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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

The Chemical Determination of Nicotinic Acid in Food Products

BY P. O. DENNIS AND H. G. REES

(*Read at the meeting of the Society on Wednesday, May 4th,* 1949)

CHEMICAL methods for the determination of nicotinic acid in the quantities normally found in foodstuffs are based on the reactivity of the pyridine ring, rupture of which is relatively easy. The most satisfactory reagent is cyanogen bromide and the observation of König[1] that a yellow colour is developed when pyridine is treated with cyanogen bromide in presence of an aromatic amine has been adapted by many workers[2,3,4,5] for the colorimetric estimation of nicotinic acid. The choice of amine is important for the development of the colour, particularly with regard to time for maximum intensity and stability.[6,7,8] Another step in the procedure that has received much attention is the preparation of the solution to be analysed; interfering substances must be removed and the solution rendered colourless or nearly so. Procedures proposed include acetone precipitation,[9] permanganate oxidation[10,11] and adsorption techniques.[7] Permanganate oxidation has proved suitable for urine analysis, but we find it to be unsatisfactory with meat products and James *et al.*[7] report indifferent results with cereals. For the past five years we have used a modification of Bandier's acetone method[9] on all materials when a purification is necessary. This is rapid and sufficiently accurate for all routine analysis, and it appears appropriate to publish the following results for workers in this field.

REAGENTS—

Nicotinic acid primary standard—Dissolve 50 mg. of nicotinic acid in 50 per cent. alcohol and make up to 200 ml. with the same solvent. This solution is stable for many months if stored in tightly-stoppered bottles in the refrigerator.

Nicotinic acid secondary standard—Prepare daily by diluting 10 ml. of primary standard to 250 ml. with water. It contains 10 μg. per ml.

Phosphate buffer solution—A 2 per cent. solution of AnalaR potassium dihydrogen phosphate in distilled water. The pH is 4·6 and the solution is stable for long periods in a refrigerator.

Cyanogen bromide reagent—Make an approximately 4 per cent. aqueous solution of cyanogen bromide by just decolorising ice-cold saturated bromine water with chilled 10 per cent. sodium cyanide solution. The bromine water and sodium cyanide solution should be made up every few days and stored in a refrigerator and the cyanogen bromide prepared just before use. Care must be taken to avoid an excess of sodium cyanide in the final solution.

Procaine hydrochloride solution—This substance is obtainable in a highly purified state for medicinal use and such material is eminently suitable as a reagent. Dissolve 3·5 g. in

80 ml. of water, add 1·5 ml. of AnalaR concentrated hydrochloric acid and make up with water to 100 ml. The solution is stable in light and air, but is best made up daily.

Acetone—Redistilled and dried over calcium oxide.

Kieselguhr—Commercial Supercel is washed with water and dried.

COLORIMETER—

A sensitive photo-electric colorimeter is recommended such as the Gallenkamp direct-reading instrument with a galvanometer calibrated logarithmically to give readings of the extinction coefficient $E_o \times 100$. A suitable filter for the colour measurement is Ilford Micro 1 Blue.

PROCEDURE—

We have found that certain materials such as meat extracts and pharmaceutical products not containing cereals and containing more than 500 μg. of nicotinic acid per gram yield light-coloured solutions after hydrolysis and neutralisation, thus enabling a simplified technique involving no solvent extraction to be used. Weigh out a quantity of the material containing between 1500 and 2500 μg. of nicotinic acid and digest it with 25 ml. of 4 *N* caustic soda on the steam-bath for 30 to 40 minutes. Cool and adjust to pH 4·0 with concentrated hydrochloric acid, using bromophenol blue as an external indicator; cool, make up with water to 100 ml. and filter through sintered glass to obtain a bright solution. The filtrate contains between 15 and 25 μg. of vitamin per ml. and, although coloured, is suitable for analysis without further treatment. One-ml. aliquots of this test solution are taken for analysis. The following mixtures are set up, preferably in 25-ml. volumetric flasks:

	T_1 ml.	T_2 ml.	S_1 ml.	S_2 ml.	S_3 ml.
Test solution	1·0	1·0	—	—	—
Nicotinic acid solution, 10 μg./ml. ..	—	—	1·5	2·0	2·5
Water	1·5	1·5	1·0	0·5	—
Phosphate buffer solution, pH 4·6 ..	5·0	5·0	5·0	5·0	5·0

Heat the flasks in a water-bath to 60° C. and add to each 2 ml. of cyanogen bromide reagent, continue heating for 15 minutes, remove, and cool to room temperature. Add 10 ml. of procaine reagent to T_1 and T_2, make up to 25 ml. with water and measure the colour in a photo-electric colorimeter within 2 to 3 minutes, for under these conditions colour development is almost instantaneous. To flasks S_1, S_2 and S_3 add 10 ml. of procaine reagent followed by 1 ml. of the test extract, make up to 25 ml. with water and measure the colour intensity.

Plot a graph of the galvanometer readings (*i.e.*, $E_0 \times 100$) of S_1, S_2 and S_3 against the nicotinic acid contents of the tubes, *viz.*, 15, 20 and 25 μg. This should give a straight or very nearly straight line.. From this the nicotinic acid contents of T_1 and T_2 may be read directly. By using this method of computation the necessity of preparing solution and reagent blanks, such as are required by the usual increment methods as employed by Wokes, is avoided, because corrections for the inherent colour of the test solution and for colour development between the extract and amine have been made by adding the equivalent amount of the extract to S_1, S_2 and S_3 after colour development and just before making up to 25 ml. Our experience has shown that no appreciable colour develops between the extract and the amine or by heating the extract with cyanogen bromide. Further, the accuracy of the increment method depends upon a strict adherence to Beer's law, a deviation from which is observed in the higher ranges of concentration of nicotinic acid.

For materials that have a low nicotinic acid content and yield highly coloured solutions after hydrolysis and neutralisation the following procedure is adopted. Weigh out sufficient material to contain between 250 and 500 μg. of the vitamin and digest with 25 ml. of 4 *N* caustic soda or 15 ml. of concentrated hydrochloric acid. Meat and yeast products are best hydrolysed with alkali, but acid is preferable for cereal products. These conditions yield lighter coloured solutions for analysis and simplify the technique of hydrolysis. In certain cases, *e.g.*, with corned beef, we have employed mild hydrolysis under pressure. After hydrolysis, cool, neutralise to pH 4·0 and make up to 50 ml. Take 5-ml. aliquots in each of two 50-ml. centrifuge tubes and treat both in the following manner to yield duplicate test solutions. Add 0·8 to 1·0 g. of kieselguhr and mix thoroughly, run in 9 to 10 volumes of dry acetone from a burette with constant stirring, close with a rubber bung and mix by vigorous shaking. The precipitate should be of a light fluffy nature; if it is not, insufficient kieselguhr has been used. Centrifuge and decant the supernatant liquid from each tube

into an 150-ml. volumetric flask, add two or three pieces of "bumper" and evaporate off the acetone under vacuum to a volume of about 5 ml., warming occasionally by immersion in warm water. To the residue in the centrifuge tube add a further 9 to 10 volumes of acetone, re-extract, centrifuge, add the supernatant liquid to the corresponding volumetric flask, and re-evaporate until there is no obvious odour of acetone in the flask, that is, to about 2 or 3 ml. Transfer the contents quantitatively to a 20 or 25-ml. flask with successive small portions of phosphate buffer solution, make up to volume and filter through a sintered glass funnel to obtain a bright test solution containing between 1 and 2 μg. of vitamin per ml. Set up the following mixtures in 25-ml. volumetric flasks.

	T_1 ml.	T_2 ml.	S_1 ml.	S_2 ml.	S_3 ml.	S_4 ml.
Test solution	5·0	5·0	—	—	—	—
Nicotinic acid solution, 10 μg./ml.	—	—	0·5	0·5	1·0	1·0
Water	1·0	1·0	0·5	0·5	—	—
Phosphate buffer solution, pH 4·5	—	—	5·0	5·0	5·0	5·0

Heat these mixtures to 60° C., add the cyanogen bromide and develop the colour exactly as before. From the average readings of S_1 and S_2 and of S_3 and S_4 a graph is plotted, and from it the nicotinic acid contents of T_1 and T_2 are read directly. We have usually found that the acetone precipitation yields a nearly colourless test extract and that on diluting 5 ml. to 25 ml. a negligible blank is produced; we also find it unnecessary to prepare a reagent blank if acidified procaine and phosphate buffer are used. If the test extract should be coloured, then a correction may be applied by adding 5 ml. of test solution to tubes S_1, S_2, S_3 and S_4 after the addition of procaine and just before making up to volume and reading the colour.

CALIBRATION CURVES—

From the many calibration curves obtained when studying the conditions necessary for optimum colour development, the following table gives an indication of the type of curve obtained with pure nicotinic acid. The conditions for colour development were essentially those recommended in the methods described above.

μg. Nicotinic acid	$E_o \times 100$
5	9·0
10	17·3
15	25·8
20	34·0
25	42·0
30	49·3
35	54·5
40	60·0

RECOVERY EXPERIMENTS—

Before the method was applied to food products, numerous recovery experiments were carried out with known quantities of nicotinic acid added to protein hydrolysates and composite meat and yeast products. Without quoting these results in detail we may state that satisfactory recoveries of the order of 98 to 101 per cent. were always obtained.

NICOTINIC ACID CONTENT OF VARIOUS FOOD PRODUCTS—

As an indication of the validity of the method we quote the following results obtained on duplicate samples of the products analysed by Wokes.[7] The results given in column 1 were obtained by our technique and those in columns 2 and 3 are taken from Wokes's paper.

	Nicotinic acid in μg./g.		
	Chemical assay		Microbiological assay
	1	2	3
Meat extract	1050	1027	1082
Yeast extract	560	478	537
Dried yeast	300	332	331
Malted barley	105	126	127
Wheat germ	135	134	129
Malt extract	96	102	95

Finally we append a series of results obtained on a variety of meat products over an extended period.

	Total creatinine, %	Nicotinic acid, μg./g.
Meat extract:		
(*a*)	7·80	1151
(*b*)	7·80	1124
(*c*)	7·52	900
(*d*)	7·45	870
(*e*)	7·40	890
(*f*)	7·00	810
Desiccated beef powder:		
(*a*)	1·22	126
(*b*)	0·90	102
(*c*)	0·90	117
(*d*)	0·88	135
Meat peptones:		
(*a*)	—	160
(*b*)	—	148
(*c*)	—	190
Meat juice:	Water	
(*a*)	58·0	362

	Water, %	Fat, %	Nicotinic acid in μg./g.	Nicotinic acid in μg./g. (dry wt. and fat-free)
Corned beef:				
Dry cure				
(*a*)	56·4	14·0	49·5	161·0
(*b*)	55·3	18·3	39·7	150·5
(*c*)	55·0	16·0	37·9	130·6
(*d*)	57·4	13·7	29·6	100·5
Wet cure				
(*a*)	64·9	2·9	40·7	126·5
(*b*)	58·4	10·4	35·7	114·5
(*c*)	53·8	14·7	29·5	93·0
(*d*)	55·8	6·8	29·5	78·7

Summary

A colorimetric method involving reaction with cyanogen bromide and coupling with procaine for the determination of nicotinic acid in food products has been presented. Rigorous purification of the extracts and compensation for reagent and extract blanks have been reduced to a minimum and are unnecessary for materials rich in nicotinic acid. To minimise the colour of the test extract, acid hydrolysis is recommended for cereals and alkaline hydrolysis for meat and yeast products.

The authors wish to thank the Directors of Oxo Limited for permission to publish these results, and to Dr. E. C. Wood for criticism of the manuscript.

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Discussion

Dr. W. F. Elvidge asked if it had been found necessary to adjust the pH of the cyanogen bromide reagent. He agreed with the authors in preferring procaine to *p*-acetophenone. He agreed also that it was necessary to make calibration curves day by day at the same time as the assays. Sometimes better results were obtained with the Beckmann instrument than with the absorptiometer plus filter. Difficulty had sometimes been experienced in obtaining a clear filtrate after addition of cyanogen bromide.

Miss C. Klatzkin stated that experience gained during thousands of assays of nicotinic acid in cereal products confirmed the authors' findings as to the advantage of acid extraction. However, even when an adsorption technique was subsequently applied the interfering substance might not be completely removed, and a blank was found helpful. The adsorption technique did, however, remove a good deal of interfering substance, as shown by the colourless solutions obtained, and in her experience did not take any longer to carry out than procedures such as that described by the authors. One of the causes of the high amine blanks she had encountered was found to be the presence of furfural. Did the authors' method obviate this difficulty without recourse to blanks? In regard to the stability of the colour given with procaine, an earlier study on cereal products by James, Norris and Wokes (*Analyst*, 1947, **72**, 327) had shown that the maximum intensity was reached in about 10 minutes after mixing, and by 15 minutes fading had set in. Timing was perhaps not so critical as with *p*-aminoacetophenone, but should not be ignored.

Mr. J. King, in answer to a question on the use of cation exchange zeolites such as Decalso for separating nicotinic acid from interfering substances, particularly amino acids and their addition products, suggested that theoretically it would be sounder to try the use of anion exchange resins of the zeo-carb 215 or 216 types containing .COOH or .SO_3H groups, as used by Consden, Gordon and Martin for the separation of acidic amino acids and by Bendall, Partridge and Westall for the separation of bases and amino acids (*Nature*, 1947, **160**, 374).

Dr. D. W. Kent-Jones said that his own earlier experiences with nicotinic acid assays using chemical methods and that of the Society's Sub-Committee working on the subject had been disappointing, and he was pleased to hear of a method for which a claim was made that it worked. Nevertheless, in view of previous disappointments, he would not be convinced of its usefulness until he had had experience of it himself.

He mentioned that arising from the work of the Sub-Committee, a note had been published in *The Analyst* (1947, **72**, 501) pointing out that some specimens of nicotinic acid contained *iso*-nicotinic acid and this complicated the problem as *iso*-nicotinic acid was not biologically active and should not be included in the assay.

Mr. W. B. Emery asked if the authors had any experience of the use of anion exchange resins in the purification of extracts for the assay. He would not expect very close agreement between the results of chemical and microbiological assays, since naturally-occuring compounds existed that gave the reaction, but had no biological activity.

Mr. J. Haslam asked if any degree of control of "room temperature" was advisable in the development of colour.

Mr. Dennis, in answer to the questions, agreed that the Beckmann spectrophotometer could be used with advantage. With regard to *iso*-nicotinic acid no great differences were observed in curves based on highly purified nicotinic acid and pharmaceutical specimens. It was also found that in presence of phosphate buffer, and under the conditions stated, no colour was produced with *iso*-nicotinic acid. The results obtained did agree with microbiological assays. When using *p*-aminoacetophenone, the very careful timing mentioned in Wokes's[7] paper was found necessary. Cyanogen bromide did not affect the pH in presence of the phosphate buffer. The authors had not been primarily concerned with cereals and had encountered no interference due to furfural. Exchange resins had not been tried as a means of purification. Provided that both test and standard tubes were cooled to "room temperature" at the same time, small differences in the final temperature were not important; however, the greater the degree of control over all the reaction conditions, the more closely did readings of successive standard curves agree.

The Use of Ether Extraction in the Determination of Uranium

By T. R. SCOTT

Although ether extraction of uranyl nitrate is now extensively used in the determination of uranium, such details of the technique as have appeared in print are scarcely adequate as a guide to the analyst. In particular, it is desirable to know to what extent and in what ways the extraction conditions may be varied without diminishing the usefulness of the method. It is the purpose of this paper to supply these details and, in addition, to describe an extraction procedure suitable for general application.

Experimental

Experiments have been conducted by shaking aqueous solutions of known composition with ether of "anaesthesia grade," measuring the volumes of the two layers after shaking has been completed and determining the composition of the ether layer by the hydrogen peroxide method (q.v.). Since the work was designed specifically to provide information for control of analytical procedures, the time of shaking was confined in all instances to 1 minute and no claim is made that results represent accurately controlled equilibrium conditions. It is nevertheless known that equilibrium is attained very rapidly in experiments of this type and the fact that repeated determinations of "percentage uranium extracted" generally agree within ±1 per cent. is sufficient indication that only small errors are introduced by restricting the time of shaking.

The extent to which extraction of uranyl nitrate has occurred is normally expressed by the partition coefficient, *i.e.*, the ratio of the equilibrium concentration in the ether layer to that in the water layer. With high partition coefficients, however, small experimental errors are unduly magnified and results in the present work are preferably expressed as the percentage of uranium extracted from the aqueous layer, when equal volumes of both layers are present after shaking. Then,

$$\text{Percentage Extracted} = \left(\frac{\text{Partition coefficient}}{1 + \text{Partition coefficient}}\right) \times 100$$

Variables that have been considered include temperature, nature and concentration of salts, concentration of nitric acid and uranyl nitrate, nature of interfering radicals and extraction of nitric acid. These will be discussed in turn.

Temperature—

Effects of temperature, over the usual range of room temperatures, are inconsiderable and could not be detected within the limits of error of the experimental method.

Nature of inorganic nitrates present—

Extraction of uranyl nitrate by ether is negligibly small unless the water layer contains quantities of inorganic nitrates or nitric acid or both. A comparison of the effects of various salts (at the same nitrate ion concentration) is made in Table I. Ferric nitrate, being most efficient in salting-out uranyl nitrate, has been used in the present work and has the additional advantage of reducing interference due to phosphate ion.

Table I

Effect of inorganic nitrates used in ether extraction

Salt	Concentration of the salt	Acid concentration	Percentage of uranium extracted
NH_4NO_3	3·0 *M*	1·5 *N*	30
$NaNO_3$	3·0 *M*	1·5 *N*	38
$Ca(NO_3)_2$	1·5 *M*	1·5 *N*	54
$Mg(NO_3)_2$	1·5 *M*	1·5 *N*	62
$Ce(NO_3)_3$	1·0 *M*	1·5 *N*	57
$Fe(NO_3)_3$	1·0 *M*	1·5 *N*	69

CONCENTRATION OF FERRIC NITRATE AND NITRIC ACID—

For analytical purposes it is essential to know the number of extractions required to recover (say) 99 per cent. of the uranium from a given solution (the actual percentage to be removed varying with the degree of accuracy of the work). Knowledge of the effects of

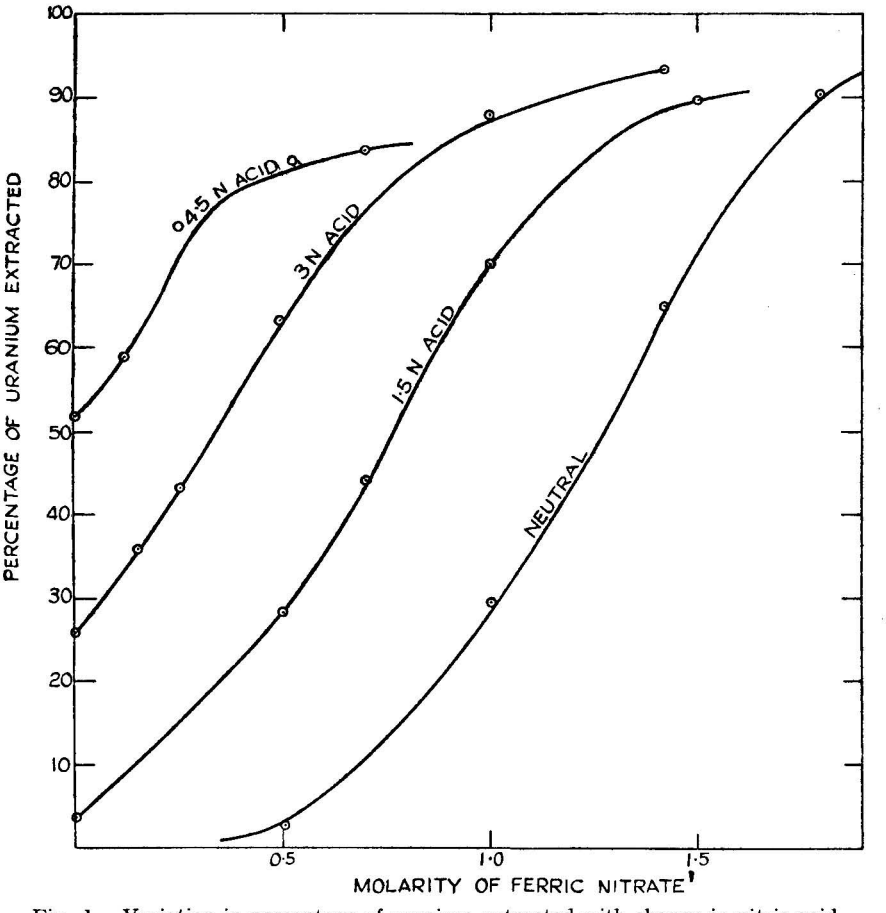

Fig. 1. Variation in percentage of uranium extracted with change in nitric acid and ferric nitrate concentrations

salt and acid concentrations on the partition coefficients is thus of great importance, especially as the range of variation is considerable. These two variables have consequently been examined in detail and the results are shown in Fig. 1.

With 4·5 *N* acid erratic values were obtained for the percentage of uranium extracted, particularly at the higher concentrations of ferric nitrate, and it is therefore recommended

TABLE II

VARIATION IN CONCENTRATION OF URANYL NITRATE

Nitric acid concentration	Ferric nitrate concentration	U_3O_8 concentration %	Percentage extracted
3·0 *N*	1·0 *M*	1·1	82·5
3·0 *N*	1·0 *M*	0·7	83·8
3·0 *N*	1·0 *M*	0·2	87·6
3·0 *N*	1·0 *M*	0·05	85·7
3·0 *N*	1·0 *M*	0·01	90·0
3·0 *N*	1·0 *M*	0·003	88·8

that acid concentrations should not exceed 3 *N* in the aqueous layer prior to extraction. With stronger acid, noticeable reaction with the ether also occurs.

CONCENTRATION OF URANYL NITRATE—

Table II shows the variation in percentage extracted over a range of concentrations. The variations are not regarded as being of importance in analytical operations.

INTERFERING RADICALS—

Anions—Although it is customary to extract uranyl nitrate from solutions containing nitrate as the only anion, large concentrations of other anions can be tolerated without modification of the routine procedure described below.

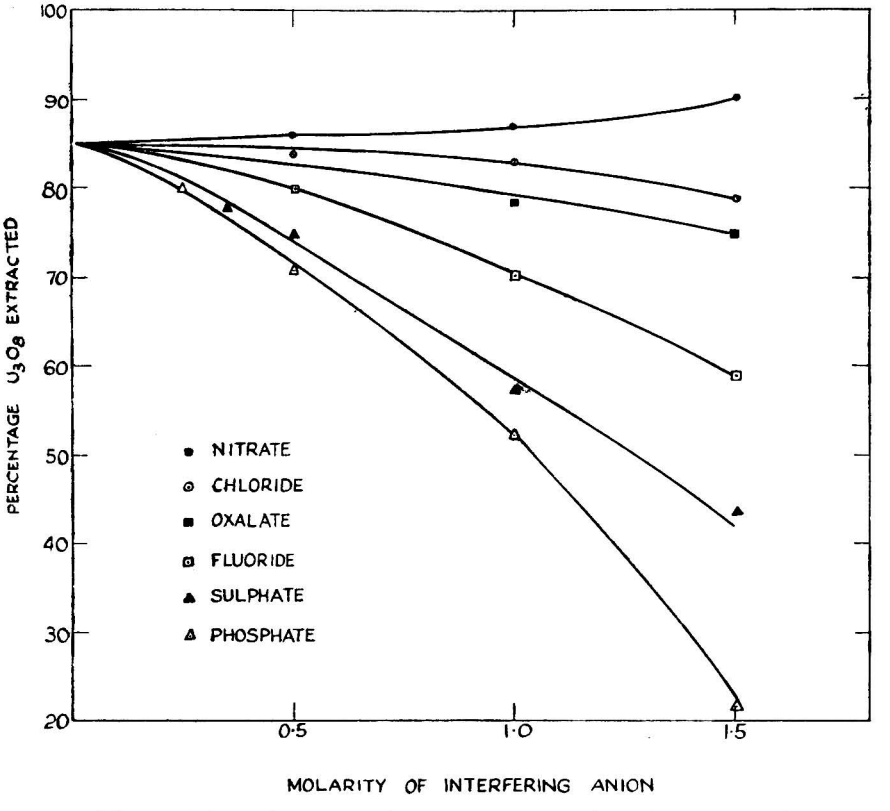

Fig. 2. Effect of various anions on percentage of uranium extracted

Solutions containing varying quantities of the common anions, introduced as the sodium salts, were made 3 *N* with nitric acid and 1 *M* with respect to ferric nitrate, and the percentage of uranium extracted was determined in the usual way. Results are shown in Fig. 2, from which it is evident that only fluoride, sulphate and phosphate can be regarded as seriously interfering radicals. The effect of acetate (not shown in the figure) is much the same as that of chloride. It should also be noted that, because of the solubility of ferric chloride in ether, significant amounts of this salt pass to the ether layer in presence of chloride ion.

