Volume 71 See Notices on p. 600 December 1946
No. 849, Pages 553—600

THE ANALYST

The Journal of The Society of Public Analysts and other Analytical Chemists

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

ENTERED AS SECOND CLASS AT NEW YORK, U.S.A., POST OFFICE
PRINTED IN ENGLAND

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A MEETING of the Society was held at 6.30 p.m. on October 31st, 1946, at the Meeting Rooms of the Geological Society, Burlington House, London, W.1. The President, Dr. G. W. Monier-Williams, occupied the chair and a lecture was delivered on "The Fundamental Laws of Polarography" by Professor Jaroslav Heyrovsky, D.Sc., Ph.D., Director of the Physico-Chemical Institute, Charles University, Prague.

NEW MEMBERS

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PHYSICAL METHODS GROUP

A JOINT Meeting of the Physical Methods Group with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry was held at University College, Cardiff, at 6.30 p.m., on Friday, October 11th, 1946. Mr. W. D. Williams, Chairman of the Cardiff and District Section of the Royal Institute of Chemistry, opened the meeting, and after welcoming the members of the Physical Methods Group to Cardiff invited Mr. R. C. Chirnside to take the chair.

The following papers on the subject of Electrometric Analysis were then read: "Recent Developments in Apparatus for *p*H Measurement and Electro-titrations," by A. D. E. Lauchlan, M.A.; "Some Applications of Electrometric Methods to Analysis," by R. J. Carter, B.Sc., A.R.I.C.; "Polarisation End-points," by D. P. Evans, M.Sc., Ph.D., F.R.I.C.

DEATH

WE deeply regret to have to record the death of

William Henry Roberts (Past President).

1:2-Cyclohexanedione-dioxime ("Nioxime") as a Reagent for Nickel

By W. C. JOHNSON and M. SIMMONS

1:2-Cyclohexanedione-dioxime was first prepared by Wallach,[1] who found it a more sensitive reagent for nickel than dimethylglyoxime and stated that it had the further advantage of greater solubility in water. In 1940, Diehl[2] drew attention to the reagent, but stated that there was no satisfactory method for its synthesis. The fact that it has now become available prompted the following investigation of its potentialities as an analytical reagent.

The pure dioxime has a melting point 195° to 200° C. with some decomposition, the actual figure depending somewhat on the rate of heating. Figures previously published have been 187° to 190° C.[1,3,4,5] Its solubility in water is 0·85 g. per 100 ml. at 20° C. It is considerably more soluble in alcohol. The name "Nioxime" has been proposed[5] for the reagent and is employed for the sake of brevity in this paper.

1:2-Cyclopentanedione-dioxime was also prepared and a solution in hot water was found to give the nickel reaction characteristic of α-dioximes. It is, however, practically insoluble in cold water.

A. Sensitivity—Wallach claimed a sensitivity for nickel of 1 in 2 millions. We found, however, that the metal could readily be detected at a concentration of 1 in 5 millions, yielding a purple-red colour when a few drops of a saturated aqueous solution of nioxime were added to a slightly acid, neutral or ammoniacal solution of nickel. At higher concentrations and in presence of salts the nickel complex forms a precipitate and small quantities are more readily observed on a filter paper after filtration.

When comparing the sensitivity with that of dimethylglyoxime it was noticed that nioxime gave a reaction in considerably more acid solution than the former reagent and this property seemed sufficiently useful to merit more precise investigation. Twenty ml. of nickel solution containing 1 part of nickel in 2 millions were acidified with 10 ml. of *N* acetic acid, 1 ml. of 0·85 per cent. nioxime solution was added and the hot solution was titrated slowly with *N* sodium hydroxide to the appearance of the characteristic colour. The nickel complex was then filtered off and the *p*H of the filtrate determined with bromophenol blue. The experiment was repeated, with α-furil dioxime and dimethylglyoxime. With the latter the nickel concentration was increased to 1 in 1 million on account of the lower sensitivity of this reagent. The conditions and results were as follows.

Oxime	Ni concentration	Indicator	*p*H
Nioxime	$0{\cdot}5 \times 10^{-6}$	Bromophenol blue	3·4
α-Furil dioxime	$0{\cdot}5 \times 10^{-6}$	Bromocresol green	4·3
Dimethylglyoxime	1×10^{-6}	Methyl red	5·1

This reaction in acid solution simplifies the conditions under which nickel may be detected in presence of certain other metals, as is evident from the following investigation.

B. Effect of other ions on the nickel reaction—To study the possible interference of other metals a series of tests was carried out in which nickel was taken at a concentration of 1 in 2 millions and the other metal at 1000 times the nickel concentration; the salts employed introduced the common acid radicals.

The following salts permitted detection of the nickel by addition of a few drops of nioxime solution without necessity of adding other reagents for complex formation or screening; those having coloured ions only necessitated comparison with a blank or filtration. $AgNO_3$, $Pb(CH_3COO)_2$, $CdSO_4$, $HgCl_2$, As_2O_3(in NH_4OH), $Al(NO_3)_3$, $CrK(SO_4)_2$, $MnSO_4$, $ZnSO_4$, $MgSO_4$, $CaCl_2$, $BaCl_2$, $Sr(NO_3)_2$, Na-K tartrate, NH_4NO_3, $UO_2(NO_3)_2$ and $Th(NO_3)_4$. Detection of nickel in presence of the salts of Al, Cr, U and Th is not possible by a direct test with dimethylglyoxime. Mercurous salts are too acid to allow a direct test with nioxime. Cupric salts give a brownish green colour but nickel can be detected by addition of nioxime in excess to an ammoniacal solution. Ferric salts give a deep brown colour and are, moreover, too acid to permit direct detection of nickel, but the reaction may be obtained in ammoniacal tartrate solution. Bismuth does not interfere when tartrate is present. Cobalt gives a brown colour and is co-precipitated with nickel from slightly acid solution. When both

metals are at low concentration, separation may be effected in ammoniacal ammonium chloride solution by using excess of reagent.

C. REACTIONS WITH OTHER METALS—With ferrous iron, bismuth and palladium, nioxime gives reactions similar to those afforded by dimethylglyoxime.[6]

D. GRAVIMETRIC DETERMINATION OF NICKEL—Nioxime was found to be unsuitable for this purpose, for a number of determinations on H.S. brand nickel metal yielded high results, presumably through occlusion of the excess reagent. Hot precipitation gave recoveries from 100·3 to 101·6 per cent. and cold precipitation produced results up to 107 per cent. The precipitate is not at all crystalline in appearance and does not filter readily.

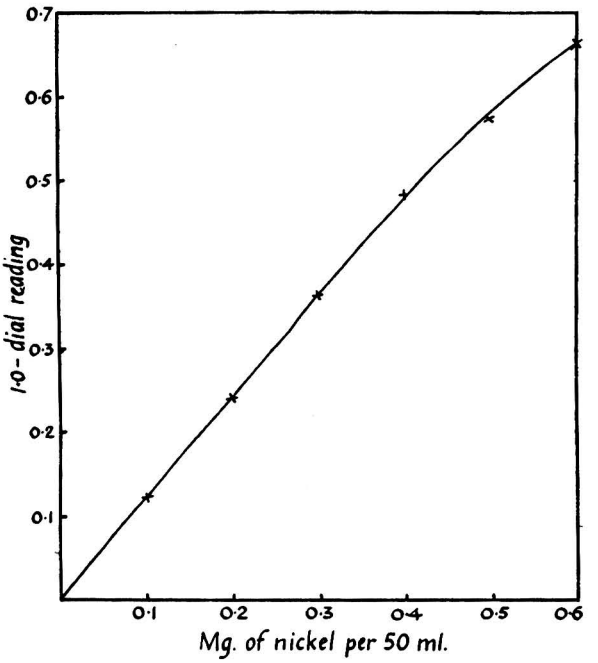

Fig. 1

E. COLORIMETRIC DETERMINATION OF NICKEL—After oxidation with bromine or iodine, nickel gives a soluble red colour with either dimethylglyoxime or nioxime. This reaction has been employed with the former reagent for the colorimetric determination of nickel,[7,8] but the colour is known to have rather unstable characteristics.[7] We found that the colour given by nioxime with nickel ions could be stabilised in a manner quite suitable for colorimetric work. After addition of gum arabic the colour due to nickel in a concentration of 1 in 100,000 was found to give the same Spekker reading just after its formation and 24 hours later.

(i) *Calibration*—The Hilger Spekker Photo-electric Absorptiometer was calibrated for the method by the following procedure. A standard nickel solution, 1 ml. ≡ 0·05 mg. of Ni, was prepared from H.S. brand nickel and pipetted volumes up to 12 ml. were mixed with 5 ml. of 5 *N* hydrochloric acid, 2 ml. of 10 per cent. gum arabic solution and 5 ml. of 0·85 per cent. nioxime solution, made alkaline with 6 ml. of 5 *N* ammonia and diluted with water to 50 ml. in a standard flask. Colour intensities were measured on the Spekker Absorptiometer, using a 1·0 cm. cell, a water setting 1·0 and a yellow green filter. The calibration curve is reproduced (Fig. 1)

(ii) *Determination of nickel in cobalt salts*—To determine nickel in cobalt chloride, nitrate or sulphate, dissolve a quantity of the salt equivalent to 0·2 g of cobalt in 5 ml. of water add 20 ml. of potassium cyanide solution (about 1·5 *M*) and oxidise the cobalt to cobalticyanide by drawing air through the solution at 25° to 30° C. until the colour changes to a lemon yellow (20–30 minutes). Add 5 ml. of nioxime solution and 10 ml. of formaldehyde

solution (35–40 per cent.) and allow the mixture to stand for 30 minutes, when the nickel-cyanide complex is decomposed by the formaldehyde[9] and the nioxime compound precipitated. It is possible to stabilise the nickel colour at this stage but the addition of gum causes a white precipitate which interferes with the colorimetric determination. Extract the compound with 10 ml. of benzene and wash the benzene extract with three 5-ml. portions of 5 *N* ammonia and then with 10 ml. of water. Then re-extract the nickel with 5 ml. of 5 *N* hydrochloric acid and wash the benzene with three 5-ml. portions of water. Boil the acid and washings, if necessary, to remove droplets of benzene, add 2 ml. of 10 per cent. gum arabic solution, 5 ml. of 0·85 per cent. nioxime solution and 6 ml. of 5 *N* ammonia and make up to 50 ml. with water. Take readings as in the calibration.

As a check on the method "synthetic" cobalt salts containing known amounts of nickel were prepared from H.S. brand cobalt spectroscopically free from nickel and nickel of the same brand. The results were as follows.

Salt	Ni added %	Ni found %
Sulphate	0·010	0·0093
Chloride	0·025	0·024
Nitrate	0·030	0·030
,, ...	0·040	0·039
Sulphate	0·040	0·039
Chloride	0·062	0·062

(iii) *Determination of nickel in steel*—Nickel was determined in a sample of standard steel by the following modification of Vaughan's procedure.[8] One-half gram of the steel was dissolved in 20 ml. of "Spekker acid" (150 ml. of sulphuric acid, sp.gr. 1·84, and 150 ml. of phosphoric acid, sp.gr. 1·75, per litre), 5 ml. of nitric acid, sp.gr. 1·20, were added, and the solution was boiled to remove nitrous fumes and made up to 500 ml. with water. Ten ml. of this solution were pipetted into a 50-ml. standard flask, 10 ml. of ammonium citrate solution (50 g. of citric acid dissolved in 50 ml. ammonia, sp.gr. 0·880, and made up to 100 ml. with water) were added, followed by 2 ml. of 10 per cent. gum arabic solution and 5 ml. of 0·85 per cent. nioxime solution, a small excess of 5 *N* ammonia was added and the whole diluted to 50 ml. The Spekker reading was taken as in the previous procedures. A reading taken on a similar solution containing no nioxime gave zero correction for the iron colour. The sample was Ridsdale's British Chemical Standard Nickel Steel No. 222 with a stated nickel content of 3·39 per cent. We found 3·40 per cent.

Summary

1:2-Cyclohexanedione-dioxime has been investigated as a qualitative, gravimetric and colorimetric reagent for nickel and has been found to offer advantages except in gravimetric work. Colorimetric method for determining nickel in cobalt salts and in steel are described.

The work was carried out in the laboratories of Messrs. Hopkin & Williams, Ltd., and thanks are due to the Directors for permission to publish.

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A Method for the Determination of Isocyanates

By H. E. STAGG

A METHOD was required for the determination of the isocyanate content of technical isocyanates, and it was expected that the materials for analysis would consist essentially of isocyanate and an inert solvent in widely varying proportions, with traces of phosgene and hydrochloric acid. However, in the course of the investigation it was found that hydrochloric acid was liable to be present in appreciable amount in the form of the carbamyl chloride, R.NH.CO.Cl, some samples of technical isocyanates containing as much as 6 per cent. of this impurity.

Principles of the Method

The methods of analysis which were developed were based on the well-known reaction of isocyanates with primary amines to form substituted ureas:

$$R.NCO + R'NH_2 \rightarrow RNH.CO.NHR \quad .. \quad .. \quad .. \quad (1)$$

At first this reaction was used as the basis of a gravimetric method; the isocyanate was mixed with an excess of aniline and after standing for a short time the excess of aniline and any solvent present in the sample were removed by steam distillation and the insoluble substituted urea was filtered off, washed free from chloride and dried to constant weight. This procedure gave closely reproducible results and a recovery of 99·0 per cent. with a sample of purified hexamethylene di-isocyanate, which contained 0·6 per cent. of inert solvent. However, the procedure was somewhat inconvenient when large numbers of samples were involved, and the need for obtaining results without delay led to the development of a volumetric procedure in which the isocyanate was allowed to react with an excess of piperidine in acetone solution, the excess piperidine being determined by titration with standard acid.

The results obtained by this method normally agreed within 0·5 unit in the percentage figure with those obtained gravimetrically, and the recovery on a purified sample of hexamethylene di-isocyanate was 99·6 per cent. Occasionally, however, it was found that the volumetric figure for samples of technical hexamethylene di-isocyanate exceeded the gravimetric figure, expressed as percentage of hexamethylene di-isocyanate, by as much as 6·0, and when this discrepancy arose the filtrate from the gravimetric determination was found to contain a considerable amount of chloride.

The absence of appreciable quantities of phosgene was demonstrated by testing with pyridine, which gives a flocculent white precipitate if phosgene is present, and aspiration with a stream of air into standard silver nitrate solution removed only traces of hydrochloric acid. It seemed likely, therefore, that the chloride present in the filtrates was due to the presence in the sample of hexamethylene dicarbamyl chloride, $(CH_2)_6(NH.COCl)_2$, which would be expected to form an intermediate stage in the synthesis of hexamethylene di-isocyanate by the normal method of phosgenation of hexamethylene diamine. The presence of this substance in the isocyanate would explain the discrepancy between the results obtained by the volumetric and gravimetric methods. In its reaction with bases the dicarbamyl chloride would be expected to behave as an addition compound of one molecule of di-isocyanate and two molecules of hydrochloric acid, and one molecule of the dicarbamyl chloride should, therefore, react with four molecules of the base giving one molecule of substituted urea and two of the base-hydrochloride. In the gravimetric procedure the aniline hydrochloride formed would pass into the filtrate and would thus have no effect upon the final result, while in the volumetric method the piperidine hydrochloride would not titrate with acid, and any hydrogen chloride present would be included in the figure returned for isocyanate. Thus, if any hexamethylene dicarbamyl chloride were present, it should lead to a discrepancy between the results by the two methods such that

$$\text{Hexamethylene dicarbamyl chloride (M.W. 241), per cent.} = 1{\cdot}435\ (b-a) \quad .. \quad (2)$$

where a = gravimetric concentration expressed as di-isocyanate, M.W. 168,

b = volumetric concentration expressed as di-isocyanate, M.W. 168.

Attempts were made to prepare a pure sample of the carbamyl chloride, by passing dry hydrogen chloride into a solution of di-isocyanate in benzene or light petroleum; white

crystals were deposited, which contained 30·8 per cent. of chlorine (theory for hexamethylene dicarbamyl chloride = 29·5 per cent.). This material was very unstable and on heating above 40° C. decomposed with evolution of hydrogen chloride.

In addition, purified di-isocyanate, free from chloride, readily absorbed up to 6 per cent. of its own weight of dry hydrogen chloride, and aspiration of the resulting solution at room temperature did not release an appreciable amount of hydrochloric acid. Analysis of the mixture gave the following results: b = 106·6 per cent.; a = 92·7 per cent.; by substitution of these values of a and b in equation (2) above, the dicarbamyl chloride content of the sample becomes 20·0 per cent. The theoretical value, calculated from the weight of hydrogen chloride absorbed, was 21·0 per cent.

It is concluded, therefore, that in certain circumstances hexamethylene dicarbamyl chloride is present in technical hexamethylene di-isocyanate and in absence of appreciable amounts of phosgene can be calculated from the volumetric and gravimetric strength figures or from the chloride formed by reaction with piperidine.

The methods described have been applied to purified and technical distilled hexamethylene di-isocyanate, fore-runnings from hexamethylene di-isocyanate distillation, and phenyl, benzyl and cyclohexyl isocyanates.

Precision and accuracy of the methods

Duplicate gravimetric determinations on a given sample usually agree within 0·1 unit in the percentage figure, while those obtained by the volumetric procedure usually agree within 1·0 unit. Analysis of a sample of purified hexamethylene di-isocyanate gave the following results:

	Inert solvent %	Hexamethylene di-isocyanate content found	
		Gravimetrically %	Volumetrically %
Hexamethylene di-isocyanate, purified . .	. 0·6	99·0	99·6

Details of methods

Reagents required—

1. Freshly distilled aniline, not deeper in colour than very pale yellow.

2. Solution of piperidine in acetone: 10 ml. of redistilled piperidine, diluted to 100 ml. with acetone and stored in a stoppered bottle.

Procedure—

Gravimetric determination—Place 3–4 g. of aniline (reagent 1) in a 500-ml. conical flask and weigh in about 0·5 g. of the sample directly from a weight-pipette. Swirl the flask gently until the contents become solid, heat on a steam bath for half-an-hour, cool and add 25 ml. of water. Break up the cake by means of a glass tube, the end of which is prevented from becoming blocked by blowing down it. Then support the flask in a fume cupboard at an angle of 45° to the horizontal and pass a rapid current of steam through the liquid for ten minutes, any great increase in volume being prevented by a low flame placed under the flask. Again break up the precipitate by means of the glass tube, and again pass steam until the odour of aniline is no longer detectable. Cool the flask in ice water, and filter the contents through a tared sintered glass crucible (1.G.3), wash with 100 ml. of cold water and dry at 130° C. (±5°) to constant weight.

$$\frac{\text{Wt. of ppt.} \times 47{\cdot}45}{\text{Wt. of sample taken}} = \text{per cent. hexamethylene di-isocyanate (M.W.168).}$$
$$= a.$$

Volumetric determination—Weigh 1·0 to 1·2 g. of the sample from a weight-pipette into a dry 250-ml. conical flask, add 25 ml. of acetone and swirl the mixture. Add 25·0 ml. of piperidine solution (reagent 2), stopper the flask and cool in a bath of ice and water for 30 minutes. Add 75 ml. of methylated spirit (64° over-proof) and 50·0 ml. of $N/2$ hydrochloric acid and then 6 drops of methyl red indicator solution and sufficient methylene blue to give a bright purple colour. Titrate the mixture with $N/2$ sodium hydroxide until it

turns bright green. Carry out a blank test simultaneously on 25·0 ml. of piperidine solution, 50·0 ml. of $N/2$ hydrochloric acid and 75 ml. of methylated spirit.

$$\frac{\text{Net titre} \times 4{\cdot}20}{\text{Wt. of sample taken}} = \text{Per cent. hexamethylene di-isocyanate (M.W. 168).}$$
$$= b.$$

Calculation of hexamethylene dicarbamyl chloride and hexamethylene di-isocyanate—

$(2a - b)$ = per cent. actual hexamethylene di-isocyanate, M.W. 168.

$(b-a) \times \frac{241}{168} = 1{\cdot}435(b-a)$ = per cent. actual hexamethylene dicarbamyl chloride, M.W. 241.

Summary

Methods have been developed for the determination of the isocyanate content of technical isocyanates. Two procedures are described: (1) a gravimetric method, based upon reaction with aniline, and (2) a volumetric method in which the isocyanate is determined by causing it to react with an excess of piperidine and titrating the excess piperidine.

Application of the methods to technical hexamethylene di-isocyanate has shown that hexamethylene dicarbamyl chloride, which would be expected to form as an intermediate compound in the preparation of the di-isocyanate by phosgenation of hexamethylene diamine, is occasionally present in the technical product. The amount of this substance present can be calculated from the difference between the figures obtained by the gravimetric and volumetric methods.

The methods have also been applied to the analysis of benzyl and cyclohexyl isocyanates.

Acknowledgment—The author wishes to thank Mr. N. Strafford for his interest and advice during the investigation, and Dr. F. S. Statham for the preparation of various samples of hexamethylene di-isocyanate and for suggesting the use of piperidine in the volumetric determination.

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July, 1946

The Adulteration of Wheat Flour with Maize and Rice Flours. A New Chemical Method for Detection and Quantitative Determination

BY M. M. TAHA EL-KATIB

IN Egypt, wheaten flour is sometimes found adulterated with maize and rice flours. The starch grains of wheat are of two forms: (1) large, lenticular grains, mostly 28–40 μ (rarely 50 μ) in size, with distinct rings and hilum; (2) small rounded or polygonal grains, usually less than 8 μ. The large grains lying on edge are more or less elliptical in outline; with polarised light indistinct crosses dividing each grain into four equal parts are evident. The small grains are detached members of aggregates, which are seldom found intact. Maize starch grains are either sharply polygonal or rounded. A distinct hilum, often with radiating clefts, is evident, at least in the larger grains. Most of the grains are from 15 to 35 μ in size. Compound forms (aggregates) of maize starch grains do not occur. Rice starch grains are 2–10 μ in diameter, often united into oval aggregates containing from two to upward of a hundred grains. Grains from the centre of a large aggregate have only flat facets, but those from the outer portion are curved on the exposed surfaces. Perfectly round grains are rare.

Hitherto the microscope has been the usual means of identifying any given cereal by the dimensions and shape of its starch grains, and the percentage of foreign grains in adulterated wheaten flour may be found approximately from the relative members of foreign starch grains counted in the field of the microscope.

In 1916, Wallis suggested a quantitative microscopical method, the lycopodium method, based on admixture of the sample with a definite quantity of the spores of *Lycopodium clavatum*.[1,2]

He observed that lycopodium spores are exceptionally uniform in size, so that one can always know that a definite number of spores represents a particular weight of lycopodium. From a large number of counts under the microscope he found that on the average 94,000 lycopodium spores weigh 1 milligram.

From this figure, one can calculate the weight of any number of the spores counted under the microscope, and, if the lycopodium has been mixed with a definite proportion by weight of a second substance, one can find immediately how much of this second substance has been examined microscopically in admixture with the counted lycopodium spores. If this second substance is wheat flour adulterated with maize starch, and one counts the number of maize starch granules present with the counted lycopodium spores, it is possible to calculate the percentage by weight of maize starch in the wheat flour provided one knows how many maize starch grains correspond to 1 mg. of maize starch.

The actual number of maize starch grains in 1 mg. of pure maize starch may be found by mixing, say, 0·2 g. of lycopodium spores with 0·1 g. of maize starch, suspending the mixture in 20 ml. of olive oil and counting the number of spores and starch grains respectively in 40 selected fields on four microscope slides. From data obtained in this way, and knowing that 1 mg. of lycopodium contains 94,000 spores, Wallis found that 1 mg. of pure maize starch contains on the average 977,000 grains.

When maize starch grains are counted in presence of wheat flour, however, some of them are indistinguishable from wheat starch grains and will therefore be omitted from the counts. The number recognisable under these conditions, per mg. of maize starch, can be found by making a 50 per cent. mixture of pure maize starch and pure wheat flour and applying the lycopodium method as outlined above. Wallis found that the number of recognisable maize starch grains per mg. of air-dry maize starch, when counted in admixture with wheat flour, was 711,000.

The following is an example of the estimation of maize starch in wheat flour by this method—0·1000 g. of lycopodium was mixed with 0·1060 g. of the sample of adulterated wheat flour and suspended in olive oil. By counting the number of lycopodium and maize starch grains in 20 fields of 2 slides, it was found that 215 lycopodium spores corresponded with 42 maize starch grains.

