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THE ANALYST

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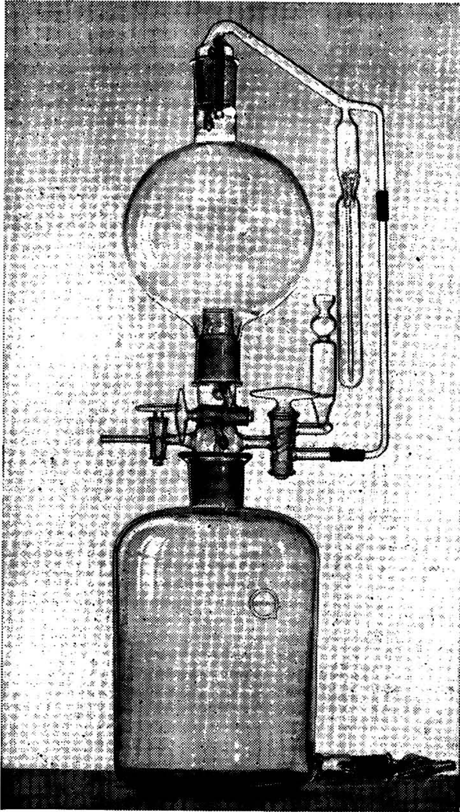

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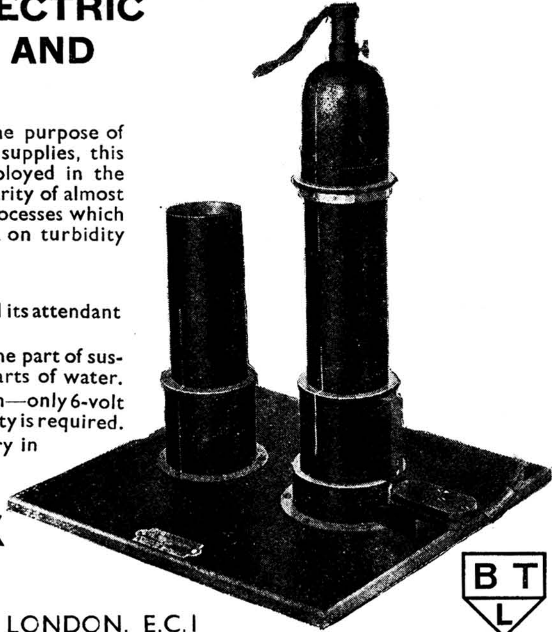

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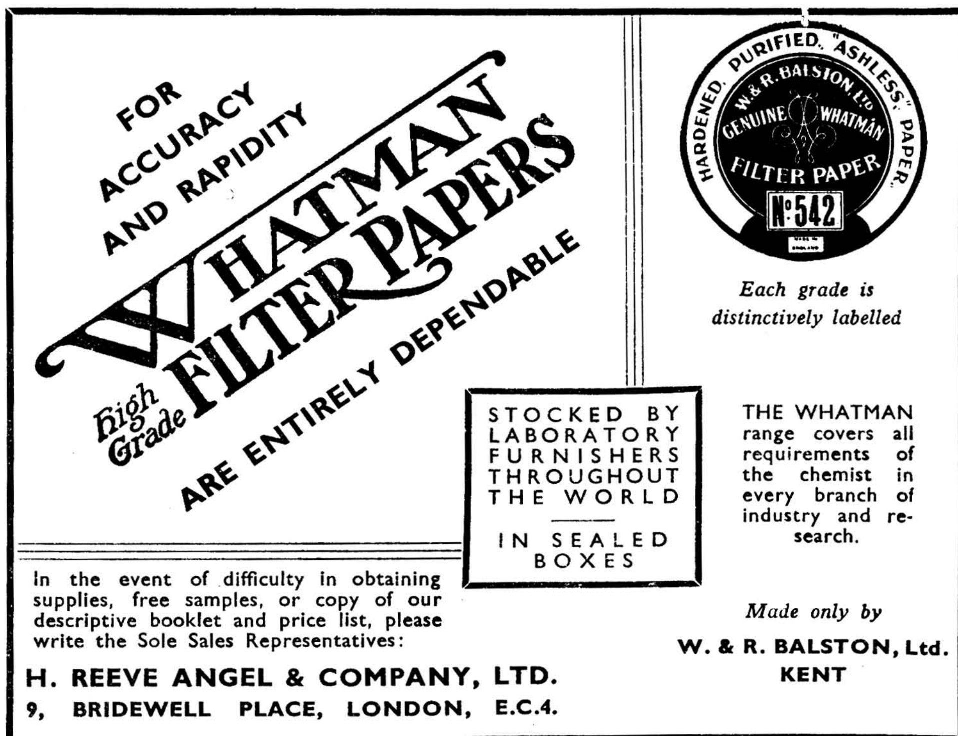

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NOVEMBER, 1946 Vol. 71, No. 848

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held on Wednesday, October 2nd, 1946, at 6 p.m., in the Chemical Society's Rooms, Burlington House, London, W.1. The President, Dr. G. W. Monier-Williams, was in the Chair. The following papers were read and discussed—"The Determination of Some Products of Sugar and Molasses Fermentation," by G. G. Freeman and R. I. Morrison; "The Estimation of Moisture in Propellant Explosives by an Improved Fischer Method," by T. G. Bonner; "The Analysis of Barium Carbide," by A. H. Edwards.

NEW MEMBERS

John Ansel Anderson, M.Sc. (Alberta), Ph.D. (Leeds), F.C.I.C.; Karl Bryce Warner Jones, B.Sc., Ph.D. (Lond.), A.R.C.S., A.R.I.C.; Reginald Dean Mason, M.Sc.(Lond.), F.R.I.C.; Richard Dennistoun Ray, B.A. (Cantab.), A.R.I.C.; Albert Lester Williams, F.R.I.C.

DEATHS

We regret to have to record the death of

Percy Faraday Frankland (Honorary Member)
Thomas McGrath
Francis Edwin Needs
Sidney Watkinson

BIOLOGICAL METHODS GROUP

A meeting of the Biological Methods Group was held at 6 p.m. on Monday, May 27th, at The Chemical Society's Rooms, Burlington House, London, W.1. Mr. A. L. Bacharach occupied the chair. The following papers were presented and discussed:—"The Genetical Requirements of Bio-assays with Higher Organisms," by Dr. K. Mather; "The Genetical Aspects of Bio-assays with Micro-organisms," by Dr. G. Pontecorvo. The papers appeared in The Analyst, 1946, 71, pp. 407–411 and 411–413, respectively, and the discussion on them will be found on pp. 535–536 of this issue.

The Direct Estimation of Polymer and Carbon Black in Vulcanised Butyl Rubber (GR-I)

By P. D. Galloway and W. C. Wake

Introduction—The normal methods of vulcanised rubber analysis consist, briefly, in evaluating the extracts in various solvents, and determining carbon black after destruction of the rubber. An estimate of the rubber is obtained either as a difference, when extracts, carbon black, sulphur and total fillers have been accounted for, or by the more recently introduced direct methods. In the case of butyl rubber (Butyl B), or GR–I, to give it its war-time designation, the difference method is not applicable, because the chemical inertness of the material prevents destruction of the rubber under conditions which leave carbon black unaffected, and no direct method has hitherto been published. The present paper seeks to fill this gap in analytical procedure by providing a means for estimating directly both the butyl rubber and the carbon black.

