised that, when the degree of proteolytic hydrolysis is to be determined, the indirect procedure is quite unnecessary. Further clarification of the respective roles of the two procedures was achieved by Van Slyke and Kirk[4] and Richardson,[5] arising out of the work of Harris[34,35] and Birch and Harris.[36]

Despite these attempts the quantitative accuracy of the direct titration is still challenged. Hence French and Edsall[24] write—

> ". . . if the titration is begun at the iso-electric point of the amino-acid and carried to an end-point near pH 9 in formaldehyde, the titration gives a measure of the free carboxyl groups. If the titration is started at pH 7 in water and carried to the same end-point, it gives a measure of the free amino-groups present. The latter method is generally the condition of choice; obviously the first can only be applied to a single amino-acid or to a mixture of amino-acids which are all of the same charge type."

These arguments would imply that the direct procedure is quantitatively inaccurate when determining, for example, the degree of proteolysis, unless the pH of digestion happened to be around 7, and are in this respect the reverse of those developed above. The existence of such differences of interpretation serves to emphasise that for proteolytic digests, mixtures of amino acids and for peptides, the behaviour of the two titration procedures has been deduced theoretically and that no experimental assessment of the accuracy of the titrations has been made. Such an assessment is now attempted with particular reference to determining the degree of proteolysis.

Experimental

Formaldehyde—AnalaR formaldehyde solution was neutralised to a pH of approximately 8·5 with 0·1 *N* sodium hydroxide, phenolphthalein being the indicator, and was used in amounts such that the final formaldehyde concentration of the titration mixture was 6 to 9 per cent.[21]

Amino acids and peptides—Amino acids and dipeptides and tripeptides were used in 0·04 *M* solution. Their purity was checked by paper chromatography.

Potentiometric titration—Acid - base titration curves were determined potentiometrically by using a Marconi (type TF 717A) pH meter with a temperature compensator. The titration unit consisted of an open 100-ml Pyrex-glass beaker into which dipped an electric stirrer, a glass electrode (Cambridge Instrument Co. Ltd.) and a pencil-type calomel half-cell modified by Mr. G. E. Newman from a design by Butler.[37] A standard pH of 4·00 at 15° C was given

by an aqueous solution containing 10·211 g of potassium hydrogen phthalate per litre (0·05 *M*). The sleeve of the calomel half-cell was detached and washed before each titration. The protein concentration in the titration vessel did not exceed 400 mg per 100 ml at any time.

Conditions simulating proteolysis—In order to investigate the accuracy of the direct and indirect procedures when the degree of proteolysis was being determined, it was necessary to produce conditions identical with those under which proteolytic digests are titrated, but in which the increase of titratable substances was accurately known. This was achieved by titrating both in water and, after adding formaldehyde, mixtures of buffer (glycine - hydrochloric acid or sodium acetate - hydrochloric acid or potassium dihydrogen phosphate - sodium monohydrogen phosphate) and protein (plasma or serum albumin or casein) and comparing them with the same mixtures to which known amounts of amino acids or peptides had been added. The titration curves of buffer + plasma simulate the buffer + protein + enzyme blanks (digestion at zero time), which should be used in determining the degree of proteolysis, and the buffer + plasma + amino acid or peptide curves simulate those of a buffer + protein + enzyme digest, but with the advantage that the increase of titratable groups is already known.

Results

Preliminary indicator titrations—

Comparison of the direct and indirect procedures was first attempted with indicator titrations under conditions simulating those of protein hydrolysis. In order to explain the results that were obtained, it became necessary to repeat the work with titration curves

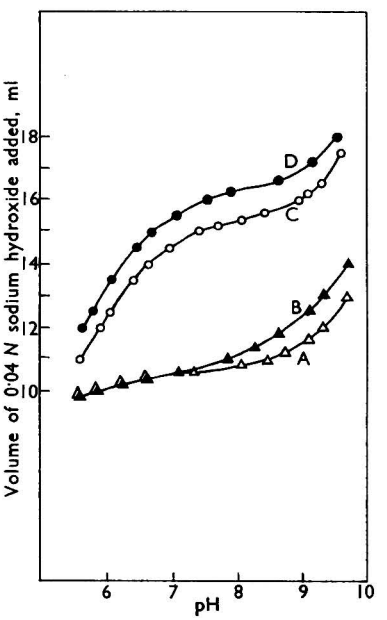

Fig. 4. Formol titration of 1 ml of 0·04 *M* glycylglycine under conditions simulating proteolytic digestion. Buffer: glycine - hydrochloric acid at pH 1·42

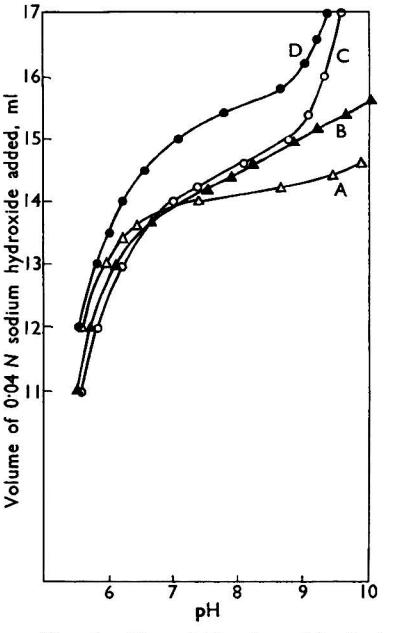

Fig. 5. Formol titration of 1 ml of 0·04 *M* glycylglycine under conditions simulating proteolytic digestion. Buffer: sodium acetate - hydrochloric acid at pH 3·61

In Figs. 4 and 5: curve A, titration of buffer + plasma; curve B, titration of buffer + plasma + glycylglycine; curve C, titration of buffer + plasma + formaldehyde; curve D, titration of buffer + plasma + glycylglycine + formaldehyde.

Direct titration is the difference at the chosen pH between curves D and C. Indirect titration is the difference, less the blank, between curves D and B at the chosen pH. The blank is the difference between curves C and A at the chosen pH

derived potentiometrically and, since these are considered fully later, only the conclusions of the indicator experiments are given, *viz.*—

(*a*) Added amino acids or peptides gave by direct titration, with phenolphthalein as indicator, values that were between 85 and 95 per cent. of the theoretical. Titration to a pale pink colour (pH about 8·5) gave slightly more consistent readings and easier matching than to a deeper colour of approximately pH 9·0. At pH 8·5 the standard deviation for the determination of glycylglycine was 0·01 ml and at pH 9·0 it was 0·02 ml.

(*b*) With the indirect method, the matching of a first end-point at pH 7 to neutral red was difficult in both control and unknown tubes and in duplicates, with consequent lack of reproducibility of results.

(*c*) When pH 9 (phenolphthalein) was used for preliminary neutralisation, very low formol titrations were obtained for peptides, from 0 to 25 per cent. of the theoretical. The titrations for amino acids were higher, but still low, being from 50 to 90 per cent. of the theoretical.

With glycylglycine, for which the theoretical titre should be 1·00 ml, the direct titre to pH 8·5 was 0·90 ml and the indirect titre (*a*) after neutralisation to pH 7 was 0·84 ml and (*b*) after neutralisation to pH 9 was 0·21 ml.

The course of both the direct and indirect procedures was next followed more fully by means of potentiometric titration curves.

POTENTIOMETRIC TITRATIONS—

Figs. 4, 5 and 6 show the potentiometric titration curves with and without formaldehyde of glycylglycine in the presence of plasma and, respectively, of glycine - hydrochloric acid buffer, sodium acetate - hydrochloric acid buffer and phosphate buffer. In each instance

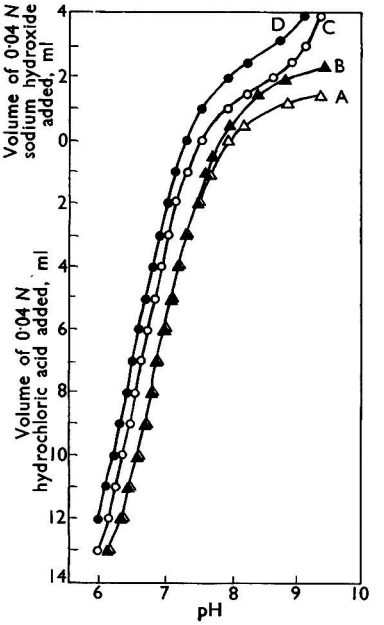

Fig. 6. Formol titration of 1 ml of 0·04 *M* glycylglycine under conditions simulating proteolytic digestion. Buffer: phosphate buffer at pH 8·04 Titration curves as for Figs. 4 and 5

curves with and without formaldehyde are given for plasma plus buffer alone in order to permit the blank titration to be accurately measured. The total volume of fluid upon which each curve has been determined has been kept the same by substituting an equal

volume of distilled water for any missing component. It is possible from these curves to calculate the formol titration by the direct method to either of the final pH values, 8·5 or 9, and by the indirect method by using any of the preliminary pH values 6, 7 or 9, and a final pH of 9. For the latter methods the curves without formaldehyde are followed as far as the selected preliminary pH value, say 7, and then the formaldehyde curves, from a point corresponding to the same amount of added alkali until the end-point of pH 9 is reached. Results calculated from Figs. 4, 5 and 6, and those obtained for several other amino acids and peptides from similar data are recorded in Table I.

TABLE I

COMPARISON OF FORMOL TITRATION OF AMINO ACIDS, PEPTIDES AND MIXTURES BY THE DIRECT AND INDIRECT PROCEDURES

Substance taken (1 ml of 0·04 *M* solution)	Buffer and protein mixture		Volume of 0·04 *M* sodium hydroxide required in titration by the direct method to—		Volume of 0·04 *M* sodium hydroxide required in titration by the indirect method, to a final pH of 9·0, from initially—		
			pH 8·5, ml	pH 9·0, ml	pH 6·0, ml	pH 7·0, ml	pH 9·0, ml
Amino acids—							
Glycine	Glycine - HCl	Plasma	0·88	0·94	0·94	0·94	0·78
Alanine	Sodium acetate - HCl	Plasma	0·99	0·96	0·98	0·95	0·81
Leucine	Sodium acetate - HCl	Plasma	1·07	1·05	0·98	1·07	0·90
Tyrosine	Phosphate	Plasma	0·93	0·86	—	—	0·47
Histidine	Glycine - HCl	Plasma	0·91	0·98	1·47	1·05	0·45
Glutamic acid	Glycine - HCl	Plasma	1·99	2·04	1·07	1·07	0·89
Glutamic acid	Phosphate	Plasma	1·89	1·90	—	0·90	0·80
Lysine	Glycine - HCl	Plasma	0·83	0·84	1·73	1·76	1·46
Lysine	Phosphate	Plasma	0·85	0·85	1·80	1·78	1·44
Peptides—							
Glycylglycine	Glycine - HCl	Plasma	0·88	0·87	0·84	0·78	0
Glycylglycine	Sodium acetate - HCl	Plasma	0·94	0·89	1·00	0·89	0·09
Glycylglycine	Phosphate	Plasma	0·86	0·85	—	0·85	0·13
Glycyltyrosine	Phosphate	Plasma	0·86	0·83	—	—	0·24
Glycylglycylglycine	Glycine - HCl	Plasma	1·05	1·07	1·04	0·97	0·14
Glycylglycylglycine	Sodium acetate - HCl	Plasma	0·93	0·95	1·05	0·96	0·16
Glycylglycylglycine	Phosphate	Plasma	0·94	0·94	—	0·90	0·02
Glycylglycylglycine	Phosphate	Serum albumin	1·00	1·05	1·05	0·95	0·17
Glycylglycylglycine	Phosphate	Casein	0·95	0·88	0·93	0·90	0
Leucylglycylglycine	Phosphate	Plasma	0·98	0·93	—	—	0·11
Mixtures—							
Alanine + leucine	Sodium acetate - HCl	Plasma	2·00	1·93	1·89	1·95	1·71
Alanine + lysine	Sodium acetate - HCl	Plasma	1·81	1·76	2·65	2·68	2·22
Leucine + lysine	Sodium acetate - HCl	Plasma	1·88	1·83	—	—	—
Leucine + lysine + alanine	Sodium acetate - HCl	Plasma	2·99	2·92	—	—	—
Glycylglycine + glycyltyrosine + glutamic acid + histidine	Phosphate	Plasma	4·40	4·54	4·30	3·62	1·60
Plasma, peptic digest	Glycine - HCl		0·92	0·86	0·79	0·54	0

DISCUSSION OF RESULTS

It will be seen from Table I that the direct method, with a buffer - protein - formaldehyde blank and an end-point of pH 8·5, gave results varying from 83 to 107 per cent. of the theoretical for all the amino acids and peptides investigated, except glutamic acid or mixtures containing it. Both carboxyl groups of this amino acid were titrated, in accordance with the predictions of Henriques[14] and Richardson[5] and the data of Van Slyke and Kirk.[4] Preliminary neutralisation to pH 6 or to pH 7 yielded very similar results, except that both amino groups of lysine were now titrated, again in accordance with the data of Van Slyke and Kirk, and that from pH 6 histidine was over-titrated.

The variability of the results obtained by direct titration and their general tendency to fall rather lower than the theoretical values may be explained partly by the error of the titration procedure (in these experiments the standard deviation was 1 per cent., *i.e.*, 0·01 ml of 0·04 *N* sodium hydroxide), partly by the fact that the ideal end-point of the titration varies slightly for individual amino acids and peptides so that the choice of any single pH, such as 8·5 or 9·0, is inevitably a compromise; and partly by the impossibility, on theoretical grounds,[3,5,12,34,35] of ever achieving a fully theoretically quantitative titration. It should be noted also that titration values for the same amino acid or peptide differ in different buffer and protein mixtures more widely than would be expected from the error of titration. The reason for this is not clear and certain possibilities such as complex formation are under investigation.

Despite variability of results caused by the factors discussed above, Table I shows that the direct method, with an end-point at pH 8·5 or 9·0, will determine amino acids and peptides, singly or in mixtures, to within the limits 83 to 107 per cent. of their theoretical value. Whichever of these final pH values is chosen would appear to make little difference to the results obtained by the direct method, the widest deviation being 7 per cent. The titration curves (Figs. 4, 5 and 6), under the conditions of these experiments, begin to rise, however, at about pH 9, so that pH 8·5 is much more nearly at the centre of the more horizontal portion of the curves, at which the pH changes most sharply with increasing volume of added alkali. For indicator titrations, therefore, pH 8·5 (a pale pink to phenolphthalein) would be preferable to pH 9, because of the potentially more sensitive end-point, and this was the finding in the preliminary indicator experiments. Since the pH range of the relatively flat part of the curve is a function primarily of the formaldehyde concentration,[21,36] it is only at the defined formaldehyde concentrations of 6 to 9 per cent. that pH 8·5 gives the more sensitive end-point. With lower final formaldehyde concentrations a higher end-point may be more desirable.

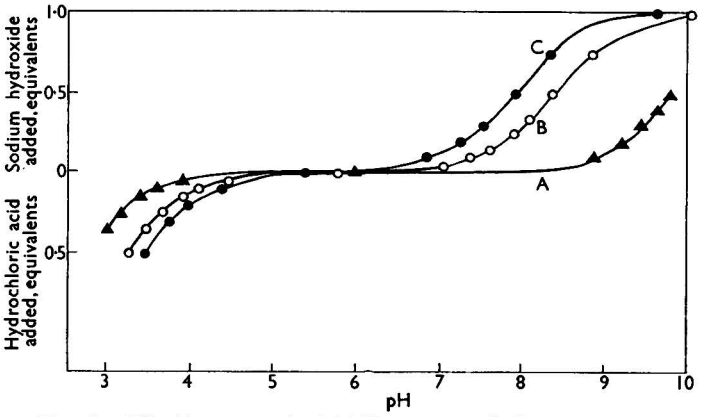

Fig. 7. Titration curves in 0·04 *M* aqueous solution at room temperature: curve A, glycine; curve B, glycylglycine; curve C, glycylglycylglycine

When the indirect techniques are compared, it is observed that the titrations of amino acids from pH 6 and from pH 7 are more or less identical (except for histidine), whereas those of peptides from pH 6 are slightly higher than from pH 7. The titrations from pH 9 are lower both for amino acids and peptides, those for peptides being particularly low. The reason for these findings is seen in Fig. 7. An amino acid such as glycine is partly titrated by the initial adjustment to pH 9 in water, so that after formaldehyde has been added a smaller than theoretical value is obtained. Peptides such as glycylglycine and glycylglycylglycine are, however, almost completely titrated at pH 9 in water, so that when formaldehyde has been added, the additional titration is very small indeed. Even at pH 7 in water, peptides, unlike amino acids, are slightly titrated[34] (see Fig. 7), so that values from pH 7 are usually less than those from pH 6. This is seen particularly clearly with a peptic digest (see Table I), which consists mainly of polypeptides. For this the titration from pH 7 was 0·54 ml and that from pH 6 was 0·79 ml of 0·04 *N* sodium hydroxide. Hence preliminary neutralisation

to pH 9, and for peptides even to pH 7, is clearly undesirable, as it results in a considerable loss of quantitative accuracy. The method still gives a "measure" of proteolytic hydrolysis and will be much more nearly quantitative for digests, such as tryptic, that consist mainly of free amino acids.

Although preliminary neutralisation to pH 6, and for amino acids to pH 7, should yield reasonably good results, it was found in the indicator titrations that the method gives poor reproducibility. The reason for this is revealed by the titration curves (Figs. 4, 5 and 6). Each final value is determined as the resultant of four indicator end-points, two for the digest and two for the blank, and none of these end-points, at pH 6, or 7 and 9 in the presence of buffer and protein, is sharp.

Despite the fact that titration commenced at widely differing pH values in the three buffers that were used, the results obtained by direct potentiometric titration appear to be as closely quantitative as those obtained by any of the indirect procedures. Nor were the values obtained with the most acid buffer (glycine - hydrochloric acid) higher than those obtained with the alkaline phosphate buffer. Further, mixtures of amino acids and peptides not "of the same charge type" were found to be titrated as nearly quantitatively as the sum of their individual components (see Table I). It would seem therefore that, despite arguments such as those of French and Edsall,[24] the direct method can in fact be used for the measurement of amino-acid nitrogen in a mixture with the same degree of quantitative accuracy as for the single pure components thereof and at least as accurately as with any indirect procedure. The theoretical principles stated earlier are thus validated.

I thank Miss Barbara Hunt for skilful assistance, and Mr. J. R. P. O'Brien and Dr. A. G. Ogston, F.R.S., for reading the initial manuscript.