Calculation—The proportion of lycopodium to flour by weight is 1:1·060. One mg. of lycopodium contains 94,000 spores. But 215 lycopodium spores correspond with 42 maize starch grains. Therefore 94,000 lycopodium spores correspond with 18,400 maize starch grains in 1·060 mg. of flour. But 1 mg. of maize starch in admixture with wheat flour shows 711,000 maize starch grains. Therefore 18,400 maize starch grains correspond to 0·0258 mg. of maize starch in 1·060 mg. of flour. Hence the flour sample is adulterated with 2·43 mg. of maize starch per 100 mg., *i.e.*, 2·43 per cent. The correct figure was 2·88 per cent. and the error is thus −15·6 per cent. of the amount of maize starch found.

APPLICATION OF THE WALLIS METHOD TO ESTIMATE RICE STARCH AS ADULTERANT—In Egypt, wheat flour is sometimes found to be adulterated with rice starch. Hence an extension of the Wallis lycopodium method is required.

(*a*) *Determination of the number of rice starch grains per mg. when counted in admixture with wheat flour*—As already described for maize starch, a 50 per cent. mixture of rice starch and wheat flour was made and mixed with lycopodium in known proportions by weight and the numbers of rice starch grains and lycopodium spores were counted. As a mean value 733×10^4 rice starch grains were found present in 1 mg. of rice starch when counted in admixture with wheat flour.

(*b*) *Determination of the percentage of rice starch in any adulterated sample of wheat flour*—Owing to the very small size of the rice starch grains, it is advisable to mix about 0·1 g. of lycopodium with about half its weight of the adulterated flour.

Example: 0·1000 g. of lycopodium was mixed with 0·0500 g. of the wheat flour and suspended in olive oil. By counting the number of lycopodium spores and rice starch grains in 20 fields of 2 slides, it was found that 212 lycopodium spores corresponded with 144 rice starch grains. Following the method of calculation used for wheat flour adulterated with maize starch, it was found that this sample contained 1·74 per cent. of rice starch. The correct figure was 1·47 per cent. and the error therefore 20 per cent. of the amount of rice starch present.

A NEW QUANTITATIVE CHEMICAL METHOD

The Wallis method is purely microscopical and like most microscopical methods is liable to large errors unless due care is taken to obtain homogeneous samples.

Since starches gelatinise at different temperatures, an attempt was made to effect a separation by this means. Lippmann and others have devoted considerable attention to the temperature of gelatinisation of different starches. Lippmann gives a table showing the temperatures at which, (1) swelling is first noticed, (2) gelatinisation begins and (3) gelatinisation is complete. For example, the respective temperatures for wheat starch are 50°, 65°, 67·5°C., and for maize starch, 45·2°, 58·7° and 62·5° C. Nyman[3] did not find so much difference in the gelatinisation temperatures but noted a decided difference in the times required for gelatinisation at a given temperature. At 53° C. rye starch required only 6 minutes for gelatinisation, whereas wheat required 24 minutes. Alsberg and Rask,[4] working with wheat and maize starches, found that there is probably no definite temperature of gelatinisation.[5] Hence it appeared difficult to make gelatinisation temperature a basis for separating maize or rice starch from wheat flour.

According to a method proposed by K. Baumann,[6] about 0·1 g. of wheatmeal adulterated with maize is mixed with 10 ml. of a 1·8 per cent. solution of potassium hydroxide and the mixture is shaken at intervals during 2 minutes. It is then treated with 4 or 5 drops of diluted hydrochloric acid (1 in 4) and again shaken; it is necessary that the liquid should remain alkaline, since the protein precipitates in acid solution. If a drop of the liquid is now placed on a slide and examined under the microscope it will be found that the wheat starch grains are completely ruptured whilst those of maize starch are unaltered.

In a study of the behaviour of wheat, maize, rice, potato and barley starch grains when treated by Baumann's technique, it was found that all except those of maize and rice are ruptured.

The method was then modified as follows to suit it for the quantitative determination of maize or rice flour added to wheat flour. About 1 g. of the sample was introduced with the help of 1·8 per cent. potassium hydroxide solution into a 100 ml. measuring flask. More of the same potash solution was added and the flask shaken. Three ml. of diluted hydrochloric acid (1 in 4) were then added and the volume was made up to 100 ml. with 1·8 per cent. potassium hydroxide solution. The whole was well shaken at intervals during 15 minutes, after which 25 ml. of the well mixed suspension was measured into a weighed dry centrifuge tube and centrifuged for 15 minutes (1000 r.p.m.). The supernatant liquid was decanted, the residue dried and weighed and the amount of added flour calculated.

A serious difficulty was encountered owing to the ungelatinised starch at the bottom of the centrifuge tube being invariably covered with a gelatinous layer which proved to be difficult to remove. After many unsuccessful attempts it was removed by washing the residue in the centrifuge tube several times with diluted hydrochloric acid (1 in 4) until the washings gave no colour with iodine; each time the deposit was well mixed with the acid and allowed to stand for 5 minutes before re-centrifuging. The starch grains left behind were then washed with dilute alkali to remove the acid and finally with water until the washings were free from alkali. The last step should be carefully done to prevent decomposition of the starch during the drying.

It must be admitted that the fibre content of the flour is capable of vitiating the result. The fibre if present will be unaffected by the treatment and will finally be carried down with the starch grains. The error, however, cannot be very large as the amount of fibre normally present in flours is very small compared with the starch content. Nevertheless an attempt was made to overcome this difficulty by passing the flour through a sifter No. 90 (38·5 threads per 10 mm. or 97 per inch).

Example—The wheaten flour was adulterated with maize starch. The sample was first passed through a sifter No. 90 and dried at 100° C. for 24 hours in an oven.

Weight of dried sample	=	0·8972 g.
Mean weight of maize starch in 25 ml.	=	0·0524 g.
Weight of maize starch in 100 ml.	=	0·2096 g.
Percentage of maize starch found	=	23·38 per cent.
Percentage actually present	=	23·21 ,,

But since wheaten flour is in practice adulterated with maize flour and not with maize starch, the method was applied to pure maize flour and the percentage of maize starch in maize flour was found to be 63·76 per cent.

Mixtures of wheat flours and maize flours in different proportions gave the following results:

Maize flour present %	Maize starch found %	Equivalent maize flour %	Error %
25·73	16·26	25·50	−0·9
25·73	15·67	24·58	−4·5
25·73	15·52	24·34	−5·4
25·73	16·55	25·96	+0·9
24·74	17·95	28·31	+13·8
24·74	16·63	26·08	+5·4
16·58	11·68	18·32	+10·5
25·73	15·45	24·23	−5·8
25·73	15·93	24·99	−2·9
		Mean	±6%

The method is applicable also to wheat flour adulterated with rice starch, as may be seen from the following table:

Rice starch present %	Rice starch found %	Error %
23·26	20·15	−13
23·26	20·93	−10
21·78	19·57	−5·5
21·78	19·40	−11
	Mean	−10%

SUMMARY OF THE NEW METHOD—First pass the sample of wheat flour through a sieve No. 90 (38·5 threads per 10 mm. or 97 per inch) and dry it at 100° C. for 24 hours in an oven. Weigh a definite quantity of the dried sample (about 1 g.), transfer it to a 100 ml. measuring flask, using 1·8 per cent. potassium hydroxide solution, and rinsing the weighing bottle with the same solution. Make up to 100 ml. with the same solution, including at last 3 ml. of diluted hydrochloric acid (1 in 4). Shake at intervals for 15 minutes.

Measure 25 ml. of the suspension into a weighed dry centrifuge tube. Centrifuge for 15 minutes, decant, wash the residue with diluted hydrochloric acid (1 in 4), stirring it well with the acid, leave for 5 minutes and again centrifuge. Repeat the process until the washing is free from paste (test with iodine). Wash with dilute alkali and then with water until no longer alkaline. Dry at 100° C. in an oven and weigh.

The author wishes to express his gratitude to Professor Ali Hassan Bey, Head of the Biochemistry Department, Faculty of Medicine, Cairo, for his kind personal guidance and to Dr. M. S. El-Ridi for his constant supervision.

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BIOCHEMISTRY DEPARTMENT, FACULTY OF MEDICINE
FOUAD I UNIVERSITY
GIZA, EGYPT

January, 1946

The Amidine-Pentacyanoferrate Reaction: A Test for Guanidines, Urea and Thiourea

BY W. R. FEARON

AN alkaline solution of urea treated with iodine and sodium nitrosoferricyanide (sodium nitroprusside) gradually acquires a stable red colour (Fearon[1]). When the reaction was first observed, an attempt was made to find the chromogen among the oxidation products of urea, but the results were so equivocal that attention was directed instead to the decomposition products of alkaline nitrosoferricyanide that were likely to be concerned in the test.

As is well known, on exposure to light and air, or to ultra-violet irradiation, an aqueous solution of sodium nitrosoferricyanide becomes able to react chromatically with guanidine

derivatives,[2] urea,[3] and thiourea.[4] In alkaline solution, a similar activation can be brought about by oxidisers such as ferricyanide,[5,6] persulphate,[7] and bromine[4]; reagents thus prepared have been used by Marston,[5] Pittarelli,[7] Weber,[6] and Grote,[4] yet no attempt appears to have been made to isolate and identify the constituent responsible for the urea and guanidine reactions.

The subject is confused by the variety of terms used by different workers. In the present paper, the nomenclature advocated by the International Union of Chemistry[8] is adopted; preference being given to the alternative terms *ammonio* and *ammonine*, instead of the phonetically ambiguous *ammine*, originally employed by Werner.

The decomposition products of nitrosoferricyanide were first prepared by Hofmann[9] and the photochemistry of several of the changes has been studied by Baudisch.[10] On being kept in aqueous solution exposed to light and air, nitrosoferricyanide is partially hydrolysed to *pentacyanoaquoferrate* (III), $[Fe^{III}(CN)_5H_2O]'''$; while, in the presence of excess of ammonia, the ammonine analogue, *pentacyanoammonioferrate* (III), $[Fe^{III}(CN)_5NH_3]'''$, is obtained. In these compounds the water molecule or the ammonia molecule can be replaced readily, and they have been used for the detection of nitroso derivatives and thio-ketones, by Schwechten,[11] and substituted hydrazines, by Feigl *et al.*[12] The ammonio compound is the easier to isolate, so it was selected for the present investigation.

Preparation of the pentacyanoammonioferrates

The following method is based on Hofmann's original procedure. Ten g. of recrystallised sodium nitrosoferricyanide are dissolved in 40 ml. of concentrated ammonium hydroxide solution (sp.gr. 0·88), and kept at about 0° C. until all the nitrosoferricyanide has decomposed. This is shown when a few drops of the mixture no longer give a red colour when added to a solution of creatinine in *N* sodium hydroxide (Weyl's test). Decomposition is complete by the end of 24 hours, by which time a greenish-yellow mixed precipitate of pentacyano-ammonioferrates (II) and (III) has separated out.

The precipitate is removed by filtration, and the residual pentacyanoammonioferrate (II) in the solution is separated by addition of absolute ethanol until no further precipitate appears. This precipitate is collected, washed with absolute ethanol until free from ammonia, and dried *in vacuo* over sulphuric acid. It can be purified by redissolving in ammonium hydroxide, and reprecipitating with ethanol, but this seems to be unnecessary.

The substance is a hydrated sodium salt of the ferrous compound, $Na_4[Fe(CN)_5NH_3].H_2O$. It is a sulphur-yellow powder, and keeps well, if stored in a desiccator over calcium chloride and in the dark. The yield is about 8 g. Kept in a bottle without special precautions, the salt gradually turns greenish-yellow and deteriorates.

Pentacyanoammonioferrate (II) dissolves freely in water, forming a bright yellow solution that is neutral to phenolphthalein. Freshly made, it gives no colour with guaiacum resin, and only a faint "prussian blue" with ferrocyanide. It forms a deep violet complex with cuprous ions and an emerald green with alkaline 2:6-dichloroquinone-chloroimide[13]; the latter reaction is very delicate and will detect a few micrograms of the ferrate.

In alkaline solution the ferrate (II) gives no colour with urea, thiourea or substituted guanidines unless an oxidiser be added or the mixture be left exposed to air for some hours. However, when a pentacyanoammonioferrate (II) solution in water is kept exposed to light, it spontaneously oxidises to the ferrate (III) form. The mixture becomes greenish-brown in colour and alkaline to phenolphthalein, gives a blue colour with alcoholic guaiacum and a "prussian blue" reaction with ferrocyanide, and reacts characteristically with thiourea and with amidine derivatives. The change is due to the formation of the equilibrium mixture:

$$2[Fe^{II}(CN)_5NH_3]'''' + H_2O + \tfrac{1}{2}O_2 \rightleftharpoons 2[Fe^{III}(CN)_5NH_3]''' + 2OH'$$

Pentacyanoammonioferrate (III) reagent—

While the ferrate (III) can be made at once by adding the theoretical amount of an oxidiser, such as iodine or persulphate, to a solution of the ferrate (II), these preparations soon deteriorate. A more stable reagent is got by exposing a 1 per cent. solution of the ferrate (II) in distilled water to light and air for a day, and then storing in a brown glass bottle in the dark. The reagent is now ready for use, and gains in potency for some weeks as oxidation proceeds, until some 16 to 18 per cent. is in the active ferrate (III) form, as shown by the titration value of the mixture. Eventually, further changes occur, the mixture deposits "prussian blue" on neutralisation and, after 6 to 9 months, loses its power of reacting with the amidine group.

Test

About 5 drops of the reagent are added to 5 ml. of the *neutral* solution to be tested. A blue colour develops with thiourea, thiocyanate, and formamidine disulphide; a greenish-blue with thiosulphate and phenylthiourea. Canavanine, the guanidino amino acid characteristic of Jack bean, develops a stable, dark magenta colour. Amidine derivatives (such as urea, guanidine, and substituted guanidines) and hydrazine derivatives fail to react unless the mixture be made slightly alkaline by addition of 0·1 *N* sodium hydroxide, or a *p*H 8–9 buffer. Under these conditions, colours ranging from orange-red to purple gradually develop and persist. On acidifying the mixture with dilute (2 per cent.) acetic acid, all types of colour are discharged, except the blue sulphur reactions, and the red canavanine and substituted hydrazine reactions.

Colour reactions of pentacyanoammonioferrate (III)

(1) In neutral solution

Hydrogen sulphide	No change
Thiosulphate	Greenish-blue
Sulphate	No change
Thiocyanate	Ultramarine
Thiourea	Dark blue
Formamidine disulphide	,, ,, immediate
Phenylthiourea	Greenish-blue
Thiouracil	,, ,,
4-Methylthiouracil	,, ,,
Cystine	No change
Cysteine	,, ,,
Glutathione	,, ,,
Canavanine	Magenta
Jack bean, aqueous extract	,,
Soya bean, aqueous extract	No change

(2) In alkaline solution, *p*H 8 to 9

Sulphur compounds	Blue colours as in neutral solution, only more rapidly developed
Acetamidine	Orange-red
Cyanamide	,, slow
Guanidine carbonate	Red
Methylguanidine	Red-purple
Glycocyamine	,,
Creatine	,,
Creatinine	No change
Dicyanodiamide	Red-purple
Gallegine	,,
Sym-dimethylguanidine	,,
Arginine carbonate	No change
Urea	Red, slow, promoted by oxidisers
Methylurea	No change
Semicarbazide HCl	Red, stable to weak acids
Allantoin	No change
Hydantoin	Red-purple
Hydantoic acid	No change
Acetamide	,, ,,
Glycine	,, ,,
Hydrazine HCl	,, ,,
Phenylhydrazine	Red, stable to weak acids
Methylphenylhydrazine	,, ,, ,, ,, ,,
Proteins:	
Egg albumin	No change
Caseinogen	,, ,,
Peptones, various commercial	,, ,,
Gelatin	Red-purple
Clupein	,,

Notes on individual types of reaction—

1. *Sulphur compounds*—These reactions are very delicate, and the test will reveal thiourea at concentrations of 1 in 10^5, and even less. The ferrate (III) is somewhat more stable than Grote's reagent, and will work in solutions sufficiently on the acid side of neutrality to inhibit completely the characteristic amidine reactions. The mixture may be warmed to hasten the test. If the acidity be too great or the mixture be boiled, it decomposes and

liberates "prussian blue," the colour of which, however, is relatively feeble when compared with that given by thiourea at concentrations between 1 in 10^4 and 1 in 10^5.

In alkaline solution, as Grote has observed, the thiourea reaction is photosensitive, and changes reversibly from blue to red on being kept for several hours in the dark. Grote ascribes this to alternate reduction and oxidation of the iron in the complex. An alternative explanation is now offered. The dichroic effect is only obtained in alkaline solutions, and only shown by thiourea and by formamidine disulphide, among all the compounds tested. Formamidine disulphide, the primary oxidation product of thiourea, appears to be the actual chromogen in both the blue tests. In neutral or slightly acid solution, it reacts instantly with the ferrate (III), and the shade and intensity of the final colour closely corresponds to that got from a solution of thiourea of twice the corresponding concentration. Formamidine disulphide is stable in acid solution and the pigment formed is permanent and suitable for colorimetry. In alkaline solution, formamidine disulphide is unstable and in presence of reducing agents can revert to thiourea, which is able to yield a red amidine type of pigment with ferrate (III).

This can be demonstrated by treating the thiourea blue pigment in alkaline solution with the appropriate amount of a reducing agent, such as ascorbic acid, whereupon the blue changes to red. If excess of reducer be added, the ferrate (III) is changed to ferrate (II) and the colour is discharged. Conversely, by careful oxidation of the red pigment in alkaline solution, the blue pigment is obtained.

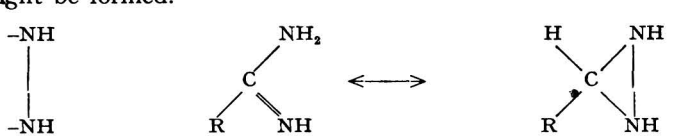
In general, the blue pentacyanoammonioferrate reaction is given by compounds containing –S–S– or :C:S groupings, but not by –SH. The failure of cystine to react may be due to its very low solubility.

2. *Amidines and Guanidines*—According to Hofmann and Baudisch, ferrate reactions in alkaline solution depend on the replacement of H_2O or NH_3 by a molecule of the reacting chromogen; thus would be formed the nitroso-compounds, $[Fe^{III}(CN)_5ON.R]'''$, described by Schwechten, and the hydrazine compounds, $[Fe^{III}(CN)_5NH_2.N:R_2]'''$, described by Feigl,[14] while Hofmann has isolated a red pigment, got by the action of nitrosoferricyanide on thiourea in alkaline solution. By analogy, the amidine and guanidine pigment ions would be: $[Fe^{III}(CN)_5NH_2.C(:NH).R]'''$. This has not yet been verified by analysis of the pigment salts. Schwechten comments on the difficulty of isolating the *iso*nitroso pigments, and although he claims to have separated at least one of them, he does not give any details of his method.

The guanidine pigment can be obtained by concentrating *in vacuo* at room temperature an equimolecular solution of pentacyanoammonioferrate (III) and guanidine carbonate until a deep red syrup is obtained. On addition of ethanol the pigment is taken up by the aqueous alcohol mixture, in which it is soluble until the concentration of the alcohol exceeds about 80 per cent. Further addition of alcohol precipitates the pigment as a tar-like mass, which, on exposure to air, absorbs carbon dioxide, and decomposes, with formation of crystals of guanidine carbonate. If, however, ether be added gradually to a concentrated solution of the pigment in the aqueous alcohol, the pigment separates out in short, dark rods, which later aggregate into amorphous globules.

A feature of the amidine reaction is the existence of an optimal *p*H for colour development. Acids inhibit colour formation completely, presumably by converting a reactive amino group into the non-reactive $-NH_3^+$. Ammonia in excess retards the reaction, by repressing the dissociation of NH_3 from the ammonioferrate. Canavanine is exceptional in that it can react in neutral solution; the other amidines require varying concentrations of alkali, but eventually all develop a colour on being kept at *p*H 8·5 to 9, a condition conveniently obtained by using saturated sodium hydrogen carbonate solution as a buffer. Among the monosubstituted guanidines, arginine is exceptional in not reacting, possibly because the amidine system is in the stable, guanidonium form as part of the zwitterion.

In general, the red pentacyanoammonioferrate reaction can be referred to the presence of an un-ionised hydrazi-linkage or an un-ionised amidine grouping, from which, by closure, the hydrazi-ring might be formed.



As a class, the amidine pigments are only stable in alkaline solution, and in neutral aqueous or aqueous-alcoholic solution resolve by hydrolysis into the free amidine compound and pentacyanoaquoferrate; for this reason the isolation of the pigments in form suitable for analysis has not yet been achieved.

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DEPARTMENT OF BIOCHEMISTRY
TRINITY COLLEGE, DUBLIN

September, 1946

Abridged Spectrophotometry with Special Reference to Carotene-containing Materials

BY R. J. TAYLOR

IN analytical work the colour produced by a substance, either in simple solution or in reaction with a reagent, is often utilised to assay the substance. If the colour of the solution, however, is due to specific absorption of light in one or more well-defined regions of the spectrum, it is fundamentally the sounder technique to assay the material spectrophotometrically by measuring the absorption of light at the wavelength of maximum absorption. The measure is then of the primary phenomenon (specific absorption) and not of the secondary phenomenon (colour) which is easily contaminated, is to some extent subjective and is not intrinsic.

The determination is commonly made in terms of an $E^{1\%}_{1cm.}$ value,[1] and the most accurate determinations have been obtained hitherto by means of absolute spectrophotometers. It is of particular interest, however, to see if results of equal accuracy can be obtained with an abridged instrument, since this type will always appeal to the analyst because of its robustness, ease of adjustment and manipulation, and economy. Consideration has therefore been given in the body of the present paper to the factors which will ensure such accuracy.

The conclusions are of general application, but an important corollary is that the plunger-type colorimeter can be converted to an abridged spectrophotometer by replacing the light correction filter by a more selective spectral filter. Such colorimeters may be considered normal laboratory equipment and their versatility is not diminished by this conversion. The modification is not of a permanent character and the instrument may be used as a colorimeter or spectrophotometer at will. The decision is governed by the proviso set out in the first paragraph above; the advantages accruing from the conversion are, in the simplest case, that a match of more uniform quality can be made and, in a more complex case, that a labile substance can be assayed in terms of the known constants of a dissimilar but more stable substance.

The technique has been applied particularly to the measurement of the $E^{1\%}_{1cm.}$ value at 460 mμ of carotene-containing materials, for which a satisfactory abridged method is lacking.

FUNDAMENTAL CONSIDERATIONS—

A spectrophotometer in the ideal sense has three principal components, (1) a spectroscope or monochromator for dispersing a source of light into its component wavelengths and selecting the required wavelength, (2) a photometer for determining the absorption of light by a test substance in terms of its optical density and itself dependent upon (3) a device (either visual, photographic or electrical) for matching or comparing the light intensities. Spectrophotometers which conform to this specification are absolute in the sense that the values obtained

are derived from the underlying theory and the design of the components, although in practice it is necessary to check the photometer by means of a standard at frequent intervals and to operate the instrument within certain limits on the optical density scale if the most accurate results are required.

In abridged instruments certain refinements are sacrificed to reduce the cost, and chief of these is the spectroscope or monochromator. This is replaced by a combination of light source and optical filter, by means of which a comparatively narrow but spectrally undispersed region of the spectrum is isolated and used as an approximation to monochromatic light.

The implication of this is that to use the photometer in the normal manner is fundamentally wrong, for the theoretical considerations which govern the design of photometers are true only for monochromatic light. This type of instrument is therefore not absolute and, in the case of those instruments of the abridged type which do incorporate photometers, the values obtained are meaningless unless corrected by a factor or series of factors (see Appendix I), or unless the photometer is adjusted arbitrarily, as in the case of the Hilger Vitameter, and used within narrow limits. Both methods require the use of a standard and there is in any subsequent measurement the chance of a double observational error (standard/photometer and photometer/sample). It is preferable therefore to match the sample directly against the standard, for which purpose the photometer should be replaced by a means for varying the amount of light absorbed either by the standard or the sample, that is, by a device for varying the depth of solution. When a suitable choice of standard has been made the determinations are exact, and accurate E values can be obtained.

The standard is therefore of prime importance. It must be a stable material with absorption characteristics similar to those of the test material in the region of the spectrum concerned, in which case the quality of the light with respect to spectral distribution transmitted by both the standard and the sample will be similar and optical matching will establish true equality of optical density. In absorption photometry one may be assaying a compound, an element or a radicle, and if the particular material can be obtained in a pure and stable form it will provide, as such, the most suitable material for comparison. On the other hand, if the material is labile then a stable standard with similar absorption characteristics must be looked for.