Butyl rubber is a co-polymer of isobutene with small amounts of butadiene or isoprene. Recent samples examined give a definite positive Weber reaction, indicating the presence of isoprene units in the chain, although earlier samples respond to this reaction by only a faint violet, which may be characteristic of the butadiene polymers.[1] The acetone extract of the rubber as received is about 1·3 per cent., and the ash 0·3 per cent., so that it contains about

98·4 per cent. of co-polymer. Kemp and Peters[2] investigated the applicability of their modified procedure for the determination of unsaturation by iodine values to butyl rubber, but they report that substitution vitiates the results. The standard procedure recommended by these authors for natural rubber and GR–S was used in the present work to compare with the results obtained by them over a varied range of conditions for both butyl rubber and polyisobutene in order to attempt an estimation of the diene component of the co-polymer by difference; it is reported[3] that this is between 1 and 2 per cent.

Rehner,[4] with ordinary butyl rubber, obtained values of 0·88 to 1·1 mole per cent. of unsaturation by the addition of iodine chloride by various methods. These values were greater than those obtained by the same author from the addition of other reagents and from viscosity determinations on ozone-degraded material.[5]

Bromine addition, with the precautions enumerated by Bloomfield[6] for the estimation of raw natural rubber, gave results, calculated as iodine values, lower than those from the addition of iodine chloride. The use of iodine chloride at or near 0° C. appears, from the figures of Kemp and Peters as well as those found in the present work, to give values only slightly lower than those obtained at room temperature. Table I summarises these results and shows that the introduction of the diene into the polymer increases its iodine value by, say, 4 to 9 units. Since the diene in the samples examined was shown to be isoprene, we obtain 1·1 to 2·4 per cent. as the amount present.

TABLE I

UNSATURATION EXPRESSED AS IODINE VALUE

Types of material	Temp. for ICl estimation	By ICl in CCl_4 (quoted from Kemp and Peters)	Values obtained in present series of experiments: By ICl in CCl_4	Values obtained in present series of experiments: By bromine addition
Polyisobutene ..	R.T.*	1·4	4·3	3·5
	Zero†	1·4	—	
Butyl Rubber (unvulcanised) ..	R.T.*	3·5–7·1	13·6	11·9
	Zero†	3·2–4·9	—	
Extract obtained in estimation of vulcanised butyl (*vide infra*)	Zero	—	3·9	3·5

* R.T. = room temperature.
† Zero = temperature obtained with ice, or 3° C. for the results quoted from Kemp and Peters.

Since polyisobutene cannot be vulcanised by the usual techniques, it follows that the vulcanisation of butyl rubber occurs through the reactivity conferred by inclusion of the diene. The latter forms reactive centres which are susceptible to attack and it was thought that oxidising agents (*e.g.*, nitric acid or hydrogen peroxide) might split the vulcanised molecules at those points, leaving what is, in effect, an inert polyisobutene substantially without cross-linking. This could then be estimated by Parker's method,[7] which consists of an extraction of the polyisobutene from the fillers by light petroleum, normally carried out after the usual 16 hours' acetone extraction.

EXPERIMENTS ON THE DEGRADATION OF BUTYL RUBBER TO POLYISOBUTENE—Various reagents were used in preliminary experiments briefly summarised here. Boiling with chromic-sulphuric acid, heating at 80° C. with 80 per cent. sulphuric acid, and heating at 80° C. for 16 hours with 100 vol. hydrogen peroxide resulted in recovery of an impure polyisobutene varying from 11 per cent., with the chromic acid treatment, to 50 per cent., with the hydrogen peroxide. Concentrated nitric acid heated above its boiling point, in a Carius tube, completely destroyed the rubber, but the same reagent seemed promising at its boiling point under atmospheric pressure; the effect of varying the duration of such acid treatment is shown in Fig. 1. It was hoped that if the acid treatment were carried out in presence of the extracting solvent (light petroleum), extraction of the liberated polyisobutene by the solvent phase might retard its further degradation, but experiments in which pure polyisobutene dissolved in light petroleum was boiled with acid, giving two phases, resulted in only 75–80 per cent. recovery. Attention was therefore concentrated on a multi-stage form of estimation in which as much polyisobutene as possible was removed by solvent

extraction after short periods of treatment with nitric acid; evidence is given later that in this way a quantitative recovery of polyisobutene is obtained without excessive degradation.

EFFECT OF NUMBER AND TIME OF TREATMENTS—The need for the multi-stage nature of the estimation is shown by some of the experiments carried out during the development of the method. Samples containing carbon black which were finely divided by buffing or rasping were found unsuitable because, whatever the nature of the washing or solvent extraction that followed the acid treatment, copious quantities of carbon black contaminated the extract obtained. The samples were therefore cut finely with scissors; if, however, the pieces were too large, an increased number of stages was found necessary to complete the estimation. About 0·5 g. of cut material was weighed into each of several flasks and boiled under reflux with concentrated nitric acid (sp.gr. 1·42) for various periods. After cooling, the acid was neutralised and the pieces of rubber were removed by filtration. These were extracted with water refluxing from a boiling solution of sodium carbonate in order to be sure of the complete elimination of acid. The rubber was then extracted with light petroleum for 16 hours. The results of these experiments are shown in the graph, Fig. 1. This shows clearly that with increasing time an increasing amount of the polymer is degraded sufficiently to render it soluble in light petroleum but, unless this degraded material is removed, further degradation occurs which results in loss of polyisobutene. Experiments showed that about 10 minutes is the optimum time of nitric acid attack at each stage, and that, with the material cut to pass a 10-mesh sieve, three successive treatments are needed. The amount of material recovered as a result of the third acid treatment is small but not negligible. Small variations in the time of reaction with nitric acid are not very important; this is indicated in Table II by experiments designed to test this point and to see if the third extraction could be rendered unnecessary by small alterations of this type.

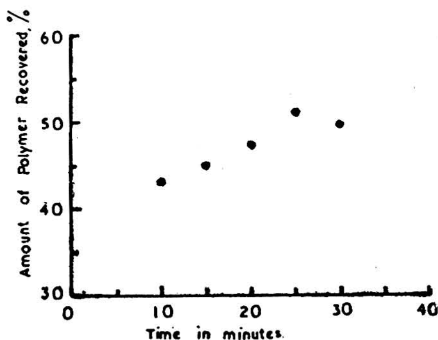

Fig. 1

TABLE II

EFFECT OF TIME OF ACID ATTACK IN MINUTES ON POLYMER RECOVERED AT EACH STAGE*

1st Stage		2nd Stage		3rd Stage	
Time min.	Polymer %	Time min.	Polymer %	Time min.	Polymer %
15	91·5	10	6·9	10	1·6
10	92·5	15	6·1	10	1·5
10	95·8	10	2·9	5	1·3

* Polymer as per cent. of total recovery of polyisobutene.

NATURE OF THE EXTRACTED MATERIAL—It is obvious from the nature of the original butyl rubber that the extract obtained in this method must be substantially polyisobutene. The agreement of the unsaturation values recorded in Table I for polyisobutene and for the extract show that the additional reactivity of butyl rubber, conferred by the diene component, has been eliminated in the extracted material. The degree of degradation was investigated by a comparison of the intrinsic viscosities (η) of the materials in toluene, where $(\eta) = \mathrm{Lt}_{c \to 0} \frac{\eta_{sp}}{c}$, η_{sp} being the specific viscosity and c the concentration in g. per 100 ml. Molecular weights are calculated from the figures given in column 2 of Table III by the modified Staudinger equation $(\eta) = KM^{\alpha}$ where $K = 3{\cdot}5 \times 10^{-4}$ and $\alpha = 0{\cdot}6$.