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DEPARTMENT OF CLINICAL BIOCHEMISTRY
RADCLIFFE INFIRMARY
UNIVERSITY OF OXFORD

January 14th, 1957

The Determination of Small Amounts of *o*-Phenylphenol

BY D. HARVEY AND G. E. PENKETH

Methods are described for the extraction and determination of the *o*-phenylphenol added as a fungicide to various materials. The determinations are made by ultra-violet spectrophotometry, colorimetry or fluorescence. The ultra-violet and colorimetric methods are rapid, sensitive and reasonably specific. In the colorimetric method use is made of a commercially available stabilised diazo compound, Brentamine fast red GG; it has advantages over the more usual colorimetric methods for phenols in rapidity and specificity. Both methods give satisfactory results with a variety of treated materials.

The fluorescence method, although specific and sensitive, is subject to serious interference and is of use mainly as a qualitative test.

o-PHENYLPHENOL is finding increasing use as a fungicide for diverse materials and the need has arisen for methods of determination that will be adequate at the rather low concentrations employed.

Various colorimetric methods have been proposed.[1,2,3,4] Tomkin and Isherwood[3] employed a method in which, after separation by steam-distillation, followed by extractions with light petroleum and sodium hydroxide solution, the *o*-phenylphenol was finally coupled with diazotised sulphanilic acid. Gottlieb and Marsh[4] made use of a reaction common to phenols unsubstituted in the *para* position, in which a colour is produced with 4-aminoantipyrine in the presence of potassium ferricyanide and sodium carbonate. However, we have found these methods lacking in either simplicity, specificity or rapidity.

Cox[5] suggested making use of ultra-violet spectroscopy and also mentioned the possibility of utilising the strong fluorescence given by *o*-phenylphenol in ethanolic sodium hydroxide solution. We have examined his suggestions in some detail, and in addition have used a stabilised diazo compound, Brentamine fast red GG, as a new reagent in a rapid and sensitive colorimetric method.

PRELIMINARY TREATMENT OF SAMPLES

The pre-treatment of the sample depends upon the nature of the material and some typical methods are described below. The quantity of sample taken will naturally be determined by the concentration of *o*-phenylphenol present and by the amount necessary to secure a representative portion. The sample weights given should be regarded as a guide only and the final solutions, however prepared, must be adjusted to contain between 5 and 25 μg of *o*-phenylphenol per ml.

"DISINFECTANT SALT" (*i.e.*, COMMERCIAL SODIUM CHLORIDE PLUS ADDED FUNGICIDE)—

Dissolve 1 g of sample in 50 ml of water. Add 10 ml of *M* sodium hydroxide solution to ensure complete solution of the *o*-phenylphenol and dilute to a suitable volume (say, 100 ml) with water. No interference has been encountered in the determination of *o*-phenylphenol in this material by any of the three methods to be described.

DISINFECTANT FORMULATIONS—

Dissolve 5 g of sample in spectroscopic-quality *cyclo*hexane and dilute to 100 ml. If the sample is aqueous, acidify it and extract the solution with five 10-ml portions of *cyclo*hexane. A spectrophotometric determination of the *o*-phenylphenol may then suffice, although if interference occurs or is suspected (see p. 500) it will be necessary to extract the *o*-phenylphenol from the *cyclo*hexane solution with sodium hydroxide solution.

CITRUS FRUITS—

The usual methods are either (*i*) steam-distillation or (*ii*) maceration with *cyclo*hexane.

(*i*) *Steam-distillation*—Roughly mince one whole fruit and transfer it to a 500-ml flask. Add 100 ml of water and 2 ml of glacial acetic acid. Connect to the usual steam-distillation apparatus and distil until the distillate is free from *o*-phenylphenol. A distillate of slightly less than 500 ml is usually sufficient, but it is advisable to collect a further 500 ml as a safeguard, the two distillates being analysed separately. Dilute the distillate with water to 500 ml in a calibrated flask and by pipette put a 100-ml aliquot into a separating funnel. Add 5 ml of approximately *N* hydrochloric acid and extract the *o*-phenylphenol (together with the oils, etc.) with five 10-ml portions of *cyclo*hexane. Combine the *cyclo*hexane layers and extract them with five 10-ml portions of 0·5 *M* sodium hydroxide solution. This separates the *o*-phenylphenol from the oils, and the determination can then be made by any of the methods to be described.

(*ii*) *Maceration*—Roughly mince one whole fruit and transfer it to the container of a high-speed mechanical macerator. Add precisely 250 ml of *cyclo*hexane (containing 1 per cent. v/v of glacial acetic acid to ensure the extractability of the *o*-phenylphenol) and macerate for 5 minutes. Allow to settle, then by pipette put 50 ml of the supernatant liquor into a separating funnel. Extract seven times with successive 5-ml portions of 3 per cent. sodium hydroxide solution. The addition of 15 per cent. of sodium sulphate decahydrate to the alkali is recommended if emulsification causes difficulty. Combine the extracts and dilute them to 50 ml with the extracting solution. Determine the *o*-phenylphenol content by any of the proposed methods. If the fruit has been treated with an alkali-soluble dye, interference will occur with all the methods and so the colorimetric procedure should be used, a correction being made for the intensity of absorption of the dye-stuff colour at the wavelength used.

TIMBER—

The best method of extraction from timber is steam-distillation. Put 1 to 5 g of sample (in the form of drillings or shavings) into a 500-ml flask. Add 100 ml of water and 2 ml of glacial acetic acid. Connect to the usual steam-distillation apparatus and distil until the distillate is free from *o*-phenylphenol. For samples containing up to 1 per cent. of *o*-phenylphenol 500 ml of distillate are usually sufficient, but a further 500 ml should be collected and tested to ensure that all the *o*-phenylphenol has been liberated. The colorimetric procedure to be described is the only completely satisfactory method of analysing the distillate, since we have found that serious interference due to naturally occurring steam-volatile substances is often encountered with the other methods.

PAPER AND CANVAS—

Weigh 1 g of shredded sample into a 250-ml flask. Add 100 ml of 0·1 *M* sodium hydroxide solution, connect the flask to a condenser and boil gently under reflux for 1 hour. Cool, filter, and analyse the filtrate by any of the three methods.

The methods of extraction outlined above are applicable to many types of sample and are those that have been most frequently used in this laboratory. However, on occasion it may be necessary or convenient to vary them. With cotton wadding, for example, treatment with sodium hydroxide produces undesirable side-effects and a Soxhlet extraction with acidified methanol or ethanol is useful. Such alcoholic solutions can be examined by any of the recommended methods.

ULTRA-VIOLET SPECTROPHOTOMETRIC METHOD

Although any ultra-violet spectrophotometer may be used, these instruments vary in, among other things, the accuracy of their wavelength scales, and so spectral data must be checked on the instrument in use. The *cyclo*hexane used must be spectroscopically transparent above 280 mμ.

The absorption spectra of *o*-phenylphenol in *cyclo*hexane and in *M* sodium hydroxide solution are shown in Figs. 1 and 2, respectively. In *cyclo*hexane solution the peak at 284 mμ is used for analysis and not, as suggested by Cox,[5] the peak at about 250 mμ, where more interference is encountered. Measurements are made at two wavelengths so that interference caused by other optically absorbing substances can be distinguished. Extraction of the *o*-phenylphenol with sodium hydroxide solution will separate it from many interfering substances, although, here again, checks at two wavelengths should be made. Interference in

the sodium hydroxide solutions may sometimes be overcome by utilising the hypsochromic effect (a shift of the absorption maxima to a shorter wavelength) that occurs when the solution is acidified, because of the change of the phenoxide to the free phenol. The absorption spectra of the interfering substances may not change with acidification, and it is in this case that the effect can be used.

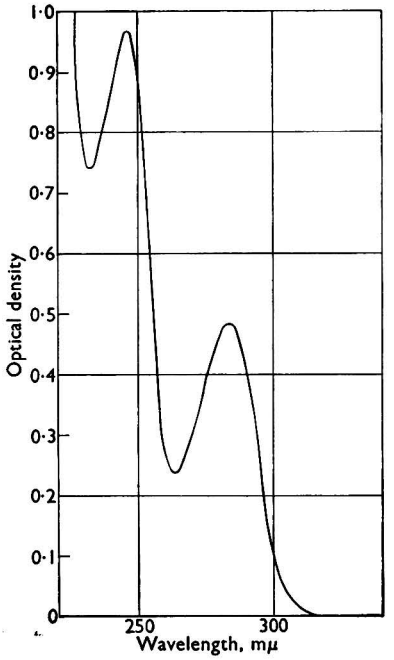

Fig. 1. Absorption spectrum of *o*-phenylphenol in *cyclo*hexane solution

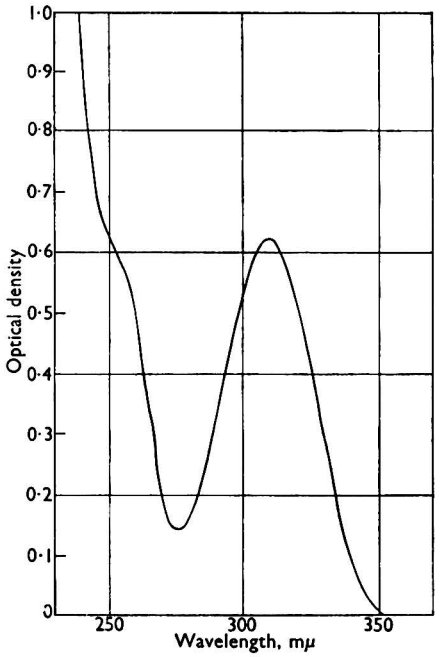

Fig. 2. Absorption spectrum of *o*-phenylphenol in *M* sodium hydroxide solution

Fig. 3 shows the absorption spectra of *o*-phenylphenol in alkaline and acid solution (it is necessary to add ethanol to the acid solution to retain the phenol in solution). Fig. 4 is a difference spectrum obtained by subtracting the optical densities of one solution from those of the other at each wavelength and plotting the results against the wavelengths. The points of maximum difference are at about 285 and 315 mμ, and these wavelengths are chosen for the determination. The detailed procedures are given below.

PROCEDURE FOR *cyclo*HEXANE SOLUTIONS—

Determine the extinction coefficients in 1-cm cells at 284 and 295 mμ of a standard solution of *o*-phenylphenol in *cyclo*hexane (2 mg per 100 ml). Measure the optical density of the sample in 1-cm cells at these two wavelengths and calculate the *o*-phenylphenol content. If the results from the two wavelengths do not agree, interference is indicated.

PROCEDURE FOR SODIUM HYDROXIDE SOLUTIONS—

Determine the extinction coefficients in 1-cm cells at 300 and 320 mμ of a standard solution of *o*-phenylphenol (2 mg per 100 ml) in sodium hydroxide solution of similar concentration to the test solution. Measure the optical density of the sample solution in 1-cm cells at these two wavelengths and calculate the *o*-phenylphenol content. Interference is indicated when results from the two wavelengths do not agree.

PROCEDURE FOR EMPLOYING THE HYPSOCHROMIC EFFECT—

Prepare a standard solution of *o*-phenylphenol containing 5 mg per 100 ml in sodium hydroxide solution of similar concentration to the test solution. Dilute a 20-ml aliquot of the standard solution to 50 ml with water and measure the optical densities of this solution at 285 and 315 mμ in 1-cm cells against a blank consisting of sodium hydroxide solution of

similar concentration. To a further 20-ml aliquot of the standard solution add 5 ml of concentrated hydrochloric acid and dilute to 50 ml with ethanol. Measure the optical densities at the same wavelengths in 1-cm cells against a blank of the reagents.

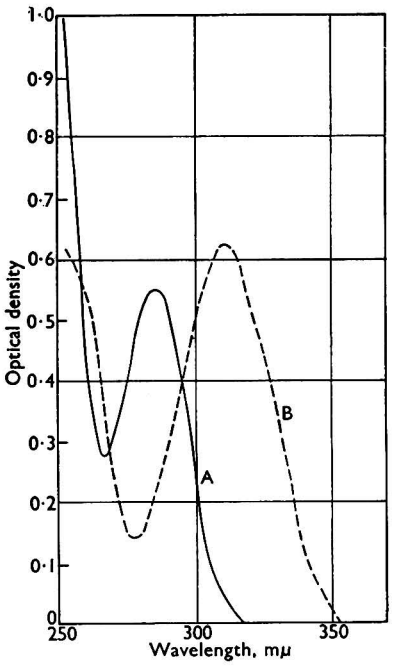

Fig. 3. Absorption spectra of *o*-phenylphenol; curve A, in acid solution; curve B, in alkaline solution

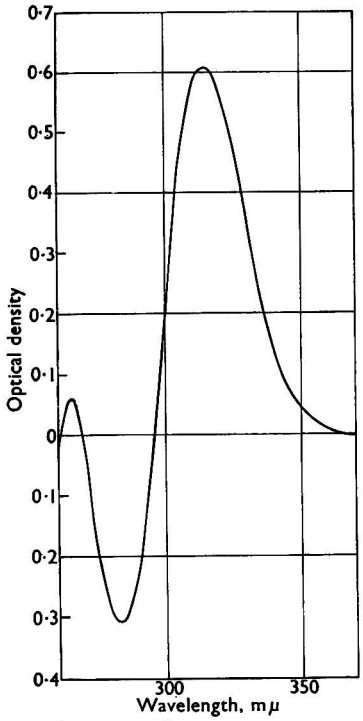

Fig. 4. Difference spectrum

By subtraction of the two sets of measurements obtain the "difference optical densities" at 285 and 315 mμ. Obtain the difference optical densities of the sample solution at these two wavelengths by treating two aliquots of the sample in the same manner as the standard solution and measuring the optical densities in 1-cm cells against the respective reagent blanks. Hence calculate the *o*-phenylphenol content of the sample. The difference optical densities are independent of background absorption that does not alter with pH; interference may occur from other phenols or aromatic acids and again this is shown by disagreement in the results from the two wavelengths.

It is important to measure the volumes of all solutions used as accurately as possible, since a small error in this respect can produce larger errors in the difference optical densities.

Fluorescence method

o-Phenylphenol in ethanolic sodium hydroxide solution exhibits a strong blue-violet fluorescence when irradiated with ultra-violet light and this can be used as a rapid method of determination. We have found that the concentration of the sodium hydroxide solution is not important provided that a similar solution is used for the standards, nor is the amount of ethanol critical.

The following procedure is recommended for sodium hydroxide solutions obtained by any of the extraction procedures that have been described.

Reagent—

Standard o-phenylphenol solution—Dissolve 0·10 g of *o*-phenylphenol in 95 per cent. ethanol and dilute the solution to 100 ml. Take 1 ml of this solution and dilute to 100 ml with 95 per cent. ethanol.

1 ml ≡ 10 μg of *o*-phenylphenol.

PROCEDURE—

Take an aliquot (*B* ml) of the sample solution and dilute it to 20 ml with 95 per cent. ethanol in a 6-inch × ½-inch test-tube. The aliquot should not be greater than 10 ml and should contain 10 to 100 μg of *o*-phenylphenol. In similar test-tubes, prepare ten standards containing 10, 20, 30, etc., up to 100 μg of *o*-phenylphenol by taking the requisite volumes of the standard solution, then adding *B* ml of sodium hydroxide solution of similar concentration to the sample solution and diluting to 20 ml with 95 per cent. ethanol. Compare the blue-violet fluorescence given by the sample under ultra-violet light with that given by the series of standards. It is essential to make the comparisons in a darkened room against a black background. Matching is rather difficult, but with experience a difference of 10 μg can be detected. The *o*-phenylphenol content of the sample is calculated from the concentration of the matching standard. Interference is indicated when the fluorescence has a yellowish tinge and when this occurs the colorimetric procedure should be adopted.

COLORIMETRIC METHOD

In experiments with various dye-stuff intermediates it was found that under the specified conditions Brentamine fast red GG reacts with various phenols (unsubstituted in the *para* position) to give colours ranging from orange to deep red. *o*-Phenylphenol gives an intense cherry red colour with an absorption maximum at 494 mμ. Brentamine fast red GG dissolved in water is a reagent suitable for the determination of very small amounts of this phenol. The reaction is carried out in strong sodium acetate solution, and the colour is developed with sodium carbonate. When gum acacia is added, the colour is stable for 24 hours and may be measured either absorptiometrically or compared visually against standards.

It was found that this method was in general less susceptible to interference than the fluorescence and ultra-violet methods and was the only one of the three to give consistently good results with impregnated timber.

REAGENTS—

Brentamine fast red GG solution—A freshly prepared 0·5 per cent. aqueous solution of Brentamine fast red GG (obtainable from I.C.I. Ltd., Dyestuffs Division). This solution is not stable for more than 24 hours.

Gum acacia solution—A 1 per cent. aqueous solution.

Sodium acetate solution—A 50 per cent. aqueous solution of sodium acetate, $CH_3COONa.3H_2O$.

Sodium carbonate solution—A 20 per cent. aqueous solution.

Standard o-phenylphenol solution—Weigh accurately 0·10 g of *o*-phenylphenol and dissolve it in 5 ml of *N* sodium hydroxide solution and dilute to 1 litre in a calibrated flask. Take 10 ml of this solution and dilute to 100 ml in a calibrated flask.

1 ml ≡ 10 μg of *o*-phenylphenol.

PROCEDURE—

Neutralise the sample to pH 7 to 9, using either an indicator paper or phenolphthalein as an external indicator, and dilute so that 1 ml contains 1 to 10 μg of *o*-phenylphenol. By pipette put 10 ml of this solution into a 6-inch × ½-inch test-tube, and add 1 ml of gum acacia solution, 1 ml of sodium acetate solution and 1 ml of Brentamine fast red GG solution in that order. After 1 minute add 2 ml of sodium carbonate solution and either compare the colour visually against standards prepared from the standard *o*-phenylphenol solution or measure it absorptiometrically at 494 mμ against a blank containing all the reagents.

DISCUSSION OF RESULTS

Of the three methods described, the ultra-violet and the colorimetric methods have been most frequently used for quantitative work.

The fluorescence method is, however, very sensitive (0·5 μg per ml can be detected) and is also specific. Our investigation has shown that of some sixty alkylphenols only *o-tert.*-butylphenol and *o-iso*propylphenol exhibit strong fluorescence (green and blue, respectively). Unfortunately the method is very susceptible to other forms of interference such as masking or quenching of the fluorescence. Moreover the accuracy of the actual fluorescence determination is such that errors of ±20 per cent. may occur and for these reasons we have used the method principally in qualitative work.

The ultra-violet spectrophotometric method has a lower limit of detection of 1 to 2 μg per ml and is the only method that we have subjected to statistical examination. In a series of experiments with oranges, which proved to be the most difficult material we have analysed, the peel and pulp were examined separately, known amounts of *o*-phenylphenol being added to each before the maceration stage. The survey covered some hundred determinations and the results of the statistical examination can be summarised as follows—

(*a*) The average recovery of *o*-phenylphenol is about 90 per cent. of the amount added, there being no significant difference in this respect between the peel and the pulp.