Of second importance is the filter. Its peak of transmission should be at the peak of absorption of the sample and it should have as narrow a spectral range and as high a transmission as possible, but in any event it should not have a broader transmission band than will cover the specific absorption characteristics of the standard and the sample. In other words, it should let through only that fraction of the light which will give identity of absorption characteristics throughout the spectral range. As a class, liquid filters provide narrower spectral bands and a higher percentage transmission than either glass or gelatine filters, but they are difficult to incorporate in instruments that are used for routine testing. Information on glass, gelatine and the newer plastic filters is to be found largely in the makers' catalogues (*e.g.*, Ilford, Wratten and Dufay-Chromex), but the range is limited. Interference filters[2] are a more recent development or, rather, a more recent technical achievement. It is too early to assess their applicability; but if the interference film can be produced to a uniform given thickness that is not significantly affected by normal variations in laboratory temperatures, then the potentialities of this type of filter are such as to make it preferable to any other type.

PRACTICAL APPLICATIONS—

The ideas which have been developed in the previous section are applicable to all types of instrument, but consideration is limited here to the visual instrument since this form is simplest in conception and other forms are mainly complementary. For ease of matching, large adjacent fields of view are required and, according to the foregoing, there is placed in one field a standard of known concentration and depth of solution, and the test sample in an adjustable cell in the other field. The two fields are illuminated by a common light source, suitably filtered.

It will readily be seen that the proposed ideas are embodied to a large extent in the plunger-type of colorimeter, and that merely by interposing a filter the colorimeter will function as an absorption photometer. The more normal spectrophotometric manipulation, involving a discontinuous change of cell thickness with a finely controlled change of optical density, is replaced by a discontinuous change of concentration with a finely controlled change

of cell thickness. Ideally a wide range of cell thickness is available for matching but, in practice, errors in the reading of thin strata and deviations from Beer's Law will limit the available range. In general it will be found, as for absolute instruments, that there is a best region of measurement which in this instance will depend on the thickness chosen for the standard solution.

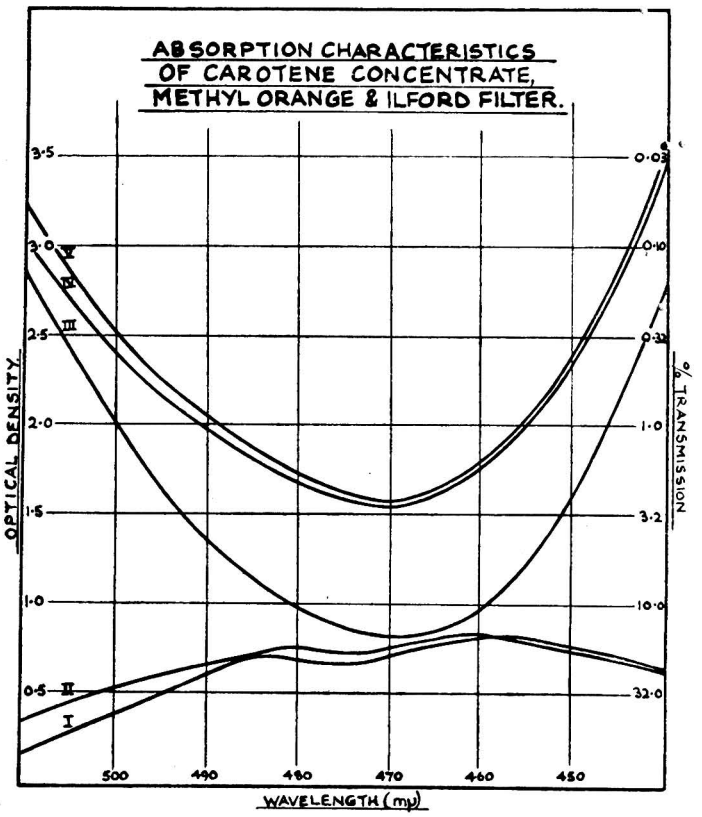

Fig. 1. Absorption Characteristics of Carotene Concentrate, Methyl Orange and Ilford Filter.

I. Absorption curve of carotene-containing oil.
II. Absorption curve of aqueous methyl orange.
III. Transmission curve of Ilford Spectrum Blue Filter 602.
IV. Transmission curve of Ilford Filter plus carotene oil.
V. Transmission curve of Ilford Filter plus methyl orange.

Thus with this type of instrument there are two conditions to be observed: (1) a standard must be used, and (2) measurements must be made between agreed limits. But, as already mentioned, these conditions must be observed in absolute spectrophotometry. Hence the procedure necessary for accurate working is no more involved in the former method than in the latter, and the limits of accuracy under such conditions similarly will be governed only by manipulative errors.

The determination of the $E^{1\%}_{1\,cm.}$ values of carotene-containing materials

The carotene extracts from these materials, when dissolved in chloroform, exhibit a broad absorption band in the blue-violet with a peak in the region of 460 mμ. A typical curve is shown in Fig. 1, Curve I. The standard chosen was methyl orange in aqueous solution. Water was used as solvent in preference to alcohol because of its lower volatility, but some care must be used in getting the crystals into complete solution. The absorption curve is shown in Curve II, which it will be seen closely simulates Curve I.

The most suitable filter to date is an Ilford Spectrum Blue 602, the characteristics of which are given in Curve III. Its maximum transparency is at 470 mμ. Curves IV and V

are drawn to emphasise the similarity of contour for light transmitted through the sample and the standard. A drawback to blue filters as a class is that their visual transmission is relatively low. It is necessary therefore to increase the strength of the source of illumination to overcome this drawback.

A standard solution is prepared from methyl orange of analytical quality. Measurements made in this department on a number of samples of methyl orange yielded a mean $E^{1\%}_{1\,cm.}$ value at 460 mμ of 756. A suitable concentration for use in the colorimeter is 0·0004 per cent., at which strength the solution has an $E_{1\,cm.}$ value of 0·302. This solution is conveniently made up in two stages, by first preparing a solution of 0·16 g. to 1000 ml. in water and diluting 25 ml. to 1000 ml. in water as required. The methyl orange does not dissolve very easily in water and it is best to stir it in a beaker, breaking up the agglomerates with a glass rod, and to decant the clear solution successively until the whole is transferred in solution to the graduated flask. A check kept on the preparation of the standard solution showed the values to be consistent within ±2 per cent.

The methyl orange solution is used to adjust the colorimeter as follows. Both cups are filled and set to an equal and suitable depth which we prefer to be half the maximum depth. If the fields are then observed to be unequal in intensity the lamp is adjusted to give equality or near equality. In general only near equality can be obtained as the lamp adjustment is relatively crude, and it is more convenient to apply a small correction than to strain after equality. In parenthesis it may be remarked that it is hard to understand why so much thought is given to the mechanics of cell adjustment in these instruments and so little to those of illumination adjustment.

When a determination is being made the left-hand cup is left adjusted to the chosen depth and the right-hand cup is cleaned, dried and filled with a solution of the sample in chloroform. The depth of solution of the sample is then adjusted until uniform intensity is again obtained over both fields, but if the depth is outside the agreed limits of matching the concentration of the sample is adjusted to come within the limits. A series of visual matches is made and the mean depth calculated.

Then, for example, if the $E_{1\,cm.}$ value of the standard $= 0{\cdot}302$

Depth of standard solution $= 30$ mm.

,, ,, test ,, $= d$ mm.

Per cent. concentration of test solution $= C$

$E_{1\,cm.}$ value of sample $= \dfrac{0{\cdot}302 \times 30}{d}$

and $E^{1\%}_{1\,cm.}$ value $= \dfrac{0{\cdot}302 \times 30}{d \times C}$

The reliability of the method has been studied by means of an inter-laboratory trial in which Ogal colorimeters were used. Replicates of five samples of carotene-containing oil were distributed to three laboratories for assay. Each sample was diluted to three different concentrations so that, with the standard methyl orange solution at a depth of 30 mm., match points were obtained for each sample at approximately 20 mm., 30 mm. and 40 mm. depth. Three operators in each laboratory made triplicate matches at each concentration. Thus there were twenty-seven match points for each sample in each laboratory, *i.e.*, a total of eighty-one match points per sample. Four of the operators had had preliminary experience of the method before the trial.

The over-all mean $E^{1\%}_{1\,cm.}$ 460 mμ values thus obtained on the five samples were 1·68, 1·49, 1·96, 1·74 and 1·33. A summary of the full statistical analysis of the data is given in Appendix II, and below, in Table I, the 5-sample percentage variations between observers, laboratories and "solution depths" are shown.

The findings of the statistical analysis (some will be obvious from inspection of Table I) were:

(i) The observed E value depends slightly but significantly on "solution depth." It is therefore advisable to stipulate 20–40 mm., and preferable to use 25–35 mm., as the working range.

(ii) The reproducibility of results by individual observers varies considerably, and it is advisable to select the best operators from those available in any one laboratory. A minimum of three readings should be taken by each observer.

(iii) Small intrinsic differences between observers exist and cannot be smoothed out by practice. It is recommended that each laboratory should always give the mean of two observers' results.

(iv) Laboratory differences (not associated with the taking of readings) between results may obtain in the early stages, but they are likely to disappear rapidly with increasing *expertise* in over-all technique.

(v) If one result is defined as the mean of two observers' triplicate readings on one solution, the difference between any two results, even when obtained in different laboratories by different observers, is unlikely (*i.e.*, will occur on only 1 occasion in 20 such comparisons) to exceed 3·3 per cent., and will usually be well below this figure.

TABLE I

PERCENTAGE VARIATIONS OF THE 5-SAMPLE MEANS OF ESTIMATES OF $E^{1\%}_{1\,cm.}$ 460 mμ

	Means at approx. "solution depth" of:			
	20 mm.	30 mm.	40 mm.	Overall means
Observer A1	107·9	104·3	99·8	103·8
,, A2	103·7	102·8	99·7	102·1
,, A3	107·0	104·6	99·9	103·8
Means for laboratory A	106·0	103·9	99·8	103·2
Observer B1	96·9	97·9	96·9	97·4
,, B2	96·9	98·0	96·1	96·9
,, B3	96·7	98·3	96·7	97·2
Means for laboratory B	96·9	98·1	96·5	97·2
Observer C1	99·7	97·9	98·3	98·6
,, C2	101·2	99·2	99·2	99·7
,, C3	101·0	99·9	100·1	100·3
Means for laboratory C ..	100·6	99·0	99·2	99·6
Means for all laboratories ..	101·2	100·3	98·5	100·0

APPENDIX I

That the use of a photometer in a normal manner with a filtered light source is fundamentally unsound can be quite easily demonstrated as follows. Suppose that a source of filtered light is divided into two beams, one passing through an absorbing medium and the other through a calibrated variable aperture. The transmission curve of the filter can be represented by Curve I in Fig. 2, and the absorption curve of the medium by Curve II. Curve IIA then represents the transmission curve of the light passing through the filter and the medium. It will be noticed that Curve IIA is flattened in relation to Curve I.

Since the photometer is non-selective, its absorption curve will be a straight line parallel to the horizontal axis, and the exact position will depend on the setting of the photometer. Suppose that it is set initially to a value *equal* to the true value of the absorbing medium at the peak of its absorption curve, that is, at the value which would be obtained on an absolute instrument. Then the line (Curve III) passes through the maximum of Curve II, and Curve IIIA, which represents the transmission curve of the light passing through the filter and the photometer, is Curve I translated to the position where its minimum (minimum referring to optical density) coincides with the minimum of Curve IIA. Since Curve IIA is flattened in relation to Curve I, Curve IIIA lies wholly within Curve IIA. That is to say that at the true match more light passes through the sample than through the photometer and an apparent inequality exists. Hence, at the observed match point, the optical density registered by the photometer will be *less* than the true value at the wavelength of maximum absorption. The amount by which it is less will depend on the characteristics of the absorption and transmission curves.

Before leaving this point, reference should be made to the work of Dann and Evelyn.[3] They have recognised the difficulties inherent in "filter" photometry and propose to resolve

them by defining the results in terms of a new unit, the "L" value, analogous and convertible to E value by a factor which is calculated from the spectral distribution curves of the filter and the sample. The concept of "L" values, however, involves an assumption that instrumental characteristics are reproducible, but work in these laboratories does not support this assumption. The method of treatment which is proposed in the body of the present paper is independent of instrumental variables, and is therefore considered to be of more universal application.

Appendix II

Statistical analysis of readings

The plan of the collaboration experiment involved a total of 405 results, *i.e.*, 5 samples × 3 laboratories × 3 observers in each laboratory × 3 "depths" × 3 readings. But as one of the observers omitted to read one sample only 396 results were finally available. This corresponds to 395 degrees of freedom in an analysis of variance. As the absolute differences between the five samples were too great to permit the variances to be treated as homogeneous, the analysis of variance was conducted on a "percentage error" basis; thus the square roots of the emergent variances give coefficients of variation directly.

The first point to note in the analysis of variance is that the second order interaction S×D×O/L has a variance significantly greater than the residual variance—which is based on the reproducibility of readings by any one observer on a given solution in the cell. This means that reproducibility of readings is not an index of the reproducibility of whole results (*i.e.*, including the measuring and diluting of the sample); in other words, some extra error must be contributed by slight differences in solution depth and strength. This is perhaps not surprising, but an important consequence is that we must use the S×D×O/L variance, and not the residual variance, as basic in assessing the significance of the other variances. Moreover, an examination of the original data showed that the residual variance differed not inconsiderably from observer to observer; while the mean coefficient of variation was 1·19, that of the best observer was 0·50 and that of the worst 2·31.

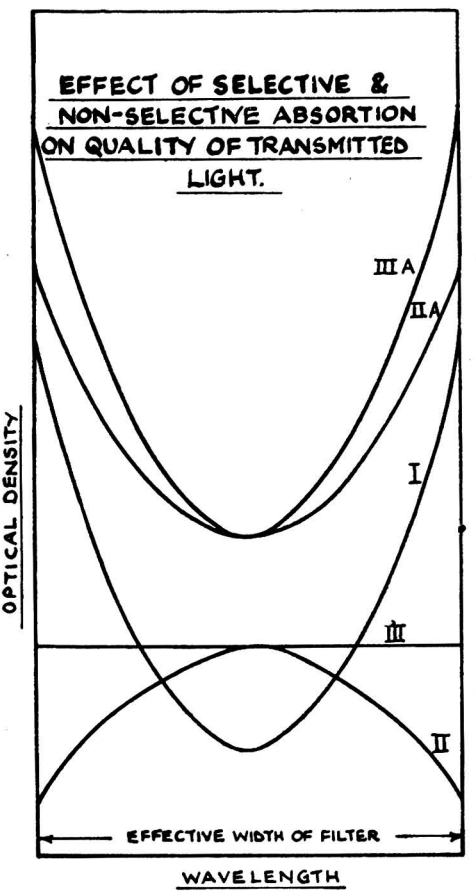

Fig. 2. Effect of Selective and Non-selective Absorption on Quality of Transmitted Light.

I. Transmission curve of light filter.
II. Absorption curve of absorbing medium.
IIA. Transmission curve of filter plus medium.
III. Absorption curve of photometer.
IIIA. Transmission curve of filter plus photometer.

The main inferences from Table II are:

(i) that the inter-depth variance is almost entirely absorbed by the hypothesis that the results decrease by 0·14 per cent. per extra mm. in solution depth.

(ii) that the inter-laboratory variance is far greater than can be accounted for by the inter-observer variance within laboratories.

(iii) that the three interaction variances, S×D, S×L and D×L, are significant.

As the five samples were tested in the same order in each laboratory, the data can be made to yield information on the effect of increasing familiarity with the technique on the size of the error. From Table II it will be noted that the "mean squares" of the deviations beween (*a*) laboratories, (*b*) observers within laboratories and (*c*) replicate readings are, respectively,

1262·635, 27·75 and 1·418. These values can be partitioned between the five samples, as shown in Table III.

The curious fact emerges that while inter-laboratory discrepancies narrow rapidly over the period of operation, neither the inter-observer nor the inter-reading discrepancies are

TABLE II

ANALYSIS OF VARIANCE OF 396 OBSERVED E VALUES

Source of variance	Sum of squares	Degrees of freedom	Mean sq.	Comparable mean sq.	Variance ratio	Probability of significance	True variance	True coeff. of variation
Main treatment—								
5 Samples	69,874·25	4	17,468·563	6·75	2588	—	—	—
3 Depths:								
linear regression ..	472·00	1	472	6·75	70	<0·001	—	—
residual	21·50	1	21·5	6·75	3·19	<0·05	negligible	—
9 Observers:								
3 Laboratories ..	2,525·25	2	1,262·625	27·75	46	<0·001	9·147	3·02
Observers in laboratories ..	166·50	6	27·75	6·75	4·11	<0·01	0·467	0·68
Interactions—								
S×D	570·00	8	71·25	6·75	10·56	<0·001	2·389	1·55
S×O:								
S×L	2,355·00	8	294·375	8·120	36	<0·001	10·602	3·26
S×O/L	186·75	23	8·120	6·75	1·20	<0·05	negligible	—
D×O:								
D×L	524·25	4	131·063	5·354	25	<0·001	2·794	1·67
D×O/L	64·25	12	5·354	6·75	<1	—	nil	—
S×D×O:								
S×D×L	12·25	16	0·766	6·75	<1	—	nil	—
S×D×O/L ..	310·50	46	6·75	1·418	4·76	<0·001	1·777	1·33
Residual error	374·46	264	1·418	—	—	—	1·418	1·19
		395						

(i) The regression coefficient (accounting for the "linear regression" variance, line 2) is −0·14 per cent. per mm. depth.

(ii) In the interaction lines S symbolises samples; D, solution depths; O, observers; L, laboratories; and O/L observers within laboratories.

affected by increasing familiarity. This suggests that the error of reading is generally intrinsic and cannot be improved but that manipulative operations which are carried out prior to matching, such as preparation and dilution of the sample, need practice before efficiency is reached. The inter-laboratory mean square narrowed finally to 28 (Table III), which value is almost exactly the same as the inter-observer mean square—27·75 (Tables II and III) —which means that at this stage true inter-laboratory differences were completely eliminated, for the recorded difference is merely due to the fact that each laboratory had its own set of observers.

TABLE III

VARIATION OF MEAN SQUARES OF 3 SETS OF DEVIATIONS FROM SAMPLE TO SAMPLE, *i.e.*, WITH INCREASING OPERATING FAMILIARITY

Sample	Set 1 (between laboratories)	Set 2 (between observers)	Set 3 (between replicate readings)
A	3,170	20·9	1·06
B	1,744	29·5	2·06
C	1,205	50·8	1·84
D	168	28·2	0·52
E	28	9·3	1·62
Means	1,263	27·74	1·42

It is now possible to estimate the magnitude of the error to be expected in normal working practice (*i.e.*, with operators who have become accustomed to the technique). If we assume that the depth of solution is always kept as near to 30 mm. as possible we can ignore this

factor as a source of variance. Let us take the case of a single sample, read in triplicate by two average observers.

Source of variance—		Variance
2 Observers	= 0·467/2	= 0·234
3 Readings each	= (1·777+1·418/3)2	= 1·125
		1·359

Now if two such tests are carried (for instance, in different laboratories) the variance of the difference between the two results will be 2 × 1·359, corresponding to a coefficient of variation of $\sqrt{(2 \times 1{\cdot}359)}$ or 1·648. Twice this coefficient gives the P = 0·95 limits of error of the difference as percentage. The conclusion is therefore: if duplicate tests (the readings being taken in triplicate by two observers) are carried out in the same, or different, laboratories, in 19 cases out of 20 the two results will differ by less than 3·3 per cent.

SUMMARY

The fundamental considerations governing the use of "non-absolute" instruments for accurate spectrophotometric measurements (abridged spectrophotometry) have been developed and discussed.

A plunger-type colorimeter can be converted to an abridged spectrophotometer by a simple replacement of the light correction filter by a more highly selective filter for the required spectral region of measurement.

A method of determining the E (460 mμ) value of carotene-containing materials with such an instrument is described. The method has so far been applied only to carotene-containing oils but it is considered applicable to all carotene-rich materials.

Thanks are due to my colleagues for helpful criticism and advice, in particular to Mr. N. T. Gridgeman for his design of the collaborative trial and statistical analysis of its results; to the laboratories which collaborated in the trial; and to the Directors of Lever Brothers & Unilever Ltd. for permission to publish this work.

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RESEARCH DEPARTMENT
LEVER BROTHERS & UNILEVER LTD.
PORT SUNLIGHT, CHESHIRE

August, 1946

Improved Method for Semi-Micro Quantitative Analysis of Some Paper Ashes and Other Similar Materials*

BY D. C. BRADLEY

INTRODUCTION—In analysing small quantities of paper ash, a limited amount of sample (not more than 50 mg.) was available, and it was necessary to devise an improved method of analysis. It was required to determine each constituent, using only a single sample. The improved method is applicable to materials containing SiO_2, Fe_2O_3, Al_2O_3, CaO and MgO, and should find use in the analysis of materials such as paper ash, plant ash, corrosion products in reinforced concrete, and other substances containing the elements iron, aluminium, calcium and magnesium present as oxides or complex silicates together with silica. Other elements, *e.g.*, copper, cadmium, cobalt, manganese, nickel, titanium, zinc and zirconium will interfere. Phosphate will also interfere if present in amount greater than 1 per cent.

A quantity of sample of the order of 25 to 50 mg. is suitable. Less than 25 mg. would probably be insufficient to give an accurate estimate of iron or aluminium unless these were present in greater quantity than 10 per cent. of the sample.

DISADVANTAGES OF PREVIOUS METHODS—In the usual method of analysis (of cement and allied materials) the sample is extracted with acid and the iron and aluminium are precipitated together as hydroxides. However, these hydroxides form gelatinous precipitates difficult

* Based on E.R.A. Report, Ref. A/T99.

to filter, especially in small quantities. The precipitate also adsorbs other ions. As the total weight of oxides may be less than 10 mg., volumetric estimation of the iron is impracticable owing to indefiniteness of end-point. Other possible variants of the usual method involve similar difficulties due to the nature and small amount of the hydroxides involved.

Kampf[1] describes a method for the volumetric determination of iron and aluminium in cement, in which the important feature is the determination of iron and aluminium by co-precipitation by means of an acetic acid solution of 8-hydroxyquinoline. He also describes a method for first titrating bromometrically the total oxine combined with the iron and aluminium and then allowing the ferric iron to oxidise potassium iodide and titrating the liberated iodine with sodium thiosulphate.

On applying this method to small samples the following difficulties were encountered. (1) Incomplete co-precipitation of the iron and aluminium. (2) The volumetric procedure was impracticable owing to indefinite endpoints. (3) Tartaric acid added in the initial stages of the procedure to prevent formation of the basic acetates of iron and aluminium caused calcium to separate as calcium tartrate.

DEVELOPMENT OF AN IMPROVED METHOD BASED ON KAMPF'S METHOD—It was decided to separate the co-precipitated iron and aluminium oxinates by means of *p*H control and weigh them separately as oxinates, thereby eliminating the unsatisfactory titrations.

The precipitation of calcium tartrate was avoided by reducing the quantity of tartaric acid and by filtering the precipitate of iron and aluminium oxinates from the hot solution.

The incomplete co-precipitation was found to be due to the fall of the *p*H of the solution below 5·0 after the addition of oxine reagent. This fall in *p*H is due to two factors:

(i) The presence of acetic acid in the reagent.

(ii) Release of hydrogen ions when precipitation of iron or aluminium takes place, *e.g.*,

$$FeCl_3 + 3C_9H_6N.OH \rightarrow 3HCl + Fe(C_9H_6ON)_3$$

Moyer and Remington[2] showed that iron is completely precipitated at *p*H values above 3·5, while aluminium begins to be co-precipitated above *p*H 4·0 and is completely co-precipitated above *p*H 5·0. Hence, if the *p*H of the solution is below 5·0 considerable error results, owing to incomplete precipitation of the aluminium. The same investigation showed that iron can be separated from aluminium by precipitating between *p*H 3·5 and 4·0.

The method due to Kampf[1] of setting the *p*H at 5·8 prior to precipitation and using methyl red indicator and ammonium acetate buffer solution is satisfactory as a preliminary rough adjustment. When only small concentrations of iron and aluminium are present, the ammonium acetate buffer prevents the *p*H from falling below 5·0 after the addition of oxine. Although more ammonium acetate might keep the *p*H above the critical value in the presence of greater concentrations of iron and aluminium, this would increase the total volume of solution and also the amount of solute present, which is undesirable. Hence it was decided to adhere to the initial procedure of Kampf, but to check the *p*H and adjust it if necessary after precipitating the iron and aluminium.

To measure the *p*H of the solution accurately requires an electrical method, indicators being useless in the coloured solution. A dipping glass electrode - calomel electrode system was used successfully.

By setting the *p*H of the solution after precipitation at 5·3, it was found possible to co-precipitate completely the iron and aluminium present.