TABLE III

VISCOSITIES AND MOLECULAR WEIGHTS

Material	(η)	Molecular weight
Unvulcanised butyl rubber ..	0·85	290,000
Extract from butyl vulcanisate ..	0·45	105,000

As would be expected, the molecular weight of the extracted material is considerably less than that of the original butyl rubber in its unvulcanised state. If substantially all the diene links are attacked, as the unsaturation indicates, then the molecular weight of the remaining material would be completely determined by the amount of diene in the original material and its distribution therein. For 2 per cent. of diene a molecular weight of about 3000 would be expected and the higher value obtained, 105,000, may be explained by some cross-linking and saturation of the few remaining double bonds by accelerator and similar substances. From the mode of obtaining the extracted polyisobutene from the butyl rubber it would be expected that the end-groups of the material would be carboxylic acid groups. Some confirmation has been obtained in experiments originally designed to test the efficacy of the washing of the material to free it from the nitric acid. Rinsing with cold water, or a fairly thorough series of washes with hot water, left relatively large amounts of nitric acid in the sample after the first treatment. This was materially reduced by washing with acetone or industrial spirit after a preliminary water wash. The spirit is preferable because turbidimetric titration of a solution of polyisobutene in light petroleum with spirit and with acetone showed that the former was the better precipitant for the polyisobutene. Even this double washing technique failed to remove the mineral acid completely, for trouble was experienced with acid fume when the extract was dried. The extent of this retention of nitric acid was determined by carrying through a stage of the estimation, *i.e.*, treating with acid, washing thoroughly with water, followed by aqueous alcohol and finally alcohol, and then extracting with light petroleum for two hours. The extract, still dissolved in light petroleum, was transferred to a stoppered bottle, shaken with water, and titrated with $N/10$ sodium hydroxide from a micro-burette. A titre of 0·20 ml. was obtained with methyl orange indicator and 1·00 ml. with phenolphthalein. The use of anhydrous alkali carbonate, as set out in the details of procedure, was found to eliminate the trouble due to this small amount of acid without introducing error, provided that the washing instructions are closely followed. However, the figures quoted above are of interest, as the difference in titre with the two indicators is, presumably, due to carboxylic acid groups in the extract. Assuming a basicity of two, the molecular weight was found to be 6000 compared with 105,000 calculated from viscosity data. This discrepancy is probably due to additional carboxylic acid groups from the oxidation of side or branched chains.

EFFECT OF NATURE OF SOLVENT ON THE EXTRACTION—Various solvents were tried for the extraction, the following points being considered: (*a*) period of extraction, (*b*) ease of evaporation, (*c*) degree of inflammability and — the most important feature — (*d*) degree of contamination of the extract with carbon black. The last was, in fact, the deciding factor since, although some slight coloration of the extract with black can be disregarded as representing negligible weight, real contamination necessitates a repeated estimation. This was found to be, very markedly, a function of the solvent used, carbon tetrachloride, for example, bringing most of the carbon black through the filter, and light petroleum (b.pt. 40° to 60° C.) never producing major trouble of this nature. Table IV gives the solvents examined, a qualitative note on the amount of carbon black extracted, and the internal pressures of the solvents. These last have been calculated from the approximate formula.[8]

$$\text{Internal pressure} = \frac{\text{surface tension}}{(\text{molar volume})^{\frac{1}{3}}}$$

and give the relative order if not the absolute magnitudes.

TABLE IV

INTERNAL PRESSURES OF SOLVENTS AND EXTRACTION OF CARBON BLACK

Solvent	Internal pressure	Contamination by carbon black
Benzene	6·5	Very black
Carbon tetrachloride	5·9	,, ,,
Cyclohexane	5·3	Slightly black
n-Hexane	3·6	Usually clear
Light petroleum	*ca.* 3·0–3·5	Usually clear

Since this contamination is not explained by any simpler property, its mechanism is probably due to the solvents of high internal pressure disaggregating the particles of carbon black which aggregate during the acid treatment. This would be in line with observations on the swelling of substances with micellar structure in solvents[9] and with the observed

state of division of the black. Light petroleum is, then, the most satisfactory solvent and is also the most volatile. Drying of the polyisobutene is usually complete in 1½ hours.

METHOD ADOPTED FOR THE DIRECT ESTIMATION OF BUTYL RUBBER

Take about 0·25 to 0·5 g. of the sample, which need not be acetone-extracted, cut it up small enough to pass a 10-mesh sieve (it must *not* be buffed or rasped), weigh it into a sintered glass crucible of porosity 2, and boil under reflux for 10 minutes with nitric acid (sp.gr. 1·42), the crucible being supported on a glass stand in the simple apparatus shown in Fig. 2. The glass stand should raise the crucible about 1½ inches from the bottom and sufficient nitric acid to flood the sintered disc should be used. After the 10-minute period, allow the crucible to drain out of contact with the hot acid and then wash it at the filter pump with two washings each of 25 ml. of water, followed by two similar washings of aqueous methylated spirit (1+1 by volume mixture) and one of undiluted methylated spirit. Then suck the crucible dry, wipe it free from spirit on the outside and return it to similar apparatus, this time with another glass stand which brings the crucible 2 inches higher in the tube and out of contact with the liquid (as shown in Fig. 2), and extract with light petroleum (b.pt. 40° to 60° C.) for 2 hours. Repeat the acid digestion, washing, and subsequent extraction with light petroleum *twice*, making a *total of three* treatments. It is advantageous to add a suitable anti-bumping agent during the extraction. Mix the three lots of extraction liquid together and add a small quantity (between 0·5 and 1 g.) of anhydrous sodium or potassium carbonate. Allow to stand for 10 to 15 minutes and then filter through a fluted filter paper (No. 1 Whatman) and wash carefully, particularly the edges of the paper, with a fine stream of boiling light petroleum. This washing is important; about 50–70 ml. of light petroleum are required to wash the paper quite free from polyisobutene. Incomplete washing will be indicated by stickiness of the edges of the filter paper. Distil off excess of light petroleum from the filtered extract and, when the liquid bulk has become small, transfer it, again with hot washing, to a tared carbon dioxide flask. Evaporate just to dryness on the hot plate and then transfer to an oven (60° to 80° C. has been found convenient) and dry to constant weight. Express the weight of extract as per cent. of polyisobutene in the original material, or multiply by 1·03 and express it as per cent. of co-polymer in the original material.

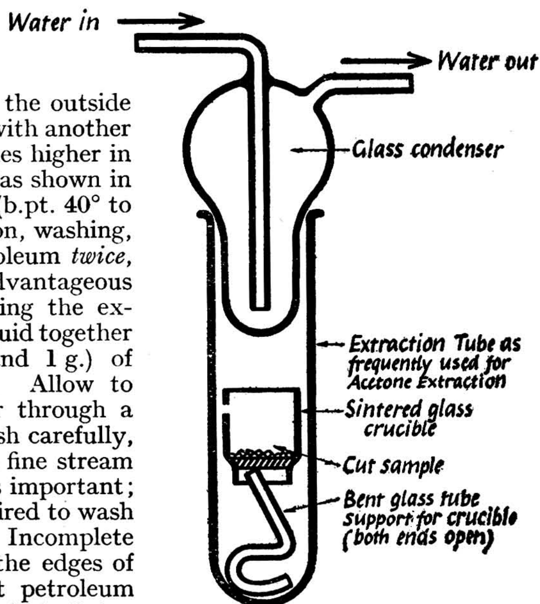

Fig. 2

RESULTS OBTAINED—Table V gives the results of 11 estimations on the same vulcanised sample (rubber A in Table VII) by two observers using this procedure. The theoretical equivalent of polyisobutene present is 58·8 per cent., corresponding with 60·5 per cent. of co-polymer, whilst the means of Table II are 58·2 and 60·0 per cent. respectively. No significant difference is obtained if the results by the two observers are considered separately, the mean figures for co-polymer then being 59·5 and 60·6 per cent. Further values obtained in duplicate determinations on samples B to F (details of composition given in Table VII) are shown in Table VI. Variation of the amount of butyl rubber present in samples C to E was obtained by admixture with a polychloroprene before vulcanisation. For technological reasons, synthetic rubbers based on polychloroprene (Neoprene types) are the only vulcanisable polymers likely to be mixed with butyl rubber. These mixtures, therefore, served the dual purpose of confirming the non-interference of the second polymer component and giving a range of butyl content; they all contained carbon black.