Provided that the presence of foreign anions does not reduce the percentage of uranium extracted below 78·5 per cent., no modification of the routine procedure (q.v.) is required to achieve 99 per cent. recovery of uranium. The same recovery can be obtained by increasing the number of extractions to four if the percentage extracted lies between 78·5 and 68·4 per cent. With greater interference, it is generally preferable to reduce the concentration of the offending anion by precipitation with CO_2-free ammonia and solution of the precipitate in nitric acid before proceeding with ether extraction.

Cations—Thorium, zirconium, cerium (quadrivalent) and vanadium are extracted to a certain extent by ether, but the interference can be dealt with by subsequent modifications of the colorimetric method for uranium (q.v.). Vanadium, zirconium and titanium can also be completely separated from sexavalent uranium by precipitation with cupferron in acid solution, the filtrate containing only the uranium. The method is less satisfactory for thorium and cerium, precipitation being incomplete except in very dilute acid. None of the other cations generally encountered in uranium analyses cause interference in the method under discussion.

EXTRACTION OF NITRIC ACID—

Most of the variables considered above also influence the degree to which nitric acid is extracted from the aqueous layer. It is thus necessary to determine partition coefficients for nitric acid, since, in repeated extractions of the same aqueous solution, the nitric acid concentration and therefore the partition coefficient for uranyl nitrate both decrease. In practice, this effect is best controlled by adding to the aqueous layer, after each extraction, an amount of concentrated nitric acid equal to that extracted. A sufficient idea of the quantities of acid to be added can be obtained from Fig. 3 which gives the same information for nitric acid as Fig. 1 does for uranyl nitrate.

ANALYTICAL PROCEDURE

Details of a satisfactory routine procedure are given below, the italic letters in parentheses referring to the discussion which follows this section.

(1) Add a suitable aliquot (*a*) of uranium solution to a separating funnel, after ensuring that the solution is 3 *N* with respect to nitric acid and 1 *M* with respect to ferric nitrate (*b*).

(2) Add an equal or slightly greater volume of ether (*c*) to the funnel and shake, with periodic releases of ether vapour, for 1 minute. Allow 1 minute for settling and drain the aqueous layer into a small beaker.

(3) Swirl the ether layer around the inside of the funnel, allow to stand for 1 minute, and drain off the small amount of aqueous layer that has collected. Transfer the ether layer to a beaker containing 30 to 50 ml. of water (*d*).

(4) Return the aqueous layer to the funnel, rinse out the beaker with the calculated (*e*) quantity of nitric acid and add this to the funnel also. Proceed with a second and third extraction (*b*) following steps (2) to (4) in each instance.

(5) Stand the beaker, containing accumulated ether layers and water, in the draught of a fume-cupboard until all the ether is evaporated (*f*).

(6) Neutralise the residual aqueous solution with sodium hydroxide, using a small piece of litmus paper as indicator, and add sufficient nitric acid to make the solution just acid (*d*). Add sodium carbonate to give a final solution containing between 1 and 5 per cent. of Na_2CO_3.

(7) To the alkaline solution add a few pieces of porous pot, and heat on the hot plate to remove dissolved ether. Finally, boil for 15 minutes (*g*) and allow to cool to room temperature.

(8) Make the cold solution up to the required volume (*a*) and filter through a Whatman 542 paper. Using a portion of the filtrate as a blank, add to another portion 0·1 ml. of 30 per cent. hydrogen peroxide per 10 ml. of solution, and measure the uranium colour photometrically (at 360 mμ. with the Coleman spectrophotometer or at 434 mμ. with the Pulfrich photometer). In the presence of Zr, Ce^{IV} and Th, the procedure requires modification (*h*).

DISCUSSION

(*a*) It is convenient for solutions to contain 1 to 20 mg. of U_3O_8 when uranium is to be estimated finally by the peroxide colorimetric method. The final volume of solution (step 7) is then brought to 50 ml. for the lower, and 200 to 250 ml. for the higher, concentrations. In this way the colours developed fall within the range 2 to 10 mg. of U_3O_8, which is most suitable for measurement with the photometer. By using a greater optical depth of solution in the Coleman spectrophotometer (with a different type of cuvette), and employing a final volume of solution as small as 20 ml., it is possible to detect 0·02 mg. of U_3O_8, though such

an amount cannot be measured with accuracy. With the Pulfrich photometer, 0·1 mg. can be detected.

(*b*) The recommended concentrations of ferric nitrate and nitric acid need not be rigidly adhered to, but for other concentrations it is necessary to work out from Figs. 1 and 2 the number of ether extractions and the amount of acid required in the course of the analysis. With the recommended concentrations, however, it is established that three extractions give 99 per cent. recovery for certain. No account is taken of other nitrates present in solution, but they help to increase the percentage of uranium extracted and ensure that the routine procedure gives even better than 99 per cent. recovery.

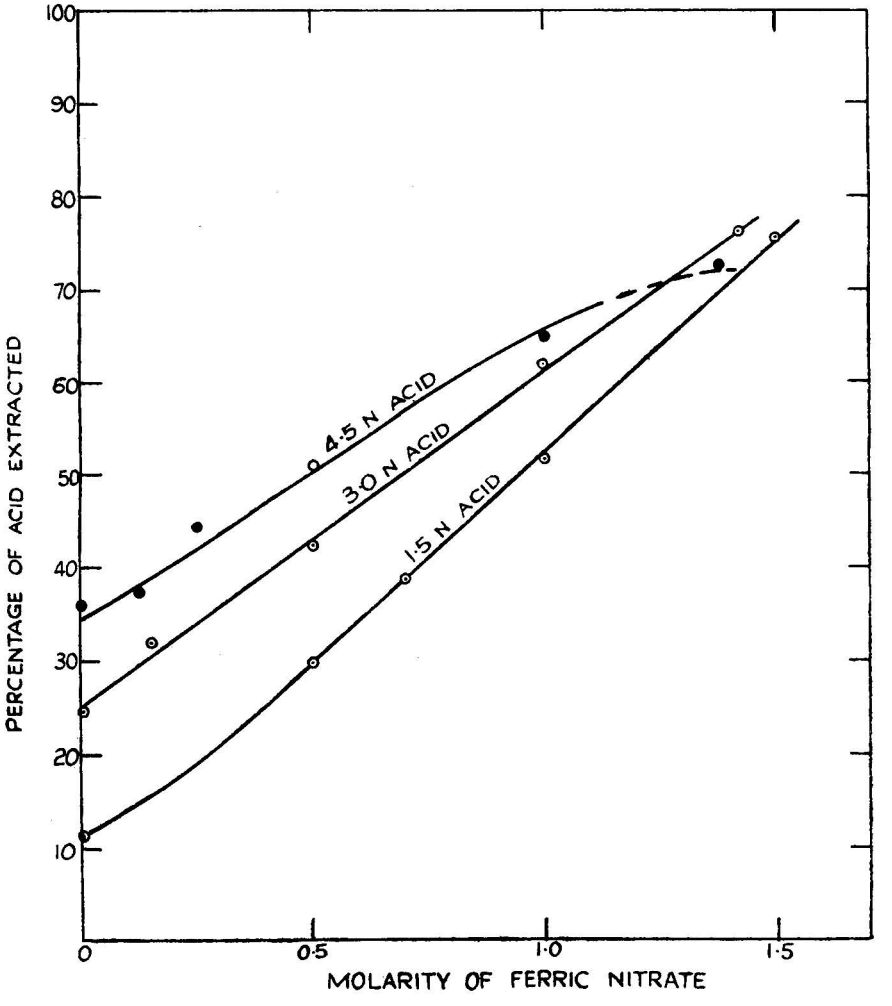

Fig. 3. Variation in percentage of nitric acid extracted

(*c*) Before the second and third extractions the beaker which has contained aqueous extract (step 4) is rinsed with the ether to be used for the next extraction. It is advisable to use a volume of ether slightly greater than that of the initial aqueous layer, so that, after shaking, the volume of the ethereal layer will not be less than the volume of the aqueous layer, despite losses due to evaporation and solution of ether in water. This ensures that efficiency of extraction is not less than that predicted by use of Figs. 1 and 3.

(*d*) If the acid ether extract is evaporated over sodium carbonate solution instead of water, bicarbonate may form and lead to large errors in the colorimetric estimation. Moreover, once sodium bicarbonate is formed under these conditions, it cannot be completely

decomposed even with prolonged boiling. The technique of step (6) ensures that this source of error is eliminated. An alternative procedure is to measure the intensity of the yellow colour in the range 440 to 450 mμ., where the adsorption is not affected by the presence of bicarbonate; the sensitivity, however, is much reduced at this wavelength.

(*e*) Using the recommended concentrations of salt and acid, 1·2 ml. of nitric acid (sp.gr. 1·41) for every 10 ml. of uranium solution should be added before the second and subsequent extractions, to restore the normality of the aqueous layer to 3 *N*. For other concentrations the quantity of acid can be estimated from Fig. 1.

(*f*) This method is rapid and convenient and eliminates the need for heating the ether layer. It is advantageous if the draught can be augumented by lowering the sash of the fume-cupboard.

(*g*) Boiling has the effect of increasing the rate of coagulation of small quantities of ferric hydroxide, etc., which may be present. These impurities are not generally extracted by the ether, but are introduced by admixture of small amounts of the aqueous layer, an effect very difficult to avoid. The quantities of precipitate are so small, however, that solutions can be made up to the desired volume before filtering, without causing significant errors. It is often useful to add 1 ml. of 30 per cent. hydrogen peroxide before boiling to ensure the precipitation of impurities such as manganese, but boiling must then be continued for 45 minutes to decompose completely the peruranate formed.

(*h*) Thorium and cerium may be separated from uranium by adding hydrogen peroxide (in step 6) before making the solution alkaline with sodium carbonate. Under these circumstances only uranium remains in solution and may be determined as described in steps (6) and (7) of the procedure. If peroxide be added to the alkaline solution complete precipitation of thorium, etc. either does not occur or occurs very slowly.

Vanadium is also extracted by ether, despite the large excess of iron salts in solution, and develops a yellow colour with alkaline peroxide. Under the conditions of the recommended procedure, however, the partition coefficient for vanadium is approximately 50 times less favourable than that for uranium—consequently the error introduced when equal quantities of both are present is only slightly greater than 1 per cent., and there are likely to be many instances where the error is no greater than the error inherent in the colorimetric method.

Summary

The variables affecting the ether extraction of uranyl nitrate from aqueous solutions have been examined and an analytical procedure capable of wide application has been described. Extractions are carried out in solutions containing 3 *N* nitric acid and 1 *M* ferric nitrate, the latter being a more efficient salting-out agent than the majority of inorganic nitrates. Under these conditions 99 per cent. recovery of uranium can be achieved by three extractions using equal volumes of ether and aqueous solution, and considerable amounts of other anions can be tolerated in solution without substantial modification of the procedure.

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Use of the Van Slyke - Neil Manometric Apparatus for the Determination of Organic and Inorganic Carbon in Soil and of Organic Carbon in Soil Extracts

By J. M. BREMNER

Introduction

In the course of a study of the chemistry of soil organic matter it became necessary to carry out numerous determinations of organic and inorganic carbon in soil and of organic carbon in soil extracts. This paper arose from a consideration of the methods available for these purposes.

Various wet and dry combustion methods have been used for the determination of organic carbon in soil, but the report of the Organic Carbon Committee of the International Society of Soil Science,[1] published in 1935, made it clear that dry combustion methods were the more reliable. The Committee found that the values obtained by a variety of dry combustion methods on nine different kinds of soil were so concordant that any choice between these methods could be made solely on grounds of laboratory convenience. The constant stream of papers on alternative methods of determining organic carbon in soil shows that although dry combustion in oxygen or air must be regarded as the standard procedure many workers on soil problems are dissatisfied with the method. The objections generally raised are that the apparatus required is expensive and occupies too much laboratory space, and that the method is too slow to be employed when a large number of samples are to be analysed. Rickson[2] recently described a modification of the Ter Meulen method for the determination of organic carbon in soil which is not so open to criticism on these points, since it can deal accurately with quantities on the semi-micro scale and is faster than the macro-methods, and the apparatus required is much more compact than those commonly used.

Various rapid titration methods[3,4,5,6] of determining organic carbon in soil have been developed which yield useful results when corrected by an appropriate factor. Unlike dry combustion procedures these methods need no modification for soils containing carbonates and can discriminate to some extent between relatively inert carbon in materials such as coal, charcoal or graphite and carbon in soil organic matter. Their use is limited, however, to comparison of related soils; they lack the precision necessary for investigations on the nature of soil organic matter.

Wet combustion methods, such as digestion of the soil with chromic and sulphuric acids, have proved unsatisfactory in the past owing to the low recoveries obtained. In only one of the wet methods tested by the Organic Carbon Committee of the International Society of Soil Science was the treatment drastic enough to achieve nearly complete oxidation of soil carbon to carbon dioxide. Some time after the Organic Carbon Committee's report was published, Van Slyke and Folch[7] studied the effect of adding various catalytic and oxidising agents to chromic acid mixtures and found that theoretical yields of carbon dioxide were obtained from all types of organic substances tested when a mixture of chromic, iodic, sulphuric and phosphoric acids was used for combustion. They employed the Van Slyke - Neil manometric apparatus[8] to collect and measure the carbon dioxide liberated on combustion and found that no modification of the method was required by the presence of any of the substances that interfere with, or require modification of, the usual dry combustion methods (*e.g.*, nitrogen, sulphur, halogens).

Since the Van Slyke and Folch wet combustion method was successful even with such difficultly oxidisable substances as cholesterol and palmitic acid, it seemed reasonable to expect that it would be applicable to the determination of organic carbon in soil. As shown below, this expectation was fulfilled. The method also proved to be suitable for the determination of carbon in soil extracts, for which no satisfactory method has previously been available. Moreover, it was found that the Van Slyke - Neil apparatus could be employed for the rapid and accurate determination of inorganic carbon in soil.

Experimental

Samples of the nine soils used in the investigation conducted by the Organic Carbon Committee of the International Society of Soil Science, and by Rickson to test his modified

Ter Meulen dry combustion method, were fortunately available in this laboratory. These samples were employed in the work described below as they represent a wide variety of soils and include some known to provide special analytical difficulties. All samples were finely ground and passed a 100 B.S. mesh. The moisture contents of the soils were determined by drying samples in an oven at 105° C. for 18 to 20 hours. The reactions involved in the determinations were all carried out in combustion tubes attached to the chamber of the Van Slyke - Neil manometric apparatus and carbon dioxide liberated was collected and measured by the procedure described by Van Slyke and Folch for micro-combustions. Briefly, this procedure is as follows. The gas is drawn over into the chamber of the manometric apparatus and is absorbed in carbon dioxide-free 0·5 *N* sodium hydroxide plus 0·3 *M* hydrazine, the hydrazine serving to reduce any halogens evolved. The unabsorbed gases are then ejected and the carbon dioxide is set free by acidification with 2 *N* lactic acid and measured manometrically.

Determination of inorganic carbon in soil

The recognised methods of determining inorganic carbon in soil all depend on measuring the carbon dioxide evolved when the soil sample is treated with dilute hydrochloric acid. If the soil were boiled with acid, decarboxylation and oxidation of some soil organic matter would probably produce carbon dioxide, especially if the soil contained manganese dioxide. It is customary, therefore, to add a reducing agent, such as ferrous chloride, to the hydrochloric acid, and to keep the boiling point as low as possible by carrying out the determination under reduced pressure. The need for these precautions has been emphasised by recent work which has shown that soils contain large amounts of uronide-like material. The technique developed by Van Slyke and Folch for determining organic carbon was found to require no modification for inorganic carbon in soil beyond substituting 2 ml. of an 0·5 *N* solution of hydrochloric acid containing 14 g. of ferrous chloride per litre for 2 ml. of combustion fluid. Decomposition of soil carbonates was carried out under reduced pressure since ejection of air from the combustion tube is the first step in the Van Slyke and Folch procedure. Decarboxylation of uronide material was minimised by limiting the boiling period to 1·5 minutes. The method is rapid, a complete analysis, weighings included, taking only 20 minutes.

Determination of organic carbon in soil

In any combustion method of determining organic carbon in soil account must be taken of the fact that many soils contain carbonates. This inorganic carbon must either be removed before the determination of organic carbon or be determined separately and subtracted from the total carbon. If a dry combustion method is to be employed it is preferable to remove carbonates beforehand, because high temperatures and a lengthy combustion period (1½ hours) are required to ensure complete decomposition of calcium carbonate. The only reagent suitable for preliminary destruction of carbonates is sulphurous acid, as it is easily removed by evaporation. It is customary to weigh the soil sample into a combustion boat, add sulphurous acid and, after a few hours, remove the excess of acid by heating the boat on a steam-bath or by leaving it overnight in an oven at 105° C. This treatment is repeated until all carbonates have been destroyed. Objections can be raised against the procedure on the following grounds—

(i) Although sulphurous acid has reducing properties which probably minimise the oxidation and decarboxylation of soil organic matter during the acid treatment, some loss of organic carbon may occur on heating, especially if several treatments with sulphurous acid are necessary.

(ii) It is often difficult to decide when all carbonates have been destroyed and the sulphurous acid treatment can be discontinued.

(iii) There is some danger of mechanical loss during the repeated additions of sulphurous acid and evaporations.

The sulphurous acid treatment can be omitted if the soil contains no inorganic carbon, but it is often difficult to decide if this is the case as some soils contain relatively inactive carbonates that are not readily detected in a qualitative test. It is generally better to determine inorganic carbon separately than to run the risk of including it in the organic carbon. As will be shown below, for the wet combustion method of Van Slyke and Folch it is neither necessary nor preferable to remove carbonates from the soil sample before analysis. Organic carbon is determined from total carbon by subtracting inorganic carbon, determined separately.

When determining the carbon contents of certain highly organic soils by the method of Van Slyke and Folch, it was found that mechanical loss of material took place during addition of the soil sample to the combustion tube, owing either to the lightness of the soil or to its tendency to adhere to the spatula during transference to the tube. This difficulty was overcome by weighing the sample in a small porcelain combustion boat and gently sliding the boat with sample into a slightly inclined combustion tube.

Determination of organic carbon in soil extracts

The Van Slyke and Folch method of determining organic carbon was found to be applicable to soil extracts. The method was tested on a solution obtained by extracting a non-calcareous Cambridgeshire fen soil with neutral 0·1 *M* sodium pyrophosphate. Samples of this extract were pipetted into combustion tubes and evaporated to dryness before analysis by leaving the tubes overnight in a large evacuated desiccator. If carbonates are present the soil extract is slightly acidified before evaporation by cautious addition of hydrochloric acid from a capillary tube.

Results

Inorganic carbon in soil—

The method was tested with the nine soils used in the investigation conducted by the Organic Carbon Committee. The results, expressed as percentages of inorganic carbon in oven-dried soil, are given in Table I, which also shows the mean values and standard deviations of the results obtained by the methods tested by the Committee.

Table I

Inorganic carbon, %

Soil No.	Previous detmns. by recognised methods: Mean	Previous detmns. by recognised methods: Standard deviation	Present method	Deviation of result by present method from previous mean
1	0·041	0·003	0·038	0·003
2	1·28	0·06	1·28	0·00
3	2·26	0·05	2·26	0·00
4	0·023	0·029	0·020	0·003
5	0·00	—	0·00	—
6	0·00	—	0·00	—
7	0·85	0·05	0·84	0·01
8	0·05	0·01	0·057	0·007
9	0·00	—	0·00	—

With calcium carbonate the maximum error of the method was found to be 1 per cent.

Organic carbon in soil—

Table II shows the recoveries obtained with the Van Slyke and Folch wet combustion method when calcium carbonate and mixtures of calcium carbonate and soil were analysed. The soil employed contained no inorganic carbon. The amount of organic carbon present in the mixtures was calculated from the weight of soil taken and the organic carbon content of the soil.

Table II

Total carbon

Carbon taken, mg.: Organic	Carbon taken, mg.: Inorganic	Carbon found, mg.
—	3·44	3·43
—	2·81	2·81
1·12	2·16	3·27
1·53	1·92	3·44

Table III gives the results of analyses carried out by the Van Slyke and Folch method on the nine soils used in the co-operative study conducted by the Organic Carbon Committee. Soil samples containing from 2 to 3·5 mg. of carbon were taken for analysis and organic carbon figures were obtained from total carbon by subtracting inorganic carbon, which was determined separately. Some of the analyses were later repeated on soil samples containing only 0·3 to 0·7 mg. of carbon; the same results were obtained. Table III also gives the mean values and standard deviations of the results obtained with the dry combustion methods tested by the Organic Carbon Committee. All results are expressed as percentages of organic carbon in oven-dried soil.

TABLE III

ORGANIC CARBON %, FOUND FROM TOTAL MINUS INORGANIC CARBON

Soil No.	Previous detmns. (12) by various dry combustion methods: Mean	Previous detmns. (12) by various dry combustion methods: Standard deviation	Present method	Deviation of result by present method from previous mean
1	0·53	0·03	0·50	0·03
2	2·91	0·12	2·85	0·06
3	0·80	0·07	0·70	0·10
4	17·83	0·27	17·77	0·06
5	2·72	0·05	2·69	0·03
6	2·74	0·06	2·83	0·09
7	2·36	0·06	2·31	0·05
8	1·86	0·06	1·89	0·03
9	2·07	0·03	2·04	0·03

The organic carbon figures obtained by the Van Slyke and Folch method after treatment of the soils with sulphurous acid are given in Table IV. Four methods of treatment were studied:

(i) The soil sample was weighed into a combustion tube and treated with 5 per cent. sulphurous acid. After a few hours the excess of acid was removed by leaving the tube overnight in an evacuated desiccator.

(ii) As above, but excess of sulphurous acid was removed by placing the tube in a boiling water-bath.

(iii) As in (i), but excess of sulphurous acid was removed by leaving the tube overnight in an oven at 105° C.

(iv) The soil sample was weighed into a small porcelain combustion boat, such as is used for semi-micro dry combustions, and treated with 5 per cent. sulphurous acid. After a few hours the excess of acid was removed by heating the boat on a steam-bath. For determination of organic carbon the boat with sample was slid gently into a combustion tube.

Soil samples containing about 3 mg. of organic carbon were taken for analysis and 2 ml. of 5 per cent. sulphurous acid were used for each treatment. All treatments were repeated until no evolution of carbon dioxide could be observed on further addition of sulphurous acid, and in some instances beyond this point. In Table IV the number of treatments performed is shown in brackets after each result; all results are expressed as percentages of organic carbon in oven-dried soil.

TABLE IV

ORGANIC CARBON %, FOUND AFTER SULPHUROUS ACID TREATMENT

Soil No.	Methods of sulphurous acid treatment: (i)	(ii)	(iii)	(iv)
1	0·51 (1)	0·50 (1) 0·49 (2)	0·49 (1) 0·48 (2)	0·51 (1)
2	2·86 (3)	2·71 (3) 2·68 (4)	2·66 (3)	2·85 (3)
3	—	0·69 (4) 0·68 (5)	—	—
4	17·75 (1)	17·0 (1) 16·7 (2)	16·8 (1)	17·2 (2)
5	2·70 (1)	2·61 (1) 2·58 (3)	2·60 (1)	—
6	—	2·73 (2)	—	—
7	—	2·24 (2) 2·23 (3)	2·20 (2)	2·28 (2)
8	1·86 (2)	1·85 (2)	1·80 (3)	1·86 (2)
9	2·03 (1)	1·90 (2)	—	2·02 (1)

ORGANIC CARBON IN SOIL EXTRACTS—

Table V gives the results obtained with the neutral sodium pyrophosphate extract of fen soil. One-ml. samples of this extract were pipetted into porcelain boats of the type used

by Rickson in his semi-micro dry combustion apparatus and evaporated to dryness by leaving the boats for a few hours in an evacuated desiccator. The organic carbon contents of the residues in the boats were determined by Rickson's method. Five-ml. samples of the extract were then diluted to 10, 20 and 40 ml., and 1-ml. samples were pipetted from each diluted solution into Van Slyke and Folch combustion tubes and evaporated. Before evaporation one of the samples was acidified with hydrochloric acid and another was treated with 1 ml. of 0·5 *M* sodium carbonate and then acidified. The carbon contents of the samples taken from the diluted extracts were determined by the method of Van Slyke and Folch.