A further development was to re-dissolve the precipitate of iron and aluminium oxinates in hydrochloric acid, add tartaric acid, and precipitate the iron oxinate alone by adjusting the *p*H to 3·6 with ammonia. After filtering off the iron oxinate, the aluminium was precipitated by making the filtrate alkaline with ammonia.

METHOD

REAGENTS—The following reagents of analytical purity are required. (i) Diluted hydrochloric acid of three different concentrations, obtained by mixing 1 vol. of the concentrated acid with 1, 2 and 10 vols. of water respectively. (ii) Ammonium hydroxide solution, sp.gr. 0·880. (iii) Tartaric acid, 2 and 20 per cent. aqueous solutions. (iv) Ammonium acetate, 25 per cent. aqueous solution. (v) Hydrofluoric acid, 40 per cent. aqueous solution. (vi) Oxine reagent prepared by dissolving 12·5 g. of 8-hydroxyquinoline in 30 ml. of glacial acetic acid and diluting to 1 litre with distilled water. (vii) Oxalic acid, saturated aqueous solution. (viii) Picrolonic acid, 2·7 g. dissolved in a litre of distilled water.

PROCEDURE.—Fig. 1 shows the essential stages of the procedure. It is advisable to follow exactly the minor details in the following description, even although some may appear to be trivial, since with the small quantities involved it is very easy to introduce unsuspected errors. It is better to carry out all heating and evaporating on a steam bath rather than over a flame, except where otherwise specified.

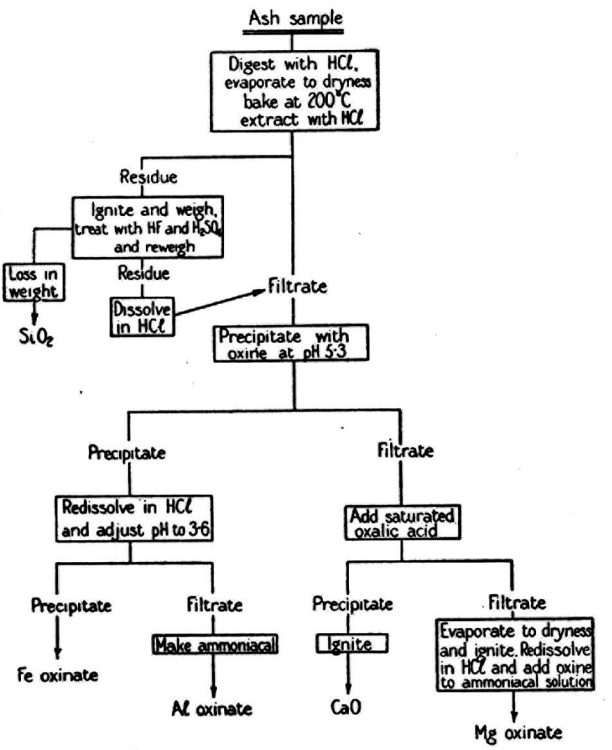

Fig. 1

Digest a weighed sample, usually 25 to 50 mg.,* in a platinum crucible with 10 ml. of diluted hydrochloric acid (1+2). If the presence of ferrous iron is suspected add bromine water to oxidise it to the ferric state. Evaporate to dryness, bake the residue for 1 hour at 200° C., cool, extract with 10 ml. of diluted hydrochloric acid (1+2) for at least 15 minutes, breaking up if necessary with a platinum wire. Cool and filter through a small Whatman No. 42 filter paper, collecting the filtrate in a 30-ml. Pyrex beaker. Evaporation, baking and extraction may be repeated if continued presence of silica is suspected. Dry the filter paper containing the residue of silica and ignite in a weighed platinum crucible. After re-weighing, remove silica by treating with hydrofluoric acid and a drop of concentrated sulphuric acid. Any residue left after evaporating to dryness is taken as unextracted metals and added to the initial filtrate.

To the filtrate add 1·0 ml. of 2 per cent. tartaric acid solution and 2 drops of methyl red indicator solution. Treat with successive drops of concentrated ammonia until alkaline to methyl red and then with dilute hydrochloric acid (1 in 10) until just acid. Add 2·5 ml. of 25 per cent. ammonium acetate solution and heat for 10 minutes. Add excess of oxine reagent with stirring, to co-precipitate the iron and aluminium. Heat for 15 minutes with occasional stirring, when the precipitate of iron and aluminium oxinates will coagulate and settle.

Cool the solution to room temperature and adjust its *p*H, using a glass electrode - calomel electrode system, to 5·3 with either ammonia or hydrochloric acid.

* A semimicro-analytical balance must be used.

Heat the solution for 15 minutes with stirring, allow to cool for 5 minutes and filter through a weighed sintered glass crucible previously dried for 1 hour at 110° C.

Wash the precipitate well with successive quantities of hot distilled water, collecting the filtrate and washings in a 50-ml. Pyrex beaker.

To collect the filtrate directly in another beaker without further transference, the apparatus shown in Fig. 2 was improvised and was found very satisfactory.

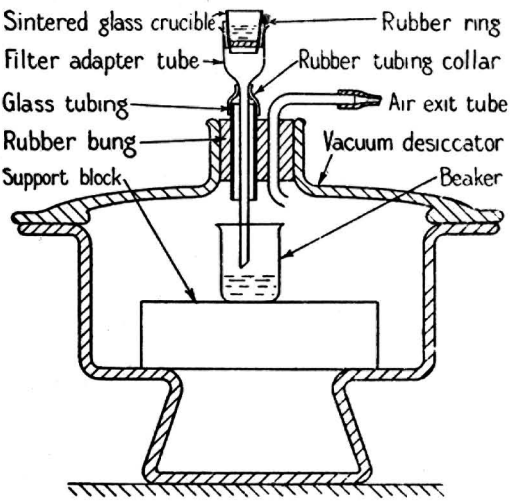

Fig. 2

SEPARATION OF IRON FROM ALUMINIUM—After drying the precipitate (which is more readily dissolved in acid if previously dried) for 10 minutes at 110° C., replace the crucible in the filtering apparatus with a fresh receiving beaker of 30-ml. capacity. Re-dissolve the precipitate by treating with the minimum quantity of almost boiling dilute hydrochloric acid (1+1) containing a drop of glacial acetic acid. Obtain the solution in the 30-ml. beaker by reducing the pressure in the desiccator and wash the crucible with hot distilled water, collecting the washings in the same beaker.

Heat the solution for 10 minutes and add 1·0 ml. of 20 per cent. tartaric acid solution, followed by concentrated ammonia, added in drops with constant stirring, until a permanent black precipitate is obtained. This should be done carefully, otherwise aluminium oxinate will be precipitated with the iron. After stirring vigorously, cool the solution to room temperature and adjust the *p*H to 3·6. Heat the solution with stirring for 15 minutes, allow to cool for 5 minutes and filter through the original sintered glass crucible.

Wash the precipitate well with hot distilled water, collecting the washings and filtrate in a 50-ml. beaker. Dry the crucible at 110° C. for 1 hour, allow to cool and weigh to obtain the weight of ferric oxinate.

Heat the filtrate, which contains the aluminium, for 10 minutes, adding a drop or two of oxine reagent to ensure an excess.

Precipitate the aluminium oxinate by adding ammonia until the solution smells faintly ammoniacal. Stir well and heat for 10 minutes. After cooling to room temperature, filter through a sintered glass crucible, previously dried for 1 hour at 110° C. and weighed. Wash the precipitate with distilled water, dry at 110° C. for 1 hour and re-weigh on cooling to obtain the weight of aluminium oxinate.

SEPARATION OF CALCIUM FROM MAGNESIUM—The calcium and magnesium present are contained in the filtrate obtained after filtering the co-precipitated iron and aluminium. Heat this to about 70° C. and slowly add saturated oxalic acid solution in excess, with vigorous stirring. When the precipitate of calcium oxalate has nearly settled, add ammonia until the solution is alkaline to methyl red. Heat for 10 minutes with frequent stirring and allow to cool. It is advisable to time the analysis so that the calcium oxalate may stand overnight, ensuring complete precipitation in crystalline form. Filter the solution through a small Whatman No. 42 filter paper and collect the filtrate and washings in a 100-ml. silica evaporating basin. Ignite the filter paper containing the calcium oxalate precipitate in a weighed platinum crucible, heating slowly until the paper has burned away, and then with the full flame to decompose the calcium carbonate formed. Full blast for 15 to 30 minutes is sufficient for small quantities. Cool the crucible in a desiccator containing both caustic soda and concentrated sulphuric acid to prevent absorption of carbon dioxide and moisture respectively, and weigh as rapidly as possible.

If only a small quantity (less than 10 to 15 mg.) of calcium oxide is present it is preferable to determine the calcium with picrolonic acid as follows. Dissolve the calcium oxide, still in the crucible, by warming with dilute acetic acid and transfer the solution to a 100-ml. beaker. To the warm (40° C.) solution add the calculated quantity of warm picrolonic acid solution

and stir well. The calcium picrolonate precipitates on standing. Allow to stand overnight and filter through a sintered glass crucible previously dried *in vacuo* and weighed. Wash the precipitate with distilled water and aspirate air through it for 5 minutes. Wash the precipitate with ether, transfer the crucible to a vacuum desiccator and maintain the vacuum for 2 hours, evacuating at least every ½ hour or preferably continuously, to remove water vapour which leaves the precipitate. (The details of drying the precipitate must be followed exactly, as the precipitate is soluble in solvents other than water and ether and cannot be dried by heating without decomposition.) Weigh the precipitate as quickly as possible, since anhydrous calcium picrolonate absorbs moisture readily. (This method is especially applicable to the determination of small quantities of calcium owing to the large weight ratio of calcium picrolonate to calcium.)

The magnesium is contained in the filtrate from the precipitation of calcium as oxalate. Evaporate this to dryness in the silica dish on the steam bath and treat the residue with concentrated nitric acid to destroy organic material. After again evaporating to dryness, heat gently to sublime off ammonium chloride and carefully ignite. It is essential to cover the silica dish with a watchglass during ignition since the magnesium oxide or carbonate formed is very light, and losses occur owing to air currents in an uncovered dish. After cooling, carefully add distilled water; the jet from a washbottle is liable to blow away some of the residue. Add 2 or 3 ml. of dilute hydrochloric acid (1 in 10) and, after warming, wash thoroughly into a 40-ml. Pyrex beaker. Heat for 10 minutes, add excess of oxine reagent (see p. 574) and precipitate the magnesium by addition of ammonia with stirring until the solution is faintly ammoniacal. Allow to stand at least 2 hours, or preferably overnight and filter the magnesium oxinate on a previously dried and weighed sintered glass crucible. Wash the precipitate with distilled water and dry for 2 hours at 105° C. Temperatures between 105° and 160° C. (at which the anhydrous compound results) cause indefinite dehydration of the dihydrate. Weigh as magnesium oxinate dihydrate.

DETERMINATION of ALKALI METALS—If it is desired to determine sodium and potassium the filtrate from the magnesium determination is evaporated to dryness and ignited in a platinum crucible. The residue is extracted with distilled water and made up to 50 ml. exactly. A 25-ml. portion is taken for determining sodium, with magnesium uranyl acetate, and potassium is determined in the remaining 25 ml., with hexanitrodiphenylamine. The details of the sodium determination are given in "*Organic Reagents for Metals*," Hopkin and Williams, Ltd., p 160.. The details of the potassium determination may be found in the "*B.D.H. Book of Organic Reagents*," 8th Ed., p. 72.

CONVERSION FACTORS—Ferric oxinate × 0·1145 = Fe; 1 mg. of Fe requires 0·625 ml. of oxine reagent.*

Aluminium oxinate × 0·0587 = Al; 1 mg. of Al requires 1·30 ml. of oxine reagent.*

Magnesium oxinate (dihydrate) × 0·0698 = Mg.; 1 mg. of Mg requires 1·00 ml. of oxine reagent.*

Calcium picrolonate × 0·0721 = Ca; 1 mg. of Ca requires 7·5 ml. of picrolonic acid solution.*

TYPICAL RESULTS OBTAINED WITH METHOD DESCRIBED—To confirm the accuracy of the improved method over a range of concentrations of the elements present, a series of synthetic solutions was analysed. Each solution contained iron, aluminium, calcium and magnesium as chlorides. Hence the analysis corresponded to the second step onwards in the scheme of the new method, *i.e.*, analysis of the extract obtained by treating the ash sample with hydrochloric acid. The concentration of the ions present was arranged so that a 10-ml. portion was taken for analysis. Results are given in the following table.

Sample No.	Fe present in mg.	Al present in mg.	Ca present in mg.	Mg present in mg.	Fe oxinate in mg. Calc.	Fe oxinate in mg. Found	% error	Al oxinate in mg. Calc.	Al oxinate in mg. Found	% error	CaO in mg. Calc.	CaO in mg. Found	% error	Mg oxinate in mg. Calc.	Mg oxinate in mg. Found	% error
I	1·76	2·61	23·16	1·51	15·3	16·2	+5·5	44·4	44·3	−0·3	32·4	33·0	+2·0	21·6	22·2	+3·0
II	0·88	2·61	23·16	3·02	7·7	7·8	+1·3	44·4	44·3	−0·3	32·4	26·7*	—	43·2	43·2	0
III	0·88	1·31	23·16	1·51	7·7	8·3	+8·0	22·2	22·3	+0·5	32·4	32·5	+0·3	21·6	20·0	−5·0
IV	0·88	0·65	23·16	1·51	7·7	8·1	+5·0	11·1	11·0	−1·0	32·4	32·4	0	21·6	22·0	+2·0
V	3·51	0·65	23·16	3·02	30·7	30·6	−0·3	11·1	11·2	+1·0	32·4	32·5	+0·3	43·2	43·4	+0·5

* Incomplete precipitation of calcium due to insufficient oxalate added.

* These values are calculated for the concentrations of oxine and picrolonic acid reagents given on p. 574, and are sufficient for complete precipitation of the stated quantities of metal.

ADVANTAGES AND DISADVANTAGES OF THE IMPROVED METHOD—The following advantages over the usual method may be claimed, when small quantities are involved:

1 When precipitated under the conditions specified in Section (5), both iron and aluminium oxinates are obtained in a crystalline form, which settles leaving a clear solution. This is a decided advantage over gelatinous hydroxides.

2. The gravimetric determination of iron and aluminium with oxine has the further advantage that the equivalent weight of the oxinate is about six times that of the oxide. This factor is particularly useful with small quantities.

3. By evaporating the solution to dryness and igniting prior to determining the magnesium, the error in the determination is decreased, (*a*) because the final volume of solution containing the magnesium is smaller, and (*b*) because the large amount of ammonium oxalate, present after the calcium precipitation, has been removed and consequently does not interfere with the precipitation of magnesium with oxine.

The following limitations may be noted:

1. An electrical method for measuring *p*H is essential.
2. The measurement of *p*H may cause slight errors, *e.g.*, precipitate adhering to the glass electrode.
3. The method is a little slower than the usual method.
4. The error in the determination of iron seems larger when small amounts of iron are present with relatively larger amounts of aluminium. Since the error appears always to be positive, it may be due to adsorption of aluminium oxinate on the precipitated iron oxinate. It may be possible to lessen or eliminate this by re-dissolving the iron oxinate and then re-precipitating at *p*H 3·6.
5. The method is limited to the analysis of substances not containing any of the interfering elements mentioned on p. 573.

CONCLUSIONS—An improved method has been developed for the semimicro-analysis of materials such as paper ash and certain corrosion products. It relies chiefly on the use of the organic reagent 8-hydroxyquinoline for separating and determining metals under controlled conditions of *p*H. When about 1 mg. each of iron and aluminium are present they can be determined with an error of about 5 per cent. and 1 per cent. respectively, whilst larger quantities can be determined more accurately without interference by a large excess of calcium.

The author wishes to thank the Director of the British Electrical and Allied Industries Research Association for permission to publish this paper.

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BRITISH ELECTRICAL AND ALLIED INDUSTRIES RESEARCH ASSOCIATION, WADSWORTH ROAD, GREENFORD, MIDDLESEX

July, 1946

The Determination of Mercury and Copper in Anti-fouling Compositions: Potassium Cobalticyanide as Complex-forming Agent in Dithizone Technique

BY H. BARNES

STUDIES of the behaviour of anti-fouling compositions carried out for the Marine Corrosion Sub-Committee of the Iron and Steel Institute have made desirable a simple method for the determination of copper and mercury when present together in small samples of such compositions. The chemical aspect of this work has been particularly concerned with leaching-rate investigations, *i.e.*, determinations of the rate of loss of copper from painted panels, and for this purpose the exposed panels are 3 in. by 1 in. microscope slides and the painted area 2 sq.in. (see Harris[2]). In addition, small samples (usually 1 sq.in.) are taken for analysis from ships after service tests. The normal limits of mercury and copper contents in anti-fouling compositions are of the order of 5–20 and 10–50 per cent., respectively, of the film; at a spreading

rate of 155 g. per sq. metre (0·51 oz. per sq. ft.) this gives 100 mg. of paint per sq.in., allowing, therefore, 5–20 mg. of mercury and 10–50 mg. of copper for analysis. Thus the amounts available in such samples are considerably less than those taken in standard methods of paint analysis. The accuracy required for the analytical method was of the order of 1–2 per cent. of the metal present, for the over-all accuracy of this type of work is limited by the greater variations arising from painting and exposure technique together with sampling errors. Recourse has, therefore, been made to micro-methods of analysis that can be used with an ordinary analytical balance.

In addition to copper and mercury, anti-fouling compositions frequently contain compounds of lead, zinc, arsenic, iron (as oxide) and barium (as sulphate); further, an anti-corrosive coat (part of which may be included in the sample) often contains quantities of chromate. The method should, therefore, be capable of determining copper and mercury in the presence of these substances.

Mercury has been determined by the use of dithizone, with which reagent copper, zinc and lead also react. The dithizone method has recently been reviewed by Sandell,[3] and it is clear that by working at low *p*H values interference from zinc and lead might be expected to be eliminated. However, even at *p*H 1·2 copper is partially extracted. Working with sea-water solutions it has recently been shown (Barnes[1]) that the rate of extraction of copper at *p*H 1·2 with *chloroform* solutions of dithizone is slow: it was also shown that the complexing action of potassium cobalticyanide could be used to suppress further the extraction of copper from these solutions. This method has been applied in the present work. Above a final copper concentration of 1 mg. per litre the addition of cobalticyanide produces a precipitate[4]; the results indicate that this has no effect on the mercury estimation.

For the determination of copper, sodium diethyldithiocarbamate has been used.

General

The Spekker Photo-electric Absorptiometer has been employed for the determination of transmittances, using a 1 cm. cell—all readings being taken in the lower part of the drum. In using the Spekker it has been the practice to set the instrument and then take an approximate reading of the unknown; the instrument is then reset and with the shutter closed the drum is moved almost to the previous position and the final adjustment of the drum rapidly made. The final stages in the determination of mercury, involving the use of the dithizone, are always carred out in a darkened room and the transmittances are determined immediately after extraction.

It has been found more reliable to standardise the dithizone at the same time and under exactly the same conditions as the unknown, rather than to use a calibration curve. The less sensitive nature of the reagent used for the determination of copper allows a calibration curve to be used, but it is almost as convenient and more reliable to standardise by the use of a stock solution at the time of the determination.

The limits of accuracy—The sample after digestion is brought into aqueous solution and the aliquot used in the final extraction with dithizone should contain about 100 μg. of mercury. With the instrument set at maximum sensitivity a difference of 25 μg. from the standard is equivalent to a drum difference of approximately 0·100; the drum may be easily read, at the lower end of the scale, to half a division, so that an accuracy of 1 per cent. should be obtainable.

For the determination of copper the aliquot in which the colour is developed should contain approximately 50 μg.; since a difference of 12·5 μg. in the range of 40–50 μg. gives a drum difference of 0·185, an accuracy of 1 per cent. is again possible.

Method

Reagents—

For the digestion—

Sulphuric acid (A.R.): sp.gr. 1·84.
Hydrogen peroxide (M.A.R.): 100 vol.
Potassium permanganate (A.R.): saturated aqueous solution.
Hydroxylamine hydrochloride (A.R.): 4 per cent. aqueous solution.

For the determination of mercury—

Mercuric chloride (A.R.): stock solution containing approximately 6·0 g. per litre. This should be diluted to give 6·0 mg. per litre and 25 ml. of the diluted solution (approximately 100 μg. of mercury) used.

Nitric acid (A.R.): 25 per cent. v/v aqueous solution.
Hydroxylamine hydrochloride (A.R.): 4 per cent. aqueous solution.
Potassium cobalticyanide: freshly prepared 10 per cent. aqueous solution.
Dithizone: this is purified by repeated extraction with ammonia (see Sandell[3]). The stock solution (1·0 g. per litre in chloroform) is stored in a refrigerator and diluted 40–50-fold just before use to give a final concentration of approximately 20 mg. per litre.
Chloroform: B.P. quality.

For the determination of copper—

Copper nitrate: approximately 0·5 g. of pure copper foil is dissolved in a little nitric acid and made up to 250 ml. with water. For standardisation this is diluted to 2 mg. per litre and 20 and 25 ml. of the diluted solution are used.
Citric acid (A.R.): 25 per cent. aqueous solution.
Ammonium hydroxide (A.R., sp.gr. 0·88): 40 per cent. v/v aqueous solution.
Sodium diethyldithiocarbamate reagent: 0·1 per cent. aqueous solution.

THE DIGESTION—

Scrape the paint from the slide, grind thoroughly, and weigh a sample of about 30 mg. (which will contain from 1·5 to 6 mg. of mercury and from 3 to 15 mg. of copper) directly into a 10 ml. flask, the neck of which is ground to fit a reflux condenser.

Add 1 ml. of concentrated sulphuric acid and attach to the reflux condenser. Heat, at first gently and then boil until the material is thoroughly charred (not more than five minutes required). Without detaching the flask, allow to cool, completing by immersion in a beaker of cold water. Partially detach the flask and rapidly add 1–2 ml. of hydrogen peroxide, allowing the latter to wash down the ground part of the condenser. Quickly replace and after warming gently boil until the digest has cleared. (If the digestion is not satisfactory at this stage it is usually expedient to weigh out another sample). Cool thoroughly, detach the flask, add a little water, washing the end of the condenser; add 1·5 ml. of potassium permanganate solution and a small piece of porous pot (the neck of the flask and the end of the condenser should be washed with the permanganate solution). Replace the flask and boil vigorously for five minutes. Cool, detach and add sufficient hydroxylamine to remove the permanganate and precipitated oxides and 1 ml. in excess. Replace the flask and boil under reflux for a few minutes. Allow to cool and after rinsing down the condenser into a standard flask add the digest and make up to the required volume. At the same time carry out a blank digestion, taking all the reagents through the procedure indicated for the unknown.

THE DETERMINATION OF MERCURY—

A trial extraction is necessary to adjust the amount used so that it is of the same order as the standard. With experience this can be done by adding the reagents to successive portions, shaking with dithizone and noting when the colour change is similar to that obtained when the standard is used.

Transfer the required amount of solution to a 250 ml. separating funnel and add sufficient distilled water to make up to 90 ml. Prepare a solution of the standard, using 25 ml. of the diluted mercury stock solution and an amount of the blank digest equivalent to the aliquot of the unknown and making up to 90 ml. with distilled water in a second 250 ml. separating funnel. Add successively to each funnel, shaking after each addition, 2 ml. of nitric acid, 5 ml. of hydroxylamine hydrochloride solution and 5 ml. of potassium cobalticyanide solution. Allow to stand for five minutes and then add 50 ml. of dithizone solution to each funnel. Holding both funnels together, shake for two minutes (3–4 shakes per second). Allow the two layers to separate and then, after inserting a roll of filter paper into the stems of the funnels, run off the required amount from each into two 1 cm. cells. Setting the instrument against the chloroform extract of the standard at 0·300, measure the transmittance of the unknown.

The dithizone is standardised by using two known amounts of mercury and proceeding as above; it is convenient to use 25 ml. and 20 ml. of the standard solution (*i.e.*, 100 μg. and 80 μg. of mercury).

THE DETERMINATION OF COPPER—

Transfer a suitable aliquot of the digest, containing approximately 50 μg. of copper, to a separating funnel and add sufficient distilled water to make up to 75 ml. Add 10 ml. of

citric acid solution followed by 10 ml. of ammonia, shaking after each addition. Add 5 ml of sodium diethyldithiocarbamate reagent with constant shaking during the addition and allow to stand for five minutes. Add 10 ml. of chloroform and shake vigorously for two minutes. Allow the two layers to separate and, after inserting a roll of filter paper in the stem of the funnel, run off the required volume into a 1-cm. cell. Determine the transmittance with the instrument set at 1·000 against pure chloroform. A blank should be run with the same aliquot of the blank digest, and for this the instrument is set at 0·400 against pure chloroform, as before. Standardise by repeating with two known solutions containing 50 and 40 μg. of copper.