(*b*) The over-all uncertainty of a single determination is represented by a standard deviation of 0·22 mg for pulp with 0 to 10 mg added, or 1·57 mg for peel with 0 to 50 mg added. The precision is sensibly the same at either of the two wavelengths used, *viz.*, 300 and 320 mμ.

The colorimetric method is not absolutely specific, but in the absence of interfering substances is capable for detecting 1 μg of *o*-phenylphenol per ml. In general, the over-all accuracy is found to be within $\pm$ 5 per cent., and even with timber, for which other methods are subject to serious interference, although the accuracy falls somewhat, the results are still satisfactory, as shown by the following—

	Pine			Beech			
o-Phenylphenol added, % ..	Nil	0·10	0·50	Nil	0·10	0·50	1·00
o-Phenylphenol found, % ..	Nil	0·12	0·54	Nil	0·09	0·55	1·00

Other phenols unsubstituted in the *para* position would no doubt cause interference, but they have not been encountered in the large number of samples that have been examined by the method.

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Research Department
Imperial Chemical Industries Limited
Billingham Division
Billingham, Co. Durham

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The Determination of 4-Aminodiphenyl in Technical Diphenylamine

By H. E. Stagg and R. H. Reed

A method is described for the determination of 4-aminodiphenyl in technical diphenylamine, or in residues from the distillation of diphenylamine, by a combination of chromatography on filter-paper and absorptiometry. Typical samples of diphenylamine contained 0·007 to 0·025 per cent. of 4-aminodiphenyl.

Technical diphenylamine normally contains a small amount, usually less than 1 per cent., of primary aromatic amines. As might be expected from the method of manufacture, aniline is often the principal constituent of the primary amines, but chromatographic examination of diazotised and coupled extracts has shown clearly the presence of other amines, and has indicated that 4-aminodiphenyl might be one of them. The carcinogenic nature of this substance to animals[1,2] and man,[3] and the increasing technical use of diphenylamine, combine to make the positive identification and accurate determination of the impurity a matter of great interest.

Experimental

Identity of the impurity—

Initial indications of the presence of 4-aminodiphenyl both in diphenylamine and in residues from the distillation of diphenylamine had been obtained by extracting the sample

with dilute hydrochloric acid, diazotising and coupling the extract with R-salt (disodium 2-naphthol-3:6-disulphonate) and chromatographing the coloured solution on discs of Whatman No. 1 filter-paper supported between glass plates,[4] with 0·1 *N* sodium hydroxide as the developing solvent. The orange-red outer band due to aniline was generally accompanied by a bluish red band lying inside and separated from the aniline band. This band was the same in colour and position as that obtained by treating mixtures of purified diphenylamine and 4-aminodiphenyl in a similar way, and exhibited the same colour change from dull brown to bluish red when the chromatogram was allowed to dry in the air.

Further evidence of the identity of the amine responsible for this band was obtained by fractional distillation *in vacuo* of a large sample of the tarry residue remaining in the still after the distillation of technical diphenylamine. The fraction boiling between 117° and 124° C at a pressure of 0·5 mm of mercury appeared to contain the largest proportion (about 1 to 2 per cent.) of the amine, and this was separated from the bulk of the non-primary amines by chromatography on an alumina column in ether solution. The amine was isolated and its acetyl derivative was prepared; this acetyl derivative melted at 168·5° to 171° C, the acetyl derivative of 4-aminodiphenyl melted at 173° C, and a mixture of the two melted at 171° to 172° C.

The similarity in chromatographic behaviour and the evidence of the melting-point and the mixed melting-point of the acetyl derivatives leave little doubt that 4-aminodiphenyl is one of the primary amines (other than aniline) that are found in diphenylamine and in diphenylamine residues.

DETERMINATION OF 4-AMINODIPHENYL IN TECHNICAL DIPHENYLAMINE—

Diazotisation and coupling of an acid extract with R-salt and then chromatography on filter-paper discs offered a convenient method of separating the 4-aminodiphenyl from other bases, but for quantitative measurement it was necessary to extract the coloured zone from the paper and measure it in an absorptiometer.

Direct extraction by cutting out the zone and lixiviating with solvents proved to be unsatisfactory, because the dye-stuff had a fairly strong affinity for the paper and large volumes of solvent had to be employed to achieve even a reasonably complete extraction. A modification of the paper-disc technique was therefore developed in which the 4-aminodiphenyl - R-salt band could be eluted from the chromatogram in such a way that the whole of the dye-stuff was recovered in a minimum volume of solvent. One gram of ground technical diphenylamine was boiled with 5 ml of approximately 0·5 *N* hydrochloric acid for 1 minute and the suspension was then cooled in ice and water and 0·2 ml of 0·5 *N* sodium nitrite was added with mixing. After 2 minutes the suspension was poured into a mixture of 2 ml of 0·05 *N* R-salt and 5 ml of *M* sodium carbonate and set aside for 5 minutes. Then 5 ml of *N* sodium hydroxide were added and, after thorough mixing, the mixture of R-salt dyes was chromatographed, with 0·1 *N* sodium hydroxide as developing solvent, by the paper-disc method,[4] except that six* 32-cm Whatman No. 1 filter-papers were used in order to accommodate the large volume of solution.

After the chromatogram had been fully developed, and while the pad of filter-papers was still wet, the outer zones were cut off to leave the inner ("4-aminodiphenyl") zone on a disc of paper generally about 17 cm in diameter. This disc of paper was then placed in the centre of a square sheet of Perspex measuring 25 cm × 25 cm × 1 cm, in which was cut a circular channel 0·5 cm deep, 1 cm wide and having an internal diameter of 18·5 cm. In the floor of the channel was drilled a hole of diameter 0·5 cm. A 25-cm × 25-cm glass plate with a central hole of diameter 1 cm was then placed symmetrically on top of the paper disc and was weighted with two steel blocks weighing about 5 lb each. The assembly of plates was supported on a flat wooden block so that a 100-ml beaker could be placed beneath the hole in the lower plate. A "chimney," consisting of a calcium chloride tube, was then fitted to the hole in the upper (glass) plate by means of a rubber stopper and was filled with 0·1 *N* sodium hydroxide for elution of the zone from the paper disc. As the eluting solvent soaked into the papers, the eluted dye dripped slowly into the 100-ml beaker; elution was complete when about 70 ml had been collected. After dilution of the eluate to 100 ml with 0·1 *N* sodium hydroxide, the optical density of the solution was read in 2-cm or 4-cm cells,

* It has been observed that in general an increase in the number of paper discs used is accompanied by an improvement in the separation and sharpness of the chromatographic zones.

according to the intensity of the colour, against a water blank, with use of a Spekker photo-electric absorptiometer fitted with a tungsten-filament lamp and Chance OB1 (No. 7) glass filters.

RECOVERY OF 4-AMINODIPHENYL BY ELUTION—

The efficiency of recovery of the 4-aminodiphenyl dye-stuff from paper discs by elution was checked by measuring the optical density of solutions of known dye-stuff content both directly and after passing through the chromatographic procedure. The results are shown in Table I.

TABLE I

RECOVERY OF 4-AMINODIPHENYL - R-SALT DYE BY CHROMATOGRAPHY AND ELUTION

4-Aminodiphenyl taken, mg	Optical density (direct reading). Reagent blank	Optical density (after elution). Water blank
With a 2-cm cell—		
0·5	0·61	0·62
		0·60
0·25	0·31	0·29
		0·29
0·1	0·13	0·13
		0·13
With a 4-cm cell—		
0·1	0·265	0·25
		0·27
0·04	0·105	0·10
0·02	0·05	0·06

It is seen that the recovery of 4-aminodiphenyl dye from the chromatogram is remarkably efficient even at very low concentrations, and the calibration graph can be based directly on the optical density of solutions of known dye-stuff content.

RESULTS

4-Aminodiphenyl was determined in samples of diphenylamine from various sources; 1-g portions of sample were taken and the optical densities were measured in 2-cm cells, with the following results—

Optical density	0·155, 0·154	0·095, 0·092	0·305, 0·328	0·117*	0·145
4-Aminodiphenyl found, % ..	0·013, 0·013	0·007, 0·007	0·025, 0·027	0·004	0·011

* This optical density was measured in a 4-cm cell.

ANALYSIS OF DISTILLATION RESIDUES—

The black tarry residues from the distillation of technical diphenylamine vary considerably in amine content: the 4-aminodiphenyl content of some can be determined directly on an acid extract of the tar, whereas others give a complicated chromatogram in which the 4-aminodiphenyl - R-salt band is difficult to delineate. In such cases it is often helpful to distil the volatile matter out of the tar at low pressure, and to test the various fractions of the distillate separately: the characteristic colour change of the 4-aminodiphenyl - R-salt band from dull brown to bluish red, on drying in air, is valuable as a means of identifying the band in these fractions. The 4-aminodiphenyl content of various samples of residue varied from 0·06 to 1 per cent.

DISCUSSION OF RESULTS

It seems clear that 4-aminodiphenyl is present in amounts up to 0·025 per cent. among the primary aromatic amines that are found in technical diphenylamine, and that the residues from the distillation of diphenylamine may contain much greater amounts. The amount present in any sample can be determined by the method described above, and the sensitivity and accuracy of the determination can be varied almost at will by increasing the amount of sample used for the initial extraction. An idea of the reproducibility of the test can be obtained from the duplicate determinations shown above for various samples of diphenylamine.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
DYESTUFFS DIVISION
HEXAGON HOUSE
BLACKLEY, MANCHESTER, 9

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The Determination of Vicinal Glycols by Oxidation with Periodate in Non-aqueous Media

BY R. J. B. REDDAWAY

A new method has been developed whereby alcohol-soluble vicinal glycols may be quickly and accurately determined by arsenite - iodine titration after oxidation with periodate in non-aqueous solution. Oxidation is rapid and specific when conducted in a mixed ethyl acetate - ethanol solvent containing triethylamine and acetic acid. The triethylammonium periodate reagent is prepared by dissolving periodic acid in this solution. The oxidations are carried out at room temperature and determinations may be completed in under 2 hours. A large number of analyses of pure batyl alcohol and of *cyclo*hexane-1:2-diol, with various reaction times, show standard deviations well below $\pm 0{\cdot}5$ per cent. Good results are also obtained with other compounds containing vicinal glycol groups.

THE need arose in this laboratory for an accurate and precise method of determining the purity of batyl alcohol and other α-glyceryl ethers, and selective oxidation with periodate was chosen. The main difficulty was the almost complete insolubility in water of the compounds to be analysed.

Hitherto most methods of determining vicinal glycols by oxidation with periodate have involved the use of aqueous solutions and metallic salts. Water-insoluble vicinal glycols have usually been determined either in solvent - water mixtures, in suspensions or in emulsions.[1,2] In a recent spectrophotometric method[3] very dilute aqueous solutions are used, and so compounds only slightly soluble may be determined, but we have found that some compounds, *e.g.*, riboflavin, will not react quantitatively with periodate at the required dilution. Karnovsky and Brumm[4] have used ethanolic solutions of periodic acid for determining batyl alcohol and other α-glyceryl ethers, but periodic acid is so readily reduced by ethanol alone that their determinations rested on measurement of the formaldehyde produced. Hartman[5] reports the use of potassium periodate in nearly non-aqueous acid media for the determination of technical monoglycerides, although thiosulphate then has to be used in the titrations owing to the strongly acid conditions.

Preliminary experiments with solvent - water mixtures and aqueous suspensions, with buffered periodate or periodic acid as reagent, soon showed that a reliable technique was not to be achieved by such means. The results of all these experiments were either low or variable. It was considered that an homogeneous medium was necessary for smooth reaction, and this led to the finding that triethylammonium periodate is soluble in a very wide range of organic solvents. Further, these solutions were found to be more stable than those of the free acid (see Table I). The solutions taken as stable enough for further investigation were those in which no obvious chemical change occurred, and no precipitate was formed, during the first few hours after preparation. Water-immiscible solvents were excluded owing to the difficulty of titrating a two-phase system. The most promising solvent appeared to be absolute ethanol, and this was shown to be so by later experiments.

TABLE I

SOLUBILITIES AND STABILITIES OF PERIODIC ACID AND TRIETHYLAMMONIUM PERIODATE IN ORGANIC SOLVENTS

Solvent	Periodic acid	Triethylammonium periodate
Methanol	S (RC)	S (RC)
Ethanol	S (RC)	S
n-Propanol	S (RC)	S (RC)
*iso*Propanol	SP:RW	S
n-Butanol	SP	S
tert.-Butanol	SP:RW	S
Methyl Cellosolve	S (RC)	S
Butyl Cellosolve	SP:RW	S
Acetone	RC	S
Ethyl methyl ketone	RC	S
Methyl formate	S	S
Ethyl formate	I	S
Ethyl acetate	I	SP
Dioxan	SP:RW	S
Tetrahydrofuran	SP:RW	S
Methyl cyanide	S (RC)	S (RC)
Dimethylformamide	S	S
Ether	I	I
Benzene	I	SP
Chloroform	I	S
Carbon tetrachloride	I	SP
Ethylene dichloride	I	SP
Pyridine	I	I
Glacial acetic acid	S (RC)	S

KEY TO TABLE—

S = Soluble (giving a comparatively stable solution if no other indication).
SP = Sparingly soluble.
I = Insoluble.
RW = Reacts fairly rapidly with the solvent on warming.
RC = Reacts fairly rapidly with the solvent in the cold.
(RC) = Reacts slowly with the solvent in the cold.

Other findings having a deciding influence on the final form of our method were as follows—

(*i*) Periodic acid is reduced more slowly by ethanol in ethyl acetate - ethanol mixtures than by ethanol alone, as found by Willard and Boyle.[6] This also applies to its triethylammonium salt.

(*ii*) The stability of triethylammonium periodate towards organic solvents (and in particular, towards the ethyl acetate - ethanol mixture chosen) is enhanced by the addition of a moderate amount of glacial acetic acid. In practice a small extra amount of triethylamine is also added, although it was found that too great an addition of this, or of the acetic acid, has a deleterious effect on the properties of the solution. The final composition of the reagent was arrived at by a series of experiments in which aliquots of solution were analysed at regular intervals for periodate content, and loss of periodate was plotted against time. With the reagent thus found, the negative error arising from consumption of periodate by the solvent is well below 0·2 per cent. under the conditions of analysis.

(*iii*) When the recommended reagent solution is used, constant values for batyl alcohol and for *cyclo*hexane-1:2-diol are obtained with large variations in the time allowed for reaction. The oxidation is rapid (over 85 per cent. in 5 minutes at room temperature) and complete in $\frac{3}{4}$ to 1 hour. Thereafter values remain constant for a further 4 to 5 hours (see Tables II and III), after which some variation occurs owing to deterioration of the reagent.

(*iv*) Further oxidation of aldehydic reaction products appears to be negligible when using the recommended reagent solution, and alcoholic sample solutions. This is a distinct advantage over reactions in aqueous media.

(*v*) Traces of water in the reagent solution (from the alcohol and the water of hydration of the periodic acid) appear to have little effect (see Table II). Replacement of

part of the acetic acid by an equivalent amount of acetic anhydride in order to remove water from the system is not beneficial.

(*vi*) The reagent deteriorates only very slowly during the first few hours after preparation, especially if stored in the dark.

TABLE II

PERIODATE-UPTAKE RATIOS FOR PURE BATYL ALCOHOL

Time of reaction, hours	Ratio found with use of dry absolute ethanol (water content 0·14%)	Ratio found with ordinary absolute ethanol (water content 0·35%)
0·5	1·003	0·992
0·67	0·994	—
0·75	0·997	1·001
	0·996	1·004
	0·996	—
	1·001	—
1·00	1·002	1·002
1·25	1·002	1·002
	1·003	0·999
	0·994	0·995
	0·999	1·000
1·5	1·000	0·999
2·0	0·991	0·995
	1·001	—
	1·000	—
2·5	1·002	1·001
	1·004	1·006
3·0	1·003	0·995
4·0	1·004	0·992
6·0	0·997	1·004
	1·001	—
Average	0·9995	0·9991
Maximum deviation, % ..	0·85	0·71
Average error, %	±0·31	±0·36
Standard deviation, % ..	0·37	0·44

TABLE III

PERIODATE-UPTAKE RATIOS FOR PURE *cyclo*HEXANE-1:2-DIOL (m.p. 104·5° C)

Time of reaction, hours	Ratio found for a once recrystallised sample	Ratio found for a twice recrystallised sample
0·5	0·987	—
	0·984	—
	0·988	—
1·0	0·989	—
	0·989	—
	0·990	—
1·25	0·989	0·993
	0·990	0·996
	0·991	0·996
1·5	0·990	—
	0·991	—
2·0	0·989	—
	0·988	—
	0·988	—
3·0	—	0·995
	—	0·995
	—	0·997
4·0	0·992	—
	0·990	—
6·0	0·981	0·996
	0·990	0·996
	0·988	0·998
Average	0·9886	0·9958
Maximum deviation, % ..	0·77	0·28
Average error, %	±0·17	±0·10
Standard deviation, % ..	0·25	0·14

(*vii*) Alternative solvents to the ethyl acetate - ethanol mixture have been examined, and results when some of these were used are compared in Fig. 1. Apart from dioxan and methyl formate, none can be recommended, and even these two are slightly inferior to the recommended solvent, whether used alone or mixed with ethyl acetate.

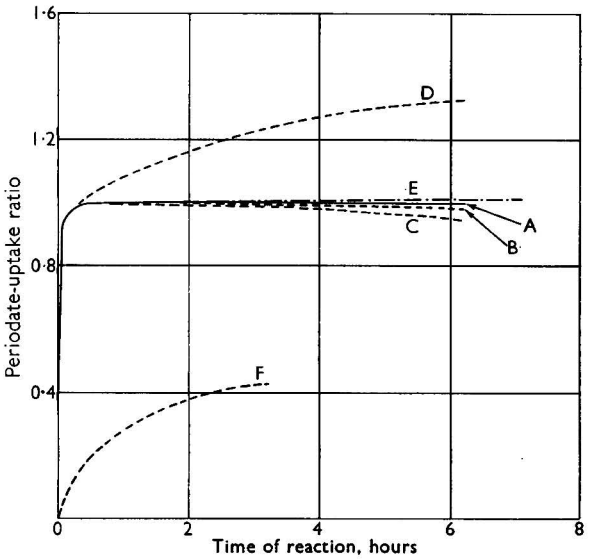

Fig. 1. Relationship between the periodate-uptake ratio and time of reaction for batyl alcohol (*x*) and *cyclo*-hexane-1:2-diol (*y*) in various solvents: curve A, standard (*x*, *y*); curve B, dioxan (*x*, *y*); curve C, *tert.*-butanol (*y*); curve D, *tert.*-butanol (*x*); curve E, methyl formate (*x*, *y*); curve F, dimethylformamide (*x*)

METHOD

REAGENTS—

Sodium arsenite, 0·1 N—Dissolve about 1·24 g of resublimed arsenic trioxide, accurately weighed, in 15 ml of 2 *N* sodium hydroxide solution, with warming. Add 15 ml of 2 *N* hydrochloric acid and then 1 ml of concentrated hydrochloric acid. Finally add 50 ml of *M* sodium bicarbonate solution, with stirring. Cool, transfer the solution to a 250-ml calibrated flask, add the washings and dilute to the mark with water.