TABLE V

RESULTS OF ESTIMATION OF BUTYL RUBBER IN SAMPLE A

Weight of sample, g. ..	0·25	0·25	0·4	0·25	0·25	0·4	0·4	0·4	0·4	0·4**	0·4
Polyisobutene, % ..	60·5	57·3	58·6	57·7	58·3	56·0	57·5	57·2	57·3	61·0	59·2
Co-polymer, % ..	62·1	58·9	60·3	59·6	60·2	57·5	59·2	58·9	59·2	62·8	61·1

TABLE VI

RESULTS OF ESTIMATION OF BUTYL RUBBER IN OTHER SAMPLES

Rubber	Experimental results %	Mean as polyisobutene %	Mean as co-polymer %	Calculated co-polymer %
B	58·0, 60·0	59·0	60·8	60·6
C	45·6, 45·6	45·6	46·9	46·4
D	23·9, 24·2	24·1	24·8	28·7
E	12·2, 12·3	12·3	12·6	11·5
F	57·7, 57·3	57·5	59·2	60·5

The figures given in Tables V and VI show the order of accuracy to be expected by following the method given above, since none of the results obtained under the varied conditions used in establishing the technique and limitations of the method are included in these Tables.

METHOD ADOPTED FOR THE DIRECT ESTIMATION OF CARBON BLACK

When butyl rubber is estimated by the method outlined above, the residue remaining on the sintered crucible after the last extraction consists of carbon black, if this was present in the mix, and any inorganic fillers not destroyed by the nitric acid. The ignition of this residue after it has been dried to constant weight gives the amount of carbon black present, subject to the usual reservations as to the presence of clay,[10] etc. A series of such carbon black estimations following the estimation of butyl was made on vulcanisate A. The following values were obtained consecutively, 31·4, 31·1, 30·9, 31·8, 28·7 and 28·0 per cent.; the mean of these is 30·3 per cent., which compares well with 30·8 per cent., the amount found by calculation from the mix formula. The standard error of the mean of the usual duplicate determinations would thus be about ±1·0 on the percentage of black, *i.e.*, about 3 per cent. of the amount of black present.

TABLE VII

COMPOUNDS USED

Ingredient	A & F	B	C	D	E
Butyl rubber	100	100	80	50	20
Neoprene GN (Polychloroprene)	—	—	25	50	80
Sulphur	2	2	2	2	2
Zinc oxide	5	5	5	10	10
Stearic acid	3	3	3	3	3
Magnesium oxide	—	—	—	4	4
Carbon black	50	50	50	50	50
Tetramethylthiuram disulphide	1	1	1	1	1
Zinc diethyldithiocarbamate	1·5	1·5	1·5	1·5	1·5
Vulcanised, minutes at 153° C.	50	40	35	35	35

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The Determination of Some Products of Sugar and Molasses Fermentations

By G. G. FREEMAN and R. I. MORRISON

(*Read at the Meeting of the Society on Wednesday, October 2, 1946*)

IN the course of studies on the 2:3-butylene glycol fermentation of sucrose and molasses, methods were investigated for the determination of the major non-gaseous fermentation products, namely, 2:3-butylene glycol, acetoin (acetomethylcarbinol), ethanol and lactic acid, in the presence of each other and of large quantities of sugar. Methods for the determination of sugars present in the initial medium and at various stages during the course of the fermentation were also examined. The work was concerned mainly with *Aerobacter aerogenes* fermentations of mashes containing sucrose, invert sugar, small quantities of mineral nutrients and calcium carbonate, and unfermentable organic matter derived from sugar cane or beet extracts, yielding a *d-meso* mixture of 2:3-butylene glycol isomers as the main non-gaseous product. The methods adopted appear applicable to a wide range of sugar fermentations by micro-organisms such as yeasts, bacteria and mould fungi in which ethanol, lactic acid and associated products are formed. They were chosen as suitably combining accuracy, rapidity and convenience for routine use.

The initial concentration of sugar in the media was usually about 10 per cent., which fell to zero on completion of fermentation. The final concentration of 2:3-butylene glycol was usually 3–4 per cent., that of ethanol 1·0–1·5 per cent., and that of lactic acid 0·5–1·0 per cent. Acetoin production was more variable, being frequently nil, but under conditions of prolonged aeration it reached as much as 2·0 per cent. or even more. In a few experiments the initial sugar concentration was 15 or 20 per cent. and the concentrations of the products were correspondingly higher.

Determination of reducing sugars

To ascertain the initial sugar concentration of fermentation media, and to follow the course of the fermentation by means of the fall in sugar concentration, reducing sugars were determined by means of a modified Fehling Soxhlet method in which cuprous oxide was determined iodimetrically by the method of Shaffer and Hartmann.[1]

The analysis of fermentation liquors involved a preliminary treatment with neutral lead acetate for clarification and removal of protein matter. This treatment proved effective in arresting fermentation and in removing the bacteria from the sample. Excess of lead was removed as the oxalate. When sucrose was present, preliminary inversion was necessary and the results were expressed in terms of invert sugar. In presence of acetoin a correction was applied to the results of sugar determinations (see p. 512).

PROCEDURE—1. *Clarification and removal of proteins*—The following method is suitable for solutions containing up to 20 per cent. of sucrose or reducing sugars. Measure 10 ml. of the sample into a 50 ml. standard flask containing 10 ml. of 10 per cent. lead acetate solution, dilute to volume and mix. A flocculent precipitate forms which coagulates and settles more or less slowly, leaving a clear supernatant liquid. Filter through a dry filter paper into a dry flask. To remove excess of lead, treat 25 ml. of the filtrate with 5 ml. of 10 per cent. neutral potassium oxalate solution, allow the mixture to stand for half an hour to ensure complete precipitation of the lead oxalate and filter through a fine paper (Whatman No. 40) into a dry flask. The filtrate may be slightly coloured but should be perfectly clear.

2. *Inversion with hydrochloric acid*—The solution for analysis usually contained both sucrose and hexoses. Inversion of the sucrose without destruction of any hexoses already present was satisfactorily effected as follows.

To 20 ml. of the clarified lead-free filtrate, in a 100 ml. conical flask, add 2·5 ml. of concentrated hydrochloric acid and dilute the mixture with water to 50 ml. Mix well, and maintain at 70° C. for 10 minutes in a water-bath. Cool, neutralise by *cautious* addition of dry potassium carbonate (2·2 g.) and dilute to 100 ml. in a standard flask. Twenty ml. of this solution is usually a suitable quantity for each sugar determination.

3. *Iodimetric determination of reducing sugars*—Full details of the method are given by Shaffer and Hartmann.[1]

RESULTS—The results summarised in Table I were obtained by weighing 2·0194 g. of pure sucrose, hydrolysing and neutralising as described above, making up to 500 ml. and carrying out determinations on aliquots of the solution.

TABLE I

DETERMINATION OF INVERT SUGAR

Volume of sugar solution taken ml.	Invert sugar taken mg.	Titration 0·1 N thiosulphate ml.	Invert sugar found mg.	Recovery %
50	212·4	60·0	209·7	98·7
25	106·2	31·9	105·8	99·6
20	84·8	25·3	83·0	97·8
10	42·4	12·9	41·5	97·8
5	21·2	6·7	21·4	101·0
			Mean	99·0

CORRECTION FOR THE PRESENCE OF ACETOIN—Acetoin is the only reducing substance produced in the fermentation of sugars by *Aerobacter aerogenes* and its determination and reducing power are discussed below. To correct the results of sugar determinations for the presence of acetoin, calculate the cuprous oxide equivalent of the acetoin in the sample from the separately determined acetoin concentration and the relationship that 1 mg. of acetoin is equivalent to 3·06 mg. of cuprous oxide. Subtract this from the cuprous oxide as found in the sugar determination and calculate the true sugar concentration from the remainder in the usual manner.