TABLE V

ORGANIC CARBON IN SOIL EXTRACT

Original extract represented by sample taken, ml.	Method of analysis	Carbon found, mg.
1·000	A	4·72
0·500	B	2·36
0·500*	B	2·35
0·500†	B	2·36
0·250	B	1·17
0·125	B	0·59

A Dry combustion method of Rickson.
B Wet combustion method of Van Slyke and Folch.
* Sample acidified with hydrochloric acid before evaporation.
† Sample treated with 1 ml. of 0·5 *M* sodium carbonate and acidified with hydrochloric acid before evaporation.

DISCUSSION

INORGANIC CARBON IN SOIL—

From Table I it can be seen that the method described for inorganic carbon is at least as accurate as established procedures. With the nine soils tested the deviations of the results given by the method from the mean values of the results obtained by the recognised techniques fall well within the standard errors of the latter.

ORGANIC CARBON IN SOIL—

The results given in Tables II and III show that the organic plus inorganic carbon content of a soil is determined by the Van Slyke and Folch wet combustion method and that the organic carbon content can be determined accurately from the total carbon figure by subtracting inorganic carbon, determined separately. Table III shows that, with two exceptions (Soils 3 and 6), the deviations of the results obtained for organic carbon by this method from the mean values of the results previously obtained by dry combustion procedures fall within the standard errors of the latter. For Soil 6 the result given by the Van Slyke and Folch method is in close agreement with the highest values given in the Organic Carbon Committee's report; these values differ from the mean (2·74 per cent.) by 0·1 per cent. carbon. Since this soil contains neither carbonates nor chlorides there should be no special analytical difficulties in the determination of organic carbon. It is therefore suggested that some variation in the sub-samples distributed to the various laboratories participating in the Organic Carbon Committee's investigation may account for the differences between the results reported for this soil. The close agreement of the results obtained with the Rothamsted sample of Soil 6 supports this explanation; Warren[1] in 1935 found 2·84 per cent. of organic carbon by the Ter Meulen dry combustion method, and Rickson[2] obtained 2·85 per cent. in 1947 by a modification of the same method; the Van Slyke and Folch method gave 2·83 per cent. Soil 3 presents special analytical difficulties to dry combustion methods since it contains less than 1 per cent. of organic carbon in presence of about 19 per cent. of calcium carbonate. Complete removal of carbonates from this soil with sulphurous acid before dry combustion is likely to prove difficult, especially if the soil contains unreactive limestone. Soil 3 also contains chlorides, which are known to lead to high results with certain dry combustion methods unless steps are taken to prevent interference. These sources of error are avoided if organic carbon is determined by the Van Slyke and Folch method, since chlorides do not interfere and it is not necessary in this case to remove carbonates.

From a comparison of the results given in Tables III and IV it is evident that treatment of a soil with sulphurous acid can lead to loss of organic carbon, the magnitude of the loss being dependent upon the method used for evaporation of the excess of acid. The loss is negligible when evaporation is carried out at room temperature (Method (i)), but can become serious if heat is used to speed evaporation. In the latter case the magnitude of the loss apparently depends upon the time of contact of the soil with hot acid. Thus it is least in Method (iv), in which evaporation of sulphurous acid is rapid, and greatest in Method (iii), in which evaporation is very slow. Method (iv) is the one generally employed to remove carbonates from calcareous soils before determining organic carbon by dry combustion methods. Although destruction of organic carbon by this method is generally slight, it is not always negligible. Clearly, Method (i), though slower, is preferable, and it is therefore suggested that this method of removing carbonates should be adopted in pretreating calcareous soils before determining organic carbon by dry combustion methods. Since the Van Slyke and Folch method, unlike the dry combustion techniques, requires no inconvenient modification for the determination of organic plus inorganic carbon, it is preferable to avoid the use of sulphurous acid by determining organic carbon from total and inorganic carbon figures.

ORGANIC CARBON IN SOIL EXTRACTS—

The results given in Table V show that:

(i) Micro and sub-micro quantities of organic carbon in soil extracts can be determined accurately by the Van Slyke and Folch method.

(ii) No loss of organic carbon by decarboxylation or other processes takes place if the soil extract is acidified with hydrochloric acid before evaporation *in vacuo*.

(iii) The organic carbon content of a soil extract can be determined accurately by this method even if a large amount of inorganic carbon is present in the extract.

This method of determining organic carbon in soil extracts has proved to be extremely useful in the study of soil organic matter, for it is rapid and can be employed with extracts obtained by treating soil with sodium hydroxide or sodium carbonate solutions, the classical humus extractants. It has patent advantages over the other methods that have been used for the purpose. In these the extract is heated in acid solution with an oxidising agent, such as dichromate or permanganate, and reduction of the oxidising agent by the carbon in the extract is measured by a titration procedure. Various factors, such as strength of acid and duration of boiling, affect the results; correction factors must be applied to all figures obtained. Since the correction factor varies with the soil and can only be determined accurately by dry combustion of a sample of the extract, it is obvious that the methods are, at best, tedious and unreliable.

CONCLUSIONS

The methods described above have several advantages over those at present employed in soil analysis. While as accurate as established techniques they are quicker and simpler and can deal with smaller quantities of material. Beyond the initial cost of the Van Slyke - Neil apparatus the reagents and apparatus employed are inexpensive and only one apparatus is required for all determinations, an important consideration if bench space is limited. The method reported for determination of organic carbon avoids the dangers involved in removal of carbonates from calcareous soils with sulphurous acid and is not affected by the presence of other substances that sometimes cause difficulty in dry combustion methods. The methods described in this paper have merits that commend their adoption for the routine analysis of soils.

SUMMARY

The use of the Van Slyke - Neil manometric apparatus for the determination of organic and inorganic carbon in soil and of organic carbon in soil extracts is described. Organic carbon is determined by the method of Van Slyke and Folch, and inorganic carbon by a modification of the same method. The methods are as accurate as established procedures and can deal with micro quantities of material. They are rapid and simple and seem well suited for the routine analysis of soils.

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The Estimation of Aldehydes, Ketones and Acetals by means of the Hydroxylamine Hydrochloride Method

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INTRODUCTION

THE first description of this method, depending on the titration of the acid liberated when hydroxylamine hydrochloride forms an oxime by reacting with the carbonyl group of aldehydes or ketones, was given by Brochet and Cambier[1] for estimating formaldehyde, methyl orange being used as indicator. Walther[2] applied the method to the estimation of citral in lemon oil, using an alcoholic solution of the reagent in presence of sodium bicarbonate, heating under a reflux condenser and titrating the experiment and a blank to both phenolphthalein and methyl orange. Bennett[3] modified Walther's method by adding enough alcoholic potash to combine with the liberated acid. Bennett and Donovan[4] extended Bennett's method to formaldehyde, acetone, benzaldehyde and cinnamic aldehyde. For formaldehyde and acetone the reaction was carried out in a stoppered bottle at room temperatures for 2 hours. Bromophenol blue was tried as indicator, but best results were obtained with methyl orange as outside indicator. Camphor gave unsatisfactory results.

Marasco[5] used aqueous reagent and methyl orange as indicator, for acetone and formaldehyde, finding that the reaction under his conditions only went to 94·4 per cent. If the liberated acid is allowed to accumulate the reaction slows down, but in neutral solution 5 minutes are sufficient for the reaction to go to the 94·4 per cent. value. A second portion of neutralised reagent is used as a blank. In the experiment the titration is incremental. Ethyl alcohol was found to have no effect when present in small amounts.

Bennett and Cocking[6] applied the method to carvone in essential oils, using dimethyl yellow as indicator, and heating in stoppered tubes to 75° C. Bryant and Smith[7] added pyridine to displace the equilibrium, but this was found to cause an indistinct end-point by Dermer, Wilson, Johnson and Dermer.[8] Mitchell, Smith and Bryant[9] used the Karl Fischer method to estimate the water formed in the reaction, excess hydroxylamine being first destroyed by the addition of sulphur dioxide and pyridine.

Doering[10] found that with acetaldehyde the reaction rate increased in presence of acid, and therefore used an aqueous acid reagent. Pevtzov[11] used bromophenol blue as indicator, finding that the reaction went to 99·5 per cent. of completion; they used an aqueous reagent for the estimation of acetone.

EXPERIMENTAL

Our first use of the method was in estimating traces of aldehyde (probably butyraldehyde) in commercial butanol made by catalytic hydrogenation of crotonaldehyde. Bromophenol blue was used as indicator, with a 2 per cent. alcoholic solution of the reagent neutralised in bulk. With 25 g. of sample and 25 ml. of reagent the first end-point indicated 0·20 per cent. of butyraldehyde and an incremental titration lasting half an hour 0·35 per cent. When the solution was left for 40 minutes before titrating, the first end-point gave 0·32 per cent., increasing to 0·37 per cent. on further incremental titration over a further $\frac{1}{4}$ hour. By adding an excess of alkali first and titrating back immediately, only 0·22 per cent. was found, increasing on standing to 0·35 per cent. Finally, by allowing to stand 2 hours before titrating, the first end-point showed 0·37 per cent., and this did not increase on standing.

Tests were carried out on 2 per cent. solutions of crotonaldehyde and butyraldehyde

in butanol. The aldehydes were 80 to 95 per cent. pure as found by several methods of analysis. The butanol was made as free from aldehyde as possible by treatments with caustic alkali, *m*-phenylenediamine and sodium, and finally contained 0·04 per cent. of aldehyde when tested by the above method. Tests on 10 ml. of the synthetic solutions and 20 ml. of reagent showed about 75 per cent. (allowing for the aldehyde content of the butanol) of the correct figure when the first end-point was used, and 95 per cent. on incremental titration. Repeat tests in which 30 ml. of reagent were used and the solutions left for 1 hour before being titrated showed about 98 per cent. of the correct figure at the first end-point, increasing to 99 or 100 per cent. on incremental titration over 5 to 10 minutes until constant.

A number of tests were then carried out with the butanol that had been freed as far as possible from aldehydes. Some of these tests used an aqueous reagent and an aqueous solution of the butanol, whilst others used an equal volume of 95 per cent. ethyl alcohol (free from aldehydes according to Schiff's test) with the alcoholic reagent. It was found that alcohols themselves apparently cause a shift in the end-point towards the acid side, whilst addition of water causes the end-point to shift towards the alkaline side. The effect is that in the above method an apparent butyraldehyde content of about 0·02 per cent. would be found in aldehyde-free butanol.

Tests were also carried out on the relative suitability of bromophenol blue and methyl orange in presence of solvents. These two indicators cover nearly the same pH range, but behave very differently in presence of solvents, as shown by Kolthoff.[12] The first tests were carried out with acetone as solvent. In anhydrous acetone both indicators will reveal traces of strong mineral acid, but in presence of water methyl orange is much less sensitive than bromophenol blue. Both, however, show the "neutral" indicator colour at the wrong place, *viz.*, methyl orange when a fair amount of acid is present and bromophenol blue when a trace of alkali is present. Increasing the acetone concentration causes the solution apparently to become alkaline to methyl orange, but acid to bromophenol blue, and the reverse occurs on diluting with water. Similar results were obtained with butanol, complicated by the fact that two layers are formed. It was also noticed that, with both solvents, both indicators could give certain colours that could not be matched in aqueous solution at any pH. Bromophenol blue also always gives a peculiar dichroic colour except when showing the fully acid (yellow) colour. Some tests were carried out by the usual method, with alcoholic reagent and dimethyl yellow as indicator, but the latter appeared less satisfactory than bromophenol blue.

The next tests were on the analysis of acetone. An aqueous solution of the sample was used, with an aqueous solution of the reagent containing bromophenol blue, neutralised in bulk. The end-point was matched against a control, in which the volume should equal the final volume in the experiment, as the end-point is much less distinct than in most titrations. The reaction was found to be incomplete if the titration was carried out immediately, possibly owing in part to the volatility of acetone. For this reason it was necessary to use a stoppered flask, shaking and allowing the solution to stand for 15 minutes before titrating. The figure obtained also increased as the excess of reagent was increased, up to a maximum, at which 97 to 98 per cent. of the theoretical figure was obtained. In spite of the relatively poor end-point very good duplication could be obtained. It was later found that the aqueous reagent neutralised in bulk developed acidity on storage; so the alcoholic reagent was afterwards used for most tests. The slight complication introduced by having to keep "solvent" concentrations similar in the experiment and the blank was more than counterbalanced by the greater speed of reaction, by the fact that the reaction apparently goes to completion under the conditions used, and as already mentioned by the fact that the alcoholic reagent neutralised in bulk is stable.

Other methods of estimating aldehydes and ketones are used in certain circumstances, when confirmation of results is required, and where the hydroxylamine method is insufficiently sensitive (*i.e.*, for traces of aldehyde in ethanol) or unsuitable (*i.e.*, for traces of aldehyde in glacial acetic acid).

The method as at present carried out is as follows.

Method

Reagent—

Twenty grams of hydroxylamine hydrochloride are dissolved in 100 ml. of water and the solution is made up to 1 litre with 95 per cent. alcohol. Twenty-five ml. of a 0·2 per cent.

solution of bromophenol blue in alcohol are added and the colour of the solution is adjusted to the neutral dichroic green/red by adding 2 *N* sodium hydroxide or hydrochloric acid (usually alkali is required). Sulphuric acid must not be used as hydroxylamine sulphate is not very soluble in alcohol.

PROCEDURE—

The reagent is added to the sample in a 250-ml. conical flask. Enough sample is taken to require from 10 to 20 ml. of 0·2 *N* alkali in the titration, *i.e.*, corresponding to 0·002 to 0·004 g.-mol. of aldehyde, and the volume of reagent used is at least three times the volume of 0·2 *N* alkali required in the titration. We aim at requiring a titration volume of about 20 ml. and use a standard volume of 70 ml. of reagent. At the same time an equal volume of reagent is taken in another flask to act as a blank and any water or alcohol added with the sample must be added to the blank in equal amount. This is because the colour of the indicator depends on the concentration of both water and alcohol.

The sample flask is now allowed to stand for about 5 minutes and then titrated with 0·2 *N* alkali to match the blank. Before final matching, a volume of water, equal to the titration volume added to the sample, must be added to the blank. Matching is best done by holding the two flasks side by side and viewing horizontally through the liquid at a good strong white light (a white cloud is ideal); in this way the end-point can be accurately judged to within less than one drop. After the two flasks have been matched they should be allowed to stand for a further 5 minutes and compared again. This is because some higher aldehydes and ketones react slowly. When the contents of the sample flask have finally ceased to fade, the total titration gives the free aldehyde or ketone. Sample and blank are then placed on the water-bath with a knob in the top for 20 minutes, after which time they are removed and cooled and the sample is re-titrated to match the blank. This second titration gives acetals and also very slowly-acting aldehydes and ketones which are sometimes not determined in the cold.

The above method is the general method. For special samples, *e.g.*, alcohol containing acetaldehyde and much acetal, modification may be necessary. Lower aldehydes (except formaldehyde, which reacts slowly) react almost instantaneously, and the lower acetals are very easily hydrolysed (again excepting formal, $CH_2(OCH_3)_2$, which is not hydrolysed by neutral reagent even on heating, but is hydrolysed on heating with acidified reagent). Hence to determine acetaldehyde and acetal in presence of each other, it is necessary to titrate in the cold as quickly as possible before heating to determine acetal.

With unknown or untried aldehydes a useful check on the completeness of reaction is to add a further measured quantity, say 10 ml., of reagent to both blank and sample after the first titration is complete. If blank and sample still match, enough reagent was originally added; if not, it is advisable to repeat with less of the sample. Paraldehyde is not acted on in the cold, providing the solution is kept neutral or alkaline. If paraldehyde is known to be present the sample should be added to a reagent made alkaline with a known amount of alkali and a similar amount of alkali added to the blank. The equivalent volume of acid is added to the blank and the sample titrated after standing 20 minutes (reaction is slow in alkaline solution) to match the blank. In this case the difference between the amount of acid added to the blank and that added to the sample is due to aldehyde other than paraldehyde present. Paraldehyde itself cannot be satisfactorily estimated by this method; a special method is necessary.

The method described has been used satisfactorily for formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, acrolein, crotonaldehyde, ethyl propyl acrolein, ethyl hexaldehyde, acetone, methyl ethyl ketone, methyl propyl ketone, methyl *iso*butyl ketone and many other less well known aldehydes and ketones.

In order to get accurate and reproducible results it is essential not to vary this procedure in any way, without good cause and without testing the effect of such variation on a known amount of aldehyde. Special attention must be paid to adding to the blank the same quantities of water or alcohol that are added to the sample in order to keep concentrations equal in both flasks.

Note—Some 95 per cent. alcohol may contain appreciable amounts of acetal; in such circumstances the reagent, after being made up, should be warmed on the water-bath for about half an hour before being finally neutralised; after this treatment the colour should remain stable indefinitely.

PROCEDURE TO BE ADOPTED FOR ACID SAMPLES—

Small amounts of very weak organic acids have no effect on the indicator, but the stronger acids such as acetic, oxalic, citric, etc., have an effect, though not a quantitative one. This effect may be allowed for as follows.

Two samples are taken, one being the normal sample treated as described above; to the other is added a volume, equal to the volume of reagent added to the first sample, of a solution containing 25 cc. of 0·2 per cent. alcoholic bromophenol blue in 100 ml. of water made up to 1 litre with 95 per cent. alcohol (this is the reagent without the hydroxylamine). This second sample, so treated, is then titrated with 0·2 *N* alkali to match the blank and the titration volume obtained is to be subtracted from the hydroxylamine titration volume as a correction for the acidity of the sample.

This modification has been found useful when analysing reaction mixtures obtained by oxidation of various aldehydes.

PROCEDURE TO BE ADOPTED FOR BASIC SAMPLES—

One example of a basic sample is pyridinised industrial methylated spirit. The sample is neutralised to bromophenol blue by adding dilute sulphuric acid, and an equal quantity of bromophenol blue is added to the blank so that the final colours will match correctly.

A special modification of the main method, in which water is used in the control test, was worked out as a limit test for aldehydes in ethyl alcohol for the British Standard Specification No. 507 (1933). This method cannot be used for the estimation of aldehydes in spirit, unless the blank of about 0·013 per cent. w/v of aldehydes is subtracted from the figure obtained.

Tests on 1 per cent. solutions of acetaldehyde in alcohol showed that in a freshly made solution all the aldehyde reacted in the cold. After keeping several months, however, only one-fifth of the aldehyde reacted in the cold, the other four-fifths being present as acetal, reacting after warming and cooling. It seems likely that in old samples of butanol the butyraldehyde may be present as butyral, and that the necessity for an incremental titration is due more to this than to slow reaction of butyraldehyde itself.

For the estimation of traces of acetone in *iso*propanol, a modification was worked out to avoid the use of aldehyde-free 95 per cent. ethyl alcohol in the control experiment. In this, 25 ml. of sample were mixed with 0·6 ml. of 0·2 per cent. bromophenol blue solution, the mixture was neutralised to the green colour, and only 5 ml. of alcoholic reagent were added. This solution was then titrated to match a control of 30 ml. of reagent. On a synthetic solution containing 0·10 per cent. of acetone, this method showed 0·096 per cent., whereas our usual method showed 0·095 per cent. On a sample of *iso*propanol the figures found were respectively 0·014 and 0·012 per cent. by the two methods. In our usual method it was found rather difficult to match the colours because of the large volume of sample that had to be taken, and better matching was obtained by adding extra indicator to both experiment and control, the figure then being found to be 0·015 per cent. of acetone. With this sample another laboratory modified the method, using bromocresol green as indicator, and matching against the neutralised reagent itself. Owing to the pH change being at the wrong place this modification gave high results. With the synthetic mixture containing 0·10 per cent. of acetone, figures of 0·16 and 0·18 per cent. were found, and with the sample in question the figures were found to be 0·046 and 0·105 per cent., the latter figure being obtained when more bromocresol green was added to the experiment.

Our method has been compared with the routine method used in another laboratory. This method uses normal aqueous hydroxylamine sulphate reagent, neutralised just before use, with methyl orange/xylene cyanol screened indicator. Normal alkali is used in the titration, and usually no control is used. Although the use of normal alkali helps to give a sharper end-point, we consider that the accuracy of the figure obtained is not as good as by our method.

It is possible to distinguish between, and estimate separately, substances that react rapidly with hydroxylamine in the cold and those that react slowly. This is done by continually matching the sample against the blank by an incremental titration. The total titration is plotted against the time. When the increase due to the slowly-acting substance becomes steady, the slope may be extrapolated back to zero time, giving the titration due to the rapidly-acting substance. Heating and cooling until no further increase takes place

will then complete the reaction of the slow reactor. In this way methyl *iso*butyl ketone and di *iso*butyl ketone were estimated separately in a mixture.

SUMMARY

A description is given of a satisfactory technique for the hydroxylamine hydrochloride method of estimating aldehydes, ketones and acetals, using alcoholic reagent. Precautions and modifications found necessary when dealing with certain carbonyl compounds and mixtures are given.

The authors wish to thank the Directors of the Distillers Company Limited for permission to publish this paper.

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The Determination of Chromium in Cast Iron and Steel

BY T. S. HARRISON AND H. STORR

AN absorptiometric method for the determination of chromium in steel was developed recently by De Lippa,[1] who used potassium bromate as oxidising agent. The main advantages of his procedure were (1) avoidance of the use of silver nitrate (as in the persulphate method) and (2) selective oxidation of Cr^{III} to Cr^{IV} with oxidation of the manganese only to the quadrivalent state, not to permanganic acid. Speed and accuracy were claimed provided that a number of precautions were taken. The procedure could be incorporated in Vaughan's composite scheme[2] if desired.

The object of this short research was to apply this method, with appropriate modifications, to the assay of chromium in graphitic cast iron and other ferrous alloys received in our laboratories.

EXPERIMENTAL

As composite schemes are not in general use in this laboratory, it was decided to take 1 g. of sample through the procedure, *i.e.*, omitting the fractionation after dissolution. This necessitated increases in the amounts of reagents subsequently added and a consequent change in the absorptiometric conditions. Filtration was introduced to remove graphite. The precautions laid down were necessary and on no account must the liquid boil down to low bulk during the oxidation. Readings taken at intervals showed the dichromate colour to be stable for several hours.

Following a number of determinations it was decided to check the results by titration. The contents of the cells were poured back, each solution was diluted to about 250 ml., an excess of 0·1 *N* ferrous ammonium sulphate was added and 0·1 *N* potassium permanganate solution was run in to give a pink colour stable for at least 2 minutes. The end-point was very clear and definite and agreement was very good. This dual ending is a distinct advantage, especially when a result is in doubt or the chromium content exceeds the ranges of available graphs.

PROCEDURE—

Graphitic irons—Dissolve 1 g. of sample in 40 ml. of Spekker acid, add a little water, oxidise dropwise with concentrated nitric acid and boil off nitrous fumes. Filter through a

Whatman No. 41 paper and wash with hot water. The volume of the filtrate and washings should be about 80 to 90 ml. Heat just to boiling on the hot plate, add 20 ml. 6 per cent. potassium bromate solution and boil gently for 5 minutes. Introduce 10 ml. of 20 per cent. ammonium sulphate solution and boil for 10 minutes. Add 8 ml. of 20 per cent. hydrochloric acid and boil gently for a further 10 minutes, replacing any evaporation losses with hot distilled water. Cool to below 19° C., dilute to 120 ml. with water, fill a 2-cm. or 4-cm. cell and measure the absorption with Ilford Spectrum Violet filters No. 601 and setting water-to-water 1·2. Convert to percentage of chromium from a graph (Fig. 1) constructed by using 1-g. quantities of a plain cast iron with increasing amounts of standard potassium dichromate solution (2·8284 g. per litre; 1 ml. ≡ 0·001 g. Cr.).