Iodine solution, 0·05 N—Standardise by titrating 10 ml of the standard sodium arsenite, after adding 10 ml of *M* sodium bicarbonate solution and 0·5 ml of starch indicator solution.

Potassium iodide solution, 20 *per cent. w/v*—A freshly prepared aqueous solution.

Sodium bicarbonate, M.

Periodic acid, $HIO_4.2H_2O$ (97 *to* 100 *per cent.*).

Acetic acid, pure glacial.

Triethylamine, redistilled.

Ethanol, absolute.

Ethyl acetate, pure.

Non-aqueous periodate reagent—Dissolve 0·56 to 0·57 g of periodic acid in about 15 ml of ethanol containing 1·0 g of triethylamine and 3·0 g of glacial acetic acid. Add 25 ml of ethyl acetate and filter the mixed solution into a 50-ml calibrated flask. Add ethanol washings through the filter to the mark; mix, and use within 2 hours.

Starch indicator solution, 1 *per cent. w/v.*

PROCEDURE—

Either weigh an amount of sample corresponding to 140 to 160 micro-equivalents of vicinal glycol into a 100-ml conical flask and dissolve in 5 ml of ethanol, or by pipette put

a 5-ml aliquot of ethanolic sample solution into the flask. Add exactly 5 ml of periodate reagent, place a stopper in the flask, swirl, and set aside in the dark for 1¼ hours. Carry out a blank at the same time, adding 5 ml of periodate reagent to 5 ml of ethanol.

Remove the stoppers and add to each flask, in order, 10 ml of *M* sodium bicarbonate, 5 ml (exactly) of standard 0·1 *N* sodium arsenite and 2 ml of 20 per cent. potassium iodide, swirling after each addition. After 15 minutes titrate with arsenite-standardised 0·05 *N* iodine from a 10-ml semimicro-burette. Starch indicator solution may be used, although the purple colour is prevented by the solvent from developing properly; however, the yellow end-point is sharp to 0·01 ml.

CALCULATION—

Let number of micromoles of sample taken $= M$

Let number of micro-equivalents of iodine per ml $= Q$

Then, periodate uptake ratio $= \dfrac{\text{(Sample titre} - \text{blank titre)}}{2\,M} \times Q = P$ (say).

$$\text{Purity, per cent.} = \frac{P_{\text{found}}}{P_{\text{theoretical}}} \times 100.$$

DISCUSSION

Although the non-aqueous oxidation technique described gives excellent reproducibility of results with batyl alcohol and *cyclo*hexane-1:2-diol, and probably also with various derivatives of sugars (Table IV), it is limited in scope not only to alcohol-soluble compounds but also apparently to simple vicinal glycols having the –CHOH–CHOH– grouping. Hence quinic acid gives anomalous results, probably on account of the carboxy group attached to the same carbon as the central hydroxy group. The method appears to be applicable to the determination of steroids having a vicinal glycol group, and to other compounds such as hydrobenzoins. It is not applicable to hydroxyethylamines or hydroxyketones, *e.g.*, ephedrine, 2-phenyl-2-hydroxyethylamine and benzoin, as the oxidation proceeds too slowly with these compounds, and is incomplete after some hours. No oxidation of unsaturated $-\overset{|}{C}=\overset{|}{C}-$ bonds[7] as in cinnamic acid occurs under our conditions, nor of piperazine.[8] These facts may be of interest when selective oxidation of mixtures is envisaged.

TABLE IV

PERIODATE-UPTAKE RATIOS FOR MISCELLANEOUS VICINAL GLYCOLS

Compound	Ratio found	Ratio, theoretical
Chloralose	1·002	1·000
	1·007	—
D-Arabinose	3·02	3·00
	2·98	—
Arabonic-*p*-anisidide	2·96	3·00
	2·98	—
Glyceryl monolaurate (technical) ..	0·832	1·000 (pure)
	0·839	—
Quinic acid	~1·5	2·00

CONCLUSIONS

A new method for determining alcohol-soluble vicinal glycols by oxidation with periodate in non-aqueous solution has been shown to be accurate and precise for two such compounds, *viz.*, batyl alcohol and *cyclo*hexane-1:2-diol. The method may prove to be applicable to all alcohol-soluble compounds having a —CHOH–CHOH– grouping, but not to any other type of compound. It could be applied to the determination of crude monoglycerides and the glycerol content of soaps and so on.

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Further Observations on Automatic Titrimetry

BY J. HASLAM AND D. C. M. SQUIRRELL

This paper describes various applications of automatic titrimeters in the general work of an analytical laboratory.

Observations are made on (*a*) the determination of nitrogen and chlorine in polymers, (*b*) the titrimetric determination of iron and aluminium, (*c*) the determination of water in organic compounds, (*d*) the determination of formaldehyde by the neutral sulphite method, (*e*) the detection of Terylene, (*f*) the determination of nylon 610 salt in moist material and (*g*) the determination of aldehydes and ketones in methyl methacrylate monomer.

SINCE publication of our previous paper[1] in 1954, we have had the opportunity of using automatic titrimeters in day-to-day work. The object of this paper is to describe some of the purposes for which we find this type of instrument very useful and particularly those that we consider to be improvements on procedures previously used, or alternatively, that have novel features. The experiments described are miscellaneous, but all have found useful application in a plastics analytical laboratory.

THE DETERMINATION OF NITROGEN IN POLYMERS

In the past it has been our practice to determine nitrogen in organic polymers by Kjeldahl digestion of the polymer with a sodium sulphate - selenium catalyst and sulphuric acid. The digestion product is made alkaline and the liberated ammonia is distilled in steam and collected in standard acid. The acid solution is boiled until free from carbon dioxide and the excess of acid is titrated with standard alkali, with methyl red as indicator. As a result of our investigations, however, it has been shown that this boiling may be avoided and the titration with standard alkali carried out to pH 4·5 if the automatic titrimeter is used. It is, of course, necessary to carry out a blank test on the reagents.

THE TITRATION OF IRON AND ALUMINIUM WITH EDTA

Following our work on the titration of bivalent metals such as copper and zinc,[2] we have shown that both iron and aluminium may be satisfactorily determined by using the titrimeter as a means of automatic maintenance of a given pH, and as a sensitive end-point detector. With a solution containing iron, an excess of disodium ethylenediaminetetra-acetate (EDTA) solution is added, after which the pH is adjusted to 5·0. The excess of EDTA is then titrated with a standard solution of a zinc salt. The procedure described below avoids any difficulties in approaching the end-point. The procedure for aluminium is similar, but our results indicate that it is necessary to boil the aluminium solution with an excess of EDTA to effect complete conversion to the complex.

METHOD FOR DETERMINING IRON

REAGENTS—

EDTA solution, 0·1 *M*—Dissolve 37·23 g of disodium ethylenediaminetetra-acetate in distilled water and dilute to 1 litre.

Zinc solution, 0·1 M—Prepare an exactly 0·1 *M* solution of zinc by dissolving the calculated amount of analytical-reagent grade zinc sulphate, $ZnSO_4.7H_2O$, previously standardised by the 8-hydroxyquinoline gravimetric procedure, in distilled water, and diluting to 1 litre.

Ammonium chloride solution, M.

Sodium hydroxide solution, 0·1 N.

PROCEDURE—

Transfer a volume of the acid iron solution under test, containing less than 0·13 g of iron, to a 250-ml beaker and oxidise to the ferric state by adding bromine water. Boil until free from bromine, cool and neutralise any excess of acid with strong sodium hydroxide solution, *i.e.*, bring the pH to just above 3·0. Add 10 ml of *M* ammonium chloride solution and 30·0 ml 0·1 *M* EDTA solution. Adjust the pH of the solution to 5·0 by the automatic addition of 0·1 *N* sodium hydroxide. From an external burette add the standard 0·1 *M* zinc solution in small increments (5 ml, decreasing to 1 ml), returning the pH to 5·0 with the automatic titrimeter between each addition. Continue the addition of zinc until a 1-ml portion no longer causes a shift in the pH of the solution from 5·0, *i.e.*, the automatic instrument will no longer switch on to add sodium hydroxide solution. At this point the solution contains an excess of zinc; immediately add 2·0 ml of 0·1 *M* EDTA solution and return the pH again to 5·0. Leave the automatic instrument switched on, and add the standard zinc solution dropwise until further addition causes no shift in pH from 5·0, *i.e.*, the indicator needle of the instrument shows no movement. The addition of the zinc solution should be stopped during the periods when the automatic instrument is making the additions of sodium hydroxide to maintain the pH at 5·0. Read the volume of standard zinc solution used and standardise the EDTA solution against the zinc solution by the same method, *i.e.*, carry out a blank titration omitting only the iron solution. Then, the difference between the blank titre and the sample titre when multiplied by 0·005584 is equivalent to the amount of iron titrated, in grams.

RESULTS—

When this method was applied to solutions of ferric chloride previously standardised by precipitation as ferric hydroxide and ignition to ferric oxide, the results were as follows—

Iron present, g	0·05297	0·1295	0·07650
Iron found, g	0·05299	0·1289	0·07631

METHOD FOR DETERMINING ALUMINIUM

The procedure for determining aluminium is similar to that for determining iron, with the exceptions that—

(*a*) the solution to be titrated should contain less than 0·065 g of aluminium,
(*b*) after the addition of 30·0 ml of 0·1 *M* EDTA solution and adjustment of the pH to 5·0 with the automatic titrimeter, the beaker is removed from the instrument and the contents are boiled for 5 minutes and cooled, and
(*c*) the difference between the blank titre and sample titre multiplied by 0·002697 is equivalent to the amount of aluminium titrated, in grams.

RESULTS—

The following results were obtained when the method was applied to an aluminium solution prepared by dissolving spectroscopically pure aluminium in sodium hydroxide and acidifying with hydrochloric acid—

Aluminium added, g ..	0·05640	0·02453	0·04878	0·06345	0·03299
Aluminium found, g ..	0·05637	0·02454	0·04882	0·06354	0·03264

NOTE—The necessity to boil the aluminium solution with an excess of EDTA is shown by the following low results obtained by carrying out the method without boiling—

Aluminium added, g ..	0·05640	0·02787	0·05574
Aluminium found, g ..	0·05590	0·02746	0·05460

THE DETERMINATION OF CHLORINE IN ORGANIC POLYMERS

It has been found that the automatic titrimeter is exceedingly useful for the determination of chlorine in organic polymers. The method used is that described in our previous paper,[1] except that we have modified the electrode system so that the silver-wire indicator electrode is held in a position touching the stirrer and is continually vibrated during the titration. This modification avoids all electrode poisoning and false potentials due to precipitated silver chloride adhering to the electrode.

Incidentally, owing to changes in the manufacture of sodium peroxide, which result in the production of a coarser product, the material now offered for sale is not suitable for chlorine fusions with 1 g of a sodium nitrate - dextrose catalyst and 15 g of peroxide. It is our experience that, if 0·8 g of dried starch (AnalaR; soluble) is used as catalyst, the fusions proceed satisfactorily.

THE DETERMINATION OF WATER IN ORGANIC COMPOUNDS BY THE KARL FISCHER METHOD

By adopting the principle of the method of van Lamoen and Borsten,[3] we have been able to use the automatic titrimeter most successfully for the automatic determination of water by the Karl Fischer method. The reagent is divided into 2 parts, one consisting of a solution of sulphur dioxide and pyridine in methanol, contained in the titration vessel, and the second a solution of iodine in methanol, contained in the burette of the titrimeter.

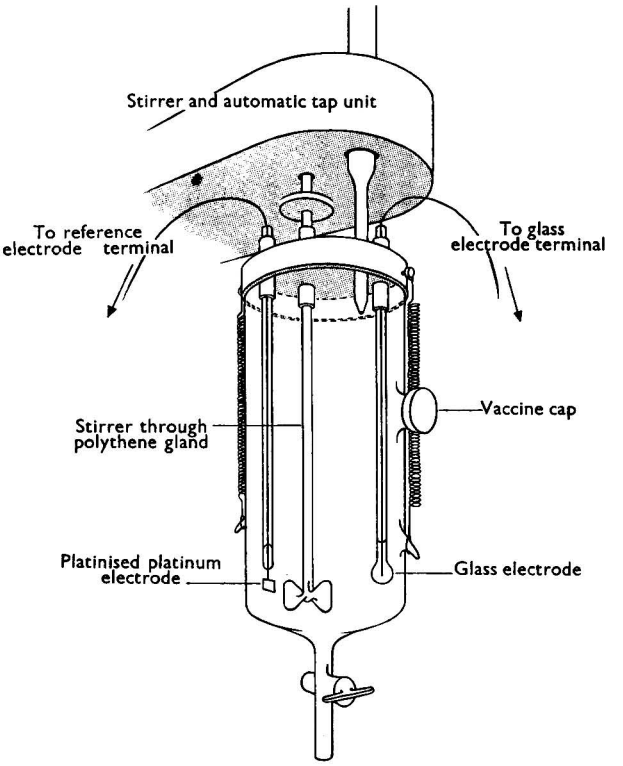


Fig. 1. Titration assembly for the determination of water in organic compounds by the Karl Fischer method

This latter solution is quite innocuous and no trouble is experienced with the burette system. Addition of the iodine solution from the burette to the sulphur dioxide - pyridine - methanol solution in the titration vessel produces Fischer reagent *in situ*. This method has proved particularly useful for the difficult determination of water in acetone. Instead of using the reference electrode of van Lamoen and Borsten, we find a glass electrode much more stable and entirely satisfactory. A diagram of the essential part of the apparatus is given in Fig. 1 and details of the application of the method to the determination of water in a plasticiser and in acetone are given below.

METHOD

REAGENTS—

Methanol.
Pyridine.

Sulphur dioxide.

Iodine - methanol solution—Dissolve 50 g of resublimed iodine in dry methanol and make up to 1 litre.

Sulphur dioxide - pyridine - methanol reagent—Prepare a solution containing 100 g of dry sulphur dioxide in a mixture of 500 ml of dry methanol and 500 ml of dry pyridine.

DETERMINATION OF THE END-POINT POTENTIAL DIFFERENCE—

When the apparatus is first set up or when a new glass electrode is being used, the end-point potential for subsequent use in automatic titrations must be found in the following way.

Add to the titration vessel 20 ml of the sulphur dioxide - pyridine - methanol reagent and close it with the vaccine cap. From the burette run in sufficient iodine solution to give the brown colour shown by an excess of Fischer reagent. Allow the solution to be stirred for 5 minutes to dry out the apparatus thoroughly. From a pipette add approximately 5 ml of a solution of water in methanol (10 g of water per litre) through the vaccine cap. Titrate with iodine - methanol solution, using the manual control of the titrimeter, and plot a potential curve of the titration. From this curve read off the e.m.f. difference at the end-point of the titration. With the two glass electrodes and automatic instrument that we have used, the end-points have occurred at scale readings of −280 and −210 mV, respectively. We have found that these potential scale readings have remained constant throughout the life of the electrodes.

STANDARDISATION OF THE IODINE - METHANOL SOLUTION—

Place approximately 20 ml of the sulphur dioxide - pyridine - methanol reagent in the titration vessel and close it with a vaccine cap. Add a few millilitres of iodine - methanol solution and turn the instrument dial to the desired end-point potential setting. Adjust the FAST - SLOW change-over control to a suitable setting and switch the instrument to automatic titration, when iodine - methanol solution will be delivered from the burette until the end-point is reached, at which point the instrument automatically switches off. No reading of the burette is taken at this stage. Switch off the AUTOMATIC switch and refill the burette. Through the vaccine cap add 5·0 ml of a standard solution of water in methanol, containing about 50 mg of water (a stable hydrate such as sodium acetate trihydrate may also be used). Again switch the instrument to AUTOMATIC and titrate the known amount of water added. Hence the relationship between the iodine solution and amount of water is simply obtained. Switch off the AUTOMATIC switch.

PROCEDURE FOR DETERMINING WATER IN A PLASTICISER—

Either after the initial titration of the 20 ml of sulphur dioxide - pyridine - methanol reagent or after the standardisation titration, refill the burette and, by means of a hypodermic syringe, inject 10 or 20 ml of the sample under test through the vaccine cap. Again switch to automatic titration, when the water in the sample will automatically be titrated with the iodine - methanol solution to the end-point potential setting. From this titration and the standardisation carried out above, the percentage of water in the sample can be calculated.

PROCEDURE FOR DETERMINING WATER IN ACETONE—

The procedure for the determination of water in acetone is similar to that described above, except that 20 ml of dry pyridine are added to the 20 ml of sulphur dioxide - pyridine - methanol reagent in the titration vessel before carrying out the determination. The end-point is quite stable (20 seconds), showing that little reaction is occurring between the acetone and very slight excess of Fischer reagent. Reaction at this stage would lead to false figures being found for water.

With a sample of acetone of low water content 0·07 per cent. of water was found; when known amounts of water were added to this acetone, the results for the percentage of water found, after being corrected for the water originally present, were as follows—

Water added, %	0·44	0·44	0·30	0·30	0·72	0·71	0·48	0·48
Water found, %	0·44	0·44	0·30	0·30	0·71	0·71	0·49	0·48

THE DETERMINATION OF THE CONCENTRATION OF FORMALDEHYDE SOLUTIONS BY THE NEUTRAL SULPHITE METHOD

A convenient method for the rapid determination of the concentration of formaldehyde solutions involves the addition of neutral sodium sulphite solution to the neutral solution of formaldehyde. The sodium hydroxide liberated is usually titrated with standard acid, with thymolphthalein as indicator. The colour change occurs over a range and is not particularly satisfactory. In our experience, titration of the liberated alkali with standard acid on the titrimeter to pH 8·95 is much to be preferred.

METHOD

REAGENTS—

Sodium sulphite solution, M—Dissolve 126 g of anhydrous sodium sulphite or 252 g of the hydrated salt in water and dilute to 1 litre.

Hydrochloric acid, N *and* 0·1 N.

Sodium hydroxide solution, 0·1 N.

Thymolphthalein indicator solution.