Discussion

1. In view of the known volatility of mercury compounds the reflux condenser and ground glass joints are essential; in addition this method ensures that any metallic soaps present in the medium are also brought into solution. That losses due to volatilisation of mercury are effectively prevented is shown by the recovery obtained using known amounts of mercury digested as described, in the presence of resinous materials (Table I).

Table I

Present 3·05 mg. of mercury

Added material mg.	Mercury found mg.
nil	3·05
6 mg. of rosin	3·00
4 mg. of Beck 1001	3·05
5 mg. of varnish	3·00

2. The amount of organic material present is, of course, small—about half the weight of the sample—and the simple wet ashing procedure given is adequate.

3. It would be more convenient to make successive additions of the reagents through the condenser; however, the danger of leaving traces of oxidising material, which would subsequently be washed into the solution, on the walls of the condenser is too great. Considerable care should be exercised to ensure that all traces of the oxidising agents used are completely removed, since dithizone is extremely sensitive to such substances.

4. The amount of mercury contained in the reagents is not usually large; the preparation of a blank digest and its addition to the standard makes tedious purification of the reagents unnecessary.

5. The potassium cobalticyanide solution should be freshly prepared each day.

6. It has been indicated that, at a low *p*H, only copper would be expected to interfere with the determination of mercury by means of dithizone; the results in Table II indicate that interference by copper, when present in very considerable excess, is completely eliminated by the cobalticyanide technique.

Table II

Present 115·5 μg. mercury

Copper added mg.	Mercury found μg.
0·11	115·5
0·33	115·3
0·55	115·5
0·76	115·0
0·91	115·3
1·27	114·3
1·64	115·3
1·82	115·3
2·18	115·5
2·91	115·5
3·64	115·5
4·37	115·5

Table III gives the results of some determinations of mercury in presence of considerable quantities of other metals, some of which react with the reagent under other conditions.

The figures in Tables II and III indicate that the method enables mercury to be determined in presence of such quantities of other metals as are likely to be encountered in anti-fouling compositions. Table IV gives the results of a number of mercury determinations, the amounts present being unknown to the analyst.

TABLE III

Added materials, mg.				Mercury, μg.	
Iron	Zinc	Lead	Copper	Present	Found
0·65	—	—	—	110·5	110·5
1·29	—	—	—	110·5	110·5
—	0·86	—	—	110·5	110·5
—	1·77	—	—	110·5	110·5
—	2·66	—	—	110·5	111·5
—	—	0·31	—	110·5	110·5
—	—	0·61	—	110·5	112·0
1·29	1·77	0·61	—	110·5	109·0
1·29	—	—	1·09	115·5	115·5
1·94	—	—	1·09	115·5	115·5
—	1·77	—	1·09	110·5	110·0
—	2·66	—	1·09	110·5	110·5
—	—	0·61	1·09	110·5	110·0
—	—	0·92	1·09	110·5	109·0

TABLE IV

Added material, mg.				Mercury, μg.	
Iron	Zinc	Lead	Copper	Present	Found
—	—	—	0·73	110·5	110·5
1·29	0·89	—	0·73	101·5	102·0
1·29	—	0·61	1·45	88·5	88·5
0·65	0·89	0·30	3·63	97·0	96·0
0·39	1·42	—	1·45	107·0	106·0

The results quoted in Tables II, III and IV were obtained directly on prepared solutions and not on materials carried through the digestion procedure.

The results in Table V were obtained on materials taken through the digestion procedure.

TABLE V

MERCURY PRESENT 5·45 mg.

Added materials, mg.								Mercury found, mg.
As_2O_3	$BaSO_4$	ZnO	PbO	K_2CrO_4	Fe_2O_3	Cu_2O	Rosin	
10·5	3·6	3·2	3·0	2·9	10·7	26·9	5·4	5·40
4·0	10·6	5·0	7·6	7·5	6·0	18·9	8·8	5·48
2·9	3·6	1·8	10·1	5·8	10·4	14·0	12·4	5·45
5·3	8·9	6·0	7·2	7·8	15·5	16·3	9·8	5·40
13·5	6·7	13·9	6·1	6·4	10·7	26·7	9·5	5·39

7. The carbamate method for the determination of copper is well established (see Sandell[3] for a review). Various solvents have been used for the extraction of the coloured complex. The advantages of chloroform in the present instance are that it is heavier than water and that it is capable, in one extraction, of taking out most of the coloured complex. A disadvantage is its relative solubility in water; but if standardisation is carried out under precisely the same conditions errors arising from this are eliminated. If the copper is determined on the same conditions errors arising from this are eliminated. If the copper is determined on the same sample as is used for the mercury determination the solution will contain large quantities of manganese. By careful work and adequate blanks allowance for any interference can be made, but the correction is rather high. It is much more satisfactory to use a separate sample for the copper, and this is more convenient from the general point of view when a large number of samples are to be analysed. When the copper is determined in this way it is not necessary to take extreme precautions for removing the hydrogen peroxide added to the digest; after the solution has cleared it is sufficient to add a little water and to boil vigorously for ten minutes. It may not be essential to use a condenser when the copper determinations are made on separate samples, but a condenser has always been used in the work here described.

8. Of the metals that are frequently present, zinc, mercury, lead and iron give insoluble or coloured products with the sodium diethyldithiocarbamate. Interference due to iron is eliminated by working in ammoniacal citrate solution. That no interference is obtained from

other metals in quantities likely to be encountered in anti-fouling compositions is indicated by the results in Table VI.

TABLE VI

COPPER PRESENT, 55·8 μg.

Added materials, μg.				Copper found, μg.
Mercury	Lead	Zinc	Iron	
70	70	180	120	56·4
260	70	350	120	56·0
260	240	350	120	55·6
70	240	180	260	56·4
70	70	350	260	56·0
260	240	350	260	56·6

SUMMARY

1. A method for the determination of mercury and copper in anti-fouling compositions has been given, using dithizone and sodium diethyldithiocarbamate. 2. A simple wet ashing procedure is described. 3. The use of potassium cobalticyanide and a chloroform solution of dithizone for the elimination of interference due to copper, even when present in considerable excess, in the determination of mercury is outlined.

Permission to publish this paper has been given by the Marine Corrosion Sub-Committee of the Iron and Steel Institute; the author wishes to thank his wife for checking the whole of the technique and Professor J. E. Harris for his interest in the work.

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THE MARINE STATION
MILLPORT, SCOTLAND

September, 1946

Notes

A MICRO CELL FOR POLAROGRAPHY AND AMPEROMETRIC TITRATION

A STUDY of polarographic cells for the examination of small volumes of liquid was made by Majer.[1] Apparatus for the same purpose has been described by several other workers.[2,3,4,5] The cell described by Langer[5] incorporates a saturated calomel reference electrode, and provision is made for the introduction of a microburette, to permit the amperometric titration of samples less than 1 ml. in volume.

The cell shown in Fig. 1*a* was devised for the polarography and amperometric titration of samples too small to be examined in the semi-micro cell previously developed.[6] Cap A, body B and solution container C are of Pyrex and are connected by ground joints. Several interchangeable solution containers are provided, so that a series of samples may be examined without dismantling the apparatus. Dropping mercury electrode D is held in position by a sleeve of rubber tubing, and fits snugly in the tube surmounting the cap. Similar sleeves retain the outlet of the micro-burette E[7] and the gas inlet tube F. The extremities of the micro-burette and the gas inlet tube are arranged tangentially as shown in Fig. 1*b*. Entry of gas produces a spinning motion in the liquid,[8] promoting mixing and elimination of dissolved oxygen. Since the immersion of the tip of the gas inlet tube need not exceed 1–2 mm., spurting is much reduced. Gas escapes through a trap attached to the side tube of A, the narrow annulus around the dropping mercury electrode being sufficient to allow this.

Mercury is introduced into container C up to a mark etched on the walls. The solution to be examined (0·5–1 ml.) is then introduced and C is attached to the body of the cell. Electrical connection to the mercury pool is made by platinum wire G. After eliminating dissolved oxygen, the amperometric titration is carried out. The container is then detached, the tips of the dropping mercury electrode, gas inlet tube and micro-burette are wiped with strips of filter paper, and another previously filled container is placed in position. For polarographic measurements the micro-burette is replaced by a plug of glass rod, or by a pencil-type saturated calomel electrode,[9] the tip of which is immersed in the liquid in the cell. The potential of the mercury pool may thus be examined.

The flow of mercury from the dropping electrode is normally insufficien to cause an excessive rise in the level in C. When the examination is prolonged, *e.g.*, when using sluggish precipitants, the solution container shown in Fig. 1*c*, designed to minimise the rise in level, is used.

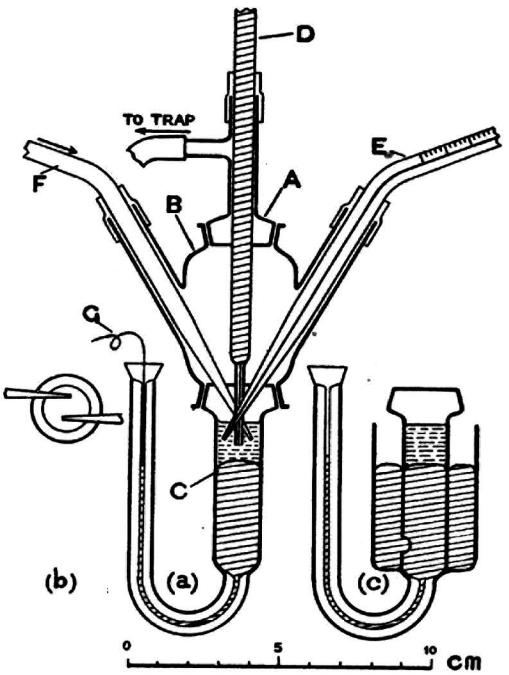

Fig. 1 (a) (b) (c)

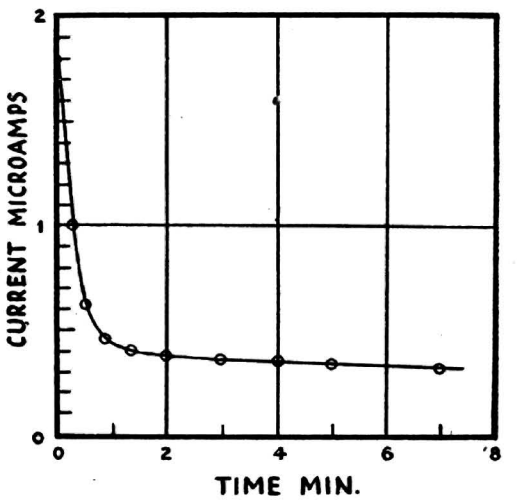

Fig. 2.

Elimination of dissolved oxygen from 0·5-ml. portions of 0·1 *N* potassium chloride solution prepared from distilled water containing air is shown in Fig. 2. The solution contained 0·02 per cent. of methyl red as a maximum suppressor, and the tip of the gas inlet tube was 1 mm. below the surface. Nitrogen was led in at 2 bubbles per sec. for a predetermined period. The current, corresponding to the sum of the heights of the two oxygen waves[10] was measured at an applied voltage of −1·4. The characteristics of the dropping electrode were as previously reported.[6] The results show that nearly all of the dissolved oxygen is eliminated in 2 min. Removal of the last traces is extremely difficult[11]; for most purposes a standard outgassing time of 5 min. was adopted.

Titrations of copper with α-benzoinoxime[12] were carried out in the cell, the solutions being prepared and standardised as previously described.[6] Dissolved oxygen was removed by a stream of nitrogen and the latter was passed in for 3 min. after each addition of reagent. Typical results are given in Table I.

TABLE I

0·01 *M* Copper sulphate solution added to 0·5 to 0·8 ml. of a supporting solution 0·1 *M* in ammonium chloride, 0·02 *M* in ammonia and 0·02 per cent. in gelatin. Titrated with 0·01 *M* α-benzoinoxime solution. Applied voltage −1·7. T = 12–13°.

Vol. of solution titrated, ml. ..	0·93	0·80	0·74	0·65	0·61
Copper taken, μg.	9·2	7·5	6·3	5·0	3·4
,, found, μg.	9·0	7·4	6·4	4·8	3·0
Error, μg.	−0·2	−0·1	+0·1	−0·2	−0·4

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CHEMISTRY DEPARTMENT
L.C.C. NORWOOD TECHNICAL INSTITUTE
KNIGHT'S HILL, S.E.27

J. T. STOCK
July 23rd, 1946

A POLARISING UNIT FOR AMPEROMETRIC TITRATION

ALTHOUGH involving similar principles, amperometric titration[1,2,3] differs from polarography in being carried out at an unvarying applied e.m.f. Since the titration is normally performed so that the diffusion current[4] of the substance to be determined, of the titrant, or of both, is obtained, the current is not greatly affected by the potential of the indicator electrode. In general, it is sufficient to adjust the e.m.f. applied to the electrodes, and hence the potential of the indicator electrode, to the nearest one-tenth of a volt. For this purpose, a simple potentiometer device is quite satisfactory.

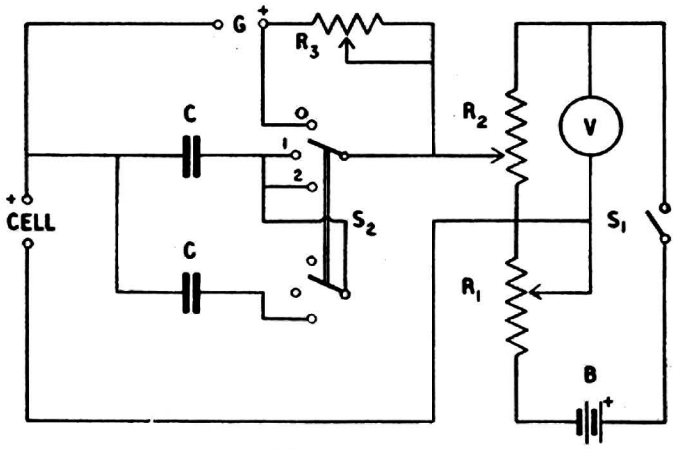

Fig. 1

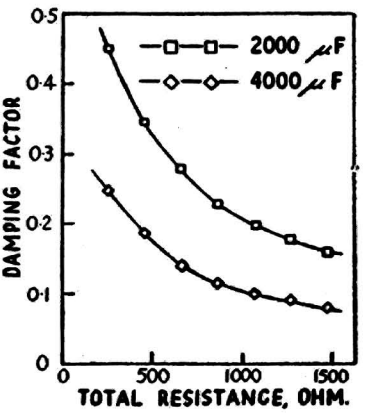

Fig. 2

R_1, R_2 500 ohm potentiometers. S_2 Double-pole three-way switch.
R_3 5000 ,, ,, V 0–3 v. voltmeter.
C, C 2000 μF. condensers. B 3 v. battery.
S_1 On-off switch. G Galvanometer terminals.

When using the dropping mercury electrode as indicator, the growth and fall of the mercury drops causes the galvanometer spot to oscillate. However, a condenser of large capacity connected across the galvanometer shunts damps the oscillations.[5,6] A galvanometer of comparatively short period (3 secs. or even less) may then be easily read. For adequate damping, the parallel resistance of the galvanometer and its shunt, which depends upon the setting of the shunt, must not be too small.[6] When the parallel resistance is too small, an auxiliary variable resistance may be connected to enable adequate damping to be obtained.[7]

The design of the unit is based upon the principles discussed above. The dimensions of the cabinet, which also contains the battery, are 9 in. × 9 in. × 5 in. The condensers and battery are secured to the bottom of the cabinet and are connected by flexible leads to the paxolin panel which carries all other parts.

Circuit arrangements are shown in Fig. 1. Most of the components are standard radio parts. A constant voltage drop, adjusted by rheostat R_1 and indicated by voltmeter V, is maintained across potentiometer R_2. The latter is provided with a graduated scale calibrated in the usual way and controls the voltage applied to the electrode system in the titration cell. Since the voltmeter is always adjusted to the same reading, it needs to be precise at this

reading only. Accordingly a comparatively cheap instrument may be used,[8] or a meter designed for other purposes may be adapted. Thus the present unit employs an available 0–500 microammeter, suitably equipped with a series resistance, R_1 being adjusted to give exactly full-scale deflection. Three-way switch S_2 enables one or both of the condensers to be connected. With the switch in position O, no damping occurs. The condensers should be of good quality and should be connected with due regard for their polarity markings. Rheostat R_3 enables adequate damping to be obtained if the parallel resistance of the galvanometer and shunt is small; when switch S_2 is in position O, this rheostat is automatically short-circuited.

The apparatus was tested by examining the diffusion currents at −1·0 volt with respect to a mercury pool anode of various concentrations of copper (as copper sulphate) in 0·1 *M* potassium chloride solution. Two Cambridge galvanometers were used in turn to measure the current. The first, of the internally-illuminated type, had a period of 2·3 sec., and was critically damped. Typical results, obtained with an electrode drop time of 4·36 sec., are shown in Fig. 2. The reduction in oscillations is here expressed as the "damping factor,"[6] *i.e.*, the ratio of the observed oscillation to that obtained with the damping device inoperative. Thus in one set of experiments, the parallel resistance of the galvanometer and shunt was 245 ohm and the oscillation with the damping device inoperative was 20·9 scale divisions (over 4 cm.). Movement of switch S_2 to bring first one, then both, of the condensers into the circuit reduced the oscillation to about one-half and one-quarter respectively. Rotation of rheostat R_3 caused a further reduction in the damping factor, as shown.

The second galvanometer had a period of 1·3 sec. and was operated at full sensitivity, its resistance being 40 ohm. The spot of light was focussed on a mm. scale 1 metre from the mirror. Using a drop time of 2·91 sec. and no damping, the oscillations were so wild that location of the extremes was impossible. Application of damping enabled useful results to be obtained. Owing to the low galvanometer resistance, the degree of damping without auxiliary resistance was small and accurate readings were difficult to obtain. Rotation of rheostat R_3 greatly reduced the oscillations and permitted easy, accurate reading.

Satisfactory amperometric microtitrations[9] of nickel, copper, etc., have been carried out using the apparatus, which has proved to be very convenient.

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CHEMISTRY DEPARTMENT
L.C.C. NORWOOD TECHNICAL INSTITUTE, S.E.27

J. T. STOCK
August, 1946

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in THE ANALYST (1946, **71**, 492).

Public Analysts	*Appointments*
ILLING, Edward Thomas	Somerset County Council.
MOIR, Daniel Donald	Borough of Surbiton.
BRANSON. Victor Cecil (Deputy)	County Boroughs of Brighton and Eastbourne and Borough of Worthing.
HAWKINS, Ernest Stephen (Additional) ..	Boroughs of Dover and Ramsgate.
CARLOS, Arthur Sydney (Additional) ..	Borough of Poole.

NOTIFICATION of the following Public Analyst appointment has been received from the Department of Health for Scotland.

Public Analyst	*Appointment*
SCOTT-DODD. Alexander	County of Inverness.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

NOTIFICATION of the following appointment of Official Agricultural Analyst has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1946, 71, 493).

Official Agricultural Analyst	*Appointment*
BRANSON, Victor Cecil (Deputy)	County Borough of Brighton.

British Standards Institution

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

Draft Specification prepared by Technical Committee FCC/4—Solvents.
CH(FCC) 7793—Draft for Ethyl Acetate (Revision of B.S. 553).
CH(FCC) 7791—Draft for Acetone (Revision of B.S. 509).
CH(FCC) 7792—Draft for Normal Butyl Acetate (Revision of B.S. 551).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Rapid Determination of Fat in Meat Products R. B. Oesting and I. P. Kaufman (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 125—The apparatus required consists of a Waring mixer, Babcock bottles (8%, 18-g, calibrated in 0·1%), a cream pipette and a Babcock centrifuge. Mix 25 g of the finely ground sample with 100 g of cracked ice (or water at 1° to 3° C.) and 2 g. of household Oakite* for 10 min. in the Waring mixer, stopping at intervals to stir the mixture and dislodge fragments from the sides of the container. Mix thoroughly, treat 10 ml. of the emulsion in a Babcock bottle with 5 ml. of glacial acetic acid, and again mix thoroughly to coagulate the protein. Add 10 ml. of conc. sulphuric acid, a little at a time, and swirl until all lumps are dissolved. Add enough hot water to form a layer over the mixture, so that the fat rises above the water and charring is reduced to a minimum. Add 5 ml. more of sulphuric acid, mix and centrifuge for 5 min. at 1000 r.p.m., then add hot water to bring the surface of the mixture just into the neck of the bottle, centrifuge for 2 min.; finally add hot water to within 1 or 2 cm. of the top of the neck and centrifuge for 1 min. Immerse the bottle in water at 70° C. and read the fat content after 2 min., reading from the top of the upper meniscus to the bottom of the lower one. Multiply the reading by 9·2 to obtain the % of fat and apply a correction if the wt. of sample was not exactly 10 g. As compared with the official A.O.A.C. method ("*Methods of Analysis*," 1940, p. 356) the probable error is ±0·5%. The method gives satisfactory results with all types of fresh or cooked meat products with the exception of those with a high cereal content. The cereal forms a layer which interferes with the reading. Satisfactory results have been obtained by immersing the bottle in boiling water, which causes the fat to rise above the cereal. A. O. J.

* *Note by Abstractor*—A sample of "Oakite A Special" recently analysed consisted of triple sodium phosphate, soda ash and caustic soda.

Spectrophotometric Method for the Estimation of Penicillin. R. M. Herriott (*J. Biol. Chem.*, 1946, **164**, 725–736)—Crystalline salts of the pure penicillins, freshly dissolved in acetate buffer of *p*H 4·6, do not absorb in the region of 290 to 360 mμ, but after being heated in this buffer they absorb strongly in this region with a maximum at 322 mμ. Impurities in crude penicillin preparations absorb in this region, but the increase on heating is due solely to the penicillin present. Thus the amount of penicillin can be estimated by measuring the change in absorption at 322 mμ.

Procedure A—To 6 ml. of 0·4 *M* acetate buffer solution of *p*H 4·6 add a 2-ml. portion of penicillin solution containing 35 to 500 Oxford units per ml. Transfer 4 ml. of the mixture to another test tube and heat one of the tubes in boiling water for 15 mins. and then cool rapidly. Evaluate the absorption density of the heated and unheated solutions at 322 mμ in a photoelectric spectrophotometer.

Procedure B—To 4 ml. of the penicillin solution containing 8 to 80 Oxford units per ml., add 0·25 ml. of a 5 *M* acetate buffer solution of *p*H 4·6 and continue as in procedure A. Procedure B is used when the concentration of penicillin is low or when little material is available.

Tests on the pure crystalline sodium penicillins G, X, K or F showed that the change in absorption at 322 mμ is independent of the nature or proportion of the various penicillins, so that crystalline sodium penicillin G can be used to construct a standard curve from which to calculate the concentration of total penicillin. The results were reproducible, individual values deviating from the average only rarely by more than 5%. Results obtained with samples of commercial penicillin were in good agreement with those obtained by microbiological assay and by the use of Scudi's colorimetric method (ANALYST, 1946, **71**, 542). F. A. R.

Biochemical

Spectrophotometric Estimation of Hexuronates (expressed as Glucuronic Acid) in Plasma or Serum. W. B. Deichmann and M. Dierker (*J. Biol. Chem.*, 1946, **163**, 753–760)—The method is a modification of that previously described (*J. Lab. Clin. Med.*, 1945, **28**, 770) for the estimation of glucuronates in urine; glucuronates can be estimated in plasma or serum without preliminary removal of proteins or glucose. The acidified sample is hydrolysed at 75° C., an excess of naphthoresorcinol is added and the mixture is heated at 50° C., the pigment is then extracted with ether and the colour of the ethereal solution is evaluated at 570 mμ. It is possible that the reagent

reacts with other compounds related to hexuronates, but in practice errors from this source do not appear to have been encountered.