DETERMINATION OF 2:3-BUTYLENE GLYCOL

Two principal methods are described in the literature for the determination of 2:3-butylene glycol. One depends upon oxidation of the glycol by bromine to acetoin, and oxidation of the latter by ferric chloride, or better by a mixture of ferric chloride with ferrous sulphate, to diacetyl, which is then converted to the nickel complex of dimethylglyoxime.[2,3,4,5,6,7,8,9] The method is, of course, also applicable to the determination of acetoin and diacetyl. We have found it inconvenient and inaccurate, and in common with Ledingham, Adams and Stanier[10] we have been unable to obtain a quantitative conversion of diacetyl to nickel dimethylglyoxime. Table II gives some results obtained by this method for acetoin under the conditions described by Kniphorst and Kruisheer.[2] Determinations of diacetyl yielded similar results.

TABLE II

DETERMINATION OF ACETOIN BY NICKEL DIMETHYLGLYOXIME METHOD

Acetoin taken mg.	Nickel dimethylglyoxime obtained mg.	Theoretical mg.	Recovery %
20·14	26·5	32·9	80·5
10·07	12·9	16·5	78·5
50·35	63·5	82·5	77·0

The alternative method, based on the quantitative oxidation of the glycol by periodic acid to two molecules of acetaldehyde, is less specific but was found to have advantages in rapidity and accuracy. It was found that, with the exception of acetoin, for which a correction could be applied, interfering substances, notably sugars and lactic acid, could be most readily eliminated by a steam distillation of the sample after saturation with sodium carbonate. Johnson[11] states that this treatment produces volatile interfering substances from the decomposition of sugars, but we have been unable to confirm this. Blank determinations on solutions of sucrose and molasses yielded zero results. It is advisable, however, to test the steam source, which may contain a small quantity of volatile interfering substances. Acetaldehyde or acetone and other low boiling ketones are not of course eliminated by this treatment but no such substance was present in significant quantities. The acetaldehyde formed by oxidation of the glycol may be absorbed in a solution of hydroxylamine hydrochloride and determined by titration of the liberated hydrochloric acid with standard sodium hydroxide.[12] This method is very suitable for routine use. A less rapid but more sensitive

method is the absorption of the acetaldehyde in a solution of sodium bisulphite and titration of the bound bisulphite with iodine.[11] The apparatus and procedure described below are based on those of Brockmann and Werkman[12] but considerably modified, so that it is convenient to describe them in full. The modified methyl orange indicator used is due to Hickman and Linstead.[13] The method has been in continuous use for routine determinations of 2:3-butylene glycol for about a year and has yielded consistently reliable results.

APPARATUS—(*a*) *Steam distillation*—The constant volume steam distillation is carried out from a 200 ml. Kjeldahl flask. The distillate passes to a double surface condenser and the condensate is collected in a 250 ml. standard flask.

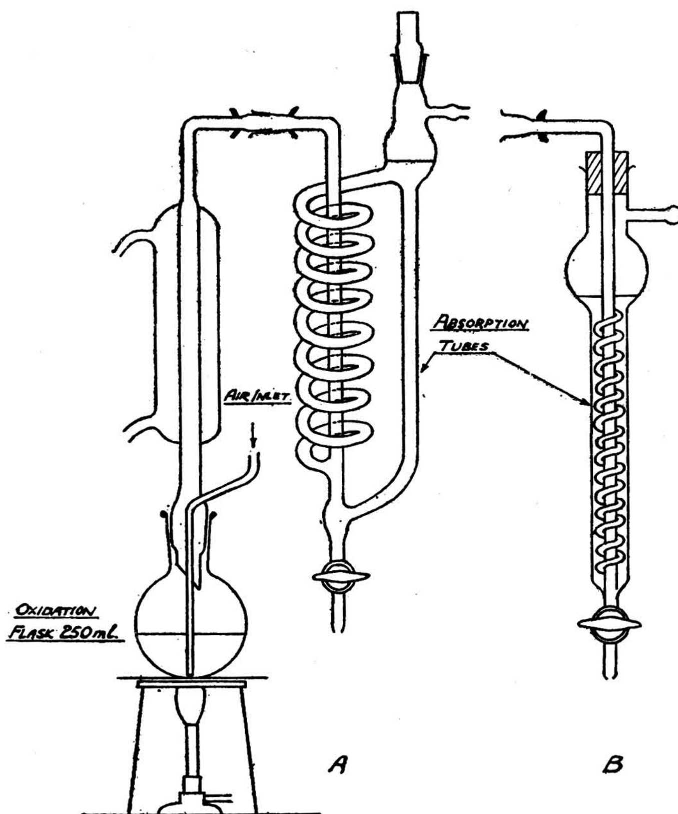

Fig. 1

(*b*) *Oxidation and absorption*—Apparatus for the oxidation of the glycol and absorption of the acetaldehyde (Fig. 1) consists of a 250 ml. bolthead flask fitted with a rubber bung or ground joint carrying a vertical condenser and an air inlet tube passing to the bottom of the flask. The oxidation takes place in the flask, which is heated over a wire gauze by means of a small flame. The condenser leads to a spiral absorption tube of about 25 ml. capacity. The type of tube illustrated in Fig. 1 A (from a design privately communicated by A. J. Williamson) has proved highly satisfactory and another suitable type[14] is shown in Fig. 1 B. Air may be forced through the air inlet from a compressed air supply, or drawn through the apparatus by means of a water jet pump.

PROCEDURE—(*a*) *Preliminary treatment*—Measure the sample (10 ml.) into a 50 ml. standard flask containing 10 per cent. neutral lead acetate (10 ml.), dilute to volume, mix and filter. This treatment effectively prevents foaming during the distillation.

(*b*) *Steam distillation*—Measure 10 ml. of the clear filtrate into the flask and add 20 g. of anhydrous sodium carbonate. Steam distil at constant volume (15–20 ml.) until about 200 ml. of distillate have been collected. Dilute to 250 ml. Time taken 15–20 minutes.

(*c*) *Oxidation and absorption of acetaldehyde* (*hydroxylamine method*)—Measure 100 ml. of the distillate, containing not more than 30 mg. of 2:3-butylene glycol, into the oxidation flask with 40 ml. of potassium periodate solution (3 g./litre) and 10 ml. of 5 *N* sulphuric acid. Place 20 ml. of hydroxylamine hydrochloride solution (7 g./litre), containing 4 drops of the indicator solution (0·10 g. of methyl orange and 0·15 g. of xylene cyanole FF in 100 ml.) in the absorption tube. Pass a slow current of air (about 4 bubbles per sec.) through the apparatus and heat the contents of the oxidation flask to boiling over a small flame. Continue heating and aeration for one hour after the contents of the flask have reached boiling-point.

(*d*) *Titration*—Drain the contents of the absorption tube into a 350 ml. conical flask and wash out the tube twice with 25 ml. of water each time. Prepare an end-point standard from 20 ml. of the hydroxylamine hydrochloride solution diluted to 100 ml. If necessary, dilute the contents and washings from the absorption tube so that the total volume at the end of the titration is approximately that of the standard and titrate with 0·05 *N* sodium hydroxide until the colour of the indicator is the same as that of the standard. At the end-point of the titration the *p*H is 4·06 and the indicator is neutral-grey in colour; on the acid side it is magenta, and on the alkaline side it becomes green.

1 ml. of 0·05 *N* sodium hydroxide ≡ 2·25 mg. of 2:3-butylene glycol.