PROCEDURE—

Accurately weigh, using a weighing pipette, a volume of solution containing about 1 g of formaldehyde into a 250-ml beaker containing 10 ml of distilled water. Cover the beaker with a watch-glass to avoid loss by evaporation. Add 2 drops of thymolphthalein indicator solution and 0·1 *N* sodium hydroxide solution dropwise until a faint blue colour is just perceptible. Measure 75 ml of the freshly prepared sodium sulphite solution into a 250-ml conical flask, add 2 drops of indicator solution and then 0·1 *N* hydrochloric acid until the blue colour just disappears. Pour this neutral solution into the previously prepared formaldehyde solution. Mix the two solutions carefully and set aside for 2 minutes. Titrate the liberated alkali automatically with *N* hydrochloric acid solution to a pH of 8·95.

1 ml of *N* hydrochloric acid ≡ 0·03003 g of formaldehyde.

The pH of 8·95 was found to be the true end-point of the titration by manual plotting of a full pH curve of the titration. It will be noted that the pH change of the indicator, *i.e.*, from blue to colourless, occurs some 5 or 6 drops of *N* hydrochloric acid before the true end-point, which would account for the low results reputed to be given by this neutral sulphite method with thymolphthalein indicator.

This method is reasonably reproducible, as shown by the results of replicate tests on a sample of AnalaR formaldehyde solution, which gave the percentage of aldehydes, as HCHO, as 35·6, 35·7, 35·9 and 35·7 per cent.

THE DETECTION OF TERYLENE BY AMINOLYSIS

In the past it has been shown that Terylene may be hydrolysed by alcoholic potash, and the terephthalic acid recovered from the hydrolysis products. The hydrolysis is quite protracted and may take from 5 to 6 hours. Moreover, the insoluble potassium terephthalate produced in the test adds to the difficulty of complete hydrolysis of the polymer.

Recently Schröder and Thinius,[4] in a paper on the analysis of the polyesters of terephthalic acid, have shown that the hydrolysis of Terylene may be readily accomplished by heating it with ethanolamine. Hydrolysis only takes about 1 to 2 hours and a measure of the ethanolamine used in the test may be determined by titration with a standard acid to bromothymol blue indicator. After this neutralisation the liquor can be filtered to yield a residue of a derivative of terephthalic acid-bis-2-hydroxyethylamide, m.p. 228° C, having a nitrogen content of 11·11 per cent.

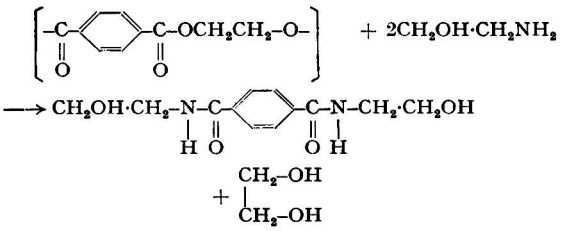
It seemed to us that an accurate determination of the ester value of Terylene might be obtained by titration of the excess of ethanolamine by means of the automatic titrimeter. Preliminary experiments indicated that at the end-point one drop caused the pH of the solution to change from 7·0 to 3·0 and hence a pH of 6·0 was chosen as the automatic end-point.

A test of the whole method was carried out with samples of Terylene chip, which were finely ground in the laboratory mill.

METHOD

Approximately 1 g of the finely ground sample was accurately weighed into a 50-ml round-bottomed flask, and approximately 3 g of redistilled ethanolamine, also accurately weighed, were added. The flask was connected to a reflux condenser, and the contents of the flask were boiled for 2 hours and then allowed to cool. A blank test was carried out on a known weight of the ethanolamine used. When cool, the contents of the flask went solid and this residue was warmed with a small amount of water and finely ground with a glass rod, before being transferred quantitatively to a 100-ml beaker, a total of 20 ml of distilled water being used for washing the flask and condenser. This slurry was now titrated, automatically to pH 6·0 with 2 *N* hydrochloric acid, with a FAST - SLOW change-over setting of 130 mV. The blank was similarly titrated, and the ester value of the sample was calculated as the percentage of polyester (Terylene) of equivalent weight 192.

The equation for the reaction is as follows—



After neutralisation the derivative terephthalic acid-bis-2-hydroxyethylamide was filtered off, washed with water and dried at 100° C and its melting-point was determined.

With two samples of Terylene the percentages of polyester found were 99·5 and 99·0 and the melting-points of the derivatives were 234° and 234·5° C, respectively.

THE DETERMINATION OF NYLON 610 SALT IN MOIST MATERIAL

Nylon 610 salt is a combination of hexamethylenediamine and sebacic acid; in moist material it may be readily determined by solution of the salt in excess of acid followed by titration of the excess of acid with standard alkali in the titrimeter.

METHOD

REAGENTS—

Hydrochloric acid, N.
Sodium hydroxide solution, N.
Ethanol.

PROCEDURE—

Accurately weigh an amount of the sample containing about 4 g of nylon 610 salt into a 250-ml beaker. Add 100 ml of ethanol and 50·0 ml of *N* hydrochloric acid. Stir until solution of the salt is effected and then titrate with *N* sodium hydroxide solution to a pH of 3·65, using the automatic titrimeter. This pH was found by plotting a full pH curve of the titration to be the pH of the solution after neutralisation of the excess of hydrochloric acid. Calculate the amount of *N* hydrochloric acid required by the sample.

1 ml of *N* hydrochloric acid ≡ 0·1592 g of nylon 610 salt.

RESULTS—

When this test was applied to known weights of dried nylon 610 salt, the results were as follows—

Salt added, g	3·906	3·016
Salt found, g	3·903	3·013

When applied to three salt solutions of unknown concentration, the following results were obtained by the proposed test and also by the lengthy determination of total solids—

Solution	A	B	C
Salt found, %	75·80, 75·72	74·96, 74·80	77·16, 77·08
Total solids found, % ..	75·8	75·2	77·0

THE DETERMINATION OF ALDEHYDES AND KETONES (CALCULATED AS ACETONE) IN METHYL METHACRYLATE MONOMER

The automatic titrimeter is of great value in this test and the principle may obviously be applied to other similar determinations. A known volume of the monomer sample is shaken thoroughly with a solution of hydroxylamine hydrochloride of known initial pH value. The mixture is then titrated automatically with standard alkali back to the pH of the original hydroxylamine hydrochloride solution. Correction is made for any methacrylic acid in the sample and the amount of alkali used is calculated in terms of acetone.

METHOD

REAGENTS—

Hydroxylamine hydrochloride solution, 2 per cent. w/v—Dissolve 20 g of hydroxylamine hydrochloride in water and dilute to approximately 1 litre. Add *N* sodium hydroxide until the solution has a pH of 4·2 to 4·3.

PROCEDURE—

Transfer, by means of a pipette, 20 ml of hydroxylamine hydrochloride solution to a 100-ml glass-stoppered conical flask. Add 20 ml of monomer sample, place the stopper in the flask and shake it for 30 minutes, using a mechanical shaker. Shake a control containing only 20 ml of hydroxylamine hydrochloride solution simultaneously with the sample. After shaking, transfer the solution to a 100-ml beaker, wash the flask with 5 ml of water and add the washings to the solution in the beaker.

Set the automatic titrimeter at the pH of the control hydroxylamine hydrochloride solution, and, with the function switch at pH RISING and the FAST - SLOW change-over switch at 40 mV, titrate the sample with 0·1 *N* sodium hydroxide solution to this pH.

1 ml of 0·1 *N* sodium hydroxide ≡ 0·0058 g of acetone.

Should the sample of monomer contain methacrylic acid, however, a correction must be applied. For each 0·01 per cent. w/v methacrylic acid present, subtract 0·001 per cent. w/v of acetone from the value obtained by the method described above.

This correction was determined by carrying out the complete procedure with 20 ml of monomer containing added amounts of methacrylic acid, with the following results—

Methacrylic acid added, % w/v	0·053	0·102	0·151	0·202
Apparent acetone content, % w/v ..	0·005	0·009	0·013	0·016

The method has been tested by determining the acetone contents of a series of monomer samples to which had been added methacrylic acid and acetone in amounts unknown to the operator at the time of test. The results were as follows—

Methacrylic acid content, % w/v	Correction for acidity calculated as acetone, % w/v	Total acetone found, % w/v	Corrected acetone found, % w/v	Acetone added, % w/v
0·049	0·005	0·090	0·085	0·095
0·129	0·013	0·077	0·064	0·071
0·020	0·002	0·023	0·021	0·023
0·080	0·008	0·050	0·042	0·048

The work described in this paper was carried out with the automatic titrimeter manufactured by Messrs. Electronic Instruments Limited, although we see no reason why other automatic titrimeters should not be used. L. H. Ruddle assisted in the work on the determination of aldehydes and ketones in methyl methacrylate monomer.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
PLASTICS DIVISION
WELWYN GARDEN CITY, HERTS.

January 30th, 1957

Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Methods for the Determination of Phenols and Sulphide

Phenols

THE term "phenols" as used in this connection covers the monohydroxy derivatives of benzene and its homologues. These compounds are used in industry in the pure state and as mixtures, and the composition of the mixture of phenols present in an effluent is unpredictable. Different phenols give slightly differing colours with analytical reagents, but the first method ("Indophenol Method") gives satisfactory results for low concentrations. For higher concentrations, a titration method ("Bromination Method") is given. In both methods the result is expressed as phenol, C_6H_5OH.

The determination should be made as soon as possible after the sample has been collected, since the phenol content tends to diminish, especially at low concentrations and at high temperatures. If delay in the analysis is unavoidable, the phenols may be "fixed" by the addition of a small amount of sodium hydroxide solution to a portion of the sample reserved for the determination.

PRELIMINARY TREATMENT OF SAMPLE

Unless interfering substances (see under the relevant "Method") are known to be absent, the following preliminary treatment should be employed. It should also be used if the sample is coloured.

REAGENTS—

Ammonium polysulphide solution—Dissolve a sufficient amount of sublimed sulphur in a solution of ammonium sulphide to produce a deep-yellow solution.

Sodium hydroxide solution, 10 per cent. w/v.

Sulphuric acid, dilute, 25 per cent. w/v.

Lead carbonate.

PROCEDURE—

Treat an appropriate volume of the sample (for the amount, see the relevant "Procedure") with a few drops of ammonium polysulphide solution to convert any cyanide present to thiocyanate, and allow the mixture to stand for about 10 minutes. Omit this treatment if cyanides are known to be absent. Remove sulphide by the careful addition of a small excess of lead carbonate and filter the sample into a distillation flask. Wash the filter-paper and precipitate with a small volume of distilled water, collecting the washings in the flask. Add 10 ml of sodium hydroxide solution and connect the flask to a water-cooled condenser. Boil the contents of the flask and distil and reject a volume of distillate equal to 20 per cent. of that in the flask. (This treatment removes amines.) Cool the flask and add to it 10 ml of dilute sulphuric acid. Distil and collect a volume of distillate equal to the original volume of the sample. A small volume of water may be added to the contents of the flask if necessary to enable this to be done. Examine the distillate by one of the methods given below, according to the amount of phenol expected.

INDOPHENOL METHOD

PRINCIPLE OF METHOD—

"Phenols" react with *p*-aminodimethylaniline in the presence of a mild oxidising agent to give a blue colour, which is measured or compared with standards.

RANGE—

The method is directly applicable to solutions containing 0·1 to 1 mg of phenols per litre. Stronger solutions may be diluted with water before being tested; weaker solutions are dealt with by a slight modification.

APPLICABILITY—

The method is directly applicable to a wide variety of solutions, but amines must be absent in the actual solution tested. (See "Preliminary Treatment of Sample.")

REAGENTS—

Carbon tetrachloride.

Sodium bicarbonate solution, 5 per cent. w/v.

Potassium ferricyanide solution, 8 per cent. w/v.

Sodium sulphate, anhydrous.

Standard phenol solution, 0·001 per cent. w/v—Prepare immediately before use by diluting 1 ml of a 0·1 per cent. w/v aqueous solution of pure phenol to 100 ml with distilled water.

p-*Aminodimethylaniline reagent*—Since the purchased base, its dihydrochloride and sulphate are unstable* and tend to discolour on being kept, the following method of preparation is recommended—

> Prepare a 0·1 per cent. w/v solution of *p*-nitrosodimethylaniline by dissolving the pure crystalline compound in water near its boiling-point. Reduce the solution with a large excess of zinc dust and a small amount of sodium bicarbonate solution (about 8 g of zinc and 2 ml of sodium bicarbonate solution per 25 ml of solution). The reduction proceeds better if the solution is warm. If difficulty is experienced, 1 drop of 10 per cent. w/v copper sulphate solution may be added. The reduced solution should be colourless. Cool and filter the solution.

PROCEDURE—

The solution for test will normally be the distillate from the preliminary treatment of the sample. A sample volume of 100 ml is convenient for distillation, unless the concentration of phenol is very low (see Note). The phenol content of the solution under test should be adjusted by dilution if necessary to lie between 0·1 and 1·0 mg per litre.

Measure 50 ml of the solution for test into a separating funnel and add 2 ml of sodium bicarbonate solution together with 2 ml of *p*-aminodimethylaniline reagent. Then add 2 ml of potassium ferricyanide solution and mix well. A blue colour will slowly develop. After 15 minutes extract the coloured solution with two successive 20-ml amounts of carbon tetrachloride. Dry the combined extracts with a small amount of anhydrous sodium sulphate and filter the mixture, washing the filter-paper with a small volume of solvent, and dilute the filtrate to 50·0 ml.

Simultaneously, using the same procedure, prepare from the standard phenol solution a series of standard solutions covering the range 0·1 to 1·0 mg of phenol.

Determine the phenol content of the sample either by visual colour comparison with the standards or instrumentally, by means of a spectrophotometer or an absorptiometer.

If an instrument is employed, use a wavelength of 6000 to 6100 A in a spectrophotometer or a suitable red filter in an absorptiometer, and read the phenol content equivalent to the observed optical density from a previously prepared calibration graph; this is established by relating the optical densities of the standards to their phenol content. Express the results as milligrams of phenol per litre of sample.

> NOTE—For samples of very low phenol content, a larger sample volume may be treated and finally extracted with the same volume (50 ml in all) of solvent as above, appropriate adjustments being made in the volumes of other reagents and in calculating the result.

* The oxalate of *p*-aminodimethylaniline, which is also available commercially, is sufficiently stable to be used for the preparation of the reagent solution.

Bromination method

PRINCIPLE OF METHOD—

The phenols in the sample are brominated with a standard solution of potassium bromate and bromide, and the excess of bromine is determined by titration.

RANGE—

For samples containing more than 20 mg of phenols per litre of sample.

APPLICABILITY—

Substances, other than phenols, that react with bromine, *e.g.*, reducing agents and unsaturated organic compounds, will interfere with the method. The preliminary treatment described above should satisfactorily eliminate most of the substances likely to interfere.

REAGENTS—

Bromate - bromide solution, 0·5 N—Dissolve 13·92 g of potassium bromate and 75 g of potassium bromide in sufficient distilled water to produce 1 litre.
Sulphuric acid, dilute, 25 *per cent. w/v.*
Potassium iodide solution, 10 *per cent. w/v.*
Sodium thiosulphate solution, 0·2 N.
Carbon tetrachloride.
Starch indicator solution.

PROCEDURE—

For samples containing between 20 and 200 mg of phenol per litre, take 200 ml of the sample.

For samples containing between 200 and 500 mg per litre take 100 ml of the sample, and *pro rata*. For stronger solutions, take such a volume as contains from 0·02 to 0·05 g of phenol and dilute to 100 ml with distilled water.

Transfer the sample under test, which will usually be the distillate from the "Preliminary Treatment of Sample", to an iodine flask and add 20·0 ml of bromate - bromide solution from a pipette; then add 10 ml of dilute sulphuric acid. Quickly re-insert the stopper and seal the neck of the flask with 10 ml of potassium iodide solution. Allow the flask to stand for 1 hour in the dark at room temperature, and then wash the sealing solution into the flask and add a further 10 ml of potassium iodide solution. Titrate the liberated iodine with 0·2 *N* sodium thiosulphate solution, adding a few drops of starch indicator solution and a few drops of carbon tetrachloride near the end-point. Standardise the thiosulphate soluion against the bromate - bromide solution, using the same procedure.

Find, by difference, the volume of bromate - bromide solution used in brominating the phenol.

1 ml of 0·5 *N* bromide - bromate solution ≡ 0·0078 g of phenol.

Express the result as milligrams of phenol per litre of sample.

Sulphide

INTRODUCTION—

The method described below is for the determination of the total sulphide present. The determination of sulphide should be made with the minimum of delay after the taking of the sample to avoid oxidation and loss of free hydrogen sulphide.

If an immediate analysis is not possible, the hydrogen sulphide must be "fixed" by precipitation of the sulphide with zinc acetate as described under "Procedure."

PRINCIPLE OF METHOD—

After acidification the free and combined hydrogen sulphide is displaced from the sample by a stream of inert gas and absorbed in a solution of zinc acetate. The hydrogen sulphide is again liberated from the zinc sulphide and oxidised with an excess of iodine. The amount of iodine used is determined by titration.

RANGE—

The method is given for concentrations above 5 mg of sulphide per litre of sample and a variation is given for concentrations below 5 mg per litre.

APPLICABILITY—

The method is generally applicable, but volatile compounds that react with iodine, and those substances that produce such compounds on acidification, *e.g.*, thiosulphate, necessitate a preliminary "fixing" (see first paragraph under "Procedure").

APPARATUS—

A wide-mouthed bottle or flask having a capacity of about 1 litre. The cork carries a small dropping funnel, an inlet tube and an outlet tube. The inlet tube has a sintered-glass diffuser on the end.

Two small conical flasks fitted with corks and inlet and outlet tubes to constitute two absorbing flasks in series.

REAGENTS—

Hydrochloric acid, diluted (1 + 1).

Zinc acetate solution, 25 per cent. w/v.

Dilute zinc acetate solution, 2 per cent. w/v—This solution should be freshly prepared.

Sodium thiosulphate solution, 0·1 N *and* 0·01 N.*

Iodine solution, 0·1 N *and* 0·01 N.*

Starch indicator solution.

PROCEDURE—

If the presence of interfering substances is suspected, or if the analysis has to be delayed, add 25 per cent. zinc acetate solution to a measured volume of sample in the proportion of 2 ml per litre, respectively. Allow the precipitate to settle. Decant the clear supernatant liquor through a small filter-paper; then transfer the precipitate to the paper. Transfer the filter-paper and precipitate to the wide-mouthed bottle or flask with 500 ml of distilled water. Proceed as described below, starting at "Connect the outlet tube of the bottle. . . ."

If the analysis is to be made immediately after sampling, and no interfering substances are present, measure 500 ml of the effluent sample (or a suitable aliquot if the sulphide content is known to be high) into the wide-necked bottle or flask.