Dilute 0·2–1 ml. of plasma or serum to 1·8 ml. with water in a glass-stoppered 50-ml. graduated cylinder and add 0·2 ml. of 19% hydrochloric acid. Leave for 45 min. in a water bath at 75° C. and then add 2·0 ml. of conc. hydrochloric acid and 1·0 ml. of a 10% solution of naphthoresorcinol in 95% ethanol. Leave the mixture for 90 mins. in a water bath at 50° C., cool and shake the solution in the cylinder with 8 ml. of ether for about 5 seconds. Allow to separate, add 5 ml. of ether and mix this with the upper layer by gentle rotation. Add a further 5 ml. of ether in the same way and leave for 3 mins. until the upper layer is clear. Pipette the coloured ether layer into a glass-stoppered flask and evaluate the colour in a spectrophotometer at a wave-length of 570 mμ, using a 10 mm. cell. Calculate the results from a graph obtained in a similar manner using aqueous solutions of pure glucuronic acid or of borneol or menthyl glucuronate. The recovery of glucuronic acid added to rabbit plasma was almost theoretical with amounts up to 40 μg., but tended to be somewhat low with greater amounts

F. A. R.

Microdetermination of α- and β-Glycerophosphates. C. F. Burmaster (*J. Biol. Chem.*, 1946, **164**, 233–240)—In this method, α-glycerophosphate is converted to glycollic aldehyde phosphate by reaction with periodate at room temperature, the excess periodate and the iodate formed are destroyed with sodium sulphite and the glycollic aldehyde phosphate is hydrolyzed with hot acid; the resulting orthophosphate is measured by the colorimetric method of Kuttner and Lichtenstein (*J. Biol. Chem.*, 1930, **86**, 671). The value for the inorganic phosphate has to be determined and subtracted from the result to give the true α-glycerophosphate value. Total (α + β)-glycerophosphate is determined on another portion of the solution by converting all the glycerophosphate to the α-form and then repeating the procedure. Estimation of total phosphorus serves as a useful check on the method.

Inorganic phosphorus—Pipette a 5-ml. sample, containing 0·01–0·05 mg. of phosphorus, into a 10-ml. glass-stoppered graduated cylinder and add in succession 1 ml. of 4% sodium sulphite solution, 1 ml. of 10 *N* sulphuric acid and 1 ml. of 7% sodium molybdate solution. After mixing, add 1 ml. of dilute stannous chloride solution (dissolve 40 g. of stannous chloride in 100 ml. of conc. hydrochloric acid and dilute 1 ml. to 200 ml. with water before use) and dilute the solution to the 10-ml. mark with water, stopper, and invert the tube 6 times. Evaluate the blue colour after 20 mins. in a photoelectric colorimeter with a No. 540 filter. Set the zero point of the instrument with a reagent blank and calculate the phosphorus content by comparing with standard solutions of KH_2PO_4 containing 0·01 mg. of phosphorus per ml.

α-Glycerophosphate phosphorus—Pipette a 5-ml. sample, containing 0·01–0·05 mg. of phosphorus, into a test tube and add 1 ml. of 0·05 *M* periodic acid and 1 ml. of 0·1 *N* sulphuric acid. After 10 mins. add 1 ml. of 4% sodium sulphite solution and 1 ml. of 10 *N* sulphuric acid and half immerse the tube in boiling water for one hour. Cool, transfer the solution to a 10 ml. graduated cylinder and make up to the mark with the washings. After half-a-dozen inversions, treat a 5 ml. portion as described for the estimation of inorganic phosphorus, except that only 0·5 ml. of additional 10 *N* sulphuric acid is added. This procedure gives the α + inorganic phosphorus.

Total (α + β)-glycerophosphate phosphorus—Pipette a 5-ml. sample containing 0·01–0·05 mg. of phosphorus, into a test tube containing 1 ml. of 10 *N* sulphuric acid and 1 ml. of 0·05 *M* periodic acid. Half immerse in boiling water for one hour, keeping the volume at 5–7 ml. by addition of water. Cool, add 1 ml. of 4% sodium sulphite solution, transfer to a 10-ml. graduated cylinder and make up to the mark with the washings. Use a 5-ml. portion for the colorimetric estimation as described above, but with addition of only 0·5 ml. of 10 *N* sulphuric acid. This procedure gives α + β + *inorganic phosphorus*.

Total phosphorus—Pipette a sample containing 0·01–0·05 mg. of phosphorus into a test tube containing 1 ml. of 10 *N* sulphuric acid and a few glass beads. Heat over a micro-burner until the solution darkens, allow to cool for a minute and then add one drop of hydrogen peroxide. Heat gently until the solution clears and then digest until fumes of sulphur trioxide appear. Cool, transfer to a 10-ml. graduated cylinder and dilute to volume. Evaluate the colour as described above, using a 5 ml. portion of the solution, but adding only 0·5 ml. of 10 *N* sulphuric acid. Although it is not usually necessary to run this determination, it is a useful check on the others. The method gave results in good agreement with the standard titrimetric method of Fleury and Paris (*Compt. rend.*, 1943, **196**, 1416), and the sum of the average values for the inorganic, α- and β-phosphorus content of a sample of sodium glycerophosphate equalled the average total phosphorus content.

F. A. R.

Effects of the Volatile Aldehydes formed on the Accuracy of the Manometric Ninhydrin-Carbon Dioxide Method in Analysis of Certain α-Amino Acids. P. B. Hamilton and D. D. Van Slyke (*J. Biol. Chem.*, 1946, **164**, 249–256)—The observation of Schott *et al.* (*J. Biol. Chem.*, 1944, **154**, 397) has been confirmed that in determining the "carboxyl-nitrogen" of valine and of the leucines by the manometric ninhydrin-carbon dioxide method of Van Slyke, Dillon, MacFadyen and Hamilton (*J. Biol. Chem.*, 1941, **141**, 627, 671), addition of hydrazine to the reagents in the gas chamber increases the accuracy by abolishing a plus error caused by volatile aldehydes formed from these amino acids. Analyses of other amino acids were not affected by the aldehydes formed and, in estimating the free amino acids in, *e.g.*, protein hydrolysates, the addition of hydrazine reduces the result by less than 1%. It is, however, recommended as a precaution that 2 g. of hydrazine sulphate per 100 ml. be added to the 2 *N* lactic acid used as reagent.

F. A. R.

Microdetermination of Glutamic Acid and its Application to Protein Analysis. B. A. Prescott and H. Waelsch (*J. Biol. Chem.*, 1946, **164**, 331–343)—Glutamic acid reacts with ninhydrin to yield β-formyl propionic acid, the 2:4-dinitrophenylhydrazone of which gives in alkaline solution a reddish brown colour with a molecular extinction coefficient of approximately 28,000 at 420 mμ. Aspartic acid interferes with the reaction as it also yields a hydrazone which is partially extracted from organic solvents by alkaline solutions, and it is therefore necessary to separate glutamic acid from aspartic acid before carrying out the ninhydrin reaction. This is accomplished chromatographically. A further source of interference arises from

the fact that ninhydrin and its decomposition product, phenylglyoxal-*o*-carboxylic acid, form dinitrophenylhydrazones, some of which may be carried into the final solution. Hence the excess of ninhydrin has to be removed; this is done by forming a complex with guanidine. When chromatographic separation is employed, interference from other amino acids is eliminated, with the exception of cystine and cysteine which, after reaction with ninhydrin, form acidic hydrazones. A correction must therefore be applied for these amino acids by subtracting 12% of the amount found to be present by independent methods.

Chromatographic adsorption—Stir 10 g. of aluminium oxide with 50 ml. of *N* hydrochloric acid for one minute, decant the acid and wash the alumina with water by decantation 8–10 times until the washings are neutral. Fill the stem of an adsorption tube 8 cm. long and of 2·3 mm. internal diameter with alumina to a height of 6 cm., fill the tube with water and centrifuge for 30 mins. at 2,000 r.p.m. Neutralise the glutamic acid solution to *p*H 7 and dilute so that 1 ml. contains 25 μg. of glutamic acid. Prepare 2 different dilutions of the solution and determine each in duplicate, together with 2 blank determinations. For each determination, filter 2 ml. of the solution by gravity through an adsorption tube and, when the surface of the liquid reaches a point about 1 mm. above the alumina, add 2 ml. of water. Discard the filtrate and washings. Elute by passing two 2-ml. portions of 0·5 *N* acetic acid through the column into a 15-ml. graduated centrifuge tube.

Reaction with ninhydrin—To each of the tubes containing eluate add 20 ± 0·5 ml. of ninhydrin and immerse the tubes in boiling water for exactly 10 minutes. Cool in ice for 2 mins. and add in succession with intervals of 5 mins. after each addition, 0·4 ml. of 14% guanidine carbonate solution, 1 ml. of 12% lead acetate solution and 0·5 ml. of 5 *N* sodium hydroxide. Dilute the mixtures to 6 ml. with water, stir, centrifuge for 10 mins. and decant the supernatant solutions into small test tubes. Add 5 ml. portions of the solutions to tubes containing 3 ml. of a 0·1% solution of 2:4-dinitrophenyl-hydrazine in *N* hydrochloric acid, cool in ice and mix by passing a stream of air through the tubes. After exactly 10 mins. add 10 ml. of capryl alcohol and stir with a vigorous stream of air for 1 min. Remove most of the aqueous phase and clarify the turbid capryl alcohol layers by centrifuging for 5 mins. Transfer 9-ml. portions to other centrifuge tubes and add 6 ml. of borate buffer (20 g. of sodium tetraborate and 5·3 g. of sodium carbonate in 1 litre of water with the *p*H adjusted to 10). Mix with a stream of air for 1 min. and centrifuge for 20 mins.

Colour development—Mix a 5-ml. portion of the borate solution with 2 ml. of absolute ethanol in a cuvette and immerse in a water-bath at 25° C. for 5 mins. Mix the solution with 1 ml. of 5 *N* sodium hydroxide and exactly 2 mins. later measure the colour in a spectrophotometer at 420 mμ against a blank containing 5 ml. of borate buffer, 2 ml. of absolute ethanol and 1 ml. of 5 *N* sodium hydroxide. Prepare a standard curve using a solution of the 2:4-dinitrophenylhydrazone of β-formyl propionic acid (prepared by dissolving 50–60 mg. in 10 ml. of absolute ethanol and 1 ml. of pyridine, diluting to 100 ml. with absolute ethanol, and then immediately before use diluting 2 ml. of the stock solution to 100 ml. with borate buffer). In the range of optical densities from 0·04 to 0·75, this curve approximates to a straight line, expressed by the formula $G = 50D - 0{\cdot}5$ (where G is the amount of glutamic acid and D is the optical density). The amount of glutamic acid present in the original sample is calculated from the expression: $1{\cdot}6 \times F_c(A - B) - 0{\cdot}12C$, where A is the amount of glutamic acid read off from the standard curve, B is the amount corresponding to the blank and C is the amount of cystine + cysteine. The factor 1·6 is derived from the dilution factors, 5/6, 9/10 and 5/6. F_c is a constant representing the retention of a portion of the glutamic acid on the alumina column and the distribution of the hydrazone between capryl alcohol and the aqueous acid and alkaline solutions; using the conditions described above, it has a value of 1·4, but varies slightly with each batch of reagents.

In the absence of interfering substances, the chromatographic adsorption may be omitted and the reaction with ninhydrin carried out in 4 ml. of 0·5 *N* acetic acid. Two blanks containing 4 ml. of acetic acid are included in each set of determinations. Instead of F_c, a constant F_p, based on the distribution of the hydrazone between capryl alcohol and the aqueous solutions, is used; this generally amounts to 1·2, but, like the constant F_c, varies somewhat with different reagents.

The glutamic acid contents of several proteins were determined, and the results were in good agreement with those obtained by other methods.

F. A. R.

Estimation of Methionine in Protein Hydrolysates with *Lactobacillus fermenti* 36. M. S. Dunn, M. N. Camien, S. Shankman and H. Block (*J. Biol. Chem.*, 1946, **163**, 577–587)—The most reliable organism for assaying methionine in protein hydrolysates was found to be *Lactobacillus fermenti* 36, using as the basal medium "Medium C" of Dunn, Camien and Shankman (*J. Biol. Chem.*, 1945, **161**, 657; ANALYST, 1946, **71**, 241). Similar assays were carried out simultaneously with *Leuconostoc mesenteroides* and with *Lactobacillus arabinosus* as previously described by Dunn, Shankman, Camien, Frankl and Rockland (*J. Biol. Chem.*, 1944, **156**, 703; ANALYST, 1945, **70**, 182) and by Shankman (*J. Biol. Chem.*, 1943, **150**, 305) respectively; these organisms gave less satisfactory results. The volume used in each tube was 3 ml., and all the solutions were adjusted to the same concentration of sodium chloride to compensate for any stimulatory or inhibitory salt effects. The tubes were incubated for 2 days at 35° C. The standard was run at 14 levels up to 28 μg. of the amino acid, whilst the amino acid test solutions and protein hydrolysates were run at 5 levels; 6 tubes were used at each level of sample and standard. The contents of the 6 tubes at each level were mixed and titrated, and the average titration value was calculated from these data. *d*(+)Methionine was found to be completely inactive in promoting the growth of *L. mesenteroides* or *L. arabinosus*, but it stimulated the growth of *L. fermenti* to the same extent as did the *l*(−)isomer. The average mean deviation from the mean values was 2·5% for 3 mixtures containing 4·81, 1·65 and 0·42% of *dl*-methionine, and recoveries of methionine from acid hydrolysates of casein or silk fibroin approximated to 100%. The probable values for the methionine contents of these 2 proteins are 3·0 and 0·14% respectively.

F. A. R.

Estimation of Threonine in Protein Hydrolysates with *Lactobacillus fermenti* 36. M. S. Dunn, S. Shankman, M. N. Camien and H.

Block (*J. Biol. Chem.*, 1946, **163**, 589–598)—The basal medium employed was "Medium C" of Dunn, Camien and Shankman (*J. Biol. Chem.*, 1945, **161**, 657; ANALYST, 1946, **71**, 241), and the assay procedure was that used in the assay of methionine (*cf.* preceding abstract), except that 3 drops of 0·8% bromothymol blue indicator solution were added to each 3 ml. of test solution to give a satisfactory end-point in presence of the discoloration produced by caramelisation of the amino acids. The standard was run at 15 levels up to 84 μg. of *dl*-threonine, whilst the amino acid test mixtures and protein hydrolysates were run at 5 levels; 6 tubes were used at each level of standard and sample. The average mean deviation from the mean values at 5 levels for mixtures containing 2·50, 1·67 and 0·98% of *l*(−)threonine was 3·5%. The recoveries of threonine from acid hydrolysates of protein were not as quantitative as with methionine, ranging from 93 to 110% of the theoretical. Unsatisfactory results were obtained when attempts were made to assay threonine by means of *Leuconostoc mesenteroides* or *Lactobacillus arabinosus*. The threonine contents of casein and silk fibroin were found to be 4·3 and 1·2% respectively.

F. A. R.

Iodimetric Micro-determination of Arsenic in Biological Material. G. A. Levvy (*Biochem. J.*, 1943, **37**, 598–601)—The first stage of the method comprises digestion of the biological material with perchloric acid to convert the arsenic into arsenic trichloride. This is concentrated, if necessary, by distillation and then reduced to arsine, which is converted into arsenite, and this is titrated with iodine. *Digestion* (*small-scale*)—Put 5 ml. of blood, 10 ml. of urine or 2 g. of tissue into a 100-ml. Kjeldahl flask and add 5 ml. of conc. nitric acid, 3 ml. of perchloric acid and 10 ml. of conc. sulphuric acid together with 2 or 3 glass beads. Heat the flask, with shaking, over a naked flame until brown fumes are given off and then heat gently until these cease to be evolved. Boil until white fumes appear and the clear liquid becomes colourless, and, after a further 10 min., cool and add 5 ml. of sat. ammonium oxalate soln. Again heat to boiling and continue the heating until white fumes appear. Cool, add 5 ml. of water and again heat to b.p., continuing until 5 min. after the first appearance of white fumes. A blank with reagents alone is carried out in the same way. *Digestion* (*large-scale*)—Put 20 g. of minced tissue wrapped up in very thin paper, or 30 ml. of blood, into a 300-ml. Kjeldahl flask with a ground-glass neck, add 30 ml. of conc. nitric acid and 2–3 glass beads and leave overnight. In tests on urine, mix not more than 150 ml. with one-fifth its vol. of fuming nitric acid and evaporate to about 10 ml. To the soln. obtained in either instance, add 15 ml. of perchloric acid and 20 ml. of conc. sulphuric acid, warm cautiously until reaction sets in, and then digest as before. When digestion is complete (more nitric acid may be required), add 20 ml. of sat. ammonium oxalate soln. and of water. *Distillation*—After large-scale, but not small-scale, digestion it is necessary to distil off the arsenic trichloride. Add 50 ml. of water to the Kjeldahl flask, cool and add through a wide-bore funnel 8 g. of hydrated ferrous sulphate, 2 g. of potassium bromide and 25 g. of sodium chloride. Immediately connect the flask, by means of a delivery tub with a ground-glass joint lubricated with sulphuric acid, to a 150-ml. Fresenius flask (containing 10 ml. of water) immersed in ice-water. Put a 200-ml. volumetric flask over the exit of the Fresenius flask and boil the liquid in the Kjeldahl flask vigorously for exactly 10 min.; then, with the liquid still boiling, disconnect the delivery tube. Transfer the contents of the Fresenius flask to the 200-ml. volumetric flask, cool and dilute to the mark. *Separation as arsine*—Fit two absorption tubes made from conical centrifuge tubes, 9·5 × 1·4 cm., with 1·5 ml. of 0·02 *N* silver nitrate and connect them together so that one tube acts as a guard tube. Stopper and connect the tubes to a small wash-tube containing 10 *N* sodium hydroxide (renewed after 12 determinations), using glycerin as lubricant for the rubber tubing. After a small-scale digestion transfer the contents of the Kjeldahl flask to a 150-ml. conical flask with 40 ml. of water, or make up an aliquot portion of the diluted digest to 50 ml. with 20% sulphuric acid. Add 10 drops of 40% stannous chloride soln. in conc. hydrochloric acid and 3 drops of 5% copper sulphate soln. Quickly introduce 15 g. of zinc shot and connect the flask immediately to the absorption apparatus. After a large-scale digestion make up a suitable vol. to 100 ml. in a 150-ml. conical flask with 20% sulphuric acid, add 10 drops of the stannous chloride soln. per 100 ml., 3 drops of the copper sulphate soln. per 100 ml. and 25 g. of zinc. Connect the flask to the absorption apparatus, and let the reaction proceed for about 30 min. *Titration*—At the end of this time connect the gas-inlet to a compressed air cylinder and add potassium iodide until the pptd. silver iodide redissolves. Add a small amount of solid sodium bicarbonate and a drop of starch soln. and slip the tube over the jet of a Conway burette, with air passing through the liquid at a gentle rate. Add 0·005 *N* iodine until a red-brown colour persists for 30 sec. To standardise the iodine soln. measure two different quantities of standard arsenite soln. into the absorption tubes, adjust the vols. to about 2 ml. with 0·02 *N* silver nitrate and titrate as described above. In 20 expts., in which 20μg. of arsenic were present, the mean recovery was 19·6μg. and the standard deviation of a single observation from the mean was 0·73μg. The average recovery of 20μg. of arsenic added to 3 ml. of blood was 18·9μg. (standard deviation 2·3μg.).

F. A. R.

Rapid Determination of Ascorbic Acid by Adaptation of Stotz's Method to Plant Materials. L. P. Pepkowitz (*J. Biol. Chem.*, 1943, **151**, 405–412)—Homogenise a 30–50 g. sample of fresh tissue (or a 5–10 g. sample of dehydrated vegetables) in 200 ml. of 1% metaphosphoric acid for 2 min. in a Waring blendor. Filter the extract through a rapid filter and discard the first turbid portion of the filtrate. Pipette a portion of the clear filtrate, containing 0·01–0·10 mg. of ascorbic acid, into a centrifuge tube and add 2 drops of 0·04% bromocresol green and 0·08 *N* sodium hydroxide dropwise until the indicator becomes green. Add 1 ml. of a buffer soln. of *p*H 4 (mix 100 ml. of 0·1 *N* citric acid and 60 ml. of 0·2 *N* Na_2HPO_4 and adjust the *p*H); mix and add exactly 2 ml. of 2 : 6-dichlorophenol-indophenol soln. (12 mg. in 200 ml. of warm water) from a rapid-delivery pipette; if there is complete decolorisation, add a further 2 ml. Next add 10 ml. of xylene as rapidly as possible, stopper and shake for 10 sec.; the complete procedure from addition of dye should be carried out within 20 sec. Centrifuge for 5 min., decant the xylene layer into the cuvette of a photoelectric colorimeter, and, with the galvanometer set at 100 and xylene in the solvent tube, note the deflection (G_S) of the galvanometer due to the dissolved dye, using a wavelength of

500$m\mu$. Prepai a control tube by putting 1 ml. of buffer, 2 drops of indicator and 2 ml. of dye in a centrifuge tube and extract with xylene as described above. Measure the galvanometer reading (G_c) due to this soln. and calculate the concentration of ascorbic acid (C) from the equation: $C = 10\,K\,(\log G_s - \log G_c)$. The value of K is ascertained with the aid of solns. of ascorbic acid of known concn. The recovery of pure ascorbic acid averaged 104·5% of the theoretical, whilst the recovery of ascorbic acid added to various plant extracts ranged from 92 to 108% in 30 estimations, with an average of 99·1%. F. A. R.

Antimony Trichloride Reaction for Vitamin D. E. M. Shantz (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 179)—The effect of varying the conditions of assay of the vitamin-D-containing fraction of fish liver oils, freed from vitamin A, sterols and other colour-producing substances, usually by a chromatographic procedure, is described. It is shown that the intensity of the yellow colour is not proportional to the amount of calciferol present except at very low concns. (5μg. or less per ml.), while the colour increases to a maximum and then slowly fades. The maximum colour development is obtained when the reaction is allowed to proceed in the dark, bright light causing results to be about 15% low The intensity of the colour increases with temp. up to 42° C. and then decreases; between 19° and 33° C. there is a difference of 40% in the colour intensity of the same solution. The following method gives reproducible results. Measure 1 ml of a chloroform soln., expected to contain about 0·07–0·25 mg. of calciferol, into a colorimeter tube which has been allowed to come to a constant temp. by inserting it in a steel tube immersed in a water-bath maintained at 30° C. Add 10 ml. of a soln. of antimony trichloride in chloroform (saturated at 20° C.), previously adjusted to 30° C., cover the steel tube and leave for exactly 4 min. Read the colour on an Evelyn photoelectric colorimeter, using a 500$m\mu$ filter, and calculate the amount of vitamin D by means of a calibration curve prepared by submitting crystalline calciferol to the test. J. A.

Organic

Analysis of Binary Mixtures of Normal Aliphatic Dibasic Acids and Esters. Use of Composition-Melting Point Relations of the Acids. D. F. Houston and W. A. Van Sandt (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 538–540)—Mixtures of similar dibasic acids often occur in oxidation products from unsaturated fatty acids, and existing methods for their separation and analysis are somewhat unsatisfactory. The results of Gantter and Hell (*Ber.*, 1881, **14**, 1545) for melting points of binary mixtures of suberic and azelaic acids indicated that this property might prove useful. An empirical method is now developed for taking melting points which provides results closely related to composition and affords its determination to within a few units per cent. This method has been applied to the systems comprising adjacent and alternate pairs of acids containing six to twelve carbon atoms. The acids used were adipic, pimelic, suberic, azelaic, sebacic, 1:11-undecanedioic and 1:12-dodecanedioic acids. One-g. samples of mixtures were prepared by weighing the component acids to 0·2 mg., bringing the mixtures to complete fusion, stirring them during cooling and powdering the solidified material in a mortar. Portions were introduced into capillary tubes of diameter 1·0 to 1·5 mm. and were liquefied by holding the tubes in a stirred and rapidly heated oil bath, air bubbles being dislodged by momentarily removing the tubes and shaking them sharply. The melted samples were quenched by quickly placing the tubes in a stream of water. Quenched samples were 3 to 5 mm. in height. Approximate melting ranges were observed during the fusion process, thus allowing rapid temperature adjustment in the final measurement. Melting ranges were determined in an electrically heated (Still, *Chem. and Ind.*, 1944, 294), mechanically stirred oil bath, the rate of heating being closely controlled by a variable-voltage transformer. Samples were placed against the bulb of the thermometer and observed through a magnifier with transverse illumination. Bath temperatures were brought rapidly (3° to 5° per min.) to a few degrees below the melting range and thereafter raised at not over 0·2° C. per min. The melting range was recorded from the first appearance of clear liquid to the disappearance of the last solid particle. Clear liquid usually appeared as a meniscus, though mixtures which were predominantly one-component systems sometimes began to melt at other peripheral places. A marked change occurred in the gross appearance of the quenched samples at temperatures below the m.pt. for all mixtures in a wide central portion of the composition range. The hard waxy surface assumed a soft matte appearance and the temperature of this phenomenon as observed during the initial rapid heating was constant within 2° or 3° C. for all mixtures of a binary system that were not grossly crystalline after quenching. The temperatures varied from system to system and microscopical observations showed that the phenomenon actually consisted of the melting of a eutectic mixture. This apparently places the binary systems in the class characterised by formation of solid solutions of limited solubility (Roozeboom's Type V). The difference between these eutectic temperatures and those obtained from "thaw-point" measurements (Rheinboldt *et al.*, *J. prakt. Chem.*, 1925, **219**, 242; 1926, **221**, 348) probably results from the intentional disturbance of equilibria by quenching. Differences occurring between the eutectic temperatures and the melting ranges of the eutectic compositions may be due partly to measurement of different stages of melting, but it is more likely that polymorphism of the acids is involved. For example, in the suberic - azelaic system it was found that the melting range at the eutectic composition was 96·7°–97·7° C. by a graphical procedure, but the eutectic temperature over a range of compositions was 91·5° to 92·5° C. by thaw-point measurement or 94°–95° C. by heating fused samples slowly. Kofler (*Ber.*, 1943, **76**, 871) has shown that suberic acid can exist in three forms, and he reported that the transition from Form I to Form III is enantiotropic, occurs at about 90° C., and is very susceptible to superheating or supercooling. Azelaic acid has also been shown to be at least dimorphic. Polymorphism would probably be encountered in binary mixtures of these acids especially in connection with intentional departures from equilibrium conditions.