The bisulphite - iodine method for determination of acetaldehyde may be used when the sample for oxidation contains 5 mg. or less of 2:3-butylene glycol. The same apparatus is used but the absorption tube contains sodium bisulphite (5 g. per litre). The remainder of the procedure is as described by Johnson.[11]

Correction for the presence of acetoin—The oxidation of acetoin by periodic acid is considered later. To obtain the true concentration of 2:3-butylene glycol, subtract from the apparent concentration the acetoin concentration multiplied by the factor 0·50. The amount of acetoin in the sample oxidised should not exceed 20 mg.

RESULTS—A sample of *d-meso* 2:3-butylene glycol, having b.pt. 182·5° C. and n_D^{20} 1·4385, was used as a standard. Portions of a solution of this material (9·092 g. per litre) were treated as described above for the determination of 2:3-butylene glycol by the hydroxylamine method, omitting the preliminary treatment with lead acetate. The results are shown in Table III.

TABLE III

DETERMINATION OF 2:3-BUTYLENE GLYCOL

Volume of solution ml.	Glycol present mg.	Glycol found mg.	Recovery %
5	45·5	45·3	99·5
10	90·9	89·9	98·9
20	181·8	180·0	99·2
		Mean	99·2

A solution was prepared containing glycol 56·4 g. per litre and 20 per cent. of High Test molasses (≡ 150 g. of total sugars as invert sugar per litre of the solution) and a number of determinations were made on 10 ml. portions—this time with the lead acetate treatment included. Three determinations gave values of 56·0, 55·8 and 56·2 g. per litre—a mean recovery of 99·2 per cent., showing that the presence of sugars and molasses impurities did not influence the determination of the glycol.

DETERMINATION OF ACETOIN (ACETYLMETHYLCARBINOL)

Acetoin is a normal product of the fermentation of sugars by *Aerobacter aerogenes* under oxidising conditions, being produced either by oxidation of 2:3-butylene glycol or by inhibition of the reduction reaction : acetoin → 2:3-butylene glycol, according to the conditions. It interferes with the determination of sugars, 2:3-butylene glycol and ethanol, and an accurate and fairly rapid method for its determination was therefore required. The method due to Langlykke and Peterson[15] was found to be the most satisfactory. This is based upon the quantitative reaction of acetoin with hypoiodite to yield iodoform, and upon the rather surprising fact that when dilute solutions of acetoin are distilled without reflux, the fraction of the total acetoin in any specific portion of the distillate is constant and independent of the original concentration. Thus by distilling three-quarters of the solution,

rejecting the first half, and collecting and using the third quarter, interfering substances, principally ethanol, are eliminated. Langlykke and Peterson found that 23·9 per cent. of the acetoin in the sample appeared in the third quarter of the solution distilled, and Blom and Efron,[16] who have obtained data on the liquid - vapour equilibria for the system acetoin - water, estimate that in the distillation of solutions containing up to 1·0 per cent. of acetoin, 24·0 per cent. of the total will appear in the third quarter of the solution distilled. Our results gave 23·4 per cent. The portion of the distillate used is treated with an excess of standard alkaline iodine solution and after a definite period acidified, and the excess of iodine is determined by titration with thiosulphate solution.

The apparatus and procedure used were essentially as described by Langlykke and Peterson, with the difference that the sample (5 ml.) was diluted to 250 ml., of which 100 ml. were taken for distillation, and instead of 0·2 *N* iodine and 0·1 *N* sodium thiosulphate the standard solutions used were 0·02 *N* iodine (25 ml.) and 0·01 *N* thiosulphate.

1 ml. of 0·02 *N* iodine ≡ 0·294 mg. of acetoin.

A small quantity of 2:3-butylene glycol appears in the third quarter of the solution distilled and reacts slowly with the alkaline iodine reagent. It was found that, under the conditions employed, the relation between the total glycol in the sample and the resulting error in the acetoin determination could be taken as approximately linear for dilute solutions. Table IV shows the results obtained by distilling 100 ml. lots of solution containing various known quantities of 2:3-butylene glycol, and treating the third quarter of the solution distilled as for acetoin determination.

TABLE IV

EFFECT OF 2:3-BUTYLENE GLYCOL ON ACETOIN DETERMINATIONS

Glycol in 100 ml. of samples mg.	Vol. 0·02 *N* iodine ml.	Acetoin equivalent mg.	Acetoin equivalent per mg. of glycol mg.
480	3·10	0·91	0·0019
240	1·48	0·44	0·0018
96	0·75	0·22	0·0023
48	0·37	0·11	0·0023

Since the weight of glycol in the sample for distillation does not normally exceed 100 mg., the error thus introduced can usually be neglected, but if desired a correction may be introduced.

RESULTS—A synthetic mixture was prepared containing in solution approximately 10 per cent. of sucrose, 1 per cent. of ethyl alcohol, 1 per cent. of acetic acid and 4 per cent. of 2:3-butylene glycol. Five-ml. samples were mixed with various volumes of standard acetoin solution (5·035 g. per litre) and diluted to 250 ml. Of each diluted sample 100 ml. were then distilled and acetoin was determined in the third 25 ml. of the distillate (Table V).

TABLE V

DETERMINATION OF ACETOIN IN PRESENCE OF OTHER SUBSTANCES

Standard acetoin solution taken ml.	Acetoin added mg.	Volume 0·01 *N* thiosulphate ml.	Diff. ml.	Acetoin determined mg.	%
2	10·07	41·86	6·35	9·90	98·5
2	10·07	41·80	6·41	9·99	99·3
5	25·17	32·01	16·20	25·25	100·4
5	25·17	32·08	16·13	25·15	99·9
10	50·35	11·63	32·58	50·8	101·1
		Blank 48·21		Mean	99·8

REDUCING POWER OF ACETOIN—Acetoin interferes with the determination of reducing sugars, but if its reducing power is determined under the appropriate conditions, a correction may be applied. With a standard solution of acetoin, the cuprous oxide equivalent was determined by the method described for reducing sugar determination. The results are shown in Table VI.

TABLE VI

REDUCING POWER OF ACETOIN

Acetoin taken mg.	Cuprous oxide mg.	Ratio: Cuprous oxide / Acetoin
4·3	12·8	2·98
8·6	25·8	3·01
12·9	39·4	3·05
17·2	53·7	3·11
21·5	68·7	3·17
25·8	80·1	3·10
42·9	130·0	3·03
64·4	201·0	3·11
85·6	258·0	3·02
	Mean	3·06

The results show that the reducing power of acetoin is constant for quantities in the range of from 4 to 86 mg., 1 mg. of acetoin being equivalent to 3·06 mg. of cuprous oxide. This is in good agreement with the results of Stahly and Werkman,[17] who used substantially the same conditions, but Langlykke and Peterson,[15] using the oxidising conditions of Stiles, Peterson and Fred,[18] found 1 mg. of acetoin equivalent to 2·39 mg. of cuprous oxide. It is thus important that the acetoin equivalent should be determined under the exact conditions used for the reducing sugar determinations.

OXIDATION OF ACETOIN BY PERIODIC ACID—Brockmann and Werkman[12] state that one molecule of acetoin yields on oxidation by periodic acid one molecule of acetaldehyde. We have tested this by oxidising aliquot portions of a standard solution of acetoin with periodic acid under the conditions used in the determination of 2:3-butylene glycol. The acetaldehyde formed was absorbed in hydroxylamine hydrochloride and determined by titration with standard sodium hydroxide. The results, calculated on the assumption that one molecule of acetoin yields one of acetaldehyde, are given in Table VII.