Connect the outlet tube of the bottle or flask to the two conical flasks arranged in series as absorbers. If a smaller volume of sample is taken, add distilled water to make the volume up to about 500 ml. In each absorption vessel place 50 ml of 2 per cent. zinc acetate solution. Connect the delivery tube and diffuser to a source of carbon dioxide, nitrogen or other inert gas, free from oxygen. Measure 50 ml of diluted hydrochloric acid into the dropping funnel and add it slowly to the sample, maintaining a slow steady stream of inert gas through the system.

The hydrogen sulphide will be carried forward and precipitated as zinc sulphide in the absorption flasks. Continue the passage of the gas for 1 hour; then disconnect the absorbers. Add to each absorber a measured volume of 0·1 *N* iodine solution (or 0·01 *N* if the sulphide content of the sample is less than 5 mg per litre) so that a small excess is present. The second flask will require a much smaller volume of iodine solution than the first. To each add 10 ml of diluted hydrochloric acid, allow the solution to stand for 10 minutes and titrate the excess of iodine with 0·1 *N* (or 0·01 *N*) thiosulphate solution, using starch indicator solution.

A blank test should be carried out using the same volumes of reagent, but omitting the sample under test.

Calculate the *total* net volume of 0·1 *N* (or 0·01 *N*) iodine solution used by subtracting the *total* volume of 0·1 *N* (or 0·01 *N*) sodium thiosulphate solution.

1 ml of 0·1 *N* iodine solution ≡ 0·0017 g of hydrogen sulphide.

Express the result as milligrams of hydrogen sulphide per litre of sample.

* The 0·01 *N* solutions do not keep and should be freshly prepared.

Notes

COMPARISON BETWEEN THREE METHODS USED FOR THE PREPARATION OF TISSUES FOR DETERMINATIONS OF POTASSIUM AND SODIUM

VARIOUS methods have been used to prepare tissues for the determination of the concentrations of potassium and sodium, namely—

(*a*) dry-ashing and then extraction with hydrochloric acid,[1]
(*b*) boiling the whole tissue in distilled water[2,3,4] and
(*c*) extracting the whole tissue with nitric or trichloroacetic acid.[5,6]

In the present investigation three different methods used for preparing various tissues of the sheep were compared.

EXPERIMENTAL

In the first series of comparative analyses, heart, liver and kidney tissues from sheep that had been anaesthetised with pentobartital or pentobarbital and chloralose and used in acute experiments were examined after preparation by methods A and B described below. In the second series, these three tissues together with spleen and skeletal muscle were taken from normal 1½-year-old Greyface (Suffolk × Scottish Blackface) wethers immediately after slaughter, and methods A, B and C were compared.

METHOD A: DIGESTION WITH TRICHLOROACETIC ACID—

An accurately weighed sample of wet tissue (approximately 1·5 g) was homogenised for 15 minutes in the cold with 25 ml of 2 per cent. w/v trichloroacetic acid (containing 1 drop of *n*-octanol). The volume was made up to 100 ml with more of the 2 per cent. acid solution, and the whole homogenate was stored in a refrigerator for at least 24 hours. Potassium and sodium were determined in appropriate dilutions of the supernatant fluid.

METHOD B: BOILING UNDER REFLUX—

An accurately weighed tissue sample (approximately 1·5 g) was gently boiled under reflux with 30 ml of distilled water in a 250-ml round-bottomed Pyrex-glass flask having a ground joint for 2 hours. The condenser was washed with distilled water and allowed to drain for 1 hour before the flask was connected. After the flask had cooled the condenser was washed with distilled water to make a final volume of 50 ml, on the assumption that the tissue had a specific gravity of 1·00. The condenser was allowed to drain for 1 hour and the stoppered flasks were stored in a refrigerator for 24 hours. Appropriate dilutions of the supernatant fluid were used for the determination of the potassium and sodium.

METHOD C: BOILING UNDER REFLUX PLUS ACIDIFICATION—

The pH of the supernatant fluid by the trichloroacetic acid method was approximately 2 and that for the supernatant fluid by the method of boiling under reflux was approximately 7. One drop of concentrated nitric acid changed this latter fluid to approximately pH 3. In method C the procedure was the same as in method B, except that one drop of nitric acid was added to the boiled tissue immediately before it was stored in the refrigerator.

DETERMINATION OF POTASSIUM AND SODIUM—

Potassium and sodium were determined by means of an E.E.L. flame photometer (Evans Electroselenium Ltd.), and appropriate corrections for interfering substances were made.

RESULTS

A comparison of the potassium concentrations in the heart, liver and kidney tissues of anaesthetised sheep after preparation by methods A and B is shown in Table I, together with a comparison of the potassium and sodium concentrations in the heart, liver, kidney, spleen and skeletal muscle of normal sheep after they had been prepared by methods A, B and C.

For the heart, liver and kidney, method A gave higher results for potassium concentrations than method B. The spleen showed a similar difference, but no significant difference was observed with the skeletal muscle. Methods A and C gave similar values for potassium in all tissues except the spleen. With this tissue method A gave significantly higher potassium concentrations than method C.

TABLE I

COMPARISON BETWEEN THREE METHODS USED FOR THE PREPARATION OF TISSUES FOR THE DETERMINATION OF POTASSIUM AND SODIUM

Tissue	Number of animals	Element found, milli-equivalents per kg (wet weight), by— method A	method B	method C	Comparison of methods† A and B	B and C	A and C
Determination of potassium—							
Heart	8*	83·32	78·26	—	0·1%	—	—
	5	85·29	80·63	85·43	2·0%	1·0%	n.s.
Liver	3*	82·16	74·68	—	5·0%	—	—
	5	83·15	78·79	83·42	0·1%	0·1%	n.s.
Kidney	8*	56·25	54·75	—	2·0%	—	—
	4	70·89	67·10	70·74	2·0%	2·0%	n.s.
Spleen	5	115·99	103·02	112·96	0·1%	1·0%	5·0%
Skeletal muscle	4	101·91	100·24	101·61	n.s.	n.s.	n.s.
Determination of sodium—							
Heart	5	37·51	37·79	38·03	n.s.	n.s.	n.s.
Liver	5	33·16	32·50	32·69	n.s.	n.s.	n.s.
Kidney	4	67·52	63·50	66·48	1·0%	1·0%	1·0%
Spleen	5	32·26	30·67	33·12	on 5% border	5·0%	n.s.
Skeletal muscle	4	21·01	20·84	21·22	n.s.	n.s.	n.s.

n.s. = not significant.

* Samples from anaesthetised animals used in the first series of comparative analyses.
† Percentages refer to the level of significance.

Concentrations of sodium are also shown in Table I, but only in the kidney and spleen were significant differences observed between the results by the three methods.

DISCUSSION OF RESULTS

It has been shown that for certain tissues the trichloroacetic acid method and the boiling under reflux plus acidification method gave similar results for both potassium and sodium. As acidification after boiling often released a further amount of potassium and gave results comparable to those by the trichloroacetic acid method, it seems evident that acidification is an important step in releasing potassium from some tissues.

In the present investigation the behaviour of sodium, which is essentially an extracellular cation, was different from that of potassium, which is essentially intracellular.

Although acidification does not appear to be necessary for complete liberation of potassium from skeletal muscle, it does appear necessary in the case of the heart, liver, kidney and spleen. This observation supports the suggestion put forward by other workers[4,7,8,9] that potassium may exist in more than one form in a tissue and that the state in which potassium is present in tissues is different from one organ to another.

One of us (M.S.M.) carried out this work while on study leave from the Faculty of Agriculture, University of Alexandria, Alexandria, Egypt.

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ROWETT RESEARCH INSTITUTE
BUCKSBURN, ABERDEENSHIRE

M. S. MOUNIB
J. V. EVANS*

January 11th, 1957

(* PRESENT ADDRESS: FACULTY OF RURAL SCIENCE, UNIVERSITY OF NEW ENGLAND, ARMIDALE, NEW SOUTH WALES, AUSTRALIA)

SPECTROPHOTOMETRIC DETERMINATION OF IRON CHELATES. PART II

In a previous Note[1] details were given of an ultra-violet spectrophotometric method for the determination of three iron chelates of the polyaminepolycarboxylic acid type, namely Fe - EDTA, Fe - HEEDTA and Fe - DTPA.

The method has now been successfully applied to three other chelates that have since become available: these are the ferric chelates of NN′-dihydroxyethylethylenediaminediacetic acid (Fe - HEEDDA), *cyclo*hexane-1 : 2-diaminotetra-acetic acid (Fe - CDTA) and ethylenediamine-NN′-bis-(*o*-hydroxyphenylacetic acid) (Fe - EDHPA).

Experimental

An examination was made of the visible and ultra-violet spectra of dilute solutions, approximately 0·0001 *M*, of the three chelates at pH values ranging from 1·5 to 11·5. Some representative results are given in Figs. 1, 2 and 3, from which it can be seen that the spectra vary greatly with the pH of the solutions.

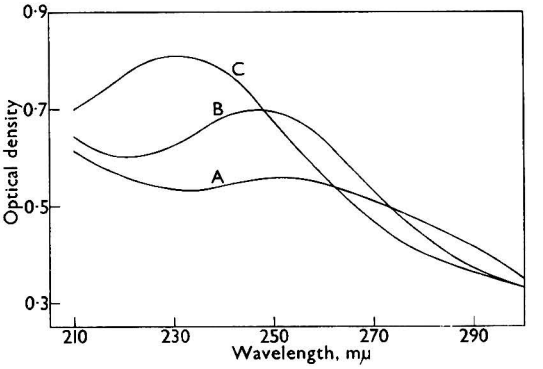

Fig. 1. Variation with pH of the ultra-violet spectrum of the iron - NN′-dihydroxyethylethylenediaminediacetic acid complex: curve A, pH 2; curve B, pH 3; curve C, pH 7 to 8

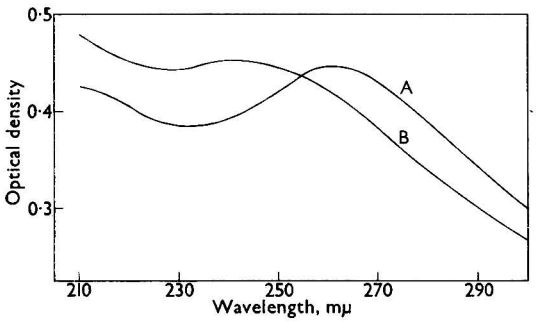

Fig. 2. Variation with pH of the ultra-violet spectrum of the iron - *cyclo*hexane-1 : 2-diaminotetra-acetic acid complex: curve A, pH 2 to 9; curve B, pH 10·5

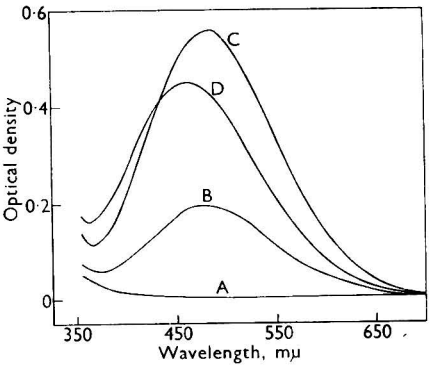

Fig. 3. Variation with pH of the visible spectrum of the iron - ethylenediamine-NN′-bis-(*o*-hydroxyphenylacetic acid) complex: curve A, pH 1·5; curve B, pH 2; curve C, pH 4 to 10; curve D, pH 11·5

For Fe - HEEDDA solutions it was found that there was no wide pH range over which the ultra-violet spectra were constant, but that solutions of pH 2·8 ± 0·1 had a peak at 250 mμ and that these conditions were suitable for quantitative determinations. In contrast, solutions of Fe - CDTA covering the range pH 2 to pH 9 all had similar spectra with a peak at 258 mμ.

There is a peak in the ultra-violet spectra of Fe - EDHPA solutions at about 280 mμ, but this wavelength is unsuitable for the quantitative determination of the iron chelate, because EDHPA alone has a considerable absorbance there. In the visible region, Fe - EDHPA solutions covering the range pH 4 to pH 10 all had a peak at 485 mμ, at which wavelength the free chelate did not interfere.

As the iron chelate solutions were found to be photosensitive, they were protected from sunlight throughout the experiments.

Standard series of these iron chelates were prepared and were brought within the appropriate pH range by the method previously described. Their optical densities were then determined at the wavelengths of the peaks mentioned above. It was found that solutions of each chelate containing 0 to 5 μg of iron per ml all obeyed the Beer - Lambert law. By plotting concentrations of iron, in μg per ml, as abscissae and optical densities as ordinates, the equations for the regression lines obtained with 1-cm cells were—

$$y = (0{\cdot}110 \pm 0{\cdot}002)x \text{ for Fe - HEEDDA;}$$
$$y = (0{\cdot}147 \pm 0{\cdot}002)x \text{ for Fe - CDTA;}$$
$$y = (0{\cdot}083 \pm 0{\cdot}001)x \text{ for Fe - EDHPA.}$$

All results fell within the 95 per cent. confidence limits.

Results

In laboratory experiments on the behaviour of iron chelates in calcareous soil, this spectrophotometric method has been used successfully in the analysis of aqueous extracts of treated soils.[2] In order to compensate for the absorbance of normal soil constituents, which is considerable in the ultra-violet region, extracts of untreated soil were made and used as controls.

The method has also been used to standardise solutions of the chelating agents themselves by what was, in effect, a spectrophotometric titration.

The gifts of HEEDDA (Versene-diol) from the Dow Chemical Company of the U.S.A., and of CDTA and EDHPA (Chel-600 and Chel-138, respectively) from the Geigy Company Ltd. of Manchester are gratefully acknowledged. I thank Mr. G. M. Clarke for his help with the statistical analysis.

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The Research Station
Long Ashton
Bristol

D. G. Hill-Cottingham
February 25th, 1957

THE ABSORPTIOMETRIC DETERMINATION OF VANADIUM IN STEEL*

Two colour reactions proposed for the determination of vanadium in steel have been examined in an attempt to determine whether the procedures described offered any improvement over the classical method with hydrogen peroxide. The reagents that have been studied are 3:3′-dimethylnaphthidine, proposed by Milner and Nall[1] for the microchemical determination of vanadium in ferrous alloys, and tungstophosphoric acid, first studied by Wright and Mellon,[2] and used more recently by Lennard[3] for the direct determination of vanadium in high-speed steels. The object of the study was to establish optimum conditions for the formation of coloured compounds in the presence of iron and to investigate the effects of interfering elements.

Experimental

Use of 3:3′-dimethylnaphthidine—

The reddish violet oxidation compound formed by the oxidation of 3:3′-dimethylnaphthidine with quinquivalent vanadium was found to obey Beer's law in the concentration range 12·5 to 250 μg of vanadium per 50 ml. The microchemical procedure[1] is subject to interference from chromium, probably owing to partial oxidation of chromium. Nall (in a personal communication) has since suggested that the acidity of the solution before selective oxidation with permanganate should be increased and states that the speed of oxidation of chromium decreases as the concentration of acid increases. The rate of oxidation of vanadium also decreases, but not to the same extent.

* Based on B.I.S.R.A. paper MG/D/69/56.

With this revision in mind, an initial approach was made with concentrations of sulphuric acid and phosphoric acid based on those suggested by Milner and Nall. The colour was found to be unstable and faded rapidly. Modifications to the conditions for colour development were investigated by varying the acid concentration, the standing time before measurement of the optical density and the temperature. The experiments showed that both the rate of colour development and the maximum optical density attained were dependent upon the final acid concentration. Warming the solution resulted in high optical densities of the reagent blanks, probably owing to partial decomposition of the excess of 3:3′-dimethylnaphthidine. A mixture of sulphuric acid, phosphoric acid and water in the proportions of (1 + 1 + 8) produced the most stable colour, which reached full intensity in 5 to 10 minutes, remained reasonably stable for 20 minutes and then faded progressively. Colour development was retarded in solutions containing sulphuric acid alone and no improvement in stability was noted. The reaction is extremely sensitive, but under the conditions investigated somewhat erratic. Reproducibility tests with standard steels gave a standard deviation of about 0·025 per cent. at the 0·5 per cent. of vanadium level. This, together with the fact that check calibration curves prepared at intervals of several weeks were widely divergent, suggested that the reagent was not suitable for the development of a precise macro-analytical method.

USE OF TUNGSTOPHOSPHORIC ACID—

The addition of sodium tungstate and phosphoric acid to a solution of a vanadate gives a tungstovanadophosphate compound, which obeys Beer's law in the concentration range 0 to 1·5 mg of vanadium per 50 ml. At room temperature a brownish yellow colour is produced, which is transformed on boiling to the true yellow colour of tungstovanadophosphate.

The interference due to coloured ions such as chromium may be compensated for by measuring the optical density of the test solution against a solution containing no added sodium tungstate. It is not possible, however, to use a compensation method if tungsten is present, since interaction between tungstate and vanadium in solution gives a yellow colour even in the absence of added sodium tungstate. Hence a correction graph for chromium must be constructed if the sample contains tungsten, as suggested by Lennard.[3]

Iron also forms a coloured compound with tungstophosphoric acid and may be compensated for by carrying out the procedure on a sample of vanadium-free iron. Trials with Lennard's procedure gave high and somewhat variable "iron blank" colours, but modification to the conditions of colour development suggested that the absorbancy due to iron could be minimised by increasing the final acid concentration. The effect of an increased concentration of phosphoric acid, variation of the concentration of tungstate and the effect of boiling time were studied. The optimum concentrations were found to be 0·025 *M* sodium tungstate with 6 *N* phosphoric acid. To reach equilibrium conditions the solution must be boiled for 5 minutes before dilution to volume. The combined colour due to iron and vanadium reaches a maximum intensity after 20 to 30 minutes and thereafter remains stable for several hours. In the experimental work reported here, the difference reading for the iron blank with 4-cm cells varied between 0·075 and 0·085 optical-density units, equivalent to about 45 μg of vanadium per 50 ml.

Cooper and Winter[4] found that the degree of interference by molybdenum was difficult to evaluate accurately. Calibration tests prepared in the presence of 5 and 10 per cent. of molybdenum confirmed this view. In fact, molybdenum gives rise to a slight additive absorption, the degree of which is variable and cannot be compensated for by a simple correction graph. This interference cannot be related to the ratio of molybdenum to vanadium. In amounts less than about 2 per cent., interference by molybdenum is not significant and may be ignored for practical purposes. In the presence of 10 per cent. of molybdenum, calibration tests indicated a positive error of between 0·02 and 0·05 per cent. of vanadium. For work of the highest accuracy with molybdenum bearing steels, the correction should be determined by using prepared solutions that correspond in composition to that of the samples.

METHOD

REAGENTS—

Sodium tungstate solution, 0·25 M—Dissolve 8·25 g of sodium tungstate, $Na_2WO_4.2H_2O$, in 100 ml of water.