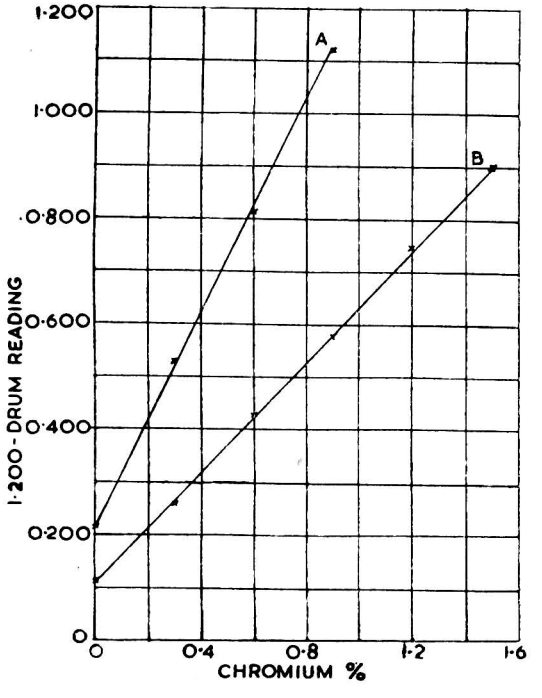

Fig. 1. Chromium in cast iron
Ilford spectrum violet filter No. 601 with Chance No. H 503
A 4 cm. cells B 2 cm. cells
Setting water-to-water 1.2

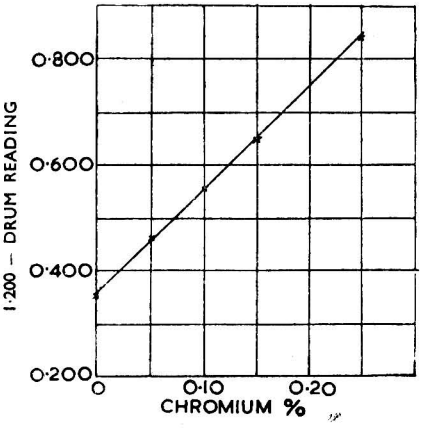

Fig. 2. Chromium in pig iron
Ilford spectrum violet filter No. 601 with Chance No. H503
4 cm. cells Setting water-to-water 1.2

Empty the contents of the cell into the beaker containing the main solution, rinsing with distilled water, and dilute to about 250 ml. Add an excess of 0·1 *N* ferrous ammonium sulphate solution and titrate back with 0·1 *N* potassium permanganate solution. 1 ml. of 0·1 *N* ferrous ammonium sulphate solution ≡ 0·001734 g. of chromium.

Alloys of higher chromium content such as ferrochrome, 30 per cent. chrome cast iron and stainless steel were analysed successfully, the titration ending only being used; 0·3468 g. of ferrochrome or 30 per cent. chrome iron and 0·6936 g. of stainless steel were convenient amounts to take. The amounts of bromate, sulphate and hydrochloric acid and the respective boiling times were increased by 50 per cent. and the solution was diluted to about 400 ml. before titration.

De Lippa's method has also been adapted to the determination of small amounts of chromium in pig iron. For this the Vignal and persulphate oxidation processes were unreliable and the diphenylcarbazide method too long for routine use. Two grams of sample were treated as above and readings taken in 4-cm. cells. The graph used is shown in Fig. 2. The above titration ending was again applicable owing to the very definite end-point.

RESULTS—

The following are typical figures. All are percentages of chromium.

(*a*) B.C.S. STANDARDS—

Description	Certificate figure	Chromium found (Spekker)
Ni–Cr–Mo C.I. "K" (No. 172)	0·41	0·40
Ni–Cr–Mo Steel "B" (No. 189)	0·68	0·70
Cr–V Steel "V" (No. 165)	0·86	0·88
Ni–Cr–Mo Steel (No. 225)	1·04	1·06

(*b*) FOUNDRY CAST IRONS—

(i) Grey cast irons containing up to about 2 per cent. of chromium, but no other alloying elements.

Vignal or persulphate volumetric method	Spekker	Volumetric ending following Spekker reading
1·03	1·02	—
1·12	1·13	—
1·30	1·30	—
1·51	1·55	—
1·75	1·76	—
—	0·50	0·47
—	0·72	0·75
—	0·97	0·97
—	1·13	1·13
—	1·19	1·18
—	1·24	1·27
—	1·32	1·32
—	1·46	1·47
—	1·58	1·56
—	1·74	1·74
1·04	1·01	1·03
1·08	1·09	1·04
1·22	1·20	1·20
1·26	1·27	1·32
1·45	1·41	1·37

(ii) 30 per cent. Chrome iron.

Persulphate, volumetric	Bromate, volumetric
30·1	30·1
30·3	30·2
31·0	31·0
33·8	34·0

(*c*) B.C.S. STANDARDS—

Description	Certificate figure	Chromium found bromate, volumetric
Stainless Steel No. 209	37·42	37·2
High carbon Ferro-chrome No. 204	69·0	68·8

(*d*) PIG IRONS—

Spekker	Volumetric ending following Spekker reading
0·22	0·23
0·22	0·23
0·23	0·21
0·25	0·23

SUMMARY

The bromate oxidation process in modified forms has been applied to the determination of chromium in a variety of ferrous alloys. The analyses may also be completed volumetrically, the end-point being very definite.

The authors thank the Directors of Messrs. Newton, Chambers & Co., Ltd., for permission to publish this work.

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The Determination of Zirconium in Minerals and Refractories by the Tannin Method

By H. HOLNESS and R. W. KEAR

The standard methods for the analysis of zirconium minerals and refractories are all subject to a number of inherent difficulties. This work has been undertaken in an attempt to devise a method in which some of these difficulties are overcome.

In previous papers the precipitation by tannin of both zirconium[1] and titanium[2] from acid chloride solution has been studied and shown to be quantitative for a definite range of acidity. Clean separations of both these elements from substantial amounts of iron, aluminium, vanadium, thorium and the rare earths were achieved. Germanium[3] and tin[4] can also be precipitated under the same conditions, but are not normally present in a zirconium analysis; they can, however, be removed by precipitation of their sulphides, or the germanium by distillation of its chloride. Titanium is the one element that interferes with the tannin precipitation of zirconium, but it interferes also with zirconium analysis by other processes.

The zirconium tannin complex includes any titanium present and this must be determined and deducted. In the cupferron method the zirconium precipitate contains the whole of the titanium and minor quantities of thorium and rare earths. The precipitate obtained by the arsenate method must be tested colorimetrically for titanium. The selenite precipitate includes any thorium present and a colorimetric test for titanium is advised. The phosphate method is the only one that yields a zirconium precipitate quite free from titanium, but it is unsuitable for substantial quantities, and the ignited precipitate is not of constant composition. On ignition the cupferron, arsenate and selenite precipitates give off poisonous fumes.

The standard cupferron procedure[5] for the analysis of a zircon mineral or refractory involves two fusions, one alkaline and one acid, double ammonia precipitation and the evaporation of a considerable bulk of liquid followed by the destruction of a large amount of organic matter before the analysis is completed; these time-consuming operations are eliminated or reduced to a minimum in the method described below.

The bisulphate fusion is avoided by dissolving the zirconia in the concentrated sulphuric acid used to dehydrate the silica, and then, after double ammonia precipitation and solution in hydrochloric acid—a step necessary to remove all traces of sulphate—the further analysis is neatly resolved by the use of tannin. The zirconium and the titanium are precipitated together from acid solution, and the iron and aluminium are recovered together from the filtrate when it is made ammoniacal. The final step, the analysis of the tannin precipitates, follows standard practice.

Experimental

The procedure described below was applied to four different zirconium materials, *viz.*, a zircon firebrick, the Brazilian zirconia rock often called baddeleyite, zircon sand from the Travancore deposits, and a sample of technical zirconium oxide. Each was analysed by the cupferron method and found to contain, in addition to zirconia and silica, minor amounts of titania, alumina and ferric oxide.

Preparation of sample—

The material was prepared by the standard methods used in ore and mineral analysis. As an additional precaution the slimings were made to pass through a G.1 sintered glass filter before they were evaporated to dryness.

Procedure—

About 0·2 to 0·3 g. of sample was mixed with 3 to 5 g. of A.R. sodium carbonate in a covered platinum crucible. Before fusing, a small bunsen flame was applied for a few minutes to remove moisture, then a low-flamed Meker burner was substituted and the blast increased over a period of 10 minutes to the maximum. Fusion was continued for 1 hour. Two methods were used for the removal of the melt from the crucible.

(*a*) The crucible and cover were, when cold, placed in a tall 400-ml. lipless beaker and covered, and then, while the cover was pushed a little to one side, a solution of 20 ml. of concentrated sulphuric acid in 100 ml. of water was poured slowly down the side and the

cover replaced. After the reaction had subsided the whole was boiled to expel carbon dioxide. The crucible and lid were then extracted and well washed with water.

(*b*) On completion of the fusion the crucible was allowed to cool slightly (to allow any drops on the lid to solidify), the lid was removed and a piece of platinum wire with a coiled end immersed in the liquid melt. After this had cooled and hardened, a Meker burner with a full blast was placed underneath, and while the crucible was held in a pair of platinum-tipped tongs, tension was applied to the wire until the melt came away cleanly. The Meker was removed and the crucible and melt allowed to cool. The melt was placed in a covered 400-ml. lipless beaker containing 90 ml. of 20 per cent. sulphuric acid and the platinum wire removed and rinsed on completion of the reaction. The crucible and lid were boiled out twice with water and a third time with 10 ml. of 20 per cent. sulphuric acid, the washings and acid being transferred to the beaker.

After solution of the melt had been effected by either one of these methods, (*a*) or (*b*), it was allowed to evaporate in the uncovered beaker over a low flame until fuming occurred. The beaker was then covered and boiled vigorously for 20 to 30 minutes to dissolve the zirconia.

After being allowed to cool it was diluted with water to about 100 ml. and boiled. The silica was filtered on an 11-cm. No. 41 Whatman paper and washed well with hot water and the filtrate, "A," reserved. The precipitate was ashed in a platinum crucible and the silica determined by treatment with hydrogen fluoride. The small residue remaining in the platinum crucible was fused with a little potassium hydrogen sulphate and leached with water into a 100-ml. beaker. A few drops of dilute hydrochloric acid were then added and the solution saturated with hydrogen sulphide; paper pulp was added and the small precipitate of platinum sulphide filtered, washed and rejected. The filtrate was boiled to remove hydrogen sulphide, oxidised with a little concentrated nitric acid, again boiled and then added to the filtrate "A."

Five grams of ammonium chloride were added and 0·88 A.R. ammonia solution (filtered free from silica) was gradually stirred in until the whole was just alkaline to methyl orange. The precipitate was filtered on a 12·5-cm. No. 41 paper with the aid of a little paper pulp, and washed with a cold 2 per cent. solution of ammonium chloride; the filtrate and washings were discarded. The precipitate was dissolved in 50 ml. of diluted hydrochloric acid (1 + 1), boiled, cooled and reprecipitated with ammonium chloride and ammonia. The precipitate was filtered and washed until free from sulphate.

The precipitate was again dissolved in 50 ml. of diluted hydrochloric acid (1 + 1), and the solution boiled and diluted to 200 ml., 5 g. of ammonium chloride were added and the solution boiled, and then a filtered solution containing 1 g. of tannin dissolved in 20 ml. of boiling water was added. To the gently boiling solution about 100 ml. of 2 *N*, filtered, A.R. ammonia solution were slowly stirred in until the liquid was only faintly acid to methyl orange. The whole was allowed to digest and flocculate, a little pulp was introduced and the precipitate was filtered on a 12·5-cm. No. 41 Whatman paper to give the first tannin precipitate (TP^1). This was washed with a hot 2 per cent. solution of ammonium chloride, ashed and weighed; it contained all the zirconia and some titania. The filtrate and washings were boiled, 1 g. of tannin (dissolved as before) was added and 2 *N* filtered A.R. ammonia solution drop by drop until the solution was just not neutral to methyl orange. The liquid was digested and filtered on a 9 cm. paper to give a minor tannin precipitate (TP^2), which was washed as before and ashed to give the remaining titania. With experience it was found that TP^2 could be included in TP^1 by making use of the dark iron tannin complex as an indicator. The ammonia was added until the darkening was just *not* permanent.

The filtrate was boiled and 20 ml. of 2 *N* filtered A.R. ammonia solution added, and the precipitate allowed to digest before being filtered on an 11-cm. No. 41 paper. It was washed with a hot 2 per cent. solution of ammonium chloride to give the final tannin precipitate (TP^3) and contained all the iron and aluminium; it was ashed and the filtrate discarded.

The ashed precipitates TP^1 and TP^2 were combined and fused with potassium hydrogen sulphate. The titania was determined either colorimetrically, or by tannin precipitation from oxalate solution, according to established practice, and the zirconia was obtained by difference.

The combined precipitate of iron and aluminium, TP^3, was also fused with bisulphate. The iron was determined colorimetrically, or by precipitation as ferrous sulphide, and the alumina obtained by difference.

It was sometimes found necessary to correct the alumina figure for a small amount of silica picked up from the glassware while the solutions were ammoniacal, *e.g.*, during the double ammonia precipitation. The presence of this silica became evident on leaching the bisulphate melt of TP[3], and when found it was filtered, ashed, and weighed to provide the necessary correction.

Results

Material analysed	Method of analysis	Loss on ignition %	SiO_2 %	$ZrO_2 + TiO_2$ %	$Al_2O_3 + Fe_2O_3$ %	Total %
Zircon firebrick	Cupferron	0·13	29·49	65·53	4·84	99·99
	Cupferron	0·13	29·40	65·89	4·67	100·09
	Tannin	0·13	29·42	65·80	4·68	100·03
	Tannin	0·13	29·49	65·62	4·94	100·18
Zirconia rock	Cupferron	—	14·31	82·77	3·11	100·19
"Baddeleyite"	Tannin	—	14·27	82·60	3·25	100·12
(Brazil)	Tannin	—	14·26	82·90	2·82	99·98
Zircon sand	Cupferron	0·10	31·61	66·16	2·28	100·15
(Travancore)	Tannin	0·10	31·39	66·37	2·34	100·20
	Tannin	0·10	31·54	66·26	2·26	100·16
Zirconium oxide	Cupferron	0·70	7·90	89·25	2·29	100·14
(technical)	Tannin	0·70	8·10	89·45	2·15	100·40
	Tannin	0·70	7·93	89·30	2·21	100·14

The procedure described above for the determination of silica possesses two commendable features, since

(*a*) there is no danger of alkaline solutions coming into contact with the glass vessels and funnels, and

(*b*) there is no liability of loss by transference of material until the silica is filtered.

In this work we have everywhere prescribed the use of filtered solutions of ammonia in order to reduce to a minimum the introduction of silica into the analysis. It is well known that dilute solutions of ammonia attack glass vessels and it has recently been shown that tannin precipitates silica from slightly ammoniacal solution,[6] hence any extraneous silica increases the amount of TP[3]. In the cupferron procedure the presence of this silica becomes apparent during the destruction of the organic matter by fuming with nitric and sulphuric acids and it can be removed before the determination of the aluminium. The presence of this hazard seems to have escaped mention in most of the published procedures on this type of work.

Summary

A modification of the standard method for determining silica in zirconium materials is described, together with a procedure for the use of tannin in the analysis of zirconium minerals and refractories.

The use of the reagent offers a comparatively rapid method for determining $ZrO_2 + TiO_2$ and $Fe_2O_3 + Al_2O_3$ in these materials.

Attention is drawn to the introduction of silica into the analysis by attack on the glassware by dilute solutions of ammonia.

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The Determination of Butter Fat, Coconut Oil and Palm Kernel Oil in Margarines

By K. A. WILLIAMS

(*Read at the meeting of the Society on Wednesday, October 6th*, 1948)

Bolton, Richmond and Revis[1] recorded the results of a large number of analyses of fats containing various percentages of butter fat, coconut oil and palm kernel oil. From these they developed two methods, one graphical and one depending on empirical formulae, for

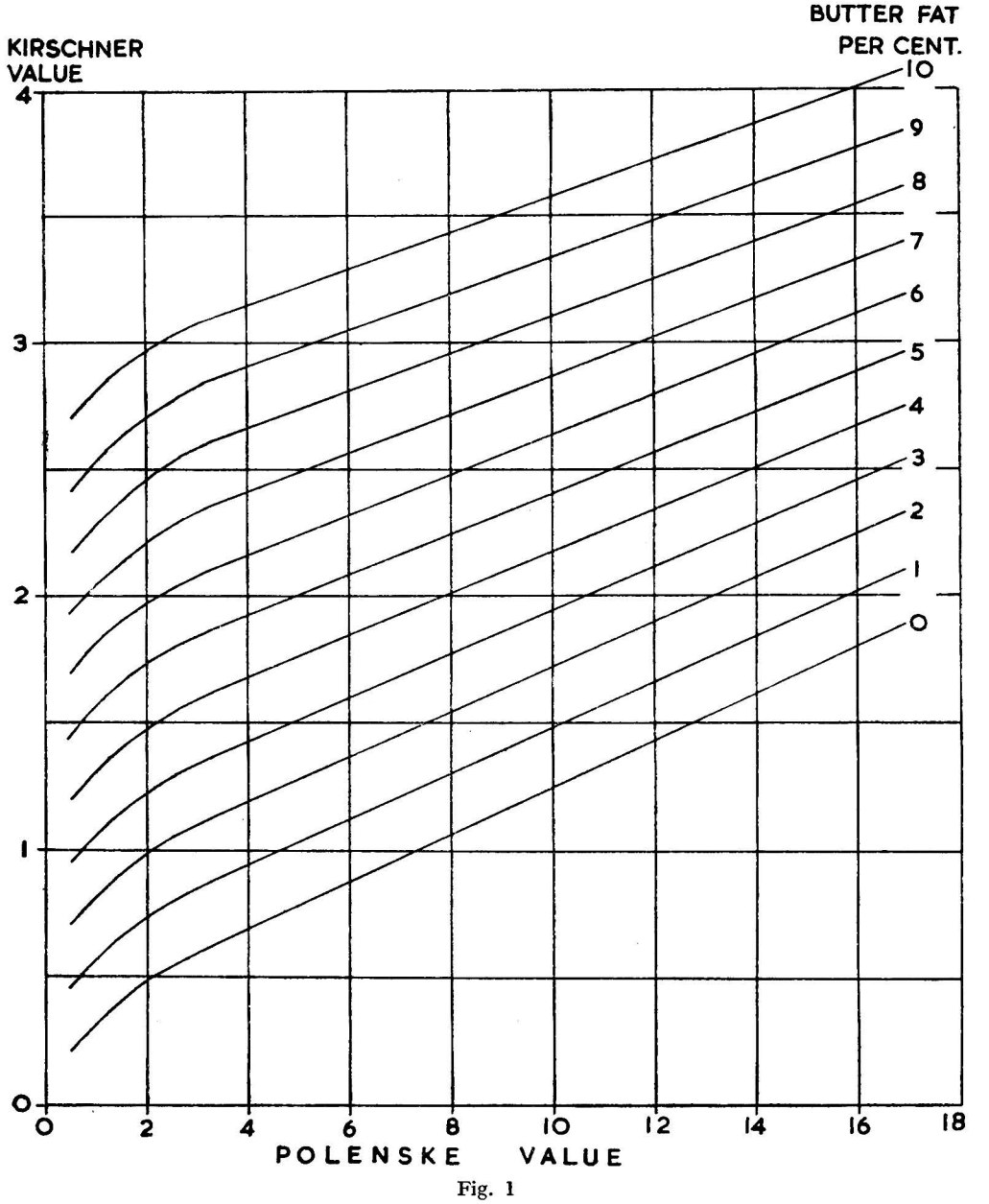

Fig. 1

calculating the percentages of these fats in a mixture from the Reichert, Polenske and Kirschner values.

Both methods have stood the test of time, but the graphical one has proved somewhat cumbersome and not particularly easy to understand. The following procedure will be found simpler in practice; it is based on the original data of Bolton, Richmond and Revis.

The Reichert, Polenske and Kirschner values of the sample having been determined, refer the Polenske and Kirschner values to Fig. 1, and read off directly from this graph the percentage of butter fat in the mixture. Subtract 0·3 from the Reichert value for *each* 1 *per cent.* of butter fat so found, and refer the corrected Reichert value and the Polenske value to Fig. 2.

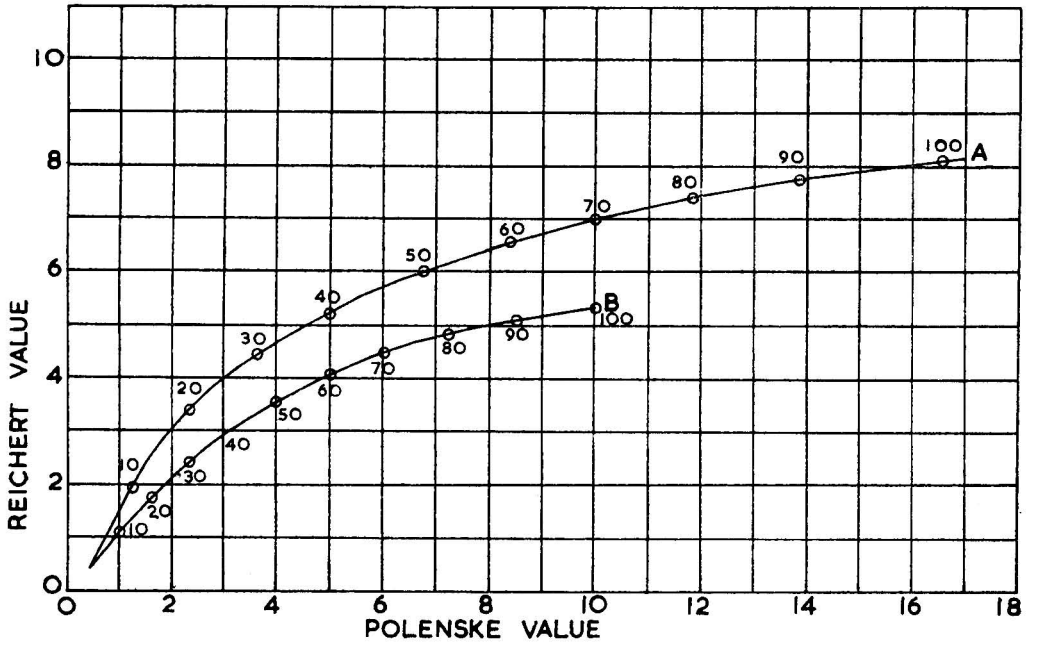

Fig. 2. Curve A.—Coconut oil. Curve B.—Palm kernel oil
Percentage of each oil corresponding to different Reichert and Polenski values

If either coconut oil or palm kernel oil is present, the point so plotted should lie reasonably closely to one or other of the two lines of the graph; then there is a clear indication of which oil is present, and the graph will show the approximate percentage. Mixtures of the two oils lead to points lying between the two lines, and the position of the points depends on the relative proportions of coconut and palm kernel oils present. Fig. 2 should not be applied if oils of the *Palmae* other than coconut or palm kernel oil are present.

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Discussion

Mr. J. King asked if Dr. Williams would kindly state what was the precise technique for carrying out the Reichert - Polenske determination adopted in the late E. R. Bolton's laboratory in compiling the original curves, of which the present curves appeared to be a much simpler version, as any significant deviation would render them far less useful. The method of B.S. No. 769 of 1938, which attains homogeneity in the aqueous phase by four or five double inversions of the 110-ml. distillate, although satisfactory for butter fat, may lead to variable results in equal mixtures of butter fat with coconut or palm kernel fats. The method suggested by the French representative in the International Commission for the Study of Fats, which requires complete homogenisation of oil and aqueous phases appears to be even more objectionable. Presumably Bolton's directions in "Fatty Foods" was adopted, *viz.*, to allow the flask containing

the distillate to stand in a water-bath at 10° to 15° C. for 15 minutes, as no doubt homogeneity of the aqueous phase is attained here or at the subsequent filtration with little disturbance of the Polenske acids.