Systems investigated comprise four classes, *viz.*, (*a*) with alternate even-carbon acids; (*b*) with alternate, odd-carbon acids; (*c*) with adjacent acids with the odd-carbon member of higher molecular weight; and (*d*) with adjacent acids with the even-carbon acid of higher molecular weight. The results are expressed graphically with the weight (%) of the acid of higher molecular wt. as abscissa and

the temperature °C. as ordinate. The melting ranges are then represented by short vertical lines. The extremities of these lines are joined by smooth curves and from these graphs tables correlating melting ranges with composition (at 5% intervals) may be constructed, and the approximate eutectic temperatures are indicated in the graph by the minimum value of the melting range. The eutectic temperatures fall into three groups, *viz.*, (*a*) below 90° C. for two odd-carbon acids; (*b*) above 110° C. for two even-carbon acids; and (*c*) between 90° and 105° C. for adjacent acids. The positions of the eutectic points occur on the side of the lower-melting component and tend to approach a central location as the acids become more nearly alike.

The precision with which the composition of a mixture of known components may be determined depends upon its relative position in the binary system. Reproducibility within ±0·25° C. corresponds to less than 2·5 per cent. w/w for most compositions, less than 1% for the most favourable proportions, and as much as 5% in the region of single components or the flat minima of even-even systems.

Distinction may readily be made between mixtures of similar melting ranges lying in opposite branches of the curve in a known binary system. When the components of the mixture are not definitely known, the possibilities will be limited by the previous history of the material. The approximate eutectic temperature may aid in classifying the mixture. for the two types of alternate-acid systems are usually distinguishable from each other and from mixtures of adjacent acids. Additional evidence from the refractive index (see next abstract) and saponification value of the methyl esters or neutralisation value of the acids will usually permit recognition of the components and composition of the mixture. Redetermination of the melting range after addition of a known proportion of one of the pure components will afford corroboration. A. O. J.

Fatty Acid Analyses of Known Mixtures of Purified Methyl Esters. A. R. Baldwin and H. E. Longenecker (*Oil and Soap*, 1945, **22**, 151–153)—A simplified procedure of fatty acid analysis, in which no separation into predominantly saturated and predominantly unsaturated groups is necessary, is described in detail as applied to the analysis of known mixtures of highly purified methyl esters. The esters are mixed in definite proportions and submitted to fractional distillation at reduced pressure, using an electrically-heated column of about 12 theorectical plates (Longenecker, *J. Soc. Chem. Ind.*, 1937, **56**, 199T) packed with glass helices and equipped with a total condensation, partial take-off, distilling head (Baldwin and Longenecker, *J. Biol. Chem.*, 1944, **154**, 255). The course of the distillation is followed by refractive index measurements on successive fractions. The iodine val., sap. val. and refractive index and the proportions of methyl linolate and methyl linolenate are determined for each fraction and the composition of each calculated by means of the equations previously described by Longenecker (*Oil and Soap*, 1940, **17**, 53). The proportions of methyl linolate and methyl linolenate are determined spectroscopically by a method essentially that developed by Mitchell, Kraybill and Zscheile (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 1).

Weigh approximately 0·1 g. of the fat into the bottom of a standard-taper, glass-stoppered Pyrex test-tube, add 2 ml. of an alkaline glycol prepared by dissolving 10 g of, pure potassium hydroxide in 100 ml of redistilled ethylene glycol, heating to 190° C., cooling and diluting to 100 ml with ethylene glycol, and place the loosely-stoppered tube in an oil-bath maintained at a constant temp. of 185° C. Mix the fat and reagent thoroughly by shaking the tubes after three successive intervals of 1 min. and continue the heating for total period of exactly 30 min. A blank test should be made in the same manner at the same time. Place the tubes in cool water and then transfer the isomerised soap and excess of reagent to a volumetric flask with triple-distilled water and further dilute the soln. until the optical density is suitable for measurement in a Beckmann quartz spectrophotometer. Measure the peak optical density of the soln. at 234 $m\mu$ and at 270 $m\mu$ against the blank diluted to the same concn. and calculate the values of $E^{1\%}_{1cm}$ from the equation $E^{1cm}_{1\%} = \frac{d}{c.l}$, where d = optical density, c = concn. and l = length of cell in cm. Calculate the proportion of methyl linolenate from the value of $E^{1\%}_{1cm}$ 270 $m\mu$ by comparison with the value obtained by submitting the pure ester to the alkaline isomerisation procedure. For the proportion of methyl linolate, the value of $E^{1\%}_{1cm}$ 234 $m\mu$, corrected for absorption due to triene material, is compared with the corresponding value obtained for isomerised pure methyl linolate.

The results of analyses of two mixtures are quoted which indicate that an accuracy of somewhat less than 1 unit % of the methyl ester concerned is attainable. The procedure has been applied to hydrogenated shortenings, with an agreement between duplicates of within 1 unit %. J. A.

Analysis of Binary Mixtures of Normal Aliphatic Dibasic Acids and Esters. Use of Refractive Indices of Dimethyl Esters. D. F. Houston and J. S. Furlow (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 541–542)—Measurement of refractive index presents a convenient and rapid means of following the progress of a fractional distillation and extends the information furnished by the observed distillation temperatures. When careful fractionation yields only single components and intermediate binary mixtures, a close estimate of composition may be obtained from a knowledge of the relation between the composition and refractive index of binary mixtures. Distillation of the dimethyl esters is one of the most satisfactory ways of separating mixtures of closely related acids, and the approximate compositions of the individual fractions can be readily determined by refractive index measurements. Further corroboration is afforded by determination of the melting ranges of the recovered acids (see preceding abstract).

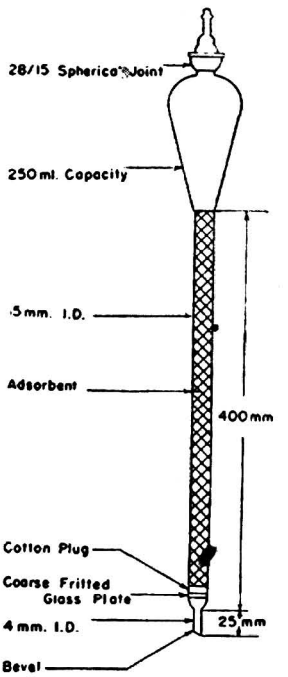
The esters used in the investigation were prepared from the acids mentioned in the preceding abstract except the C_{12} ester, which was fractionally crystallised from an original methyl ester distillation fraction. The refractive index was measured with an Abbe-type refractometer which could be read to 0·0001 index unit. Temperature was controlled to within ±0·05° C. by rapid circulation of water from a constant temperature water bath through the prism jackets of the instrument. Measurements were made at 5° C. intervals for all temperatures at which the esters were liquid. The expected linear relationships were found and are expressed by the following equations in which t represents °C. Dimethyl adipate, 1·4205–0·00040

($t-40$); dimethyl suberate, $1{\cdot}4262-0{\cdot}00039_5$ ($t-40$); dimethyl azelate, $1{\cdot}4284-0{\cdot}00039_5$ ($t-40$); dimethyl sebacate, $1{\cdot}4307-0{\cdot}00039$ ($t-40$); dimethyl 1,11-undecanedioate, $1{\cdot}4329-0{\cdot}00038_5$ ($t-40$); dimethyl 1,12-dodecanedioate, $1{\cdot}4345-0{\cdot}00038$ ($t-40$). Several series of binary mixtures were prepared which represented various possible types and their n_D values were measured at 40° C. The relation between n_D^{40} and composition could be represented as a straight line in all mixtures when the composition was expressed as weight percentages. The relation was non-linear for molecular percentages. As the difference in n_D^{40} of adjacent homologous esters varies from about 0·0030 between C and C_7 to 0·0016 between C_{11} and C_{12}, a difference of 0·0001 in n_D^{40} corresponds to a 3 to 6 per cent. change of composition in binary mixtures of adjacent esters. This is about the precision obtainable from equivalent wt. determinations in which a difference of 0·2 unit corresponds with about 3 per cent. change in composition.

One possible source of error is the presence of dissolved gases, the refractive index of liquid dimethyl esters falling several fourth-place units when the ester is exposed to air for a day or so. Original values can be obtained after redistillation or removal of dissolved gases but are not obtained by use of desiccants. This error can be avoided by determining the refractive index immediately after distillation or by storing the liquid esters under reduced pressures. Methyl esters of monobasic acids, such as myristic or palmitic acids, may be present in products of oxidative cleavage of unsaturated acids from various sources (Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 43T), although they would normally be removed by partitions before analysis of the dibasic acids. If any remained they would interfere with the determination of composition by refractive index. Methyl myristate, for example, distils in the temperature range between dimethyl sebacate and undecanedioate, but has a refractive index almost as low as dimethyl azelate. In presence of this the calculated composition would indicate too large a proportion of the component of lower molecular weight. However, the apparent composition derived from determination of the saponification value would err in the opposite direction and, accordingly, agreement between the results obtained by the two methods would confirm the absence of monobasic esters and enhance the validity of the analysis.

A. O. J.

Analysis of Oil-soluble Petroleum Sulphonates. Extraction - Absorption Method. F. Brooks, E. W. Peters and L. Lykken (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 544–547)—Petroleum sulphonic acids are formed in the treatment of petroleum distillates with sulphuric acid in refining processes, or by treatment of petroleum stocks with oleum in the manufacture of medicinal white oils. Some of the sulphonic acids formed remain in the oil layer ("mahogany acids") and the others dissolve in the acid layer (green acids). After the sulphonation process, the acid oil layer is separated from the sludge containing the green acids and is neutralised with caustic alkali and the crude mahogany soaps are extracted with diluted alcohol. These crude soaps consist principally of approximately equal amounts of sulphonic acid soaps and mineral oil with small amounts of water, carboxylic acid soaps, free alkali and inorganic salts. A method has been devised which removes certain shortcomings of the A.S.T.M. method (*Standards on Petroleum Products and Lubricants*, Designation D855–45T, September, 1945), the Archibald and Baldeschwieler method (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 608) and the adsorption method of Koch (*Id.*, 1944, **16**, 25).



The sulphonic acids, carboxylic acids and mineral oil are separated from the inorganic salts by extracting an acidified aqueous suspension of the sample with chloroform. The inorganic salt content is calculated from the sulphated residue obtained from the aqueous phase, correction being made for the alkali residue contributed by the sulphonate, carboxylate and free alkali. A portion of the residue obtained from the chloroform extract is titrated potentiometrically for strong and weak acids to determine the respective equivalents of sulphonic and carboxylic acids present, and another portion is neutralised and the mineral oil is isolated by adsorbing the sulphonates and carboxylates on Attapulgus clay. The percolation apparatus of Koch (*loc. cit.*) has been modified somewhat to permit the application of pressure and cause a more rapid rate of flow through the adsorbent. The modified percolator is shown in the figure. It was found that a pressure of 0·5 to 1 lb. per sq. in. forced the liquid through sufficiently rapidly. The adsorbent was that recommended by Koch, *viz.*, 30- to 60-mesh Attapulgus clay calcined at 487° C. (Attapulgus Clay Co., 260 South Broad St., Philadelphia, Pa.). Inverted-rim beakers of the type described by the A.S.T.M. (*loc. cit.*) were used for evaporations where there was danger of loss by creeping.

To separate *Organic* and *Inorganic Constituents* disperse 10 g. ± 0·1 g. of the sample in 25 ml. of chloroform in a 100-ml. beaker, transfer into a separating funnel with 25 ml. of chloroform, rinse the beaker into the funnel with 50 ml. of 6 *N* hydrochloric acid, shake the funnel vigorously, rinse the

beaker with 50 ml. of acetone and add the rinsings to the contents of the funnel. Mix intimately and draw the lower chloroform phase into a tared inverted-rim beaker, extract the aqueous phase with three 25-ml. portions of chloroform, evaporate the combined chloroform extracts to dryness on the water bath, heating for 15 min. after disappearance of the odour of chloroform, cool to room temp. and weigh.

To determine *Free Inorganic Salts* draw the aqueous layer into a tared platinum dish, evaporate to dryness on the steam bath, add 2 ml. of 36 *N* sulphuric acid, rotating the dish so as to moisten all the residue, heat carefully over a small flame until fuming ceases and repeat the operation with 1 ml. of sulphuric acid. Finally heat in a muffle furnace at 800° ± 50° C. to constant weight.

To determine *Sulphonate* and *Carboxylate Soaps* determine the number of equivalents of strong and weak acids present in 100 g. of the chloroform extract by electrometric titration (A.S.T.M. D664–44T) of an accurately weighed 1- to 2-g. sample. Also analyse the residue for water content by the Karl Fischer method. (Place 20 ml. of anhydrous pyridine and 10 ml. of anhydrous ethyl ether in a 100-ml. glass-stoppered flask and titrate to the red-brown Fischer end-point. Add 0·5 to 1 g. of the sample in a glass weighing boat, slide the boat into the flask, swirl the liquid until the sample has dissolved and titrate the soln. with Fischer reagent (A.S.T.M., D268–44T) to the original red-brown end point.)

To determine *Mineral Oil* treat 2 g. of the chloroform extract in a 100-ml. beaker with enough 0·5 *N* alcoholic sodium hydroxide (calculated from the electrometric titration) to neutralise completely the strong and weak acids present and add 0·1 ml. in excess. Remove the alcohol on the steam bath, add 25 ml. of light petroleum and stir to dissolve the sample completely. Transfer the soln. into the reservoir of the percolation apparatus, rinsing the beaker and stirring rod with an additional 25 ml. of light petroleum. Apply a pressure of 0·5 to 1 lb. per sq.in. at the top of the percolator and force the soln. through a 40 in. column of adsorbent clay. Force an additional 150 ml. of light petroleum through the column in the same way. Collect the percolate in a tared inverted-rim beaker and evaporate to dryness on the steam bath, heating for 15 min. after disappearance of the odour of the solvent. Heat for 15 min. intervals at 110° C. until the weight lost between successive heatings is less than 1 mg.

Determine the *Water Content* of the original sample by the A.S.T.M. method (D75–40) or, if the free alkali content is low, by the Karl Fischer method.

To determine *Free Alkali* treat 10 g. of the sample with 50 ml. of ethyl alcohol and 50 ml. of benzene and swirl until dissolved. Add 6 or 7 drops of thymolphthalein indicator and titrate with standard 0·5 *N* sulphuric acid until the colour matches that of a standard prepared in the same way with omission of the indicator. This is essentially the A.S.T.M. method.

To determine the *Specific Gravity* fill a 25-ml. Weld-type pyknometer fitted with a special stopper having a bore of diam. 4 to 5 mm. with the original sample, warm to 70° or 80° C. until all air bubbles have risen, cool to 15° to 20° C. adding more sample if necessary, insert the stopper and warm the neck of the flask with a small bunsen burner if the sample is very viscous. Place the pyknometer with the cap in place in a bath at 25° ± 0·1° C., and remove excess of sample by wiping with a cloth moistened with benzene and alcohol. Replace the cap and weigh.

If A is the wt. of chloroform-soluble organic residue (g.), C is the wt. of organic residue used for mineral oil determination, D is the wt. of mineral oil found, W is the wt. of original sample taken for analysis, B is the wt. of sulphate ash obtained from the aq. residue, E is the number of equivalents of strong acids per 100 g. of organic residue and e the corresponding figure for weak acids, G is the free alkali content (%), X is the mineral oil content of sample (%), Y is the water content of the organic residue (%), F is the sodium sulphonate content (%), V is the ml. of sulphuric acid of normality N required to neutralise the free alkali, W_1 is the wt. of sample used in free alkali determination, W_s the wt. of sample in the pyknometer at 25° C. and W_c the wt. of water contained in the pyknometer in calibrating, the amounts of the components present in the sample, and related values, are given by the following equations.

Mineral Oil %, $100AD/CW$

Inorganic salt content %, $\dfrac{100B-71A(E+e)}{W}-\dfrac{71G}{40}$

Sodium sulphonate content %,

$$\frac{A(100+22E)}{W}-X-\frac{A(Y+312e)}{W}$$

Carboxylate soap content %, $334Ae/W$

Combining wt. of sodium sulphate, FW/AE

Free alkali content as NaOH %, $4VN/W_1$

Specific gravity, W_s/W_c.

The method is inapplicable, without sacrifice of accuracy, to the analysis of samples containing volatile oils. Approximate values for the components of samples of this type can be obtained by application of the procedure described, elution of the sulphonates from the Attapulgus clay with methanol to recover the adsorbed sulphonates and carboxylates and evaporation of the methanol to obtain the wt. of sulphonate and carboxylate. The mineral oil content is then ascertained by difference. Owing to the insolubility of the chloroform extract from green acid soaps in light petroleum, attempts to apply the method to green acid sulphonates have been unsuccessful. A. O. J.

Iodimetric Method of Analysis for Organic Peroxides. K. Nozaki (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 583)—Owing to its accuracy, reliability and general applicability the iodimetric method for analysis of organic peroxides has been extensively used but different workers rarely use the same solvent, primarily because the solvents used heretofore have not been completely satisfactory. Acetic acid requires an inert atmosphere, acetone tends to react with iodine in presence of water and with alcohols liberation of iodine is slow and the soln. must be heated to incipient boiling. Acetic anhydride is now recommended as an excellent solvent for iodimetric peroxide analyses. It is a good solvent for both peroxides and sodium iodide, it does not react with iodine, precautions against atmospheric oxygen are not necessary, the reaction is rapid in its presence and, after dilution with water, starch indicator may be used.

To the peroxide sample in a glass-stoppered Erlenmeyer flask add 5 to 10 ml. of acetic anhydride and 1 g. of powdered sodium iodide, swirl the mixture to dissolve the iodide and set aside for 5 to 20 min. Add 50 to 75 ml. of water, shake vigorously for about 30 sec. and titrate the liberated iodine with standard sodium thiosulphate soln. using

starch indicator. With very dilute solns. a slight positive correction of the starch end-point may be necessary. When polymers or other water-insoluble substances are present in the peroxide sample, it is advantageous to add a small amount of chloroform to the initial reaction mixture. The two-phase system may then be titrated to the starch end-point or to the disappearance of the iodine colour in the chloroform layer.

The acetic anhydride should not contain much acetic acid, which accelerates atmospheric oxidation. Reagent grade acetic anhydride from a recently opened bottle gave a blank reading of less than 0·01 ml. of 0·1 *N* sodium thiosulphate after standing for 20 min. with sodium iodide in air. Technical acetic anhydride may be used, but it sometimes requires a small correction factor. With purified benzoyl peroxide it was found that 50-mg. samples can be correctly analysed to within 0·2%, and other acyl peroxides gave similar results. When the method is applied to peroxygen in ethers and ethylene derivatives, slightly longer reaction times must be allowed.

Pyridine interferes with the method when starch indicator is present but not in its absence. Presence of phenols causes slightly low results (98·5%) and some ethylene derivatives react rapidly enough with iodine to affect the results. Styrene and oleic acid react slightly with iodine, and cyclohexene causes results as low as 80%. Thus, peroxygen determinations in presence of ethylene derivatives, *e.g.*, vegetable oils, require preliminary tests to determine the extent of the reaction of these derivatives with iodine. A. O. J.

Identification of Amino Acids Derived from Cystine in Chemically Modified Wool. R. Consden, A. H. Gordon and A. J. P. Martin (*Biochem. J.*, 1946, **40**, 580–582)—Methods are given for the identification by partition chromatography on paper, as previously described by the authors (*Biochem. J.*, 1944, **38**, 224; ANALYST, 1945, **70**, 31) of cysteic acid, lanthionine, djenkolic acid, thiazolidine-carboxylic acid and S-methylcysteine in protein hydrolysates. F. A. R.

Inorganic

Volumetric Determination of Aluminium (in Silica-Alumina Catalysts). M. N. Hale (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 568–569)—The method is a modification of that of Snyder (*Id.*,) 1945, **17**, 37; ANALYST, 1945, **70**, 189). An aluminium sulphate soln., containing tartrate, is neutralised with lithium hydroxide soln. and potassium fluoride is added in excess. The hydroxyl ion released by the reaction is titrated with hydrochloric acid.

Reagents—Potassium fluoride, 30% soln., neutralised to phenolphthalein and stored in a wax-lined bottle. Sodium tartrate, 25% soln. Thymolphthalein, 0·1% soln. in ethyl alcohol. Aluminium sulphate, 0·5 *N*, prepared by dissolving 55·535 g. of $Al_2(SO_4)_3.18H_2O$ in 1 litre of water. Hydrochloric acid 0·5 *N*, standardised against the aluminium sulphate soln. Lithium hydroxide 0·5 *N*, standardised against the hydrochloric acid. (The ratio ml. of hydrochloric acid/ml. of lithium hydroxide soln. is *R*.)

Method—Weigh 1 to 1·5 g. of the sample into a 100 ml. platinum dish and ignite to remove carbon and ammonium salts. Cool, moisten, add 10 ml. of hydrochloric acid, and then add hydrofluoric acid cautiously until the sample dissolves completely. Evaporate the soln. to dryness and heat the dish to redness for a few seconds. Fuse the residue with about 3 g. of potassium bisulphate, cool, and dissolve the melt in 80 ml. of water. Transfer the soln. to a beaker and stir mechanically. Titrate with lithium hydroxide soln. until slight pptn. indicates that the free acid is nearly neutralised. Add 6 to 8 drops of thymolphthalein soln. and 25 ml. of sodium tartrate soln. (If much magnesium or lead or more than 0·3 g. of aluminium oxide is present use 50 ml. of tartrate soln.) Titrate until a blue colour persists for 30 seconds and add 0·1 to 0·2 ml. of lithium hydroxide soln. in excess. Titrate with 0·5 *N* hydrochloric acid until the blue colour just disappears. Record the readings A_1 and B_1 of the acid and hydroxide burettes respectively. Add 25 ml. of potassium fluoride soln., titrate with 0·5 *N* hydrochloric acid until the restored blue colour is discharged and add 1 ml. in excess. Titrate with lithium hydroxide soln. until the blue colour reappears. Record the burette readings A_2 and B_2. The weight per cent. of Al_2O_3 in the sample =

$$\frac{(A - BR) \times N \times 0{\cdot}017 \times 100}{\text{Sample weight}}$$

where $A = A_2 - A_1$, $B = B_2 - B_1$, R is the ratio $\frac{\text{ml. of HCl}}{\text{ml. of LiOH}}$ and N is the normality of the hydrochloric acid.

Manganese, ammonium and tin ions interfere, and coloured ions obscure the end-points when their concn. is high. The following materials, in amounts equivalent to approximately 5% of the sample, did not cause significant interference: iron, nickel, copper, magnesium, calcium, sodium, potassium, lead and chromium. If large amounts of metals that form complexes with tartrate are present (*e.g.*, iron, lead and magnesium) additional sodium tartrate soln. must be used to avoid low results. The method is claimed to be quicker and more accurate than the conventional gravimetric determination. L. A. D.