TABLE VII

OXIDATION OF ACETOIN BY PERIODIC ACID

Acetoin taken mg.	*N*/20 NaOH ml.	Acetoin found mg.	%
10·38	2·30	10·1	97·4
10·38	2·32	10·2	98·2
20·76	4·58	20·2	97·2
20·76	4·54	20·0	96·5
41·52	8·61	38·0	91·8
41·52	8·70	38·3	92·4
83·04	13·39	59·0	71·0

The results show that under the conditions employed the oxidation is practically quantitative for amounts of acetoin up to 20 mg. Above this the degree of conversion to acetaldehyde becomes progressively lower. Therefore in correcting results of 2:3-butylene glycol determinations for the presence of acetoin the amount of the latter in the sample oxidised should not exceed 20 mg. This corresponds to a concentration of 25 g. of acetoin per litre in the original fermented liquor, which was in practice never exceeded unless at least half of the 2:3-butylene glycol was oxidised to acetoin.

DETERMINATION OF ETHANOL

For the determination of ethanol, which frequently forms a considerable part of the products of the fermentation of sugars by *Aerobacter aerogenes*, oxidation by excess of acid potassium dichromate was found satisfactory, the excess of dichromate being determined by reduction with potassium iodide and titration of the iodine formed with standard thiosulphate.[19] Non-volatile interfering substances were eliminated by distilling the sample, suitably diluted, without reflux. Corrections were applied for the presence of acetoin and 2:3-butylene glycol, both of which are quantitatively oxidised by the reagent. Preliminary experiments showed that, when half the original liquid in the distillation flask had distilled over, the distillate contained all the ethanol, 59·7 per cent. of the total acetoin and a proportion of the 2:3-butylene glycol. It was found that for dilute solutions of the glycol (0·1 per

cent. or less) this proportion could be taken as constant at 9·4 per cent. of the total in the sample taken for distillation. Values obtained by distilling 100 ml. samples of glycol solutions and collecting the first 50 ml. of distillate are shown in Table VIII.

TABLE VIII

DISTILLATION WITHOUT REFLUX OF DILUTE SOLUTIONS OF 2:3-BUTYLENE GLYCOL

Glycol in 100 ml. sample taken mg.	Glycol concentration %	Glycol in first 50 ml. of distillate mg.	Glycol % of total
480	0·48	37·5	7·8
240	0·24	19·6	8·2
96	0·096	9·0	9·4
48	0·048	4·5	9·4

Using the quantities described with normal fermentation samples the concentration of glycol in the solution distilled for ethanol determination does not exceed 0·1 per cent.

The apparatus and procedure for preparation of the sample, and for the distillation were the same as described for the determination of acetoin. The volume taken for distillation was 100 ml. The first 50 ml. of distillate were collected and an aliquot used for determination of the alcohol using the following procedure.

Prepare the acid dichromate solution by mixing 100 ml. of 0·2 *N* potassium dichromate solution with 100 ml. of concentrated sulphuric acid and 50 ml. of water. To standardise, pipette 25 ml. into 500 ml. of water in a flask, add 2·0 g. of potassium iodide, stopper the flask for 10 minutes and then titrate with 0·1 *N* sodium thiosulphate. For the determination mix a suitable aliquot of the distillate with 25 ml. of the acid dichromate solution in a 100 ml. flask. Stopper and allow to stand for 12 hours (overnight), then transfer to a flask with 500 ml. of water and 2 g. of potassium iodide and titrate with 0·1 *N* thiosulphate.

When the concentrations of acetoin and 2:3-butylene glycol are known, the ethanol concentration in the original liquor can be calculated.

1 ml. of 0·1 *N* thiosulphate ≡ 1·152 mg. of ethanol
≡ 2·203 mg. of acetoin
≡ 1·502 mg. of 2:3-butylene glycol.

RESULTS—100 ml. portions of a standard solution of ethanol (0·974 g. per litre) were distilled, and the first 50 ml. of distillate collected. Ethanol was determined on aliquots of the distillate. Results are shown in Table IX.

TABLE IX

DETERMINATION OF ETHANOL

Volume of distillate taken ml.	Volume 0·1 *N* thiosulphate ml.		Ethanol found mg.	Ethanol present mg.	Recovery %
5	8·37 8·41	8·39	9·65	9·74	99·1
10	16·79 16·75	16·77	19·28	19·48	99·0
20	33·15 33·42	33·29	38·4	38·96	98·9
				Mean	99·0

One hundred ml. of a standard solution containing ethanol (0·974 g./litre) and acetoin (1·007 g. per litre) was distilled and 10 ml. portions of the first 50 ml. of the distillate were oxidised. The results are shown in Table X.

TABLE X

DETERMINATION OF ETHANOL IN THE PRESENCE OF ACETOIN

Vol. 0·1 *N* thiosulphate used ml.		Vol. thiosulphate required by acetoin ml.	Diff.	Ethanol found mg.	Ethanol present mg.	Recovery %
21·91 22·32	22·12	5·45	16·67	19·20	19·48	98·6

Determination of lactic acid

The standard method for the determination of lactic acid is based on oxidation of the acid to acetaldehyde by potassium permanganate in boiling sulphuric acid solution,[20] and, as modified by Friedemann *et al.*[21] by addition of manganous sulphate to increase the rate of oxidation of the lactic acid and prevent further oxidation of acetaldehyde, the method has been found suitable for the determination of lactic acid in mashes fermented by *Aerobacter aerogenes*. The use of ceric sulphate as an oxidising agent for lactic acid, as described by Gordon and Quastel,[22] was not examined, but that reagent may have advantages over permanganate.

It is necessary to eliminate a number of interfering substances, principally protein, 2:3-butylene glycol, acetoin, and sugars. Probably the most effective method involves steam distillation of the alkaline solution to remove the volatile substances and then, after acidification, extraction of the lactic acid by ether in a continuous apparatus. This method is, however, very slow; a more rapid procedure involves the removal of protein by tungstic acid,[23] of 2:3-butylene glycol and acetoin by steam distillation, and of sugars by treatment with copper sulphate and lime.[24] When acetoin is present, it must be removed before the lime and copper sulphate treatment, otherwise non-volatile products are formed which interfere seriously with the subsequent determination of lactic acid. The following procedure was found satisfactory in preparing the sample for oxidation.

Mix the sample, containing not more than 100 mg. of lactic acid, with 10 ml. of 10 per cent. sodium tungstate solution and 10 ml. of 0·67 *N* sulphuric acid in a 50 ml. standard flask. Warm to 60–70° C. if necessary to induce coagulation of the precipitate. Cool and dilute to volume. Filter through a dry paper into a dry flask.

Take 25 ml. of the filtrate and make alkaline by adding 5 ml. of 2 *N* sodium carbonate, add 10 g. of anhydrous sodium sulphate and steam distil at constant volume (20 ml.) until 200 ml. have been distilled. Wash the residue into a 100 ml. standard flask and add 15 ml. of 10 per cent. copper sulphate solution and 15 ml. of 10 per cent. calcium hydroxide suspension. Dilute to 100 ml. and shake well. After four hours, filter through a dry paper and use an aliquot portion of this filtrate, containing not more than 5 mg. of lactic acid, for the determination.

Suitable apparatus and procedure are described by several authors.[21,25,26] We have found that the apparatus here described for the determination of 2:3-butylene glycol gives satisfactory results when used for lactic acid determination, air being drawn through the apparatus and permanganate introduced dropwise through the suitably modified air inlet tube.

Analysis of synthetic mixtures

The results of determinations by the above methods on synthetic mixtures containing sucrose, 2:3-butylene glycol, acetoin, ethanol and lactic acid are shown in Table XI.