Dr. WILLIAMS, in reply, said that the S.P.A. method was drafted by a committee which included the late E. R. Bolton and which discussed his technique in detail with him in his own laboratory. It had also the advantage of having before it much of the original apparatus used by Bolton and his collaborators in putting together the tests of Reichert, Polenske and Kirschner to form the composite whole now called the Reichert - Polenske - Kirschner test. Some of the original apparatus was still in fact in existence in the author's own laboratory. He believed that the S.P.A. method agreed closely with the original technique of Bolton, Richmond and Revis; it certainly agreed with the technique taught to him by Bolton. The British Standards Institution had been careful, in formulating B.S. No. 769, to adhere strictly to the details of the S.P.A. standard method.

He agreed with Mr. King that the fullest possible description of the test was absolutely necessary if different operators were to obtain substantially the same results. The method recently circulated by the International Commission was not nearly sufficiently detailed. Particular attention had been directed by the S.P.A. Committee, after the publication of its report, to the problem mentioned by Mr. King. This difficulty arose from a necessity to homogenise the aqueous portion of the Reichert distillate, without dispersing the insoluble fatty acids floating in it. If the receiving flask is shaken after it has been cooled and just before filtering, there is a probability that liquid insoluble fatty acids will become dispersed and will pass through the filter. The S.P.A. Committee has tried many methods of preventing this accumulation of some liquid insoluble acids in the aqueous phase, but has not found any method offering complete success, especially if the test is carried out on mixtures containing a large percentage of coconut oil. In such cases it is best, at present, to omit the mixing of the contents of the receiver. That is to say, the flask and contents should be cooled as described in the S.P.A. text, and the contents then filtered through the dry, specified filter paper. The liquid should be poured on to the filter in such a way that aqueous liquid reaches it first, the globule of fatty acid following later. In this way it is generally possible to avoid direct contact of insoluble fatty acid with the dry paper, and the amount of insoluble acids contaminating the aqueous phase in the filtrate is kept at a minimum. His experience was that, if as much as possible of the aqueous phase was filtered and then mixed, its composition was very close to that of the whole aqueous phase distilled.

Note added by the author, September, 1949—The French text referred to by Mr. King had been used by analysts in a number of European countries on a sample circulated by the International Commission for the Study of Fats. The results of these tests were discussed at the recent meeting of the Commission in Amsterdam, when it was immediately apparent that the above criticism of the text was justified. As a result of this discussion, further samples are to be examined by members of the Commission, who will use and compare the English and Dutch formulations of the method.

Notes

SUGGESTED MECHANISM OF POISONING BY LIQUID TETRACHLOROETHANE

FEW cases of poisoning by the drinking of liquid tetrachloroethane (acetylene tetrachloride) have been recorded. Elliott[1] reports a case where a man died approximately 12 hours after drinking "a small quantity" of the liquid; he appears to have been in a deep coma for the whole of the period. Hepple[2] gives details of a case where the victim went into a coma within 1 hour of drinking the liquid and died 8 hours later; 12 ml. (20 g.) were recovered from the stomach contents.

In one such case where this laboratory performed the toxicological work, 25 g. of liquid tetrachloroethane was recovered by steam distillation from the stomach contents and intestines, and identified by boiling-point and chlorine determinations. The history of the case is very similar to that of those reported. Coma occurred shortly after the taking of the liquid (exact time unknown, but certainly less than 1 hour) and death followed about 9 hours later without consciousness being regained.

The pathological report states that the stomach contents were a dark brown liquid material with a pronounced odour resembling that of chloroform. Moderate congestion of the stomach lining with slight congestion of the liver was also noted.

Lehmann and his associates[3] have shown that the vapour of tetrachloroethane has a toxic action similar to that of chloroform but about four times as potent. Of liquid chloroform,[4,5,6,7] 1 to 2 oz. (28 to 56 g.) is generally considered a minimum lethal dose, although recoveries have followed larger doses.

In view of the high toxicity of tetrachloroethane as compared to chloroform and related chlorinated hydrocarbons, it was decided to find if any metabolic products could be isolated. Examination of the urine (200 ml.) showed it to be very acid in reaction and from it was isolated 0·14 g. of free oxalic acid, although none could be detected in the stomach contents. It would appear probable that this is formed from the tetrachloroethane by hydrolysis followed by oxidation and its disturbance of the calcium metabolism would in part account for the high toxicity of tetrachloroethane.

The writer is indebted to Dr. C. N. Partington, Assistant County Pathologist, Dorset County Council, for permission to quote from his post-mortem report, also to Mr. H. Campbell for assistance with the experimental work and to Mr. E. B. Parkes, M.Sc., F.R.I.C., Director of this laboratory for permission to publish.

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December, 1948

A RAPID METHOD OF ESTIMATION OF AMMONIA NITROGEN IN SEWAGES AND EFFLUENTS

AMMONIA nitrogen in sewage and sewage effluent is normally estimated either by distillation or by direct nesslerisation after some form of preparation of the sample. There is a divergence of opinion as to which is the more suitable or accurate method to use for sewage liquors. Distillation is the choice of the Ministry of Health,[1] which states that "direct nesslerisation . . . cannot be recommended for quantitative purposes but may be found useful for giving an approximate result quickly." On the other hand, the American Public Health Association[2] prefers direct nesslerisation, after a preliminary clarification treatment at pH 10·5, as it avoids errors from hydrolysis of urea and other organic compounds. Even so, distillation by the American method is under closer control than the British approved method since use is made of a phosphate buffer solution which maintains a constant pH of approximately 7·4 during the process.

Apart from the need for accuracy, other factors influencing the choice of method may have to be taken into consideration. For instance, where large batches of routine samples are involved, a direct method can save time, apparatus, and bench space and can be operated in a laboratory where there is no gas supply.

A simple, rapid, direct nesslerisation method applicable to sewages and effluents has been developed in this laboratory. Results with it have been confirmed in the laboratories of the Northern Outfall Works and at County Hall, Westminster Bridge, and are in close agreement with those obtained by the American controlled distillation method. Besides effecting economy of time, space, and materials, it reduces previous sources of error arising from the preparation of samples for direct nesslerisation and is thought to be as accurate as any method hitherto described.

EXPERIMENTAL

Influence of sodium hydroxide on the Nessler colour—Normally, to prepare samples for direct nesslerisation, sodium hydroxide is used to remove substances which otherwise would give a turbidity with Nessler solution. A study of the effect of the concentration of sodium hydroxide in the final matching solution on the Nessler colour complex showed that the yellow colour deepened progressively with increasing of concentration of sodium hydroxide, and that unless the standard contained the same alkali concentration as the prepared sample, serious error could arise.

This effect is shown in Table I, relating to 100-ml. quantities of ammonium chloride solution (1 ml. ≡ 0·000001 g. of nitrogen) containing various concentrations of sodium hydroxide matched against a standard of the same concentration of ammonium chloride containing no sodium hydroxide. Results are expressed as per cent. increase in depth of colour due to the sodium hydroxide.

TABLE I

Concentration of NaOH, g./100 ml.	Increase in depth of colour per cent.	Concentration of NaOH, g./100 ml.	Increase in depth of colour per cent.
0·008	0·0	0·16	6·5
0·016	1·0	0·20	9·5
0·024	1·0	0·32	13·5
0·032	2·0	0·40	16·5
0·040	2·0	0·60	30·0 (*a*)
0·060	4·5	0·72	50·0 (*b*)

All solutions were optically clear except (*a*), hazy, and (*b*), cloudy. Solutions containing more than 0·5 g. of sodium hydroxide per 100 ml. were useless for matching, owing to the turbidity produced.

Avoidance of sodium hydroxide treatment—In testing large batches of samples it is more convenient to use one standard, if possible, than numbers of standards treated with sodium hydroxide to correspond with the concentrations present in the prepared samples. Attention was therefore given to reducing the caustic soda concentration to a minimum, common for all samples. The work of Gledhill and McCanlis[3] had shown that sodium hexametaphosphate (Calgon) solution prevented the development of cloudiness in chalk well waters with Nessler solution. This reagent was therefore tried, and it was found that a small quantity of Calgon solution not only suppressed all cloudiness and haze in the matching solutions, but it eliminated the need for any sodium hydroxide whatsoever to prepare the samples and thereby rendered the intermediate filtration stage unnecessary. All samples of sewages and effluents treated by this Calgon

technique have given solutions optically clear. (This does not apply to saline river water samples.) The presence of Calgon solution had no significant effect on the maximum depth of colour produced (unlike sodium potassium tartrate, which also gives optically clear solutions with sewages and effluents prepared without treatment with sodium hydroxide but which deepens the matching colour considerably).

PROCEDURE

Pipette 2 ml. of the filtered sewage or effluent into a 100-ml. measuring flask, partially dilute with ammonia-free distilled water, add 1 ml. of 10 per cent. Calgon solution and complete the dilution to the 100-ml. mark. Add 2 ml. of Nessler solution, allow to stand for 15 minutes and then match against a standard made up by diluting 10 ml. of ammonium chloride solution (1 ml. = 0·00001 g. of nitrogen) and 1 ml. of 10 per cent. Calgon solution up to 100 ml. (*Note*—In our experience optically clear solutions are obtained even with only 0·2 ml. of 10 per cent. Calgon solution for 2 ml. of sewage.)

Comparison of methods—Table II shows ammonia nitrogen contents of sewages and effluents prepared in this manner compared with results obtained by distillation using phosphate buffer solution according to the American Public Health standard method. The solutions were matched in a Klett colorimeter at the Northern Outfall and by a photo-electric colorimeter at the Southern Outfall. The samples tested were average daily sewage and effluent samples; all results are expressed as ammonia nitrogen in parts per million.

TABLE II

	Sewage		Effluent	
	Calgon method	Distillation	Calgon method	Distillation
Southern Outfall 1	39·8	39·4	39·8	38·8
2	39·5	40·2	37·0	36·5
3	43·0	43·0	46·0	45·5
4	45·0	45·0	43·3	42·8
5	39·3	40·5	42·0	42·5
6	37·8	39·3	38·0	39·3
7	47·0	47·7	45·5	46·5
7 (*a*)		46·5		44·5
8	40·3	41·0		
8 (*a*)		41·0		
8 (*b*)	51·5	51·0		
8 (*c*)		50·5		
Northern Outfall 9	30·4	30·0	30·8	29·6
10	31·0	30·5	33·1	32·7
11	27·8	26·7	29·4	28·6
12	31·3	29·6	31·7	29·4
13	28·8	27·8	31·5	29·8

Nos. 7 (*a*), 8 (*a*) and 8 (*c*) were duplicate samples of Nos. 7, 8 and 8 (*b*), but matched against standards not containing Calgon solution.

Nos. 8 (*b*) and 8 (*c*) were sewage of samples No. 8 to which ammonium chloride had been added equivalent to an additional 10 p.p.m. of ammonia nitrogen.

The experiments indicate a close agreement between the two methods, practically within experimental error. It will be seen that added known quantities of ammonium salts have been wholly recovered. The process has been adopted for routine samples in the Southern Outfall laboratory and has given uniformly satisfactory results with a great saving of time.

SALINE RIVER WATERS—

It has been noted above, parenthetically, that this technique is not suitable for tidal river water samples. The salts in such river waters continue to give turbidities even in presence of large quantities of Calgon solution and some form of treatment with caustic soda solution is necessary to obtain clear solutions for matching. If, however, the amount of sodium hydroxide used in preparation is kept very low, in fact is insufficient by itself to give complete treatment, then a small quantity of Calgon solution will suppress any haze or cloudiness that would have developed. Tidal waters vary considerably in their sodium hydroxide requirements and further work is necessary to see if they can be adapted to the simplicity of the technique as it applies to sewages and effluents.

SUMMARY—

A simple, rapid, direct nesslerisation method of estimation of ammonia nitrogen in sewages and effluents gives results comparable with those obtained by controlled distillation.

Sodium hydroxide, normally used in the preparation of samples, can give rise to serious errors: its use, together with the filtration stage, is avoided by using a small quantity of sodium hexametaphosphate (Calgon).

The method has limitations with tidal river waters.

Thanks are due to Dr. R. J. Stephenson, Mr. P. E. L. Farina and Mr. H. G. Haynes for their part in the experimental work, and to Mr. C. J. Regan, Chemist-in-Chief of the London County Council, for advice and encouragement.

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LONDON COUNTY COUNCIL
PUBLIC HEALTH DEPARTMENT
SOUTHERN OUTFALL WORKS
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J. E. HOULIHAN

March, 1949

CONTAMINATION OF WATER BY TRICHLOROETHYLENE

CASES of contamination of wells by trichloroethylene have come to our notice. In the first, the well was situated beside a factory that used large quantities of trichloroethylene as a solvent. During a fire at the factory a tank of the liquid burst and the ground was saturated with the solvent. After more than four years the water in the well still had an odour of trichloroethylene and the well had to be abandoned. The well was sunk in gravel only about 20 feet from a river and one might have expected that the movement of water through the gravel would have removed the contaminant.

In the other case, the well was situated 150 to 200 yards from a pit in an open field where waste trichloroethylene had been dumped. It was in valley gravel and in the direct line of flow towards the river. The water in it had a slight odour of trichloroethylene and was said to cause stomach disorders, giddiness, etc. The amount of trichloroethylene in the water was found to be 18 parts per million when estimated by the following method, a modification of the Fujiwara pyridine - sodium hydroxide reaction.

From these two cases it is evident that contamination by compounds of this nature is likely to be very persistent and there is some evidence of toxicity at very low concentrations.

METHOD—

Standard solution—A convenient standard can be prepared by first dissolving 1 ml. of commercial trichloroethylene in alcohol and making up with alcohol to 100 ml., and then diluting 1 ml. of this solution to 500 ml. with water, to give a solution containing 20 parts per million.

Procedure—Place 5 ml. of the sample in a test tube, add 2 ml. of colourless pyridine and 5 ml. of a 50 per cent. w/v solution of sodium hydroxide in water, shake thoroughly and stopper with cotton wool. Place the tube, and a similar tube containing the standard solution treated in the same way, in a boiling water-bath for 5 minutes. Cool and compare the orange colour obtained in the supernatant liquid with that of the standard. For a more accurate estimation prepare a series of tubes containing different amounts of trichloroethylene and match with the sample treated in parallel. It should be possible to estimate 5 parts per million with ease and the method would probably detect 1 part per million.

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March, 1949

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Direct Determination of the Volume Occupied by the Husk in the Analysis of Malts. J. R. Gwilt (*J. Inst. Brew.*, 1947, **53**, 152–154)—In the standard method for the determination of extract in brewing malts in this country (*Ibid.*, 1933, 517) a volume of 15 ml. is allowed for husk and other insoluble matter present in 50 g. of malt.

Warren (*Brew. Trade Rev.*, 1924, **38**, 148) has shown the allowance of 15 ml. to be too high. Bishop (*J. Inst. Brew.*, 1934, 187) allows for variation in the volume occupied by the insoluble solids and finds an average of about 10 ml. for British two-rowed malts, and later (*Ibid.*, 1944, 140) gives a method for correcting apparent extract to find the true extract. In the present work the volume occupied by the grains is measured directly.

Method—Mash 50 g. of malt as in the Institute of Brewing's standard method (*Ibid.*, 1933, 517), and at the end of the mashing period transfer the material quantitatively to a litre-beaker and heat it to boiling-point. Allow the grains to settle and decant the supernatant liquid through a 24-cm. Whatman No. 12 fluted paper. Repeatedly extract the grains with boiling water and wash by decantation until all soluble matter has been removed. (In the investigation this was checked by determination of the sp.gr. of the washings.) Rinse the residue on the paper back into the beaker with hot water, remove most of the water by boiling, and dry the residue, first to apparent dryness at 98°

to 100° C., then by two evaporations with acetone and finally at 100° C. to approximately constant weight. Weigh the dried grains and transfer them quantitatively to a 150-ml. graduated flask containing about 80 ml. of acetone, swirl the flask to remove air bubbles, dilute to the mark with acetone, and weigh. Weigh the flask empty and full of acetone and again full of water (to obtain the density of the acetone), making all determinations at 68° F. as a convenient standard temperature. Thus, the weight of acetone displaced by the grains can be calculated and hence its volume, *i.e.*, the volume occupied by the grains.

Heron (*Ibid.*, 1902, 668) gave 13 and 17 ml. as the outside limits for the volume of insoluble solids in the malts he had examined, whereas Brown (*Ibid.*, 1943, 195; *Analyst*, 1943, **68**, 309) found the volume to be 9·5 to 10·7 ml. for British two-rowed malts. Determinations by the method given above with some British two-rowed malts gave results ranging from 9·7 to 10·3 ml., *i.e.*, in good agreement with Brown's figures. Hence, although the standard method of analysis gives results consistent among themselves, it is confirmed that the apparent extract for average brewery malts is nearly 1 lb. low compared with other extract-yielding materials. A correction for a wide range of malts can be obtained from Bishop's tables (*loc. cit.*).

For comparison, determinations were made with crystal (12·6), brown (12·0) and chocolate (13·4) malts and with oat malt (19·0) and with unmalted flaked barley (12·1), the results in brackets being the volume (ml.) of the grains from 50 g. of the material. A. O. JONES

Data for the Oils of Some Common Oleaginous Seeds. G. Loew (*Industria y Quim.*, 1948, **10**, 5–6)—Healthy onion seeds (*Allium Cepa*) contained 8 per cent. of moisture and 17·90 per cent. of fatty material. Oil obtained from the crushed seeds by expression, and by extraction with naphtha gave, respectively, the following data: d^{15} 0·9354, 0·9289; n^{25} 1·4738, 1·4730; solidifying-point −15° C., −15° C.; α_D^{2} (ethyl sulphate) −1·8, −1·5; deviation (Jean) +30°, +26·8°; acidity as oleic acid 1·78, 1·92 per cent.; saponification value 187·7, 197·5; iodine value (Rosenmund and Kuhnhenn) 107·7, 111·9; unsaponifiable matter (Spitz and Hönig) 2·37, 1·20 per cent.; modified Bellier test—became turbid on cooling from 60° C. to 11°, 24° C., respectively. A. F. STURGESS

South African Fish Products. Part XXIX. The Composition of the Liver Oil of the Soupfin Shark (*Galeorhinus canis*, Rond.). M. L. Karnovsky, A. W. Lategan, W. S. Rapson, and H. M. Schwartz (*J. Soc. Chem. Ind.*, 1948, **67**, 193–196)—The iodine value of the liver oil of the mature fish increases with the oil content of the liver. This is due to the greater unsaturation of C_{16}, C_{18}, C_{20}, and C_{22} acids.

In the mature fish vitamin A, α-glyceryl ethers, and sterols are major components of the unsaponifiable matter; sterols predominate in the foetus. The unsaponifiable matter of four samples of liver oil from mature fish was from 1·99 to 7·84 per cent. Results are given in the accompanying table.

	I	II	III	IV	V
Liver oil from	Mixed oils	Fat females	Thin females	Male	Foetus
Oil in liver, %	—	83·0–83·2	26·2–36·0	60·2	46·1
Unsap. in oil, %	1·99	2·52	7·12	7·84	8·68
$E^{1\%}_{1\,cm.}$ 328 mμ. of oil	3·78	9·16	48·4	63·6	0·15
Neovitamin A as % of total vitamin A	30·9	34·1	24·1	29·0	—
Composition of unsaponifiables.					
Total vitamin A, %	11·2	21·5	39·7	47·7	0·1
Cholesterol, %	28·7	19·6	24·9	6·5	88·3
α-Glyceryl ethers,* %	21·7	20·5	15·7	17·8	3·4
Squalene, %	3·8	1·4	1·1	0·6	0·5
Satd. hydrocarbons, %	2·2	—	0·7	—	0·2
Total	67·6	63·0	82·1	72·6	92·5
Sapon. value of unsap. after acetylation**	195·5	221·3	—	199·0	162·8
do. (calc.)**	134·9	137·9	165·6	161·4	139·7
Fatty alcohols in unsap., % (calc. as oleyl alcohol)	29·0	39·9	—	17·9	11·1

* Calculated as selachyl alcohol.
** Calculated as mg. of KOH required to saponify acetates from 1 g. of unsaponifiable.

E. B. DAW

Biochemical

Oxidase Activity in Potato Tubers. IV. Quantitative Method for the Determination of Peroxidase. J. S. Wallerstein, R. T. Alba, M. G. Hale, and H. Levy (*Biochim. et Biophys. Acta*, 1947, **1**, 327–334)—A method is described for the determination of peroxidase by a reaction with *o*-phenylenediamine in presence of hydrogen peroxide under standard conditions. The reaction is stopped by adding acetone, the mixture is extracted with butanol, and the butanol extract assayed colorimetrically and fluorometrically.

Method—Wash and peel the potatoes, cut into dice of about 1-ml. volume and wash briefly in running water. Homogenise a suitable weight of dice (normally 40 g.) with water in a Waring blendor at high speed in a total volume of 300 ml. After 60 sec. reduce the speed of the blendor, and add

a few drops of caprylic alcohol. Pipette 20 ml. of the homogenate from the blendor for the test. Where indicated, blanch the dice in a colander by placing in boiling water for the required time, and immediately cool in ice-water. Prepare purified horse-radish peroxidase by the method of Willstaetter and Stoll (*Annalen*, 1918, **416**, 21).

Procedure—Add 100 ml. of *o*-phenylenediamine solution (made by dissolving 25 g. of *o*-phenylenediamine dihydrochloride in water, diluting to 2 litres and adjusting the pH to 5·0 with sodium hydroxide) to 200 ml. of water, and stir slowly in a beaker. Add 0·7 ml. of 30 per cent. hydrogen peroxide and 20 ml. of homogenate. Stir continuously for 4·5 min., remove 50 ml. of the reaction mixture and, at 5 min., add 50 ml. of acetone to stop the reaction. Pipette 5 ml. of the acetone mixture into 95 ml. of water and 100 ml. of butanol in a separating funnel. Shake vigorously, separate the butanol layer, filter through a Whatman No. 5 filter paper, and measure in a Klett - Summerson colorimeter, using standard Klett test tubes, with a number 42 blue filter against a butanol blank. Also measure the fluorescence on 2 ml. of the butanol filtrate after mixing thoroughly with 48 ml. of butanol, against a standard of 1 part of fluorescein in 4 million parts of 0·01 *N* sodium hydroxide, using a Pfaltz and Bauer fluorophotometer. For comparative purposes a purpurogallol peroxidase assay was carried out on aliquots of the same batch of homogenate used for the *o*-phenylenediamine test. The technique was a modification of the method of Willstaetter and Stoll as applied by Stern and Kegeles (unpublished communication, Yale University, 1941). The method depends on the extraction by ethyl acetate of purpurogallol formed from pyrogallol, and photometric determination of the optical density of the extract with correction for the tyrosinase blank.

Results—Results by the *o*-phenylenediamine method correspond closely to the Willstaetter pyrogallol procedure. Reproducibility is comparable in the two methods, whereas sensitivity and ease of assay is substantially greater with the *o*-phenylenediamine procedure. Under the conditions of assay the amount of colour and fluorescence developed is linearly proportional to time for about 15 min., and thereafter shows a sharp decline. The temperature optimum for the reaction is about 60° C. J. S. HARRISON

Infra-Red Analysis of Crystalline Penicillins. R. B. Barnes, R. C. Gore, E. F. Williams, S. G. Linsley, and E. M. Petersen (*Anal. Chem.*, 1947, **19**, 620–627)—A method, based on the presence in the infra-red spectrum of characteristic absorption bands, is presented for the analysis of each of the different varieties of penicillin. Owing to the insolubility of the salts of penicillin in all the solvents that are of use in infra-red measurements, and to the risk of loss and degradation in converting the salt to the free acid and transferring to a suitable dry solvent, it was necessary to record the spectrum of the crystalline salts.