Sulphuric - phosphoric acid mixture—Cautiously add 150 ml of concentrated phosphoric acid and 150 ml of concentrated sulphuric acid to 600 ml of water. Cool and dilute to 1 litre.

Phosphoric acid, diluted (1 + 2).

Potassium permanganate solution, 0·01 N.

Standard vanadium solution—Dissolve 1·485 g of ammonium metavanadate in water and dilute to 1 litre in a calibrated flask.

1 ml ≡ 0·5 mg of vanadium ≡ 0·1 per cent. of vanadium.

PROCEDURE FOR STEELS FREE FROM TUNGSTEN—

Dissolve 0·5 g of the sample in 25 ml of sulphuric - phosphoric acid mixture. Oxidise with concentrated nitric acid and evaporate to fumes. Cool and dilute to 30 ml, warming if necessary to dissolve soluble salts. After cooling, dilute to 100 ml in a calibrated flask, mix well and transfer two 10-ml aliquots to 250-ml beakers. One aliquot is for the test solution; to it add 0·01 *N* potassium permanganate solution from a burette until one drop produces a pink colour that persists for 1 minute and then add two drops in excess. Add 20 ml of diluted phosphoric acid and 5·0 ml of 0·25 *M* sodium tungstate solution, and dilute to 50 ml. Add two glass beads, cover the beaker with a clock-glass and boil the solution gently for 5 minutes. Cool, dilute to 50 ml in a calibrated flask, mix well and set aside for 30 minutes. The second aliquot is for the compensating solution; treat it as for the test solution, but omit the addition of sodium tungstate solution.

Measure the optical density of the test solution against that of the compensation solution, using a balanced two-cell type (selenium photocells) absorptiometer with a mercury-vapour lamp, Ilford No. 601 violet filters and Calorex H503 heat filters. Deduct the optical density obtained from a sample of vanadium-free iron similarly treated as an "iron blank" and convert to the percentage of vanadium from a prepared calibration graph. To compensate for the reduced-iron content of samples containing more than a total of 20 per cent. of alloying elements, the weight of vanadium-free iron taken for the "iron blank" must be reduced to correspond to the iron content of the sample under test.

A calibration graph may be prepared by adding measured amounts of standard vanadium solution to a series of samples of vanadium-free pure iron.

PROCEDURE FOR STEELS CONTAINING TUNGSTEN—

If tungsten is present, omit the compensating solution, and measure the optical density of the test solution, and deduct the sum of the "iron blank" and the "chromium blank." The latter may be found from a correction graph relating percentages of chromium to optical density. The interference is linear and approximately equal to 0·015 per cent. of vanadium for each 1 per cent. of chromium present.

TABLE I

RESULTS WITH THE TUNGSTONVANADOPHOSPHATE METHOD FOR LOW-ALLOY STEELS

B.C.S. No.	Composition			Certified value for vanadium, %	Vanadium found	
	Nickel, %	Chromium, %	Molybdenum, %		Mean,* %	Mean deviation, %
251	5·2	—	—	0·03	0·02	±0·01
252	4·1	—	—	0·46	0·46	±0·01
253	2·9	—	1·0	0·22	0·22	±0·015
254	2·1	—	1·3	0·52	0·52	±0·005
255	—	1·0	1·4	0·26	0·27	±0·01
256	—	2·3	—	0·36	0·36	±0·01
257	—	1·7	—	0·12	0·11	±0·005
258	—	3·1	—	0·65	0·65	±0·01
275	—	—	—	0·05	0·055	±0·005

* Mean of 6 determinations.

RESULTS

In Table I the results obtained with a range of low-alloy steel standards are compared with accepted values determined by the standard volumetric method.[5] These results were considered to provide satisfactory evidence of the reliability of the procedure. A further series of results, shown in Table II, obtained with steels containing tungsten are compared with B.C.S. certificate values and with values obtained by the B.I.S.R.A. Methods of Analysis Committee.[6]

The reaction between vanadium and tungstophosphate is less sensitive than the oxidation reaction with 3:3′-dimethylnaphthidine, but is more reliable and offers a fivefold increase in sensitivity over the method with hydrogen peroxide.

TABLE II

RESULTS WITH THE TUNGSTOVANADOPHOSPHATE METHOD FOR TUNGSTEN STEELS

Sample No.	Composition				Acepted value for vanadium,* %	Vanadium found	
	Chromium, %	Tungsten, %	Molybdenum, %	Cobalt, %		Mean,† %	Mean deviation, %
MGS 48	5	22	0·5	12	1·52	1·55	±0·03
MGS 166	4	10	—	—	4·02	4·05‡	±0·07
MGS 176	3	10	—	—	0·54	0·55	±0·015
MGS 212	4·5	8	2	—	2·17	2·15	±0·04
BCS 220	4·6	6·7	4·2	—	1·35‖	1·37§	±0·02
BCS 241	5·2	20·3	—	5·8	1·54‖	1·54	±0·02

* Obtained by the B.I.S.R.A. Methods of Analysis Committee.
† Mean of 6 determinations.
‡ For 0·25-g sample and 0·25 g of pure iron.
§ Corrected for interference by molybdenum.
‖ B.C.S. certificate values.

I thank Miss D. V. Swindell for carrying out a considerable portion of the analytical work, and also Mr. W. R. Nall (Bragg Laboratory) for many helpful suggestions. This Note is published by permission of the British Iron and Steel Research Association.

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METALLURGY (GENERAL) DIVISIONAL LABORATORIES
THE BRITISH IRON & STEEL RESEARCH ASSOCIATION
HOYLE STREET
SHEFFIELD, 3

P. H. SCHOLES
November 22nd, 1956

THE ABSORPTIOMETRIC DETERMINATION OF TRACES OF IRON IN BISMUTH

IN the course of investigations into dissolution in liquid metals, it became necessary to develop a method for the determination of traces of iron (5 to 50 p.p.m.) in bismuth metal. Colorimetric methods appeared the most promising, and the use of ammonium thiocyanate, thioglycollic acid and *o*-phenanthroline was considered. Bismuth interfered seriously with the first two reagents and this interference could not be easily overcome. However, it was found that with *o*-phenanthroline the effect of bismuth was masked by the addition of ethylenediaminetetra-acetic acid (EDTA) without preventing the development of the coloured iron complex.

EXPERIMENTAL

Sandell[1] states that, if sodium citrate is used to adjust the pH of a solution, hydroquinone must be used to reduce iron to the ferrous state. However, hydroquinone produces a strong yellow colour with EDTA; in view of this, hydroxylamine hydrochloride was used as a reducing agent and was found to be satisfactory. In the presence of nitric acid, *o*-phenanthroline showed a very low sensitivity to the presence of iron and so all solutions were prepared by first dissolving the metal in nitric acid and then evaporating to dryness several times with hydrochloric acid.

To ensure complete reduction of the iron the reducing agent must be added before the other reagents. It was found that in order to obtain stable solutions, the EDTA, citrate and *o*-phenanthroline must be added together, and the pH raised to 5·5, otherwise precipitation occurred. Under these conditions the solutions were stable for several hours, the colour taking 1 hour to develop.

ACCURACY AND PRECISION OF THE METHOD—

The iron in two samples, one of pure and one of commercial bismuth, was determined ten times. The results for pure bismuth *A* were as follows—

31·0, 30·0, 31·1, 31·2, 31·0, 30·0, 31·0, 31·1, 31·7 and 31·9 p.p.m.,

which gave a mean value of 31·0 p.p.m., with a standard deviation of 0·60 or 1·93 per cent. of the mean.

For commercial bismuth *B* the results were as follows—

90·6, 92·5, 94·0, 91·5, 92·0, 91·6, 91·4, 91·5, 90·8 and 91·4 p.p.m.,

which gave a mean value of 91·7 p.p.m. with a standard deviation of 0·96 or 1·09 per cent. of the mean.

The iron contents of commercial bismuth *B* and of another sample of pure bismuth *C* were determined spectrographically. For sample *B* the result was 80 ± 15 p.p.m., whereas the result by the proposed colorimetric method was 91·7 p.p.m. Similarly, for sample *C* the results were 10 p.p.m. and 7·45 p.p.m., respectively. Samples *A* and *B* originated from Mining and Chemical Products Ltd., and sample *C* was specially prepared.

Method

Reagents—

All solution should be prepared in redistilled water.

EDTA solution—A solution containing 37·2 g of the disodium salt per litre.

Sodium citrate solution, 50 per cent. w/v.

o-Phenanthroline solution, 0·25 per cent.

Hydroxylamine hydrochloride solution, 5 per cent. w/v.

Bismuth solution—Dissolve 50 g of Specpure bismuth in nitric acid and evaporate to dryness three times with hydrochloric acid; re-dissolve the residue in 5 ml of concentrated hydrochloric acid, transfer to a 100-ml calibrated flask, dilute to the mark with redistilled water and shake well.

1 ml ≡ 0·5 g of bismuth.

Standard iron solution—Dissolve 0·04 g of Specpure iron in aqua regia and evaporate to dryness; dissolve the residue in 50 ml of 20 per cent. hydrochloric acid, transfer to a 1-litre calibrated flask (N.P.L. class A tolerance), dilute to the mark with redistilled water and shake well.

1 ml ≡ 40 μg of iron.

Procedure for calibrating the absorptiometer—

By pipette place 2 ml of bismuth solution in a 100-ml calibrated flask. Add to the flask the appropriate aliquot of standard iron solution. Simultaneously carry out a blank determination, omitting the standard iron solution. If, at any stage, precipitation of bismuth occurs, add hydrochloric acid dropwise until the precipitate just redissolves, avoiding an unnecessary excess. Add 1 ml of hydroxylamine hydrochloride solution. In a 100-ml beaker mix together 10 ml of EDTA solution, 30 ml of sodium citrate solution and 2 ml of *o*-phenanthroline solution. Add the contents of the beaker to the 100-ml calibrated flask. Dilute the contents of the flask to the mark with redistilled water, mix well and set aside for 1 hour. Using a Spekker absorptiometer, measure the absorption against that of the blank, using Ilford No. 603 (Spectrum blue-green) filters and a 4-cm glass cell.

Procedure for determining iron in samples of bismuth—

In a 100-ml beaker dissolve the sample (up to 2 g) in nitric acid. In another beaker, take the same amounts of reagents and give an identical treatment for the blank. Evaporate to dryness three times with hydrochloric acid. Add hydrochloric acid dropwise to redissolve the sample and then add 1 ml of hydroxylamine hydrochloride solution. Transfer to a 100-ml calibrated flask and proceed as described above for the calibration procedure.

I am indebted to Mr. W. H. Hardwick for helpful advice and discussion.

Reference

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Chemical Engineering Division
Atomic Energy Research Establishment
Harwell, nr. Didcot, Berks.

D. G. Holmes
January 2nd, 1957

THE ABSORPTIOMETRIC DETERMINATION OF URANIUM IN SOLUTIONS BY AN IMPROVED THIOCYANATE METHOD

An intense yellow colour is produced when a concentrated solution of ammonium thiocyanate is added to a slightly acid solution of uranium.[1,2,3] The intensity of the colour depends greatly

upon the concentration of thiocyanate and to a lesser extent upon the amount of acid present. The method is most suited for use with sample solutions from a uranium plant that contain sufficiently small concentrations of impurities as not to warrant any chemical separation, but it is also applicable to solutions of solids from which interfering elements have been removed. The method cannot be applied when molybdenum, vanadium, titanium, chromium, osmium or ruthenium is present.

Interference from ferric iron was reported by Steele,[1] who used stannous chloride solution to reduce the ferric iron to the ferrous state. This was satisfactory provided the amount of ferric iron present in the solution was less than 5 mg. As ferric iron is often in excess of this, or its concentration is unknown, an alternative method was required that would overcome the difficulties encountered when a stannous chloride solution is used for the reduction of the ferric iron.

Ascorbic acid was found to be preferable to stannous chloride for the reduction of ferric iron. Larger amounts of ferric iron could be tolerated and the reaction was almost instantaneous. The concentration of sulphuric or hydrochloric acid in the sample had no marked effect on the intensity of the colour in the range pH 0·5 to 2·5. Solutions of either the chloride or sulphate can be used for this method.

Method

All measurements were made in a Unicam SP600 spectrophotometer with balanced 1-cm cells. The glass apparatus used was calibrated by the conventional methods.

Reagents—

Ammonium thiocyanate solution—Dissolve 500 g of analytical-reagent grade ammonium thiocyanate in distilled water, dilute to 1 litre and filter.

Ascorbic acid solution—Dissolve 20 g of L-ascorbic acid, B.P., in distilled water and dilute to 1 litre.

Procedure–

Transfer a carefully measured aliquot of the sample (containing approximately 1 mg of uranium) to a 25-ml calibrated flask, and add 5 ml, or more if required, of ascorbic acid solution. Shake the flask gently, then add 7 ml of ammonium thiocyanate solution and dilute to the mark with distilled water. Prepare a blank from the same amounts of the reagents as used in the test solution. Measure the optical density in a 1-cm glass cell at a wavelength of 365 mμ with the reagent blank in the reference cell. As the thiocyanate colour fades, measurement should be made within 1 hour of the colour development.

To calibrate the instrument, take suitable aliquots of an accurately prepared uranium solution, develop the colour and measure the optical density as described above. An aqueous solution of the reagents is again used as a reference blank. Some typical results for the calibration of the instrument were as follows—

U_3O_8 per 25 ml, mg ..	0·100	0·200	0·300	0·400	0·500	0·600	0·700	0·800	0·900	1·000
Optical density ..	0·894	0·790	0·702	0·626	0·554	0·490	0·436	0·391	0·345	0·305

Note that, if required, a more concentrated solution of ascorbic acid may be used, but the amounts stated are adequate for the reduction of well over 5 mg of ferric iron.

Conclusions

The method described is rapid and more reliable than the method of Steele[1] and can be used in the presence of far greater concentrations of ferric iron. The various difficulties connected with the use of stannous chloride for the reduction of ferric iron have also been overcome by the use of ascorbic acid.

I thank the Manager and Consulting Engineer of the Hartebeestfontein Gold Mining Company Limited for their permission to publish this Note.

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Hartebeestfontein Gold Mining Co. Ltd.
Stilfontein, Transvaal

H. T. Tucker
December 27th, 1956

THE ANALYSIS OF BISMUTH - URANIUM ALLOYS

SEN SARMA and Mallik[1] have shown that uranyl uranium can be quantitatively precipitated with oxine in the presence of various cations, including bismuth, after the addition of ethylenediamine-tetra-acetic acid (EDTA) to form soluble metal - EDTA complexes. This has been confirmed with solutions containing bismuth and uranium, and it has been noted that low results are obtained with less than about 20 mg of uranium; above this figure the recoveries are quantitative in the presence of up to 5 g of bismuth (this being the largest amount of bismuth taken).

These observations are made from the following results for uranium in experiments in which a 10 to 20 per cent. excess of EDTA was present over that required for the formation of a (1 + 1) complex with bismuth—

Bismuth taken, g ..	1·0	2·0	3·0	3·0	3·0	3·0	4·0	5·0
Uranium taken, mg ..	39·1	39·1	39·1	31·3	23·5	15·6	7·8	39·1	39·1
Uranium found, mg ..	39·0(5)	39·0	39·1(5)	31·3	23·4	15·3	7·2	39·2	39·2

Solutions for analysis were prepared from weighed amounts of bismuth metal (99·99 per cent. pure), aliquot portions of a standardised solution of uranyl nitrate (prepared from uranium metal turnings) and nitric acid; both metals were supplied by A.E.R.E., Harwell. The uranium turnings were cleansed of oil and cutting compound by immersion in molten phenol at about 60° C, and were then pickled in an acetic acid - nitric acid mixture. After solution of 4·0 g of clean metal in nitric acid and dilution to 1 litre, the uranyl nitrate solution so obtained was standardised gravimetrically by both the oxine and diuranate procedures and 10 ml of this solution were found to contain 39·1 mg of uranium.

A direct adaptation of Sen Sarma and Mallik's procedure[1] gave a method for the determination of uranium in bismuth-rich alloys from a lower limit of about 0·5 per cent. of uranium and covering the composition range of interest as liquid-metal reactor fuel. The fact[2] that the bismuth - EDTA complex is hydrolysed at pH values greater than 8 may be exploited for the determination of bismuth with oxine, after removal of the uranyl oxinate by filtration, simply by making the necessary pH adjustment. The use of this procedure to determine both uranium and bismuth would best serve the analysis of those alloys in which the bismuth to uranium weight ratios lie between about 3 to 1 and 1 to 3.

METHOD

PROCEDURE FOR PREPARING THE SOLUTION—

Dissolve the sample in diluted nitric acid (1 + 1) and evaporate to dryness; dissolve the residue in concentrated hydrochloric acid and again evaporate to dryness. Dissolve the residue in concentrated hydrochloric acid, dilute with water and add an excess of EDTA; the amount added may conveniently be taken as twice the sample weight. Finally dilute to about 150 ml.

PROCEDURE FOR PRECIPITATING URANIUM—

The following procedure is adapted from that of Sen Sarma and Mallik.[1] Adjust the pH of the solution to 5·5 ± 0·3 by neutralising with diluted ammonia solution (1 + 1), using methyl red as indicator, and then add 1 ml of diluted acetic acid (1 + 1) and 25 ml of 20 per cent. ammonium acetate solution. Warm to about 60° C and add a 4 per cent. solution of oxine in ethanol; set aside in a warm place for 10 minutes and then collect the precipitate on a sintered-glass crucible of porosity No. 3. Wash the precipitate with warm water containing 25 ml of the oxine solution per litre and dry it at 105° C for 1 hour.

PROCEDURE FOR DETERMINING BISMUTH—

Add a further volume of the oxine solution to the uranium-free filtrate, warm to about 50° C and make alkaline with sodium hydroxide solution. Allow the precipitated bismuth oxinate to coagulate and collect it on a fluted hardened filter-paper. Wash the precipitate and dissolve it in hot diluted hydrochloric acid (1 + 1), add 2 ml of the oxine solution, warm to 60° C, and re-precipitate bismuth by adding diluted ammonia solution (1 + 1) until a precipitate just forms, and then a further 5 ml of the ammonia solution. Set the mixture aside in a warm place for 10 minutes, and then collect the precipitate in a sintered-glass crucible and wash and dry it.

RESULTS

The recoveries of uranium and bismuth from solutions containing uranyl and bismuth ions are shown in Table II.

TABLE II

RECOVERIES OF URANIUM AND BISMUTH

Uranium taken, mg	Bismuth taken, mg	Uranium found, mg	Bismuth found, mg
78·2	192·4	78·2	193·0
117·3	150·7	117·8	149·9
156·4	101·0	155·9	100·8
195·5	60·2	195·3	60·0

I thank the Director, A.E.R.E., Harwell, for permission to publish this Note.