Fluorimetric Determination of Aluminium in Steels, Bronzes and Minerals. A. Weissler and C. E. White (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 530–534)—The dye Pontachrome Blue Black R, also known as Superchrome Blue Black (Colour Index 202, sodium or zinc salt of 4-sulpho-2-hydroxy-α-naphthalene-azo-β-naphthol) is used as a reagent for the determination of aluminium. The red fluorescence obtained when the test solution is irradiated with ultra-violet is measured photoelectrically. Pontachrome Violet SW (Colour Index 169) has also been found to be a useful reagent but requires more careful *p*H control and is more susceptible than the Blue Black to traces of iron which destroy the fluorescence. The results of experiments to determine the best test conditions are given in detail. Briefly the conclusions are that 1·5 ml. of 0·1% soln. of dye in 95% alcohol (the sol. is allowed to stand for a few days before use) are used in 50 ml. of soln. buffered to *p*H 4·8 with 0·5 g. of ammonium acetate and 0·05 ml. of sulphuric or acetic acid. The soln. is prepared at room temp. and left for at least 1 hr. before measurement. A Lumetron fluorescence meter is used, with a Corning 5874 filter in the primary radiation (transmission near zero at 300 and 390 mμ, about 38% at the peak at 360 mμ) and a red plastic filter (transmission near zero below 600 mμ, about 80% at 650 mμ and about 90% above 700 mμ) to isolate the fluorescent light. A straight line relationship between scale reading and the aluminium content

of 50 ml. exists in the range 0 to 20 μg. of aluminium. Interfering elements, including iron, are removed by electrolysis with a mercury cathode.

Methods—A, for steel—To determine acid-soluble aluminium, dissolve 1 g. of sample in 25 ml. of diluted sulphuric acid (1+9) and dilute to 500 ml. (Take 0·1 g. of sample if the aluminium content exceeds 0·1%). Electrolyse a 10 ml. aliquot portion in a small stirred Melaven mercury cathode cell for 1 hour at a current of 0·5 amp. Transfer the soln. to a 50 ml. graduated flask containing 5 ml. of 10% ammonium acetate soln. and 1·50 ml. of dye soln. and dilute to the mark. After at least 1 hour measure the fluorescence, calibrating the meter with standards prepared by the same method (including the electrolysis) from a suitable pure aluminium soln. of known concn., *e.g.*, one made from pure potassium aluminium sulphate. As the calibration curve is a straight line only two standards are needed for ordinary work.

To determine acid-insoluble alumina in steel, dissolve 1 g. of sample in diluted sulphuric acid (1+9), filter, and wash the residue thoroughly with diluted hydrochloric acid (1+19) and then with water. Burn off the paper in a platinum crucible and heat the residue with 1 ml. of diluted sulphuric acid (1+1) and 5 ml. of hydrofluoric acid until copious fumes are evolved. Cool, rinse the sides of the crucible and evaporate and fume again. Cool, transfer to a platinum dish with 50 ml. of water and heat until the salts dissolve. Make alkaline to methyl red with 10% sodium hydroxide soln. (prepared in platinum and kept in a ceresin bottle), make just acid with diluted sulphuric acid (1+9) and add 25·0 ml. in excess. Dilute to 500 ml. in a graduated flask, take a 10 ml. aliquot part for electrolysis and continue as above.

B, for bronzes—Take 1 g. (or other appropriate weight) of the sample, dissolve in 10 ml. of diluted nitric acid (1+1), add 20 ml. of diluted sulphuric acid (1+1) and evaporate to fuming. Cool, wash the sides of the beaker with 15 ml. of water and again evaporate to fuming. Cool, dilute to 100 ml., neutralise and continue as in the method immediately above.

C, for minerals—For siliceous samples weigh a suitable amount into a platinum crucible, add 2 ml. of diluted sulphuric acid (1+1) and 10 ml. of hydrofluoric acid and evaporate to fuming. Cool, wash the sides of the crucible and again evaporate to fuming. Add the soln. to 100 ml. of water in a platinum dish and neutralise and continue as above.

For other types of mineral apply ordinary methods of analysis and determine the aluminium in the R_2O_3 ppt.

Interferences—Titanium, vanadium and zirconium, which are frequently found in steel, are not removed by the electrolysis. Zirconium does not affect the fluorescence, but titanium, and especially pentavalent vanadium, cause low results. The determination of aluminium in steels can, however, be carried out when the sample contains these metals, as their carbides are insoluble in diluted sulphuric acid.

The sensitivity of the method is claimed to be better than 1 part of aluminium in 10^8. Evidence is given showing that the fluorescent substance contains one molecule of aluminium hydroxide and two molecules of dye. L. A. D.

Absorptiometric Determination of Iron and Copper in Red Phosphorus. J. A. Brabson, O. A. Schaeffer, A. Truchan and L. Deal (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 554–556)—The reagents chosen are 1:10-phenanthroline for iron determination and sodium diethyldithiocarbamate for copper. The sample used for the copper determination is dissolved in diluted nitric acid, but this reagent results in formation of some pyro- and metaphosphoric acids, which interfere with the determination of iron. Whilst these acids can be hydrolysed to orthophosphoric acid (which does not interfere) the process involves the possibility of attacking glassware and extracting iron from it. A soln. of bromine in carbon tetrachloride is therefore used to prepare the soln. for iron determination.

Method for iron—Weigh 1 g. of sample into a dry 250 ml. beaker and add 75 ml. of carbon tetrachloride, 75 ml. of water and 15 ml. of nitric acid. Add slowly (*e.g.*, from a burette) 20 ml. of bromine - carbon tetrachloride mixture (1 to 3 by volume) and stir until the formation of yellow crystals of $PBr_5.2CCl_4$ ceases. Nest the beaker in a 400 ml. beaker, which serves as an air-bath, and evaporate on a hot plate until frothing and evolution of dense brown fumes occurs and the volume of liquid is 5 to 6 ml. If the liquid is coloured add a few crystals of potassium chlorate and heat until the colour is removed. Avoid prolonged heating and the consequent attack on the beaker. Dilute to 100 ml., cover the beaker and boil the liquid for 45 min. so that the vol. is reduced to 30 ml. Rinse into a 100 ml. graduated flask and dilute to 70 ml. The further treatment depends on whether the sample had been stabilised by addition of hydrated alumina or not. If no alumina is present add 1 ml. of hydroxylamine hydrochloride soln. (10 g. per 100 ml.) and leave for 15 min. Add 5 ml. of 1:10-phenanthroline soln. (0·1 g. per 100 ml.) and then ammonia soln. until the *p*H is 6. Dilute to the mark, leave for 1 hour, and then measure the absorption, using a green filter (525 mμ). If alumina has been added use 4 ml. of hydroxylamine soln., and after 15 min. add 1·0 ml. of citric acid soln. (10 g. of monohydrate per 100 ml.), shake, and cool to room temp. Continue as above, except that the soln. is left for 2 hr. before measuring. Make a calibration curve, using standard ferrous ammonium sulphate soln. (1 ml. ≡ 0·01 mg. of iron). Correct for the blank on the reagents. Nickel and copper (up to 100 p.p.m.) do not interfere. Lead (up to 1,500 p.p.m.) does not interfere if the citric acid procedure is used.

Method for copper—Weigh 2·5 g. of sample into a 300 ml. tall-form beaker, add 100 ml. of nitric acid (1+1), heat to start the reaction and allow solution to proceed without further heating. Evaporate the soln. until brown fumes are no longer evolved, cool, add 2 to 5 ml. of conc. nitric acid and heat until the evolution of brown fumes ceases. Repeat this treatment until the operation fails to produce any brown fumes and then evaporate the liquid to a syrup. If the syrup is coloured heat it for a few min. with about 0·2 g. of potassium chlorate. Cool, add 10 to 20 ml. of water and boil. If the soln is coloured after boiling for 10 min., evaporate and repeat the potassium chlorate treatment. Transfer the soln. to a 100 ml. graduated flask and add 2 ml. of citric acid soln. Add ammonia soln. until the *p*H is >8·5, add 10 ml. of gum arabic soln. (1 g. boiled with 100 ml. of water, filtered and diluted to 200 ml., prepared freshly each day) and dilute to 70 ml. Add 10 ml. of sodium diethyldithiocarbamate soln. (0·2 g. per 100 ml.), dilute to 100 ml. and measure the absorption, using a blue filter (425 mμ). Nickel and lead (>4 p.p.m.) interfere. Nickel may be removed with dimethylglyoxime and a correction applied

(about 6 p.p.m. of copper) to the result, which is found to be high, probably because of the slight solubility of nickel dimethylglyoxime. L. A. D.

Differential Reduction of Iron and Tin. A. C. Simon, P. S. Miller, J. C. Edwards and F. B. Clardy (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 496–497)—Iron and tin are determined titrimetrically on the same sample of material and the method is suitable for bronze and other copper alloys containing less than 1% of antimony. After separation from copper the iron is reduced in an inert atmosphere with metallic nickel in cold dilute hydrochloric acid and titrated with standard potassium dichromate. The tin is then reduced with metallic nickel in hot concentrated acid and titrated with standard iodine.

Method—Dissolve the sample (containing not more than 0·1 g. of tin and 0·05 g. of iron) in diluted nitric acid (1+1) in a 300-ml. conical flask. Boil, dilute with water to approx. 100 ml. and add 3 ml. of aluminium nitrate soln. (10%). Add ammonium hydroxide until the blue copper complex is formed and then 10 ml. in excess. Boil and filter off iron, aluminium and tin hydroxides. Wash 4 or 5 times with ammonium hydroxide (2%). Return filter paper and ppt. to the original flask, add 10 ml. of perchloric acid (sp.gr. 1·67) and 5 ml. of nitric acid and heat gently to destroy the paper. (To avoid risk of explosion add the nitric acid drop by drop if the soln. darkens.) Dilute to 100 ml., re-ppt. with excess ammonia, filter and wash thoroughly. Destroy the paper as before and then add 10 ml. of sulphuric acid and evaporate to "fuming." Cool.

Iron determination—Dilute to 150 ml. with water, add 15 ml. of hydrochloric acid and boil to complete solution. Cool, add solid carbon dioxide and then cool below 20° C. in an ice-bath. Add about 30 g. of small nickel shot and, as the last lumps of carbon dioxide disappear, stopper the flask and shake vigorously (mechanically) for 15 min. Rechill in the ice-bath, decant the soln. from the nickel shot into a 500-ml. extraction flask and wash the shot twice by decantation. Add 10 ml. of mercuric chloride soln. (saturated) and titrate with standard potassium dichromate. For high-tin alloys subtract a "blank" correction of 0·02%. Return the nickel shot to the soln. and add 60 ml. of hydrochloric acid.

Tin determination—Fit the flask with a stopper and tube suitably bent to dip into a beaker of water. Bring rapidly to the boil and allow to boil gently for half-an-hour. Replace the beaker of water by one containing sodium bicarbonate soln. (10%), then cool the flask to below 20° C. Remove the stopper, drop in a small piece of solid carbon dioxide and titrate with iodine soln. using starch as indicator. Standardise the iodine soln. against pure tin reduced in the same way as the sample. C. F. P.

Determination of Manganese in Caustic Soda. R. F. Moran and A. P. McCue (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 556–557)—The method employs visual comparison of the permanganate colour with standards, and is arranged so that accidental traces of reducing substances which could destroy the colour are not introduced.

Reagents—Phosphoric acid (1+1): equal volumes of pure 85% orthophosphoric acid and water. Treated phosphoric acid (1+10): mix 1 litre of water with 100 ml. of 85% phosphoric acid, add 0·8 g. of potassium periodate and boil vigorously for 20 to 25 min.; cool and keep in a very clean bottle rinsed with the reagent. Do not add water after boiling. Standard manganese soln.: dissolve 0·3077 g. of pure $MnSO_4.H_2O$ in water, add 10 ml. of 85% phosphoric acid and dilute to 1 litre. Dilute 10 ml. to 1 litre, so that 1 ml. of the second soln. contains 1 μg. of manganese.

Method—Weigh a sample containing 10 ± 0·5 g. of sodium hydroxide into a 250 ml. conical flask and add 50 ml. of water. Add 50 ml. of phosphoric acid (1+1) and 0·8 g. of potassium periodate and boil vigorously for 20 to 25 min. Cool, transfer to a cylinder and dilute to 100 ml. with treated phosphoric acid. For comparison with standards, which are similarly prepared, transfer the solns. to 12 in. 100 ml. Nessler cylinders which have been rinsed with about 10 ml. of treated phosphoric acid. Standards should not be used for more than one day. The accuracy and precision of the results when the manganese content is about 0·3 p.p.m. are comparable with those of methods employing photoelectric instruments. L. A. D.

Volumetric Determination of Magnesium in Magnesium Carbonate Ores. L. R. Williams (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 542–544)—Calcium is partially removed as sulphate from the soln. of the sample and magnesium is pptd. as hydroxide by adding excess of standard sodium hydroxide soln. The excess of alkali is titrated with sulphuric acid, with methyl red as indicator. The sodium hydroxide soln. need not be free from carbonate.

Reagents—Sodium hydroxide, 0·50 *N*, standardised against sulphamic acid, with methyl red as indicator. Sulphuric acid, 0·25 *N*, standardised against the sodium hydroxide soln.

Method—Cover a 0·5 g. sample of ore with water, slowly add 10 ml. of 6 *N* sulphuric acid and boil until the sample is decomposed. Evaporate until all the sulphuric acid is expelled, add 25 ml. of hot water, boil to dissolve all soluble matter and filter, washing the residue about 5 times with hot water. Dilute the filtrate to 100 ml., neutralise with 6 *N* sodium hydroxide and stir in 0·5 g. of sodium peroxide. Boil for about 20 min. to decompose excess of peroxide. If no manganese is present omit this treatment with sodium peroxide. Acidify with 6 *N* sulphuric acid and boil for a few min. Add 3 or 4 drops of methyl red indicator soln. (1%) and adjust carefully to the end-point, using 0·50 *N* sodium hydroxide and 0·25 *N* sulphuric acid. Boil for a few min. and filter into a 250 ml. graduated flask, washing the residue of hydroxides (Fe, Al, etc.) with hot water. Just acidify the filtrate with 0·25 *N* sulphuric acid and add 25·0 ml. of 0·50 *N* sodium hydroxide. Mix the contents of the flask thoroughly, cool, dilute to the mark and pour into a large beaker. After allowing the pptd. magnesium hydroxide to settle for 25 to 30 min., pour the supernatant liquid through a filter into a 250 ml. graduated flask containing 50·0 ml. of water until the total volume is exactly 250 ml. Transfer the soln. to a beaker, add one drop of methyl red soln. and titrate with 0·25 *N* sulphuric acid. If a drop of phenolphthalein soln. is also added a colour change from red to yellow takes place shortly before the methyl red end-point.

The method is claimed to give results comparable in accuracy with those of the gravimetric pyrophosphate method in less than 4 hours. Smaller samples may be used if the carbonate ore is rich in magnesium, but the accuracy is reduced.

L. A. D.

Note on the Determination of Zinc. S. Sen (*J. Indian Chem. Soc.*, 1945, **22**, 94–95)—Two indicators are recommended for the determination of zinc by titration with potassium ferrocyanide: (1) ammonium molybdate (internal) and (2) ferric chloride (external).

(1) *Method*—After removal of all interfering heavy metals, neutralise the hydrochloric acid or nitric acid solution, containing about 0·03 g. of zinc oxide in a volume of about 100 ml., with ammonia, phenolphthalein being used as indicator. Add 5 ml. of glacial acetic acid, 10 ml. of 10 per cent. ammonium chloride, and 0·5 ml. of 2 per cent. ammonium molybdate solution, and titrate with standard potassium ferrocyanide (34 g./litre) to a chocolate-brown end-point. Traces of iron give an azure blue to chocolate-brown end-point, but large amounts of iron give a variable end-point and must be removed.

(2) Ferric chloride indicator—Oxidise a 10 per cent. solution of ferric chloride with a few drops of nitric acid, boil for 5 min., and cool. Discharge the yellow colour by the drop-wise addition of phosphoric acid, and then add 1 ml. of nitric acid. Use, soaked on a piece of filter paper, as an external indicator.

Method—Neutralise the zinc solution (free from interfering ions) to methyl orange and add 1 ml. of hydrochloric acid in excess. The volume should be about 100 ml. Add a measured excess of ferrocyanide solution, and titrate back with *N*/50 potassium permanganate until a drop of the solution placed on the prepared filter paper no longer gives a blue colour. Standardise the ferrocyanide solution in a similar manner, a standard solution of zinc being used instead of the test solution. These methods are recommended for the determination of zinc in pigments and non-ferrous alloys.

C. F. P.

Microchemical

Quantitative Separation of Tervalent from Quinquevalent Arsenic Derivatives on the Micro Scale. T. B. B. Crawford and G. A. Levvy (*Biochem. J.*, 1946, **40**, 455–458)—Quantitative removal of phenylarsenious acid, but not of phenylarsonic acid, from aqueous solutions was obtained by means of a 0·4% solution of ethane-1:2-dithiol in carbon tetrachloride.

Acidify the solution of the arsenical and extract by vigorous shaking for 2 mins. with two 20 ml. portions of the dithiol reagent, followed by three 20 ml. portions of pure carbon tetrachloride. After each treatment, add 5 ml. of carbon tetrachloride to the funnel and run off, without shaking, in order to wash out the stopcock and stem of the funnel. Analysis of the aqueous and carbon tetrachloride layers showed that arsenious acid and phenylarsenious acid were quantitatively removed into the chloroform layer, whilst arsenic acid and phenylarsonic acid were completely retained in the aqueous layer.

F. A. R.

Physical Methods, Apparatus, etc.

Simple Polarographic Cell. J. B. Gisclard (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 196)—A 5-ml glass syringe barrel closed at the bottom by a wired-on rubber stopper is used. A 20-gauge 5-cm. stainless steel hypodermic needle thrust upwards through the stopper will make contact with the mercury pool while permitting admission of a stream of nitrogen. When the soln. is being deoxygenated the tip of the needle is withdrawn below the surface of the liquid so that the gas bubbles through the liquid. After this, it is pushed above the surface and prevents re-entry of oxygen.

J. T. S.

Reviews

AN INTRODUCTION TO CHROMATOGRAPHY. By TREVOR I. WILLIAMS, B.A., B.Sc., D.Phil. Pp. viii + 100. London and Glasgow: Blackie & Son, Ltd. 1946. Price 10s. net.

This well-produced little volume is intended, so the author states in his preface, to meet the "needs of the student who, with an already crowded syllabus, requires a briefer and simpler account" than that given in the two standard textbooks, by Zechmeister and Cholnoky and by Strain, respectively. Admittedly these older books are rather detailed and perhaps, for the student, over-burdened with references, but at the same time they are full of practical wisdom about the best method of carrying out chromatographic separations under a variety of circumstances. The book under review must be judged by the extent to which it succeeds in presenting the subject in a form that the student can assimilate quickly and thoroughly. With this criterion in mind, the chapters on general principles, methods and applications deserve high praise; they are admirable summaries of the older literature and include many practical hints that are obviously the result of first-hand experience. "Disc chromatography" is described for the first time and there is a useful chapter on partition chromatography. An excellent account is also given—the first in English with the exception of a passing reference in a recent paper by the reviewer—of the method of Tiselius, which enables chromatography to be carried out with the aid of that most useful adsorbent, charcoal.

The one chapter in the book that might perhaps be better is that entitled "Examples of the Use of Chromatography." The examples, although illustrating several different methods of detecting the individual bands in a chromatogram, admittedly the most critical step in chromatography, are a trifle esoteric. Surely in writing a book expressly for the student the opportunity might have been taken to choose examples that the student can try out for himself. What better illustration of the basic principles of chromatography could have been found than Tswett's classical experiment on the separation of leaf pigments? This might have been followed by such exercises as the separation of a mixture of dyestuffs or the isolation of carbazole from anthracene, each illustrating different techniques, with the aid of substances that are familiar and readily available.

Apart from this, there is little in this book that deserves criticism, though the statement on p. 62 that "no use yet seems to have been made of organic adsorbents even for ordinary inorganic chromatography" is inaccurate, Erlenmeyer and his colleagues having used 8-hydroxyquinoline, violuric acid and 5-oxo-4-oximino-3-phenyl-isoxazoline for the separation of inorganic ions.

In mitigation of these adverse comments a special word of commendation should be given for the high quality of the illustrations, both photographs and coloured plates being excellent. Indeed the standard of publication is so much higher than we have been forced to accept in recent years that one hopes the days of austerity for British publications are at last drawing to a close and that they will soon be able to compete on equal terms with their opposite numbers in the United States. F. A. ROBINSON

INDUSTRIAL EXPERIMENTATION. By K. A. BROWNLEE, M.A. Pp. 116. London: H.M. Stationery Office. 1946. Price 2s.

That very active volcano, the Stationery Office, is in continuous eruption these days. So great is the flow that few subject it to more than a distant and cursory scrutiny for fear of being overwhelmed; and yet amid the arid waste (which, however, could hardly be called *form*less) there lies "full many a gem of purest ray serene" to reward the intrepid explorer.

The particular specimen now sparkling in the light of publicity is a good example. Sponsored by a Directorate—Royal Ordnance Factories (Explosives)—which the average chemist would not immediately associate with such matters, it is a clear account of the principles that should be followed in designing industrial experiments if the maximum return is to be obtained for a given expenditure of time and money, and of the arithmetical processes which enable sound and objective conclusions to be drawn from the resulting data. The emphasis throughout is on practice rather than theory, as is appropriate in a book for practical people; and a knowledge of mathematics, other than arithmetic and the ability to substitute in a formula, is not required.

There is, of course, a limit to the extent to which theory can be compressed or omitted in a subject so full of pitfalls as the design and computation of experiments, and the discussion in Chapter II of such fundamental concepts as "standard deviation" and "significance" may be too brief for many readers. Moreover, the imperative necessity for randomness in sampling is not explicitly dealt with, and in view of the ease with which an experiment can be ruined by lack of attention to this point, the omission is rather serious. If, for example, the output of a machine engaged on repetition work is sampled otherwise than genuinely at random, variation due to slow cyclic changes may be obscured or even suppressed.

In other respects the book contains much of value. A lucid exposition of the merits of "factorial" as compared with "classical" designs is followed by a detailed description of the main techniques likely to be useful in process control and development, with the appropriate computations in each case. There is no mention, however, of factorial experiments involving more than two levels of a controlled variate. The final chapters suggest many questions that would well repay statistical investigation in the field—from this point of view largelyunexplored —of chemical industry. Incidentally, the foreword by Dr. R. C. Bowden, Director of Ordnance Factories (Explosives), is admirable in its terse good sense, as may be illustrated by a quotation that epitomises the whole matter. "In carrying out an industrial experiment the choice is not between using a statistical design . . . or the ordinary methods; the choice is between correct or incorrect methods."

The cost of the book is so low as to be not significant ($£ = 0{\cdot}1$). It is recommended—with the reservation that it would be advisable for the purchaser to learn more of the fundamental theory of the subject elsewhere—to all whose work involves study of an industrial process, for it will at least indicate how to increase the usefulness of an investigation, the applicability of the resulting conclusion and hence the prestige (and it is to be hoped the remuneration!) of the investigator. ERIC C. WOOD

MICROCHEMISTRY GROUP

THE Annual General Meeting of the Microchemistry Group will be held on January 31st, 1947, at 4.30 p.m. at the Sir John Cass Technical Institute, 31, Jewry Street, Aldgate, London, E.C.3.

Tea will be provided after the meeting, and then a number of papers dealing with microchemical analysis will be read. Details will be announced later.

PHYSICAL METHODS GROUP

Preliminary Notice of Meetings

ON Tuesday, February 11th, 1947, at 6 p.m., a Meeting will be held in the Rooms of the Chemical Society, Burlington House, London, W.1, at which papers on Fluorimetric Analysis will be read by Mr. E. J. Bowen, F.R.S., Dr. E. Kodicek and Dr. D. M. Simpson.

On Friday, May 2nd, 1947, at 6 p.m., a Meeting will be held in the Chemistry Lecture Theatre, King's College, Newcastle-upon-Tyne, at which papers on Physical Methods of Gas Analysis will be read by Dr. C. E. Ransley, Mr. W. J. Gooderham and Dr. M. Akhtar.

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No. 849, Pages 553—600

Contents

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Printed for the Society of Public Analysts and other Analytical Chemists by W. Heffer & Sons Ltd., Hills Road, Cambridge. **Communications to be addressed to the Editor, J. H. Lane, 7-8, Idol Lane, London, E.C.3.**

ERRATA:

VOL. 71, 1946:

p. 42. Col. 2, lines 7–8 from the bottom of the page, *for* "2-aminopyridine" *read* "2-aminopyrimidine."

p. 82. Col. 1, line 42, *for* "8 g." *read* "18 g." of pure barium hydroxide.

p. 176. Line 21, *for* "potassium silicofluoride" *read* "fluosilicic acid."

p. 177. Line 4 from bottom of page, *delete* "from" now in front of "2 ml. to 50 ml."

p. 178. Graph I, along ordinate axis, *for* "0.009 *N*" *read* "0.0009 *N*."

p. 179. Graph II, along ordinate axis and in heading, *for* "Th/F" *read* "Th/F_4" and in heading *delete* brackets round "Range."

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