The sample was prepared by mulling a small quantity (0·5 to 3·0 mg.) of crystalline sodium penicillin with liquid petrolatum (Nujol) directly upon the rock-salt plates of the absorption cell. A recording spectrometer (Perkin - Elmer Model 12-B) was used. Many of the absorption bands found are associated with the crystal structure of the material and the nature of the cation of the salt affects the spectrum; the results given here are for crystalline sodium salts only. All types of penicillin show a characteristic absorption band at 1770 cm.$^{-1}$. The frequencies of bands characteristic of individual types and sufficiently free from mutual overlapping to be used for analysis are: G (benzyl) 703 cm.$^{-1}$; X (*p*-hydroxybenzyl) 831 and 1220 cm.$^{-1}$; F ($\triangle^2$-pentenyl) 971 cm.$^{-1}$; K (*n*-heptyl) 1330 cm.$^{-1}$; dihydro F (*n*-amyl) 715 and 1166 cm.$^{-1}$

For quantitative analysis it is necessary to know the thickness of the sample under examination. In effect, this is done by mixing intimately with the penicillin a solid internal standard in known concentration. The standard should have a strong absorption band convenient to the spectral regions of interest, but have no absorption interfering with the analytical bands; it should be easily weighable and mullable with the sample, and easily obtainable in a pure state. As internal standard, DL-alanine was chosen, and measurements were made on the band at 851 cm.$^{-1}$

Procedure for penicillin G—Establish a calibration curve as follows. Weigh out 15 mg. of thoroughly ground, pure penicillin G and 7·5 mg. of DL-alanine. Mix the two dry with a small mortar and pestle. Place a drop of Nujol on one of the polished rock-salt plates of the absorption cell, and add about 10 mg. of the dry mixture. Using the second rock-salt plate of the absorption cell, mull the sample thoroughly. Adjust the cell thickness to give about 50 per cent. transmission at 851 cm.$^{-1}$ Set the spectrometer at 925 cm.$^{-1}$ with the cell in the beam, and adjust the slits and amplification factor to give a full-scale deflection. Record the spectrum from 925 to 775 cm.$^{-1}$, the zero being determined at the beginning and end of the run. Set the spectrometer at 760 cm.$^{-1}$, adjust for full-scale deflection and record from 760 to 695 cm.$^{-1}$ Note the slit settings and amplification factors, which must be duplicated in all successive runs. From the record of the spectrum, measure I and I_o (the intensities, respectively, of the transmitted and incident radiations) at 703 cm.$^{-1}$ and 851 cm.$^{-1}$ Obtain I_o by linear interpolation of the background radiation. Repeat on samples successively diluted with an inert solid, such as magnesium oxide, and plot a calibration curve of $R = \log (I_o/I)$ at 703 cm.$^{-1}$ $\div$ $\log (I_o/I)$ at 851 cm.$^{-1}$ *versus* percentage of penicillin G. Using this calibration curve, analyse the unknown by the same procedure. An accuracy to within ± 2 per cent. is claimed.

Other types of penicillin are analysed similarly by using the appropriate absorption band. In some determinations, the values of I_o were determined by using the cell in - cell out technique.

Interference in the analysis may be caused by impurities in the penicillin if these have absorption bands close to those used for analysis. The band at 703 cm.$^{-1}$ in sodium penicillin G is due to the

monosubstituted phenyl group, and any impurities, such as sodium phenyl acetate, having this grouping will interfere with the analysis of penicillin G. The presence of such impurities may be detected by the presence of additional bands in the spectrum, and for this reason the complete spectrum of a sample is recorded before analysis.

Effects due to crystal orientation were found with one sample of penicillin G, the crystals of which were in the form of flat plates. On pressing the Nujol mull between the rock-salt plates, orientation of the crystals took place; analysis gave a low value. Orientation was revealed by rotating the absorption cell at an angle to the beam of radiation. Orientation affects only some of the absorption bands and its presence can be detected by an abnormally low value of the ratio log (I_o/I) at 805 cm.$^{-1}$ ÷ log (I_o/I) at 900 cm.$^{-1}$ Orientation can be prevented by more thorough grinding of the sample, and a grinding time of 5 min. is recommended. G. H. TWIGG

Polarographic Estimation of Steroid Hormones 4. Determination of 3 (α)- and 3 (β)-hydroxy-17-ketosteroids. W. R. Butt, A. A. Henly, and C. J. O. R. Morris (*Biochem. J.*, 1948, **42**, 447–452)—A method is given for the separation of 3 (α)- and 3 (β)-hydroxy-17-ketosteroids in urine. The urine is first hydrolysed and extracted (Barnett, *et al.*, *ibid.*, 1946, **40**, 445).

Reagents—(1) *Digitonin solution*—Use a 1 per cent. solution of B.D.H. digitonin in warm 90 per cent. ethanol. The digitonin can be kept in solution by storing the reagent at 37° C. (2) *Pyridine*—Dry over barium oxide and distil frequently.

Procedure—Put into a graduated centrifuge tube a benzene solution of urine extract containing 1 to 1·5 mg. of 17-ketosteroid. Evaporate to dryness under reduced pressure. Add 0·75 ml. of digitonin solution and quickly heat to boiling over a microflame to dissolve as much material as possible. Stopper the tube and keep overnight in a refrigerator. Add in portions, and with stirring, 10 ml. of peroxide-free ether. Allow the precipitate to flocculate before adding the last 2 to 3 ml. Centrifuge for 5 min. at 2000 to 3000 r.p.m. and decant the supernatant liquid into a 50-ml. separating funnel. Wash the precipitate three times with 5-ml. portions of ether, stirring and centrifuging as before. Wash the bulked liquid three times with 5-ml. portions of water, transfer to a small flask, evaporate the ether under reduced pressure, and dry the residue *in vacuo* over calcium chloride for 1 hr. This constitutes the α-fraction.

Dissolve the residue in the centrifuge tube in 0·25 ml. of dry pyridine and place in a water-bath at 60° to 70° C. for 3 min. Cool, and then add 5 ml. of ether in small quantities as before. Centrifuge for 5 min. and transfer the supernatant liquid to a small separating funnel. Wash the residue in the centrifuge tube with pyridine and ether and finally wash twice with 5-ml. portions of ether. Wash the combined extracts twice with 5-ml. portions of 2 *N* sulphuric acid, and then three times with 5-ml. portions of water. Transfer to a small flask and dry the residue exactly as above. This constitutes the β-fraction.

With normal urines use about one-tenth of the α-fraction and the whole of the β-fraction. Oxidise with permanganate - periodate, treat with Girard's reagent T, and take a polarogram (*Idem, ibid.*).

W. S. WISE

Agricultural

Determining Small Amounts of Calcium in Plant Materials. A Colorimetric Method. E. H. Tyner (*Anal. Chem.*, 1948, **20**, 76–80)—*Reagent—Chloroanilic acid solution*—Add 1 g. of chloroanilic acid to 500 ml. of distilled water, dissolve by warming to 50° C., cool, and dilute to 1 litre.

Procedure—Place 1 g. of the finely ground plant material in a 50-ml. Pyrex beaker and heat to 450° C. in a muffle furnace. Moisten the cold ash with a few drops of distilled water, dissolve in 5 ml. of diluted hydrochloric acid (1 + 3), and evaporate to dryness. Add 5 ml. of 0·1 *N* acetic acid, warm for a few minutes, cool, transfer to a 100-ml. graduated flask, and dilute to the mark. Filter the liquid into a dry Erlenmeyer flask. Pipette a portion of the filtrate containing from 0·4 to 1·1 mg. of calcium into a 50-ml. graduated flask, dilute to 20 ml. and add from a pipette 10 ml. of a 0·1 per cent. chloroanilic acid solution. Allow to stand for at least 3 hr. Dilute to 50 ml., mix, and filter the liquid into a dry flask. Using a spectrophotometer and light of wavelength 550 mμ., compare the light transmission of the liquid with that of a water blank. To calibrate the instrument treat solutions of known calcium content with 10 ml. of the chloroanilic acid solution and proceed as from that point in the determination.

Iron, aluminium, barium, strontium, and manganese in the amounts usually present in plants do not interfere. As magnesium chloroanilate is occluded in the calcium chloroanilate precipitate the presence of magnesium may give high results. Results obtained by the method are usually higher than those obtained by the standard volumetric method (*Assoc. Off. Agric. Chem., Official and Tentative Methods of Analysis*, 5th Ed., 1940, p. 127).

B. ATKINSON

New Method for Determination of Small Amounts of Molybdenum in Plants. C. S. Piper and H. S. Beckwith (*J. Soc. Chem. Ind.*, 1948, **67**, 374–379)—The method developed for microgram amounts of molybdenum involves preliminary extraction of molybdenum with cupferron and chloroform and the subsequent formation of the complex of molybdenum with dithiol (4-methyl-1 : 2-dimercaptobenzene). The optimum pH for extraction of molybdenum from acid solutions containing citrate is 0 to 0·5, and precipitation of the molybdenum - dithiol complex in 15 to 25 per cent. (v/v) sulphuric acid prevents interference by tungsten. For the extraction of the dithiol complex, amyl acetate proves to be a better solvent than amyl alcohol.

METHOD—*Reagents—Dithizone solution*—Dissolve 0·25 g. in 600 ml. of carbon tetrachloride, warming

to 50° C. to assist solution, extract the dithizone as the ammonium salt by shaking with 350 ml. of water containing 3 to 4 ml. of concentrated aqueous ammonia and wash the separated aqueous layer with three 75-ml. portions of re-distilled carbon tetrachloride, discarding the carbon tetrachloride layer each time. Add 600 ml. of re-distilled carbon tetrachloride, slightly acidify the aqueous layer with re-distilled hydrochloric acid, shake thoroughly, and wash the carbon tetrachloride solution of dithizone in another separator with three 150-ml. portions of water, discarding the aqueous layer each time. To the carbon tetrachloride solution of dithizone in a stoppered Pyrex reagent bottle add 750 ml. of carbon tetrachloride and store at 2° to 5° C.

Cupferron solution—Prepare fresh daily by dissolving 3 g. in 50 ml. of water, and filter if necessary.

Dithiol solution—Dissolve 1 g. of dithiol from a freshly opened tube in 500 ml. of a 1 per cent. solution of sodium hydroxide. The dithiol dissolves more readily if the tube is warmed to 30° to 35° C. before opening. When completely dissolved add thioglycollic acid from a pipette with constant stirring until a slight haze forms (8 to 9 ml.). Completely fill several 50- or 100-ml. stoppered reagent bottles (preferably of Pyrex glass) with the reagent and store at 2° to 4° C. The reagent is then stable for about 3 months.

Sodium perchlorate solution—Dissolve 550 g. of pure sodium perchlorate in water and dilute to 1 litre. Make the solution alkaline to phenol red (used externally) with sodium hydroxide solution, filter if necessary, and shake with 20- to 30-ml. portions of dithizone solution (*supra*) until no more heavy metals appear to be extracted. Shake finally with 50 ml. of carbon tetrachloride, separating as completely as possible and discarding the carbon tetrachloride layer. Acidify with a few drops of sulphuric acid and store in a Pyrex bottle. The excess of dithizone remaining in the solution does not interfere with its use.

Ammonium citrate solution, 12 per cent.—Dissolve 100 g. of citric acid in water, neutralise with aqueous ammonia solution to pH 7 to 8, and dilute to 1 litre. If this reagent is used for copper determinations, purify it with dithizone as described for sodium perchlorate.

Procedure—To 2 to 4 g. of the material in a Kjeldahl flask add 4 ml. of perchloric acid and enough nitric acid to ensure oxidation of all the organic matter. The perchloric acid may be replaced by 8 ml. of sodium perchlorate solution (*supra*) and an additional ml. of sulphuric acid. Add 2 to 5 ml. of sulphuric acid (preferably 5 ml. if it does not interfere with subsequent work) and maintain the presence of at least 2 ml. of sulphuric acid to prevent local overheating with consequent risk of explosion after the nitric acid has been expelled. Mix well, and heat the flask at low heat (120 to 150 watts) on a hot-plate or over a Bunsen burner turned very low. When dense brown fumes appear, remove the flask from the heater for 5 min., then resume digestion slowly and at low heat until dense fumes of sulphuric acid appear. Continue digestion for 5 to 10 min. and finally for 1 to 2 min. at the full heat of the plate (500 to 600 watts). If the liquid is not now colourless, add 1 or 2 ml. of nitric acid and heat to fuming. Dilute the digest with water, add ammonium citrate solution, neutralise to pH 3, and extract with dithizone solution to remove copper. One extraction suffices unless the copper content is being determined. To the aqueous residue add 8 ml. of diluted sulphuric acid (1 + 1), thereby reducing the pH to 0·3 to 0·5, and shake well. Add 1 to 1·5 ml. of 6 per cent. cupferron solution and shake again to precipitate all iron and molybdenum, using the larger amount if the iron content appears to be high. Add 10 ml. of chloroform and shake for 40 sec. to extract the metal - cupferron complex. After 3 to 5 min. separate the chloroform phase, add 0·5 ml. more of the cupferron solution and 10 ml. of chloroform, shake for 30 sec., and separate after allowing to stand as before. If this second extract does not show a blue tinge, repeat the extraction with 3 drops of cupferron solution and 5 ml. of chloroform. Remove the solvent from the combined extracts by boiling in a micro-Kjeldahl flask containing 2 glass beads. Remove the flask from the heater, add 3 ml. of concentrated sulphuric acid, and digest for 1 to 2 min. until all the chloroform vapour has been expelled. Add 5 drops of perchloric acid and again digest until the liquid is nearly clear. Remove the flask from the heater to allow the condensate to rinse down volatilised matter in the neck, and, if necessary, rinse the neck of the cooled flask with 1 ml. of water. Re-heat until the neck is perfectly clean (5 min.).

Dilute the cold digest with 5 ml. of water, boil gently for 1 to 2 min. to dissolve all the ferric sulphate, transfer the cold solution to a 25-ml. graduated test mixer with three 2- or 3-ml. portions of water, adjust the volume to 17 ml., mix thoroughly and, when the mixture is quite cold, add 15 drops of the dithiol reagent, shake immediately twice and allow to stand for 30 min. to ensure complete precipitation of molybdenum. Add, by means of a pipette, 7·5 ml. of *iso*amyl acetate (b.p. 135° to 142° C.), shake thoroughly to extract the complex, and set aside for 4 to 6 hr. The colour is stable for at least 24 hr. Remove the amyl acetate phase and determine the intensity of the colour in a photo-electric colorimeter, using a waveband of 670 to 690 mμ. With a filter photometer use a filter combination of Ilford spectral orange (607) and spectral red (608).

To prepare the calibration curve add to suitable amounts of a standard molybdenum solution in a series of micro-Kjeldahl flasks 0·5 mg. of iron in the form of ferrous sulphate solution, 3 ml. of concentrated sulphuric acid and 2 glass beads and digest until fuming occurs. Cool, dilute, transfer to 25-ml. test mixers and develop the colour as already described. Make a blank determination, adding 0·5 mg. of iron during the digestion with nitric, sulphuric, and perchloric acids.

The transmission curve for the molybdenum - dithiol complex shows strong absorption at 670 to 690 mμ. and at 430 to 440 mμ., the former wavelength range being the more suitable for photometric measurements in the presence of other metals.

In the latter range, tin, when present, causes interference. Satisfactory determinations can be made in the range of 0·2 to 25 μg. of molybdenum. For larger amounts it is desirable to use 0·5-cm. cells or to increase the volume of amyl acetate used to extract the dithiol complex.

Of the other elements extractable by cupferron and chloroform at pH 0·5, copper, tin, and tungsten are the only ones reported to give colour complexes with dithiol. The copper complex collects as a bluish-black precipitate at the interface, and 19 μg. of copper give an absorption comparable with 2 μg. of molybdenum. It is desirable therefore to remove most of the copper by a single extraction with dithizone solution as described. Tin gives a bright pink precipitate with dithiol and is readily detected in more than trace amounts. It rarely occurs naturally in plant material in amounts sufficient to interfere, although material stored in contact with tin plate may contain 50 p.p.m. or more. When dissolved in amyl acetate the tin complex gives a very pale yellow solution, its effect on the transmission of the molybdenum - dithiol complex being negligible at 670 to 690 mμ. When tin is present in undue amounts (> 100 μg.) it can be removed, together with iron, by a preliminary extraction with cupferron and chloroform at pH 4·5. Tungsten gives a bluish-green precipitate with dithiol but not in the strongly acid conditions of this method. Iron in 2-mg. amounts (*i.e.*, more than is likely to be present from plant material) causes no significant error. Titanium, vanadium, zirconium, thorium, and uranium are extractable by cupferron, but give no coloured complexes with dithiol and have no effect upon the transmission of the molybdenum complex. A. O. JONES

Determining Total Replaceable Bases in Soils. C. J. Schollenberger (*Anal. Chem.*, 1948, **20**, 1121)—The residue, obtained by igniting the evaporated ammonium acetate "leachate" of a soil, according to Bray and Willhite (*Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 144), is sometimes grey to black and not completely soluble in boiling *N* hydrochloric acid. The insoluble residue can be dissolved, and its basicity included in the determination, by adding 2 to 3 drops of 30 per cent. hydrogen peroxide solution and allowing to stand for a few minutes before boiling. No error is incurred if the indicator, methyl red, for the back-titration of the excess of acid is in alcoholic solution. M. E. DALZIEL

Water Analysis

Two New Total Alkalinity Indicators. M. Taras (*J. Amer. Water Works Assoc.*, 1948, **40**, 468–472)—Two indicators are recommended, particularly for alkalinities less than 150 p.p.m., *viz.*, *A*, disodium 4 : 4′-*bis*(*m*-tolyltriazeno)-2 : 2′-stilbenedisulphonate and *B*, disodium 4 : 4′-*bis*(*p*-methylaminophenylazo)-2 : 2′-stilbenedisulphonate. They give yellow to pale brown and orange to brown colour changes, respectively, at pH 5·4 to 5·2. Both change to mauve at pH 4·8, become purplish at pH 4·6 and a deeper purple up to pH 4·0. *B* is insoluble at the neutral point and gives rise to purple specks as the end-point is approached. *A* is prepared by tetra-azotisation of 4 : 4′-diaminostilbene-2 : 2′-disulphonic acid and coupling with *m*-toluidine; *B* is prepared by coupling the same azo compound with monomethylaniline (see *Anal. Chem.*, 1947, **19**, 339). The indicators are used in 0·1 per cent. solution. *A* is best dissolved in acetone. C. F. HERBERT

Organic

Detection of Diphenylamine in Commercial Petrol. G. E. Mapstone (*Chem. and Ind.*, 1948, 807)—The possibility of detecting diphenylamine in petrol was examined by extracting the sample with a concentrated acid and then adding a strong oxidising agent. Concentrated hydrochloric and sulphuric acids and 70 per cent. phosphoric acid were tried, solid potassium dichromate and sodium nitrite being used as the oxidising agents. With sulphuric acid, side reactions interfered, even when the mixture was poured into water. With phosphoric acid the colours obtained ranged from yellow to red, whereas with hydrochloric acid they were green to blue. In general, sodium nitrite gave better results than potassium dichromate.

A study of the conditions of the test with concentrated hydrochloric acid and sodium nitrite led to the following procedure. Shake 10 ml. of the sample with 1 ml. of concentrated hydrochloric acid, add a small crystal of sodium nitrite, and shake. The presence of diphenylamine is indicated by a blue colour in the acid layer. The test is not as sensitive as that with ammonium vanadate (the basis of the official test) and requires the presence of 40 to 50 p.p.m. of diphenylamine to yield a light blue colour. It has the advantage of dispensing with the preliminary washing of the petrol with sodium hydroxide solution, and of using reagents immediately available without preparation.

The test was compared with the original form of the official test (*Ibid.*, 1948, 383). This form of the official test, however, has now been replaced by an improved modification. A. O. JONES

Determination of *cyclo*Pentadiene and Methyl*cyclo*pentadiene in Admixtures with other Hydrocarbons. J. S. Powell, K. C. Edson, and E. L. Fisher (*Anal. Chem.*, 1948, **20**, 213–215)—An apparatus for the quantitative depolymerisation of the dimers of *cyclo*pentadiene and methyl *cyclo*pentadiene is described, together with a method for determining the monomers that is based on measurement of the optical densities of solutions of the fulvenes formed from the monomers by reaction with acetone and benzaldehyde. The determination is unaffected by other unsaturated or aromatic hydrocarbons.

Method—The depolymerisation apparatus is shown in Fig. 1. By means of a preliminary trial adjust the power input of this apparatus so that the depolymerised sample vapour leaves the heating coil at 340° to 360° C.

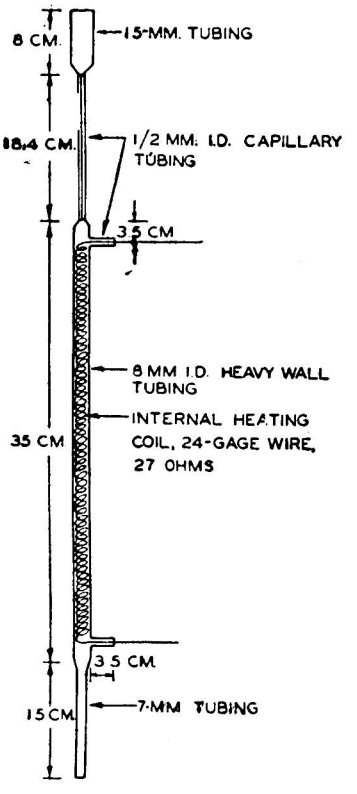
Preparation of sample—Data for suitable flasks, dilutions, and volumes of sample are given in Table I. Dry the sample with sodium sulphate. Place

approximately 20 ml. of toluene (*C.P.*) in an appropriate volumetric flask and weigh. Pipette a suitable volume of the sample into the flask, re-weigh, and dilute to the mark with toluene.

Table I

Approximate percentage w/w of dimer in sample	Required dilution of sample	Volume of mixture to depolymerise ml.	Volume of receiver ml.
0·0 to 0·9	None	15	50
0·8 to 2·8	None	5	50
2·6 to 9·0	3/10	5	50
9·0 to 28	1/5	5	100
28 to 90	3/50	5	100

Place the outlet tube of the depolymerisation apparatus in a second volumetric flask acting as receiver and containing 20 ml. of toluene and cooled by acetone and solid carbon dioxide. Pass



the diluted sample through the apparatus and wash through with 5 ml. of toluene. Remove the receiver, allow its contents to come to room temperature, and then dilute to the mark with toluene. Aliquot parts of the solution are used in the determination.

Determination of cyclo*pentadiene and methyl*cyclo*pentadiene monomers*—The depolymerised sample as prepared has the correct dilution for reaction with acetone, but requires diluting 1 to 5 for the reaction with benzaldehyde.

Prepare four 125-ml. glass-stoppered bottles as follows.

In (1) and (2), mix 5 ml. of acetone and 5 ml. of alcoholic potassium hydroxide solution (5 g. in 100 ml. of 95 per cent. ethanol). In (3) and (4), mix 5 ml. of a 20 per cent. v/v solution of C.P. benzaldehyde in 95 per cent. alcohol and 5 ml. of alcoholic potassium hydroxide solution. Prepare bottles (2) and (4) to serve as blanks by adding 10 ml. of 4 per cent. acetic acid solution to each. Immerse all four bottles in a water-bath and allow them to reach the bath temperature of 32° C. Add 5 ml. of the correctly diluted sample to each bottle and swirl the liquids without allowing them to touch the stoppers. Add 10 ml. of 4 per cent. acetic acid to bottle (1) after exactly 15 min., and to bottle (3) after exactly 20 min. Add 20 ml. of toluene to each bottle, mix thoroughly, decant the hydrocarbon layers into 50-ml. beakers, add approximately 1 g. of sodium sulphate to each beaker and cover with watch glasses. After dehydration, determine the optical densities of the toluene solutions with a spectrophotometer at a wavelength of 425 mμ. and with a slit width of 0·03 mm.

Calculation of results—First calculate the extinction coefficients, K, from the observed optical densities, D, after allowing for the densities of the blanks, from the equation $K = D/C\,L$, where C is the concentration of the sample in grams per litre, and L is the optical path in cm. The weight per cent. of *cyclo*pentadiene then equals $100\,(k_1K_1 - k_2K_2)$, and that of methyl*cyclo*pentadiene $100\,(k_3K_2 - k_4K_1)$, where K_1 and K_2 are the extinction coefficients for the acetone and benzaldehyde reaction products, respectively. The constants k_1 to k_4 are determined by replication of the analysis with pure *cyclo*pentadiene and methyl*cyclo*pentadiene separately, and determining the extinction coefficients for the two compounds in both reactions. Then, $k_1 = K_dA$; $k_2 = K_bA$; $k_3 = K_aA$; and $k_4 = K_cA$; and where $A = 1/(K_aK_d - K_bK_c)$, K_a refers to *cyclo*pentadiene and K_b to methyl*cyclo*pentadiene in the acetone reaction, and K_c to *cyclo*pentadiene and K_d to methyl*cyclo*pentadiene in the benzaldehyde reaction, respectively.