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METALLURGY DEPARTMENT
UNIVERSITY OF MANCHESTER

R. A. J. SHELTON
April 17th, 1957

Book Reviews

METHODS OF CHEMICAL ANALYSIS AS APPLIED TO SEWAGE AND SEWAGE EFFLUENTS. Issued by the Ministry of Housing and Local Government. Pp. vi + 96. London: Her Majesty's Stationery Office. 1956. Price 10s. 6d.

This eagerly awaited Second Edition was published in December. At a time when interest in the problems of river pollution is rapidly increasing, an official booklet on such a subject is certain to be welcome, not only because the first edition, issued in 1928, has been out of print for a long time, but also because there is now much wider recognition of the need for detailed standardisation of the empirical methods on which most effluent analysis is based.

The booklet was compiled by a distinguished committee of experts, under the chairmanship of Dr. Key, and the considerations that guided them in their choice of recommended methods are clearly and concisely set out in the preliminary Report. Here the chemist will find reason and authority for modifying some time-honoured methods, and the over-worked will find sanction (all too rare among "official" methods) for the sacrifice of some accuracy when speed is really the more important consideration. In short, the Committee have attempted to supply analysts with a thoroughly realistic set of methods covering all the main features of routine sewage and sewage-effluent analysis; they have admirably succeeded.

Although each of the sections into which the booklet is divided contains much of interest and value, the 17 pages devoted to determinations of dissolved oxygen and B.O.D. are perhaps the most important, since wide divergence between different laboratories now occurs all too frequently. It is clearly desirable, therefore, that the recommendations of the Committee for standardising this test should be adopted in detail, even if, in individual cases, adoption may involve modification of well established routine. Particularly is this important in relation to the composition of dilution water for the B.O.D. test: there may be justification for divergencies from the recommendation in special cases, but there is none for routine samples. The procedure and precautions that are necessary to obtain concordant results are well described, although the Committee's recommendations for "seeding" sterile effluents may not always be easy to carry out. Warning is given of the uncertainties that attend the estimation of B.O.D. in chlorinated effluents (can a chlorinated effluent really have a B.O.D.?) and under "General Observations" quite extensive consideration is given to the erratic effects of nitrification. It is rather surprising, therefore, that, although the release of oxygen by suspended algae under the influence of light is pointed out, the reverse effect that may increase the B.O.D. of river samples incubated in the dark is not mentioned. Also, the information that can often be obtained by comparing the B.O.D. values of an unknown effluent at different dilutions is not referred to in this section, although it may be very useful.

The sections, new in this edition, that deal with determinations of metals, phenols, cyanides, sulphides and anionic detergents invite special attention. It is not to be expected that they will escape some detailed criticism, but in the Report the Committee recognises that methods of analysis will have to be varied to suit individual cases. Nevertheless, the recommended procedures will not always find general approval. For example, the directions for estimating sulphides

by converting them to methylene blue are formidable, involving as they do the preliminary distillation in an inert atmosphere of the unstable *p*-aminodimethylaniline. The corresponding nitroso compound, which does not require preliminary distillation, can easily be reduced in a few minutes when required (a fact which the Committee recognises elsewhere) and it is a much more convenient and equally effective reagent.

Recommendations for determining nitrogen in its various forms follow conventional lines, but might have been extended to include directions for dealing with chlorinated effluents. The section on acid permanganate value has been entirely rewritten and the detailed instructions for making this important test are now admirably precise. Adequate procedures for standardising methods of determining total, suspended and settleable solids are given and there are notes on the strength of sewage, which include a method for determining the $N/8$ permanganate demand.

It is quite remarkable how much useful information has been compressed into the 96 pages of this booklet, which is adequately bound and attractively presented. Fallible mortals will be mildly encouraged to note within its pages that the printers' devil is still humanly frail and automation has not yet overwhelmed H.M.S.O. J. G. SHERRATT

GAS CHROMATOGRAPHY. By COURTENAY PHILLIPS. Pp. x + 105. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1956. Price 25s.; $4.50.

Now that gas chromatography is firmly established as an analytical tool, there will be many analysts who are either about to use this technique for the first time, or who are responsible for training juniors in its application. To them this book is confidently recommended; it gives a complete introduction to the field in the space of only a hundred pages, by surveying the types of problem that can be solved by gas chromatography, and the methods that have been used in practice. In addition to a valuable theoretical treatment of the subject there are concise descriptions of a number of different types of apparatus. The book is well illustrated by clear diagrams, both of apparatus and of actual chromatograms. It would, however, have been an improvement if a diagram of the Martin gas-density balance could also have been included, for it is difficult to follow a purely verbal description of this instrument.

Although the greater part of the book, as might be expected, is devoted to gas - liquid partition chromatography, there is a short section on gas-adsorption methods, a much older technique. A very brief comparison of adsorption and partition methods is included, which might usefully have been extended.

The references given are not exhaustive; but they have evidently been carefully selected, and considerably enhance the value of the book. N. H. RAY

OXINE AND ITS DERIVATIVES. Volumes III and IV: DERIVATIVES OF OXINE—Parts 1 and 2. By R. G. W. HOLLINGSHEAD, M.A., A.R.I.C. Pp. vi + 617–896 + Index to Vol. III, pp. 1–16; vi + 897–1212 + Index to 4 volumes, pp. 1–40. London: Butterworths Scientific Publications. 1956. Price 42s; 42s.

The first two volumes of this work (*Analyst*, 1955, **80**, 569) discussed 8-hydroxyquinoline, its preparation, properties and uses. The main topics for the present pair are the various derivatives, treated on similar lines. Since the literature coverage is very substantial, the number of pages allotted, indicated in parentheses, gives a fair idea of the amount of active interest aroused by the various subjects; these are, the salts of oxine (33), monohalogen (38), di- and tri-halogen (69) and methyl (65) derivatives, sulphonic (74) and carboxylic (15) acids, phenyl and substituted phenyl derivatives (7), nitroso, nitro and amino derivatives (27), azo (21), hydroxy (9) and miscellaneous (37) derivatives.

Oxine and its substitution products have been used as antibacterials for many years. For example, bandages impregnated with Vioform have been used in the Swiss army since 1904; some were encountered in the 1939 war. A report on modern investigations, admittedly still incomplete, into antibacterial and antifungal action in this group is therefore given.

The remaining 152 pages comprise an addendum to the previous chapters of recent papers; by this device coverage to early 1956 has been achieved. Indeed, in some places, information appeared herein before publication in the journals. The nuisance of dual location has been mitigated to some extent by the inclusion of good indexes, although at least two Swiss patents are not shown in the index of patents, possibly because they are cited in the main text and not among the collected references.

Varying nomenclature has caused some malarrangements. There is a heading (p. 644) for oxine sulphosalicylate, but the 2-hydroxy-5-sulphobenzoate is classified under "miscellaneous salts." Similarly, on pp. 840 and 841, in connection with oxine-5-sulphonic acid, 1-phenyl-2:3-dimethyl-4-dimethylaminopyrazolone is treated as though it were different from pyramidone (which incidentally is described as a *de*hydropyrazole).

For people working in any way with oxine, this text affords an excellent jumping-off ground. It gives the impression that it has been largely a labour of love; it is to be hoped that love will not go unrewarded. B. A. ELLIS

TABLES OF PHYSICAL AND CHEMICAL CONSTANTS. Originally compiled by G. W. C. KAYE, O.B.E., M.A., D.Sc., F.R.S., and T. H. LABY, M.A., Sc.D., F.R.S. Now prepared under the Direction of an Editorial Committee. Eleventh Edition. Pp. vi + 233. London, New York and Toronto: Longmans, Green & Co. Ltd. 1956. Price 25s.

One of the most difficult classes of book to review is the reference book. It cannot, strictly speaking, be "read," and to attempt to check its correctness can be exceedingly difficult (unless the errors be glaringly numerous). When the book has reached its eleventh edition, such a check can be fairly omitted: and a glance at the names of the editors and contributors to this newly revised "Kaye and Laby" provides evidence of the quality of the workmanship.

The reviewer of the present edition can only excuse his presumption in commenting on this standard work by pleading that he, too, met G. F. C. Searle at Cambridge. Kaye and Laby, in the preface to their first edition, acknowledge the encouragement and suggestions of Mr. G. F. C. Searle, F.R.S., in 1911; Professor Feather, in 1955, acknowledges Professor Searle's encouragement in similar terms; and the reviewer will always remember with delight the way Professor Searle "looked in" on wartime university lectures on electronics—and was heard to comment that electronics wasn't physics!

Professor Feather's preface gives the background against which this edition must be judged. When the tenth edition was published, the original authors were both dead; the publishers then set up an editorial board, at first of three, and later of four (Dr. H. Barrell, Dr. E. A. Coulson, Professor N. Feather, F.R.S., and Mr. J. M. C. Scott), who co-ordinated the work of the 38 contributors engaged in completely revising the various sections. The essential features, particularly the "brief *résumé* containing references" to which attention was drawn in the preface to the first edition, are retained as indispensable; alterations are mostly in the detail.

Much information has been added since the tenth edition of 1948, and many of the less necessary tables have been omitted. Much has been gained by this—and much that received criticism from earlier and better qualified reviewers has been excised in keeping the increase in size of the volume to a mere 20 per cent., so that little of value has been lost. "Viscosities of solids," on p. 38, still, however, includes Golden Syrup: and perhaps the twelfth edition (when it comes) will provide an opportunity for adding a word or two to the title of this table. Until the twelfth edition comes, however, the eleventh is an essential to any laboratory bookshelf, and should certainly replace any copy of the tenth edition that may still be there. J. B. ATTRILL

THE CONDENSED CHEMICAL DICTIONARY. Fifth Edition. Revised by ARTHUR and ELIZABETH ROSE. Pp. xx + 1201. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1956. Price $12.50; 100s.

A reference book of a very different type, but no less valuable, is this dictionary. The fifth edition has been completely revised and is printed by photolithography from typescript, a method that considerably simplifies the printing procedures; the previous edition was printed from type in the more conventional way.

This is, as the publishers state, a mine of information for chemists, engineers, consultants, purchasing agents and executives: they might well have added authors and editors. It may give little information of value to the "pure" analyst; but the consultant, who may want information on shipping restrictions (in the United States, at any rate), hazards (fire and toxicity), uses, sources of supply, trade-names and many other facets of the chemical trade, should find it worth buying. Authors could well use it to check nomenclature: although the warning must be added that this is primarily American, and is not always identical with usage in this country. Nevertheless, the fourth edition has been well thumbed in this editorial office, and the fifth edition is a much needed replacement. J. B. ATTRILL

METALLURGICAL ANALYSIS BY MEANS OF THE SPEKKER PHOTOELECTRIC ABSORPTIOMETER. By F. W. HAYWOOD, B.Sc., Ph.D., F.R.I.C., F.I.M., and A. A. R. WOOD, A.R.I.C. Second Edition. Pp. viii + 292. London: Hilger & Watts Ltd. 1957. Price 40s.

In 1944, when the first edition of this book appeared, some metallurgical analysts were still opposed to the new absorptiometric methods of analysis pioneered by E. J. Vaughan and his collaborators. Since then, the large number of publications in this field bear witness to the almost universal adoption of absorptiometric methods and, in the routine laboratory, the verb "to Spekker" is heard at least as frequently as the verb "to titrate." A new edition of Haywood and Wood is therefore overdue.

The plan of the book follows closely that of the first edition and is in two parts; the first concerned with the role of absorptiometric analysis and with the manipulation of the Spekker absorptiometer; the second giving detailed instructions for the more important determinations in metallurgical analysis.

In the first part, the description of the old and new models of the instrument with the thorough exposition of the means for their efficient operation is excellent. If there is a fault to be found, it is in the incompleteness of the account of the physical basis of absorptiometry. For a proper understanding of the subject, it is essential to know the energy distribution of the light source as well as the response of the photocells and the transmission of the filters over a wider wavelength region than given in this text. The value of the book to the research analyst would be considerably enhanced if this aspect were treated more thoroughly.

In the second part of the book, methods for determining certain constituents of zinc alloys have now been included, and substantial additions and modifications have been made to the chapters on the analysis of steel, copper and aluminium alloys. The authors are justified in claiming that the methods chosen have given reliable service and that the selection affords a useful introduction to the subject. However, the reader would be well advised to note that the most recent references are to publications in 1954, since when several of the methods given have been displaced by faster, more selective or more reliable procedures.

A most useful innovation is a selected bibliography on absorptiometric methods, but it is to be hoped that this feature will be expanded in future editions to cover a less limited range of publications.

For training the young analyst in the proper use of the Spekker absorptiometer, Haywood and Wood is invaluable. For anyone who found the first edition useful, the large amount of new material in this second edition would justify its purchase. C. H. R. GENTRY

QUANTITATIVE CHEMICAL ANALYSIS. By CUMMING and KAY. Eleventh Edition. Revised by R. A. CHALMERS, B.Sc., Ph.D. Pp. 540 + xvi. Edinburgh and London: Oliver & Boyd Ltd. 1956. Price 30s.

For an elementary textbook of quantitative inorganic analysis to run through eleven editions in just over forty years is in itself a sufficient recommendation of the basic design of its founder authors, which was to ensure for the student a sound training in classical methods of chemical analysis.

"Cumming and Kay" has always retained this distinguishing characteristic, but owing to the rapid advances in chemical methods during recent years had, in the last edition or so, begun to wear an antiquated look. This aspect has now been largely rectified, and the book rejuvenated, by the reviser of the eleventh edition.

The improvement has been effected by a general overhaul of the book, which has involved much rewriting, many omissions of effete matter, the inclusion of methods that are more in line with modern practice and guidance to the literature for techniques that are prevented by the limitations imposed by a book of 540 pages from receiving full treatment.

With the exception of a procedure for the reduction of stannic ion by means of antimony powder, the methods described are sound and likely to be useful to a student in his future career.

The erratic behaviour of powdered antimony as a reducing agent is well known to assayers of tin; it was fully examined by B. S. Evans and D. G. Higgs (*Analyst*, 1944, **69**, 291).

The misapplied use of the word *case* in various phrases and connotations that could, with a little thought on the part of a writer, be expressed in words of more precise meaning, leaves a regrettable impression of careless writing in a book that is in other ways commendable. The reviewer noted 120 *cases*—most of them misapplied.

There are some misprints, mostly trivial; but "official density" for optical density and "2 mg." instead of 2 μg, in defining the concentration of a standard arsenic solution in the Gutzeit test, may be misleading to a student. F. L. OKELL

INTRODUCTION TO STRUCTURE IN ORGANIC CHEMISTRY. By C. K. INGOLD, D.Sc., F.R.S. Pp. viii + 200. London: G. Bell & Sons Ltd. 1956. Price 20s.

Professor Ingold has been persuaded to print the first four chapters of his monumental work "Structure and Mechanism in Organic Chemistry" (reviewed in *The Analyst*, 1954, **79**, 659) as a simple introduction to the general principles of molecular structure for first-year undergraduates . . . and at a price within their means. While this little book will certainly achieve this purpose, it can also be strongly commended to practising analysts who wish to brush up their knowledge of the fundamentals of contemporary theoretical organic chemistry, but have so far failed to find a suitable source book. H. IRVING

Publications Received

ORGANIC SYNTHESIS. Volume I: OPEN-CHAIN SATURATED COMPOUNDS; Volume II: OPEN-CHAIN UNSATURATED COMPOUNDS, ALICYCLIC COMPOUNDS, AROMATIC COMPOUNDS. By VARTKES MIGRDICHIAN, Ph.D. Pp. xxx + 833; xvi + 835–1822. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1957. Price $35.0; £14 the set.

HANDBOOK OF SOLVENTS. Volume I: PURE HYDROCARBONS. By IBERT MELLAN. Pp. vi + 249. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1957. Price $6.50; 52s.

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Quatrième Série. Pp. vi + 209. Paris: Masson et Cie. 1956. Price 2400 fr.

MATHEMATICS AND STATISTICS FOR USE IN PHARMACY, BIOLOGY AND CHEMISTRY. By L. SAUNDERS and R. FLEMING. Pp. x + 257. London: The Pharmaceutical Press. 1957. Price 27s. 6d.

Published by direction of the Council of The Pharmaceutical Society of Great Britain.

METHODS OF BIOCHEMICAL ANALYSIS. Volume IV. Edited by DAVID GLICK. Pp. x + 362. New York and London: Interscience Publishers Inc. 1957. Price $8.50; 68s.

VITAMIN A. By THOMAS MOORE, Sc.D., D.Sc. Pp. xx + 645. Amsterdam: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd.; New York: D. Van Nostrand Co. Inc. 1957. Price 76s.; $14.00.

LABORATORY AND WORKSHOP NOTES 1953–1955. Compiled and edited by RUTH LANG, Ph.D., A.Inst.P., *for* The Institute of Physics. Pp. xii + 248. London: Edward Arnold (Publishers) Ltd. 1957. Price 30s.

A fourth selection reprinted from the Journal of Scientific Instruments.

INSTRUMENTAL ANALYSIS. By PAUL DELAHAY. Pp. xiv + 384. New York and London: The Macmillan Company. 1957. Price $7.90; 55s. 6d.

ANALYTICAL MICROSCOPY: ITS AIMS AND METHODS IN RELATION TO FOODS, WATER, SPICES AND DRUGS. By T. E. WALLIS, D.Sc., F.R.I.C., F.P.S., M.I.Biol., Hon.F.R.M.S. Second Edition. Pp. viii + 215. London: J. &. A. Churchill Ltd. 1957. Price 25s.

TECHNIQUE OF ORGANIC CHEMISTRY. Edited by ARNOLD WEISSBERGER. Volume X: FUNDAMENTALS OF CHROMATOGRAPHY. By HAROLD GOMES CASSIDY. Pp. xviii + 447. New York and London: Interscience Publishers Inc. 1957. Price $9.75; 78s.

JOURNAL OF MICROBIOLOGY, EPIDEMIOLOGY AND IMMUNOBIOLOGY. Original Russian publication edited by I. I. ELKIN. Scientific Translation Editor, D. J. BAUER, B.A., M.B., B.Chir., Ph.D. Volume 28, No. 1. 1957. Pp. iv + 164. London, New York and Paris: Pergamon Press Ltd. *for* Pergamon Institute. Subscription £17 14s.; $50 per year.

First number of a complete translation, issued in monthly parts, of the Russian journal.

MODERN CEREAL CHEMISTRY. By D. W. KENT-JONES, Ph.D., B.Sc., F.R.I.C., and A. J. AMOS, Ph.D., B.Sc., F.R.I.C. Fifth Edition. Pp. x + 817. Liverpool: The Northern Publishing Co. Ltd. 1957. Price 105s.; $15.25.

Erratum

JUNE (1957) ISSUE, p. 455, 4th line from foot of page. *For* "Potter" *read* "Pottier."