Results—The results of analyses on synthetic mixtures of the hydrocarbons show that the method is accurate; the greatest deviation from the correct value over a range of 8 to 21 per cent. of *cyclo*pentadiene and 0·4 to 10 per cent. of methyl*cyclo*pentadiene, was 0·3 per cent.

Indene, which also condenses with acetone and benzaldehyde to form fulvenes, reacts too slowly with acetone to cause appreciable interference, but, owing to its reaction with benzaldehyde, does introduce an error, in the methyl*cyclo*pentadiene content, of approximately one-fourth of the indene content. W. C. WAKE

Spectroscopic Determination of *cyclo*-Pentadiene and Methyl*cyclo*pentadiene. J. S. Powell and K. C. Edson (*Anal. Chem.*, 1948, **20**, 510–511)—An ultra-violet absorption method is described for determining *cyclo*pentadiene and methyl*cyclo*pentadiene in presence of each other. The interference of aromatic hydrocarbons and conjugated di-olefines is allowed for by comparison of the absorption of monomeric and dimeric forms of the two substances. The absorption peak for *cyclo*pentadiene is at 240 mμ. and that for methyl-*cyclo*pentadiene at 247 mμ. The absorption of the dimers of both substances is very small and is negligible at 240 mμ. and beyond. The absorptions determined are those of solutions in *iso*octane at 240 and 258 mμ.

Preparation of sample—Pipette 5 ml. of sample into a tared 100-ml. volumetric flask and weigh. Dilute to the mark with *iso*octane that has been purified by passage through a chromatographic column of silica gel until it shows no absorption of ultra-violet light at 240 mμ. Place the outlet tube of the depolymerisation apparatus (see previous abstract) in a 100-ml. volumetric flask containing 30 ml. of *iso*octane. Cool the flask with acetone and solid carbon dioxide. Transfer 5 ml. of the diluted sample to the funnel of the depolymerisation apparatus and, after depolymerisation, wash through with 5 ml. of *iso*octane. Remove the receiver, allow its contents to reach room temperature, and then dilute to the mark with *iso*octane.

If aromatic hydrocarbons or conjugated di-olefines are present, prepare a blank by heating the sample at 100° C. for 6 hr. in a closed container capable of withstanding a pressure of 10 atmospheres. This treatment dimerises the *cyclo*pentadiene and methyl*cyclo*pentadiene.

Dilute a portion of the sample and of the dimerised material, if a blank is necessary, according to Table I.

TABLE I

Weight of *cyclo*pentadienes in sample %	Dilutions: For measurement at:— 240 mμ.	258 mμ.
82–100	5/100	5/50
28–89	5/100	10/50
11–33	5/100	25/50
6–17	5/100	—
3–8	5/50	—
0–3	15/50	—

Determination of absorption—Determine the optical density of the diluted depolymerised sample at 240 and 258 mμ. with the spectrophotometer set at 100 per cent. transmittance on the diluted blank at each wavelength. If a dimerised blank is not necessary, then pure *iso*octane is used in its place.

Calculations—As in the method given in the previous abstract, determine the extinction coefficients (K); the weight per cent. of *cyclo*-pentadiene is given by $100\ (k_1K_1 - k_2K_2)$, and that of methyl*cyclo*pentadiene by $100\ (k_3K_2 - k_4K_1)$, where K_1 and K_2 are the extinction coefficients at 240 and 258 mμ., respectively. As in the previous method, the values of the constants k_1 to k_4 are determined by experiment with pure compounds. The same equations apply, $k_1 = K_dA$; $k_2 = K_bA$; $k_3 = K_aA$; $k_4 = K_cA$; and $A = 1/K_aK_d - K_bK_c$). In this case, however, K_a refers to *cyclo*pentadiene, and K_b to methyl*cyclo*pentadiene at 240 mμ., whilst K_c and K_d refer to the corresponding extinction coefficients at 258 mμ.

Results—The results of analyses on synthetic mixtures of the hydrocarbons show that the method is accurate, the greatest deviation from the actual value observed over a range of 32 to 91 per cent. of *cyclo*pentadiene and 9 to 68 per cent. of methyl-*cyclo*pentadiene, was 1·3 per cent.

Indene also interferes in this second method and, if present to any extent, should be removed by fractional distillation. W. C. WAKE

Polarographic Determination of Nitrobenzene. I. A. Korshunov, A. V. Ryabov, L. N. Sazanova, and A. S. Kirillova (*Zavod. Lab.*, 1948, **14**, 519–522; *cf.* I. A. Korshunov and A. S. Kirillova (*J. Gen. Chem. Russ.*, 1948, **18**, 785–792)—A polarographic wave suitable for the determination of nitrobenzene in concentrations not greater than $2 \times 10^{-3}\ M$ in the supporting electrolyte is obtained in acid, neutral, or alkaline medium. The supporting electrolyte is a buffer—citric acid and disodium hydrogen phosphate, acetic acid and sodium acetate, hydrochloric acid and sodium chloride, or sodium hydroxide solution. The nitrobenzene is added to the buffer in the form of an alcohol, acetone, or dioxan solution, the volume of which does not exceed 5 per cent. of that of the buffer solution.

The half-wave potential at 20° ± 1° C. *versus* the saturated calomel electrode varies with the pH as follows:

pH	Half-wave potential (v.)
2	— 0·15
4	— 0·27
6	— 0·40
7	— 0·47
8	— 0·53
10	— 0·60
12	— 0·63
14	— 0·63

These figures were obtained with a capillary passing 2·75 mg. of mercury per sec., and having a drop-time of 3·2 sec. The nitrobenzene concentration was varied between 5×10^{-5} and $3 \times 10^{-3}\ M$ without affecting the results. The relation between wave-height and concentration is not strictly linear. [The results do not differ greatly from the values of the "reduction potential" at 25° C. *versus* the normal calomel electrode,

obtained by Shikata (*Trans. Faraday Soc.*, 1925, **21**, 42), but they differ appreciably from the half-wave potentials at 25° C. *versus* the saturated calomel electrode, obtained by Pearson (*Ibid.*, 1948, **44**, 683)].

A sharply-peaked maximum appears with concentrations of 10^{-3} *M* and above. Gelatin eliminates it and causes the half-wave potential to become more negative. Thus the half-wave potential at pH 1·0 is −0·1 v., but with a gelatin concentration of 0·01 per cent. it becomes −0·24 v., and with a concentration of 0·02 per cent., −0·27 v.

Because of the high volatility of nitrobenzene the indifferent electrolyte should preferably be de-oxygenated before the sample is added. The sample is therefore delivered from a special burette (see Heyrovsky, *Polarographie*, 1941, p. 259).

In presence of aniline or benzidine the determinations can be carried out with the hydrochlorides of these bases as indifferent electrolytes (4 volumes of aniline or 2 volumes of benzidine to 1 volume of concentrated hydrochloric acid). In nitric acid of 1·0 to 1·5 *N* or less, no added indifferent electrolyte is required.

The dinitrobenzene isomers in buffer solutions of pH 6·5 to 8·5 give two waves, the first coinciding with that of nitrobenzene, and the second at a potential 0·2 v. more negative. A mixture with nitrobenzene can be analysed by measuring the two wave-heights and adjusting the first for the amount of dinitrobenzene as found from the second.

G. S. SMITH

Advantages of Butyl Rubber in Organic Analysis. A. H. Corwin and C. Karr (*Anal. Chem.*, 1948, **20**, 1116)—The permeability of butyl rubber to air is only a tenth of that of natural rubber, and so butyl rubber is clearly superior for use in connections in the Dumas apparatus for the determination of nitrogen. Diffusion constants for natural, butyl, and silicone rubbers with respect to carbon dioxide and water vapour are given and, although not highly accurate, they indicate the advantage of connections of butyl rubber in carbon and hydrogen determinations. The constants are: for carbon dioxide, natural rubber $25{\cdot}2 \times 10^{-10}$, butyl rubber $0{\cdot}47 \times 10^{-10}$, and silicone rubber $25{\cdot}8 \times 10^{-10}$; and for water vapour, natural rubber $6{\cdot}41 \times 10^{-10}$, butyl rubber $4{\cdot}66 \times 10^{-10}$, and silicone rubber $36{\cdot}4 \times 10^{-10}$. M. E. DALZIEL

Inorganic

Iodimetric Micro-determination of Bromide and Iodide. E. Schulek (*Analyt. Chim. Acta*, 1948, **2**, 74–87)—Halogens react with alkali cyanides to form halogen ion and the corresponding halogen cyanides, from which free iodine is liberated easily and bromine more slowly, whilst chlorine cyanide is stable. By utilising this difference in behaviour, iodine and bromine can be separated from chlorine and determined iodimetrically. They can be determined in presence of one another within given limits.

Micro-determination of iodide—Chlorine method—Neutralise the solution (containing 1 to 10 μg. of iodide in 2 to 3 ml. in a small glass-stoppered vessel) to methyl red. The solution should be free from iron, manganese, and bromide. Add 2 to 5 drops of freshly prepared, saturated chlorine-water. After 5 min., add 2 drops of freshly prepared, 5 per cent. potassium cyanide solution and shake well three times, loosening the stopper, to ensure thorough mixing of the liquid. Acidify the mixture with 5 drops of 10 per cent. hydrochloric acid solution, and add 1 drop of 1 per cent. potato-starch solution and 3 drops of 20 per cent. potassium iodide solution. Cool the solution to 0° C. and after 10 min. titrate with 0·001 or 0·0005 *N* sodium thiosulphate from a micro-burette.

For 10 to 150 μg. of iodide, use a volume of 8 to 10 ml. in a 50-ml. glass-stoppered Erlenmeyer flask, and add 6 to 10 drops of chlorine-water. Add 3 to 5 drops of potassium cyanide solution (half the volume of chlorine-water), 8 to 10 drops of hydrochloric acid, 1 drop of starch solution, and 3 drops of potassium iodide solution, and titrate with 0·002 *N* thiosulphate.

Make a blank determination on the reagents.

Results are accurate to within ±1 per cent.

Hypobromite method—To the neutral solution containing up to 10 μg. of iodide, add 3 to 4 drops of sodium hypobromite solution, prepared by treating 20 ml. of 5 per cent. sodium hydroxide solution with 10 ml. of freshly saturated bromine-water. After 15 min., add in a jet 0·5 ml. of a 5 per cent. solution of phenol to remove the excess of hypobromite. Shake three or four times with occasional releasing of the stopper and acidify the solution with 5 drops of 10 per cent. hydrochloric acid solution. Add 1 drop of starch solution, 3 drops of freshly prepared, 20 per cent. potassium iodide solution, and titrate as above, after 5 min.

For 10 to 150 μg. of iodide, use a volume of 8 to 10 ml., 10 drops of hypobromite solution, 1 ml. of phenol solution, 10 to 12 drops of hydrochloric acid, 1 drop of starch, 3 drops of potassium iodide solution, and 0·002 *N* thiosulphate.

The accuracy is ±1 per cent., a blank determination being necessary on the reagents.

Micro-determination of bromide—Neutralise the solution (1·5 to 20 μg. of bromide in 3 to 5 ml.) to methyl red, add 2 to 3 drops of freshly saturated chlorine-water, and shake the mixture. Add 1 drop of freshly prepared, 5 per cent. potassium cyanide solution and shake thoroughly to convert the bromine chloride and the excess of chloride into bromine cyanide and chlorine cyanide and chloride, loosen the stopper occasionally. Add 4 to 5 drops of hydrochloric acid solution, 2 drops of freshly prepared, 20 per cent. potassium iodide solution, and 1 drop of starch solution. After 10 min. titrate as above, but carefully, as the reaction between bromine cyanide and potassium iodide is slow.

20 to 250 μg. of bromide should be contained in 8 to 10 ml., 8 to 15 drops of chlorine-water and half this volume of potassium cyanide solution, 10 to 15 drops of hydrochloric acid, 3 to 4 drops of potassium iodide solution, 2 drops of starch solution should be added, and the titration made with 0·002 *N* thiosulphate.

The accuracy is to within −9 per cent. on 1·5 to

3·5 μg., and ±1 per cent. on 16 to 240 μg. Iodide must be absent.

Small quantities of bromide and iodide together—Iodide must be between 10 and 150 μg. and bromide between 16 and 240 μg. Determine the sum by the chlorine method, and then iodide according to the hypobromite method; calculate bromide by difference.

The results for bromine are correct to within −3·5 per cent., and for iodine, to within ±1·85 per cent. M. E. DALZIEL

Volumetric Determination of Small Amounts of Soluble Sulphates. C. L. Ogg, C. O. Willits, and F. J. Cooper (*Anal. Chem.*, 1948, **20**, 83–85)—The end-point of the titration of sulphate with barium chloride in presence of tetrahydroxyquinone disodium salt, dipotassium rhodizonate, or disodium rhodizonate is uncertain owing to the local formation of the red barium salt, which reacts only slowly. An apparatus and titration technique which identify the end-point colour and permit continuous comparison of the solution with a standard colour filter are described.

The apparatus consists of a rectangular titration vessel (25- × 45- × 50-mm. high optical absorption cell), a standard 25- × 45-mm. glass colour filter, two 5-ml. burettes graduated to 0·01 ml., and a titration stand. The titration vessel and the light filter are mounted side by side on an opal glass plate. Illumination, preferably fluorescent, is from below and all opal glass is masked except that covered by the titration vessel and the filter. Results are best when no overhead artificial illumination is used.

Procedure—Adjust the solution to pH 6·5 to 7·5, first boiling the acidified solution to expel carbon dioxide if the carbonate content is high. Transfer the solution to the titration vessel and adjust the volume to about 15 ml. Add about 0·08 g. of the indicator and dilute the solution with an equal volume of 95 per cent. ethanol. Titrate with 0·02 *N* barium chloride solution until a permanent colour that matches the colour filter is obtained. Continue stirring for 1 or 2 min. at the end-point to ensure that the colour does not fade, and use a rubber-tipped stirrer to prevent scratching the bottom of the cell. The end-point is taken when an added drop of barium chloride causes a deeper coloration than the colour filter.

Standardise the barium chloride solution against potassium sulphate solution (1·8140 g. per litre), so avoiding the correction factor used when standardisation is made gravimetrically. The titre should not be smaller than 3 ml. in the determinations or in the standardisation, otherwise the apparent normality varies. In the analysis of micro-samples, add a known amount of standard potassium sulphate, if necessary, to give a titre greater than 3 ml. The addition can be measured more accurately than the error incurred by a smaller titre.

The standard colour filters of polished glass with 37 per cent. transmission at 550 ± 2 mμ. need not be identical, but the same filter must be used for standardisation and determination. Different turbidities due to the formation of different amounts of barium sulphate in titrations can be compensated for by covering the colour filter by one of a series of microscope slides ground, by means of an aqueous suspension of carborundum, to produce different amounts of diffused light.

Titrations of equal aliquots of a sulphate solution are reproducible to within ±0·02 ml. of the mean value; the average deviation is 0·003 mg. on 1 mg. of sulphur. M. E. DALZIEL

Determination of Calcium and Magnesium in Iron Ore by Means of a Cation Exchanger. Yu. I. Usatenko and O. V. Datsenko (*Zavod. Lab.*, 1948, **14**, 1323–1327)—The separation of iron before the determination of calcium and magnesium in iron ores causes difficulties because re-precipitation of ferric hydroxide precipitated by aqueous ammonia solution is essential to avoid loss of calcium and magnesium even when the adsorptive properties of the precipitate are reduced by using concentrated solutions. The use of a cation exchanger (Wofatit R) to absorb calcium and magnesium from solutions containing iron and aluminium in presence of tartaric acid gives a simple and satisfactory separation. [The Wofatit series of ionic exchange resins are referred to in a recent review by Duncan and Lister, *Quarterly Reviews, Chem. Soc.*, 1948, **2**, 307.—G. S. S.]

Preparation of Wofatit filter—To remove cations, Wofatit R was treated five times with concentrated hydrochloric acid at 70° to 80° C. over a total period of 4 hr., and then washed to remove chloride ions. The product gave 0·54 per cent. of ash. About 60 to 65 ml. of the moist Wofatit were placed in a 100-ml. burette over a layer of glass wool.

Tests for absorption—30 ml. of neutral calcium chloride solution, containing the equivalent of 0·0440 g. of calcium oxide, *i.e.*, an amount equivalent to 8·8 per cent. in 0·5 g. of iron ore, were passed through the filter at the rate of 5 ml. per min. The once-filtered solution contained no calcium ions. Similar amounts of calcium chloride solution were treated with 1, 2, 3, 5, and 10 ml. of concentrated hydrochloric acid, each solution was diluted to 50 ml., and passed once through a Wofatit filter, followed by 3 or 4 washes with water, and the filtrates analysed for calcium. With 1 and 2 ml. of acid no calcium was left in solution, but with the other amounts 1·0, 2·5, and 31·0 mg. respectively of calcium oxide were found in the filtrate. Reduction of acid concentration before filtration could not be effected by addition of ammonia, since ammonium ions are absorbed by Wofatit R and the filter then allows calcium ions to pass through.

Mixtures of calcium salts and iron salts in presence of tartaric or citric acid were also passed through Wofatit R filters. All the iron passed through and all the calcium was absorbed. Tartaric acid solution, which often contains calcium as impurity, was purified before use by passing it through a Wofatit filter. Citric acid was less suitable than tartaric acid, being more difficult to wash out of the absorbent. Similar results were

obtained with aluminium in place of iron, and with magnesium in place of calcium.

With a mixture of 0·0440 g. of calcium oxide, 0·0320 g. of magnesium oxide, 1 ml. of concentrated hydrochloric acid, and 50 ml. of water, one filtration left 0·2 to 0·5 mg. of calcium oxide and 0·3 to 0·7 mg. of magnesium oxide in the filtrate. In presence of tartaric acid and iron, the amounts in the filtrates increased to 2·2 and 2·8 mg. respectively. Second filtrations, however, gave complete absorption.

Extraction—When a used filter was treated with 25 ml. of concentrated hydrochloric acid, introduced in small portions, and then washed 3 or 4 times with water, only 33·2 mg. of calcium oxide instead of 44·0 mg. were extracted. After two further treatments with 15-ml. portions of acid, the total extracted was still only 42·8 mg. Treatment with 100 ml. of diluted hydrochloric acid (1 + 4) gave complete extraction of calcium oxide and magnesium oxide in four operations.

Procedure—Place 0·5 g. of the finely divided ore in a 100-ml. beaker, moisten with water, and heat under cover with 15 ml. of concentrated hydrochloric acid until there are no dark coloured particles on the bottom of the beaker. Remove the cover and evaporate the solution to a syrup, add about 30 ml. of hot diluted hydrochloric acid (1 + 4), stir, filter, wash the residue with acidified water until the washings are no longer yellow, evaporate the filtrate to 2 to 3 ml., dilute to 30 ml. with water, and add 20 ml. of 20 per cent. tartaric acid solution. Run the solution at the rate of 5 ml. per min. through a Wofatit R filter, test the filtrate for absence of calcium by means of ammonium oxalate solution, wash the filter 3 or 4 times with water and reject the washings, treat the washed filter with four 25-ml. portions of diluted hydrochloric acid (1 + 4), and then wash 3 or 4 times with water. In the filtrate determine calcium and magnesium by the usual gravimetric or volumetric methods.

With contents of about 1 per cent., the results for percentages of calcium or magnesium oxide differed from the mean by up to 5 parts in 100, but with higher contents (up to 6·7 per cent.) the variation was only about ±1 part in 100.

G. S. SMITH

Removal of Manganese in the Determinations of the Zinc, Calcium, and Magnesium of Manganese Ores and Products. R. L. Evans (*Anal. Chem.*, 1948, **20**, 87)—The standard procedure (Hillebrand and Lundell, *Applied Inorganic Analysis*, John Wiley and Sons, 1929) is modified. After adding potassium perchlorate, evaporate the suspension cautiously to dryness on a low-temperature hot-plate or steam-bath; bumping is avoided by heating below the boiling-point. Moisten the residue with a few millilitres of nitric acid and digest in 50 to 100 ml. of cold water. Filter with or without suction, and wash the beaker and residue with an equal amount of water. The combined filtrate and washings then have a suitable nitrate concentration for the direct determination of zinc, calcium, and magnesium without a further evaporation.

With good filtration the method removes up to 1 g. of manganese leaving a clear solution of manganese content equivalent to less than 3 drops of 0·1 *N* potassium permanganate. Re-precipitation of manganese from the combined residues of six samples gave a filtrate containing less than 1 mg. of calcium or magnesium.

M. E. DALZIEL

Spectrographic Determination of Beryllium in Light Industrial Alloys. A. C. Pruig (*Informacion Quim. Analit.*, 1946, **1**, 21–23)—A rapid method for determining beryllium in alloys rich in magnesium by means of a spark discharge between suitable electrodes is described.

Method—Dissolve 0·165, 0·330, and 0·660 g. of trihydrated beryllium nitrate by warming in 50-ml. portions of hydrochloric acid. Cool the solutions to room temperature and dilute each one to 100 ml. with water. Prepare three solutions each containing 1 g. of beryllium-free Electron alloy dissolved in 40 ml. of diluted hydrochloric acid (1 : 1). Dilute each solution to 100 ml. with water and add to each 1 ml. of the beryllium solution; mix well.

Use a regular discharge from a Feussner generator between high-purity carbon electrodes prepared by Gatterer's method, and the following excitation conditions: FF4, C1/5, L1/1, I 0·75 amp., distance between electrodes 2 mm., standard electrodes of 5 mm. diameter, simple type diaphragm (Zeiss Spectrograph Q–24), focus 3031 A., aperture 0·03 mm., and time of preliminary discharge 1 min. between the electrodes before the test solution is added.

Place 2 drops of test solution on each electrode by means of a thin platinum thread, 0·2 mm. in diameter, having one end bent into a circle 3 mm. in diameter. Allow the electrodes to dry for 1 min., pass a spark discharge for 1 min., the optimum time for small concentrations of beryllium. Use Agfa Phototechnicher Film B, a developer containing Metol 2 g., hydroquinone 4 g., sodium sulphate 25 g., sodium carbonate 18·5 g., potassium bromide 2 g., and water up to 100 ml., a development time of 4 min. at 18° C., and a fixing solution containing 400 g. of sodium thiosulphate, 100 ml. of sodium hydrogen sulphite (38° Bé), and water up to 1 litre.

Use a microphotometer to measure the difference in photographic blackening of the lines Be 3130·4 and Mg 2915·5, and plot the logarithms of the concentrations of beryllium against the logarithms of the ratios of intensities of these lines.

The graph obtained can be used to standardise electrodes made from Electron alloy containing beryllium, and these can, in turn, be used as standards of comparison for beryllium alloys, the necessity of preparing special electrodes by fusion of the component elements thus being eliminated.

A. F. STURGESS

Volumetric Micro-determination of Arsenic and Iron. G. F. Smith and J. S. Fritz (*Anal. Chem.*, 1948, **20**, 874–876)—The volumetric micro-determination of oxalate by titration with perchloratoceric acid in perchloric acid solution, 5-nitro-1 : 10-phenantholine ferrous complex being

used as indicator, has been extended to include the micro-determination of iron and of arsenic.

Standardisation of 0·001 *N* perchloratoceric acid in 2 *M* perchloric acid against sodium oxalate in 2 *M* perchloric acid is effected according to the method of Salomon, Gabrio, and Smith (*Arch. Biochem.*, 1946, **11**, 433; *Analyst*, 1947, **72**, 310) for calcium except for the use of weight burettes for sampling. The end-point is marked by a red to faint blue colour change of the indicator. Similar titration of a known arsenite solution gave values ranging from 0·0042 mg. low to 0·0007 mg. high on samples containing from 0·270 to 0·153 mg. of arsenic trioxide. On a known iron solution, reduced by passage through a Jones micro-reductor, the values were from 0·0007 mg. low to 0·0022 mg. high on samples containing from 0·197 to 0·314 mg. of iron.

The values for arsenic are predominantly low, and for iron, predominantly high. M. E. DALZIEL

Determination of Mercury in Organic and Inorganic Compounds. Stannous Chloride Reduction Method. J. N. Bartlett and W. M. McNabb (*Anal. Chem.*, 1947, **19**, 484–487)—By modifying the Krieckhaus method (Low, *Technical Methods of Ore Analysis*, John Wiley and Sons, 1919, p. 181), errors due to loss of mercury during digestion and to incomplete washing of the free mercury are reduced, and the range of useful accuracy is increased to between 0·03 and 0·3 g. of mercury.

Inorganic mercury—Treat 50 ml. of solution containing about 100 mg. of mercury with 10 ml. of concentrated hydrochloric acid and 10 ml. of stannous chloride solution in a 125-ml. Erlenmeyer flask. Prepare the stannous chloride solution by dissolving 125 g. of stannous chloride dihydrate in 70 ml. of concentrated hydrochloric acid and diluting to 250 ml., the solution being heated over tin until clear and then stored over tin. Insert a "cold finger" into the flask to about 2 cm. above the liquid surface and heat the solution until the mercury forms into globules. Filter through a Gooch crucible and wash the metal by decantation with 2 *N* sulphuric acid and water until freed from chloride ions. Transfer the mercury and the asbestos mat to a 125-ml. Erlenmeyer flask and by means of 5 ml. of hot, concentrated nitric acid dissolve the finely divided mercury remaining on the walls of the crucible. Collect the acid in the flask and dissolve the remainder of the mercury. Dilute the solution to 35 ml., add dropwise a slight excess of saturated potassium permanganate solution, and destroy the excess by means of a 10 per cent. solution of ferrous sulphate in 0·3 *N* sulphuric acid. Cool the solution to about 15° C., add 0·4 ml. of ferric alum indicator, and titrate with standard potassium thiocyanate.

The results given are all low but correct to within 1 part in 330.

Procedure for determining semi-micro amounts of mercury—Weigh a sample containing between 30 and 100 mg. of mercury into a 15-ml. centrifuge tube and add 5 ml. of water and 2 or 3 drops of hydrochloric acid. Heat to 50° C. and add a mixture of 3 ml. of concentrated hydrochloric acid and 3 ml. of stannous chloride solution, also at 50° C. Allow to stand for 5 min., re-heat to 50° C., and centrifuge for 5 min. at 1500 r.p.m. Repeat the heating and centrifuging if the mercury is still not globular. Remove the supernatant liquid by means of a fine-porosity filter-stick of about 10-mm. diameter. Wash the mercury three or four times with 2-ml. portions of 2 *N* sulphuric acid and then with water until free from chloride, removing the wash-liquids by means of the filter-stick. Disconnect the filter and place it in the centrifuge tube with 2 ml. of concentrated nitric acid for 3 to 4 min. to dissolve the mercury, and transfer the solution to a 125-ml. Erlenmeyer flask; wash the filter by drawing water through it in the reverse direction, and collect the washings in the flask. Add potassium permanganate, dilute, and titrate as in the macro-method.

Results of 14 analyses of 5 compounds gave values ranging from 1 part in 500 low to 1 part in 300 high, 10 of the values being low.

Organic mercury—Precipitation of mercury without preliminary oxidation of the organic matter necessitates the use of a water-soluble solvent so that addition of the reducing mixture does not precipitate the compound itself. Acetone, ethanol, glacial acetic acid, dioxan, and hydrochloric acid were tried.

Procedure—Place a sample containing 50 to 70 mg. of mercury in a 200 × 25 mm. Pyrex test tube and add 3 ml. of dioxan containing 2 drops of hydrochloric acid to rinse the sample into the base of the tube. Add a mixture of 2 ml. of stannous chloride solution and 2 ml. of hydrochloric acid, and insert a 180 × 15 mm. cold finger to a point directly above the liquid surface. Lower the test tube into an oil-bath and digest at 115° C. until the mercury becomes globular (25 to 30 min.). Add 2 ml. of water and if the liquid remains clear for 3 min., the reduction may be regarded as complete, otherwise digest for a further period. Remove and rinse the cold finger, and filter and wash the mercury as before. Add 2 ml. of concentrated nitric acid to the test tube and transfer the resulting solution to a 125-ml. Erlenmeyer flask, rinsing the crucible with 2 ml. of hot nitric acid; dilute the solution and complete the determination as for inorganic compounds.

Twenty-five values were obtained for 8 compounds ranging in mercury content from 23 to 64 per cent.; and the values for any one compound agreed to within 1 part in 250; the maximum deviation of a mean value from the value obtained by the method of Sloviter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 890–893) or of Rauscher (*Ibid.*, 1938, **10**, 331–333) was 1 part in 320. M. E. DALZIEL

Determination of Copper in Steels by Colorimetric Titration with Dithizone. I. V. Suprunovich and A. B. Konovalova (*Zavod. Lab.*, 1948, **14**, 1061–1063)—From a solution containing citric acid and disodium hydrogen phosphate dithizone extracts copper, platinum, palladium, gold, silver, mercury, bismuth, and stannous tin. Of these elements platinum, palladium, and gold are not

found in steels, tin can be oxidised to the stannic form, which does not interfere, and the other elements give dithizonates that are decomposed by shaking the extract with potassium iodide solution. The oxidising action of ferric iron is prevented by its combination in a complex. Copper can be determined by colorimetric titration of the extract, the mixed colour of the dithizonate and free dithizone being used. The mixed colour gives more accurate results than that of the pure dithizonate since in the latter case the excess of reagent may be incompletely extracted or the dithizonate may be partly decomposed; and, furthermore, the sharpest colour change is obtained with a 50 per cent. excess of the reagent present. The colour is a mixture of reddish-violet and green giving a violet or grey.

Reagents—Buffer solution—Dissolve 38 g. of citric acid and 21 g. of disodium hydrogen phosphate in water, shake with a concentrated solution of dithizone in carbon tetrachloride, run off the lower layer, wash the aqueous solution with carbon tetrachloride, and dilute with twice-distilled water to 250 ml. *Potassium iodide solution*—Dissolve 10 g. of potassium iodide in 450 ml. of water acidified with 5 ml. of *N* hydrochloric acid, shake with dithizone solution as with the buffer solution, and dilute to 500 ml. *Standard copper solution*—Dissolve 0·5 g. of electrolytic copper in 20 ml. of 6 *N* nitric acid, evaporate nearly to dryness, add 2 or 3 drops of glacial acetic acid, and dilute to 500 ml. in a graduated flask. Before use dilute a portion to give a solution containing 1 μg. in 1 ml.

Procedure—Dissolve a suitable weight of steel in 100 ml. of diluted sulphuric acid solution (1 + 9), and add nitric acid dropwise to the boiling solution until the colour becomes light yellow. Cool, dilute to 250 ml., and place an aliquot portion containing 10 to 20 μg. of copper in a 150-ml. separating funnel. Dilute to 25 ml. with 10 per cent. sulphuric acid solution, add 2 drops of 0·02 per cent. cresol red solution, add aqueous ammonia carefully until the colour changes from red to yellow, and add 2 ml. of the buffer solution. Then add 4- or 5-ml. portions of a solution of 15 mg. of dithizone in 100 ml. of carbon tetrachloride, shaking after each addition for 2 to 3 min., and pouring the extract into a 50-ml. graduated colorimeter tube. Continue the extractions until the last is green, and add to the solution in the tube a volume of dithizone solution equal to about half that used in the extractions. Shake the contents of the tube for 2 min. with 10 ml. of the acidified potassium iodide solution. In another 50-ml. tube place a similar volume of dithizone solution, add a similar amount of sulphuric acid and cresol red, neutralise with aqueous ammonia, add the buffer solution, and then run in the standard copper solution with shaking until the colours match.

The determination takes 25 to 30 min. The method was tested on copper-free steels with added copper, on alloy steels containing chromium, nickel, and molybdenum, and on cobalt steels. Errors with copper contents of 0·1 to 0·2 per cent. are insignificant. G. S. SMITH

Polarographic Determination of Copper and Nickel in Steel. A. G. Stromberg, R. V. Dityatkovskaya, and N. V. Milovanova (*Zavod. Lab.*, 1948, **14**, 919–925)—Adsorption of copper and nickel on ferric hydroxide precipitated by ammonia renders many published methods for the polarographic determination of copper and nickel inaccurate. Experiments to test the degree of adsorption in 50 ml. of a solution containing 0·5 g. of iron in presence of various concentrations of ammonium chloride and ammonia have now shown that when the ammonium chloride concentration is 0·7 *N* the adsorption of copper becomes nil at ammonia concentrations of not less than 2·5 *N*, and of nickel at concentrations of not less than 3·0 *N*.

Copper and nickel in steel—Procedure—Dissolve 0·5 g. of steel in 25 ml. of diluted hydrochloric acid (1 + 1) with 1 to 2 ml. of concentrated nitric acid, evaporate to dryness, add 4 ml. of diluted hydrochloric acid (1 + 1) and 5 ml. of water, and heat to dissolve the residue. Cool, transfer to a 50-ml. graduated flask, add 15 ml. of concentrated aqueous ammonia, dilute to the mark, mix, and leave for 10 to 15 min. for the precipitate to settle. Pipette an aliquot portion of the clear solution into a polarographic cell, add to it 4 ml. of saturated sodium sulphite solution and 1 ml. of 0·25 per cent. gelatin solution, mix, and obtain the polarogram between −0·4 and −1·2 v. Measure the separate copper and nickel waves.

Comparison of results with those of chemical determinations showed that differences did not exceed 0·02 per cent. with copper and nickel contents of 0·1 to 0·5 per cent. With nickel between 1 and 4 per cent., the differences for nickel were between 0·03 and 0·10 per cent. G. S. SMITH

Determination of Phosphorus Pentoxide in Phosphate Rock. J. L. Kassner, H. P. Crammer, and M. A. Ozier (*Anal. Chem.*, 1948, **20**, 1052–1055)—When ammonium molybdiphosphate is determined volumetrically by dissolution in an excess of standard sodium hydroxide solution and back-titration of the excess of alkali with phenolphthalein as indicator, the results obtained for phosphorus pentoxide are 0·3 per cent. higher than by the normal gravimetric determination as magnesium pyrophosphate. The alkalimetric method is improved by using a mixed indicator that changes colour at the stoicheiometric end-point, and by precipitation of the ammonium molybdiphosphate under conditions that ensure a uniform composition of the product.

Reagents—Indicator—Dissolve 0·1000 g. of phenol red and of bromothymol blue in an excess of sodium hydroxide solution, adjust the pH of each solution to 7·5 with nitric acid, and dilute each accurately to 250 ml. Mix 40·0 ml. of the bromothymol blue solution and 25·0 ml. of the phenol red solution for the indicator and use 0·5 ml. for each 100 ml. of solution at the end-point.

Citromolybdate solution—Dissolve in 1360 ml. of water, without heating, 54 g. of ammonium nitrate, 52·6 g. of citric acid, and 68 g. of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, and pour the

solution into a mixture of 253 ml. of concentrated nitric acid (sp.gr. 1·42) and 310 ml. of water. Clarify the solution by adding filter paper pulp or a few drops of diammonium hydrogen phosphate solution and boiling for 5 to 10 min.; allow to stand overnight before siphoning off the clear solution.

Procedure—Treat 1 g. of the sample with 15 ml. of water, and add 5 ml. of concentrated nitric acid and 10 to 20 ml. of 60 to 70 per cent. perchloric acid; heat to copious fuming. Cover the container and boil the solution gently for 20 min. to dehydrate the silica. Allow to cool somewhat, add 75 ml. of water, and heat to boiling; then filter the solution into a 250-ml. flask, and wash the filter with hot dilute nitric acid and hot water. Treat the silica with hydrofluoric acid and nitric acid and fuse the residue with sodium carbonate; transfer the extracted melt to the bulk of the solution to ensure complete recovery of the phosphate. Dilute the solution to 250 ml.

Add 80 ml. of the citromolybdate solution to a 25-ml. portion of the solution, heat to boiling, and maintain at this temperature for 2 to 3 min. before filtering either hot or cold. Wash the precipitate 4 or 5 times by decantation with 20-ml. portions of neutral, cold, 1 per cent. potassium nitrate solution or water, and transfer it to a filter crucible and wash ten times more. Place the crucible in the original beaker and dissolve the precipitate in an excess of about 15 ml. of 0·3 *N* sodium hydroxide (carbonate-free). Add 1 ml. of the mixed indicator solution and titrate the solution with 0·1 *N* nitric acid until yellow. Remove the crucible and wash it with water. Adjust the volume of the solution to 200 ml., and titrate back the excess of acid with 0·3 *N* sodium hydroxide to a permanent light purple colour, viewed by direct sunlight or a fluorescent daylight lamp.

Results—The maximum deviation in 30 consecutive samples was 0·1 mg. of phosphorus pentoxide. M. E. DALZIEL

Spark Technique in Spectrographic Analysis of Slags. R. H. Steinberg and H. J. Belic (*Anal. Chem.*, 1948, **20**, 72–73)—A rapid spectrographic method by which the lime/silica ratio of open-hearth steel-furnace slag can be estimated is given. With the spark technique used an extremely high vapour temperature is obtained and the electrodes are comparatively cool, hence calibration curves are stable.

METHOD—*Apparatus*—The spark source is a commercial 2-kVA high-voltage spark unit with an added inductance of 0·045 millihenry and a capacitance of 0·021 microfarad. The unit has a Fuessner synchronous rotating gap. The spectrograph used is a 1·5-m. instrument with a 24,000 lines per inch grating providing a dispersion of 7 A. per mm. A graphite electrode 0·25 in. in diameter cut flat at one end and with a hole 0·1 in. in diameter and 0·125 in. deep cut in the flat surface is used.

Procedure—Pack the cup of the electrode by pressing the electrode into a pile of the powdered slag. Add a drop or two of a 2 per cent. solution of Ethocel (ethyl cellulose ether) in butyl acetate to the slag to lengthen the time of "blow away." Other organic cementing compounds may be satisfactory. Remove the solvent by aeration or heating. Use a graphite rod of the same size but with a cone-shaped tip as the other electrode and spark for about 10 sec. After developing the film by standard technique, use a microphotometer to measure the density of the relevant lines on the film. For lime/silica ratios from 0·6 to 3·0 calculate the logarithm of the relative intensity of the 3905·53 A. silicon and the 4302·53 A. calcium lines, and for lime/silica ratios from 2·5 to 8·5 compare the 3905·53 A. silicon line with the 4318·65 A. calcium line. From analyses of standard samples prepare a graph of the logarithms of the lime/silica ratios against the logarithms of the relative intensities for each of the pairs of lines.

The reproducibility of results is approximately to within 10 per cent. If free lime is present, the figure for the lime/silica ratio is high.

B. ATKINSON

Physical Methods, Apparatus, etc

Crystallographic Data. Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 1124–1125)—Crystal data for 1 : 3 : 5-tri-(*p*-chlorophenyl)-benzene are given under the titles proposed in the original paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

Crystallographic Data. Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 1249–1250)—2-Methylnaphthalene is described under the headings listed in the original paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579).

M. E. DALZIEL

Reviews

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Vol. IX: Oils, fatty—Pituitary Body. Pp. 671. London: Longmans, Green & Co., Ltd. 1949. Price 80s.

Perhaps one does not take down a Dictionary of Applied Chemistry from the shelf for entertainment or general reading, yet one may do so with this volume of "Thorpe." It is quite fascinating. If one reads on there is a chance of getting a sort of mental indigestion as the change from, say, the phase rule to photosynthesis is rather great, but it's all very readable and absorbingly interesting. Moreover, this volume seems to contain major articles one or more of which must be of interest to almost any variety of chemist. Who is not interested either in oils and fats, oxidising enzymes, petroleum, phosphorus, pigments, photography, particle size or photosynthesis? And he could browse on pituitary body or orthobaric density just as a *hors d'oeuvre*, and then turn to pest control for "afters"; opium is there if he needs it, and by way of liqueur, there is perillyl alcohol.

The aim of the Dictionary is to provide a balanced treatment of modern chemistry, including both original work and industrial aspects. It must be said that the editors and contributors have done this task very well indeed in the present volume and the index adds to its utility. One could, of course, comment on the inclusion of this or the exclusion of that, but the outstanding fact is that it is most useful and contains just that information and those references that the busy chemist, specially the analyst and consultant, will most need. It is difficult to compare one article with another. The analysis of fatty oils is useful and informative; photosynthesis or pest control are inevitably more interesting as well as being equally useful.

Petroleum provides a major article, almost a book in itself, which is full of interest as well as of utility. So one might go on. The fact is that the whole volume is excellent and one that every chemist must either possess or at least have access to. Besides those major articles already mentioned, perhaps one should draw attention to paper-making (S. F. Edge), Vitamin P (Bergel), penicillin, patulin, oxalic acid, phthalic acid, oxazines and oxygen as further examples of the matter available in this ninth volume of the best chemical dictionary in the English language.

The answer to anyone reading a review to help him to decide if he should buy the book is—of course you must, it is excellent.

H. E. Cox

PRACTICAL METHODS IN BIOCHEMISTRY. By F. C. KOCH and M. E. HANKE. Fifth Edition. Pp. ix + 419. Baltimore: The Williams & Wilkins Co. London: Bailliere, Tindall & Cox. 1948. Price 16s. 6d.

The precise nature and scope of biochemistry is still somewhat of a mystery to chemists working in non-biological fields, and many still apparently think of it as the type of chemistry that deals—to paraphrase a famous saying—with blood, sweat and urine. The book now under review appears to give colour to this limited concept, for it deals exclusively with that branch of the subject known as physiological or clinical biochemistry. In this respect it is an excellent student's manual, and he who has worked through the 283 experiments described in it can reckon himself to be a competent physiological biochemist. But there are other aspects of biochemistry not covered by this book—a biochemistry of nutrition, of therapeutics, of micro-organisms and plants—and this ought surely to be made clear by authors who claim to write textbooks of biochemistry.

Considered as a textbook of practical methods used in clinical biochemistry, the book could hardly be bettered. The first part covers the chemistry of cell constituents and the five chapters comprising it deal respectively with carbohydrates, fats, proteins and amino acids, nucleoproteins and nucleic acids, and hydrogen ion activity and pH. The second part is on the chemistry of the digestive tract and the third, the longest of the three, is on blood and urine. Every conceivable test of importance in clinical diagnosis appears to be referred to, and several of Van Slyke's manometric methods are given in detail, together with instructions for using the apparatus and the requisite tables for calculating the results. A chapter on colorimetric and fluorimetric methods of estimating vitamins and another on microbiological assays are included for the first time in this edition; the instructions are precise, useful and up-to-date.

The book is intended primarily as a practical companion to a particular American textbook of theoretical biochemistry, but it can obviously be used independently. One is left wondering, however, to what extent it is good for a student to be given such detailed instructions. Would it not be more profitable for him if he were made to search through the original literature for the methods to be used—at all events in the later part of his course?

This very objection, however, makes the book of value to others, and many analysts, especially those employed in hospital laboratories, would find this book useful, since it collects together in a handy form most of the standard methods he is likely to need.

The only criticism that may perhaps be offered is that some of these methods have not been brought up-to-date, for some of the references given are comparatively old, and for most methods, however well tried, refinements have been introduced in recent years. Surely these ought to be taken note of in a detailed textbook of analytical methods. Apart from this one point, however, the book has few blemishes; it is singularly free from printer's errors, it is well printed and bound, it has a good index and its price is moderate.

F. A. ROBINSON

ANALAR STANDARDS FOR LABORATORY CHEMICALS. The British Drug Houses Ltd. and Hopkin & Williams Ltd. Fourth Edition. Pp. xviii + 302. London. 1949.

The object of the publication of this latest edition of AnalaR Standards is to provide chemists with a revised and up-to-date series of specifications for laboratory chemicals; apart from this object it will be found of considerable value in the application of its assays and methods of test to other analytical problems. It has been considerably revised and enlarged with specifications for 58 new "AnalaR" chemicals; compounds of ten elements not previously in the range is a feature of the additions. In order to obtain the correct interpretation of the significance of the impurity tests, the user of the book should be familiar with the introductory explanatory notes.

The introduction in this edition of newer methods such as the determination of water by the Karl Fischer technique, electrolytic deposition and polarographic methods, breaks away from the classical standardisation necessary in more authoritative works; the polarograph is particularly adaptable to estimation of small amounts of contaminants in "pure" chemicals. The compilers of the book point out that introduction of newer methods of great sensitivity may show that a figure previously quoted as the maximum limit of an impurity has had to be revised, but that the purity of the product has not, in fact, been lowered.

Very little criticism of the book can be made; one minor editorial point might be mentioned of the unnecessary selection of arsenic for transcribing the maximum impurity limit from percentage to parts per million in each monograph.

The book is very well produced, the print is clear and the binding robust, and no printing errors were detected. It is very useful and this new edition should find a place on the working bench shelf of every laboratory.

D. C. GARRATT

Erratum: August (1949) issue, p. 479.

In the 7th line below the figure, *for* "between the two slides," *read* "between the two solids".

THE DETERMINATION OF RIBOFLAVINE BY CHEMICAL MEANS

THE attention of analysts is drawn to the following communication which has been received from the Sub-Committee on Vitamin Estimations of the Analytical Methods Committee:

The Chemical Panel of the Sub-Committee on Vitamin Estimations of the Analytical Methods Committee was asked to investigate existing chemical methods for the determination of riboflavine and to report whether any of them or a modification of any of them could be accepted as reliable for a range of foodstuffs embodying cereals, yeast and yeast products, meat products and malt products.

This Panel has made a full investigation of the problem; it has come to the conclusion that, although one modified technique shows distinct promise, none of the methods studied can at present be recommended to the practising analyst as reliable for so wide a range of foodstuffs. The formulation of a technique capable of general application even in the defined field appears to depend upon the development of a fluorimeter of better performance than those at present available, in order to secure increased sensitivity and a greater degree of reproducibility in results, particularly between one observer and another.

CROWN AGENTS FOR THE COLONIES

SCIENTIFIC OFFICER (ANALYTICAL CHEMIST) required by the East Africa High Commission for the East African Industrial Research Board for one tour of 4 years with prospects of permanency. Commencing salary will be between £760 and £935 a year according to qualifications and experience in a scale rising to £1,320 a year. Free passages and liberal leave on full salary. Candidates, preferably under 45, must possess a University Degree with Honours in Chemistry or an equivalent qualification. They should have had good experience in Chemical analysis, particularly of clays, rocks and mineral ores. Apply at once by letter, stating age, whether married or single, and full particulars of qualifications and experience and mentioning this paper to the Crown Agents for the Colonies, 4, Millbank, London, S.W.1, quoting M/N/22686/3E on both letter and envelope. The Crown Agents cannot undertake to acknowledge all applications and will communicate only with applicants selected for further consideration.

BOROUGH AND COUNTY OF THE TOWN OF POOLE

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APPOINTMENT OF CHEMIST

APPLICATIONS are invited for the appointment of Chemist in the above Undertaking.

The salary will be at the rate of £420 rising (subject to satisfactory service) by annual increments of £15 to £450 per annum.

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The water of the Undertaking is derived from Well and Boreholes and is softened, filtered and sterilised.

Candidates should possess not less than three years' analytical experience, and some knowledge of water bacteriology would be an advantage.

Applications, giving full particulars of qualifications and experience, accompanied by copies of two recent testimonials, should be forwarded to the undersigned not later than Monday, 24th October, 1949.

Canvassing will be a disqualification.

WILSON KENYON,
Town Clerk.

Municipal Buildings,
Poole, Dorset.
22*nd September*, 1949.

Ministry of Supply invites applications from
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CANDIDATES should have a good honours degree or equivalent, with post-graduate experience an advantage. Some posts require a good knowledge of spectroscopy, radiation chemistry or modern physico-chemical methods of analysis.

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Write quoting F.697/49 B.O. to Technical and Scientific Register (K), York House, Kingsway, London, W.C.2, for application forms, which must be returned by 28th October, 1949.

ANALYTICAL CHEMIST wanted in Research Laboratory of industrial establishment near Cambridge. Good opportunity for development work in analytical methods. Interest in preparation of pure compounds desirable and experience in physical methods of analysis essential. Salary £600-£800 per annum. Write, stating age and full particulars of experience, to Box No. 3720, THE ANALYST, 47, Gresham Street, London, E.C.2.

BOOTS PURE DRUG CO., LTD. have a vacancy for a qualified laboratory assistant in their Development Department, to work on formulation of veterinary and horticultural products. Salary according to age, experience and qualifications. Write, Personnel Manager, Boots Pure Drug Co., Ltd., Station Street, Nottingham.

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