Table XI

Analysis of synthetic mixtures containing 2:3-butylene glycol, sucrose, acetoin, ethanol and lactic acid

Substance	Concentration taken g./litre	Concentration found g./litre	Concentration found Mean	Recovery %
Synthetic mixture No. 1				
Sucrose (as invert sugar) ..	52·20	50·8 50·5 50·4	50·6	96·9
2:3-Butylene glycol	40·30	40·0 39·2	39·6	98·2
Acetoin	20·18	20·1 20·1	20·1	99·5
Ethanol	25·91	25·5 25·2	25·3	97·6
Lactic acid	9·55	9·57 9·89 9·92 9·90	9·8	102·7

Substance	Concentration taken g./litre	Concentration found g./litre	Mean	Recovery %
SYNTHETIC MIXTURE No. 2				
Sucrose (as invert sugar) ..	5·27	4·88 5·05 5·38 5·74	5·3	100·6
2:3-Butylene glycol	40·61	39·0 39·2	39·1	96·3
Acetoin	15·10	15·0 15·0	15·0	99·3
Ethanol	18·91	18·4 18·6	18·5	97·8
Lactic acid	12·45	12·61 12·39 12·60 12·48	12·5	100·5

The percentage recovery was in general slightly low except for acetoin and lactic acid. The recovery of 2:3-butylene glycol was rather lower than was expected from results quoted in Table III, but was considered sufficiently good in view of the complexity of the mixture and the cross corrections involved. Lactic acid recovery in Mixture No. 1 is rather high, possibly because of incomplete elimination of sugar. In practice, however, lactic acid was not determined in mixtures with high sugar concentrations, and Mixture No. 2 is in this respect more typical of the kind of mixture analysed.

Table XII gives results of some determinations made on liquors fermented under widely different conditions, to demonstrate the range of concentrations of sugar and of the fermentation products which are encountered in practice. In optimal fermentations conducted for the production of 2:3-butylene glycol as the main product (Nos. 38 and 45/4), the carbohydrate is practically completely removed and acetoin production is very small compared with that of the glycol. Under these conditions determination of 2:3-butylene glycol gave results of over 99 per cent. of the theoretical, as shown in Table III, rather than the somewhat lower recoveries obtained in synthetic mixtures containing high relative proportions of acetoin (Table XI).

TABLE XII

ANALYSIS OF FERMENTED LIQUORS

Fermentation No.	Conditions	Concentration of constituents in g./litre: Initial sugar as invert	Final sugar as invert	2:3-butylene glycol	Acetoin	Ethanol	Lactic acid
38	Sucrose fermentation under anaerobic conditions	115·0	nil	34·0	nil	16·1	6·4
44	Fermentation of crude commercial glucose by unacclimatised organism with aeration	114·6*	6·8*	29·2	0·9	13·1	10·4
45/4	Fermentation with high initial sucrose conc. Aeration unrestricted until sugar almost completely fermented	200·4	3·6	77·2	4·7	—	—
41/4–5	Sucrose fermentation. Unrestricted aeration for 40·5 hours	111·5	nil	12·6	27·0	—	—
47	Unaerated fermentation of beet blackstrap molasses	79·4	4·9	22·7	nil	—	—

* Reducing sugar calculated as glucose

SUMMARY

Methods are described for the determination of sugars, 2:3-butylene glycol, acetoin, ethanol and lactic acid in fermentation liquors in the presence of each other. After preliminary clarification and removal of proteins, reducing sugars were determined iodimetrically by the Shaffer - Hartmann Fehling Soxhlet method. When sucrose was present a preliminary inversion with hydrochloric acid was necessary. 2:3-Butylene glycol was determined by oxidation to acetaldehyde by periodic acid. Acetoin was determined by the iodoform reaction after removal of more volatile interfering substances such as ethanol. Ethanol was determined by oxidation with standard potassium dichromate sulphuric acid mixture at room temperature after being separated by distillation. Lactic acid was determined by oxidation to acetaldehyde by acid permanganate after the sample had been freed from protein by

treatment with tungstic acid, from 2:3-butylene glycol, acetoin and other volatile neutral substances by steam distillation, and from sugars by treatment with lime and copper sulphate. A correction for acetoin was applied to results of sugar and 2:3-butylene glycol determinations, and corrections for acetoin and 2:3-butylene glycol were applied to results of ethanol determinations. Results are given of determinations on synthetic mixtures, containing the five components in various proportions and on actual fermentation mashes obtained under a variety of conditions.

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IMPERIAL CHEMICAL INDUSTRIES LTD., EXPLOSIVES DIVISION
RESEARCH DEPARTMENT
STEVENSTON, AYRSHIRE

July, 1946

Discussion

Mr. L. EYNON asked (1) if the nature or proportions of the products of fermentation by *Aerobacter aerogenes* were substantially the same whether cane, beet or "high-test" molasses were used; (2) if in the fermentation of "high-test" molasses addition of nutrients was necessary; (3) if the methods of determination described would be applicable to the products of ordinary alcoholic fermentation of molasses.

Mr. F. C. HYMAS asked if the authors had considered the use of dialysed iron in preference to lead acetate for clarifying their solutions. It would avoid necessity of precipitation of excess lead, though whether it would also inhibit further fermentation remained to be tried.

Dr. G. G. FREEMAN, in reply to Mr. Eynon's questions, said that—(1) In this paper the term molasses was meant to include: "high-test" molasses (containing 70–75 per cent. of total sugars as invert sugar) as well as cane and beet blackstrap molasses (containing 45–55 per cent. of total sugars as invert sugar). The products of fermentation of high-test molasses and their proportions in relation to the initial carbohydrate differed a little from those obtained in sucrose fermentations, described in Table XII. Individual batches of cane and beet blackstrap molasses varied greatly in their readiness of fermentation, particularly with unacclimatised strains of the organism, but in all cases the products were the same and their proportions in terms of sugar fermented did not differ significantly. (2) Fermentations of high-test molasses as well as those of sucrose and blackstrap molasses required addition of the following inorganic salts as nutrients for *A. aerogenes*: ammonium sulphate, magnesium sulphate, dipotassium hydrogen phosphate, potassium chloride and ferrous sulphate. Some of the early workers used complex nutrients, such as peptone, as a basis for their media, but it is now known that this organism grows well on synthetic media containing citrate or glucose as source of carbon and nitrates or ammonium salts as sources of nitrogen (Koser, S. A., *J. Bact.*, 1924, **9**, 59). We found that addition of growth factors, such as yeast extract, yeast autolysate and malt rootlet extract, had no effect on either the rate or the products of fermentation of sucrose and high-test molasses. (3) The methods described would be suitable for the determination of initial or unfermented sugar and ethanol in alcoholic molasses fermentation mashes, though other methods for the determination of the latter would then probably be preferable. Alcoholic fermented liquors also contain glycerol and small amounts of lactic acid and 2:3-butylene glycol, *e.g.*, in the wine industry. For determination of 2:3-butylene glycol and lactic acid, the methods described above would be satisfactory, particularly if the sensitive method of absorption of the liberated acetaldehyde in sodium bisulphite and iodimetric titration of the bound bisulphite were used. According to Brockmann and Werkman[12] glycerol has no effect on the determination of butylene glycol by the periodic acid oxidation method. In reply to Mr. Hymas, Dr. Freeman said that the use of lead acetate as a clarifying agent had proved so satisfactory and straightforward that other reagents had not been investigated for this purpose. He agreed that the use of dialysed iron seemed attractive and would be worth trial.

The Analysis of Barium Carbide

By A. H. EDWARDS

(*Read at the Meeting of the Society on Wednesday, October* 2, 1946)

THE analytical work which is the subject of this paper was undertaken as a complement to a research in the Technical Chemistry Laboratories of The British Coal Utilisation Research Association on the rate of formation of barium carbide. Most of the samples submitted for analysis were between 0·5 g. and 30 g. in weight. Routine analyses of alkaline earth carbides have been mainly confined to calcium carbide, and the difficulties of sampling have necessitated the use of large quantities (100 g.–10 kg.) for its analysis. New methods, therefore, had to be devised and, in view of the possibility of their wider application, they were considered worthy of record.

The scope of the analysis required can be judged by reference to Tables I and II.

TABLE I

TYPICAL ANALYSIS OF BARIUM CARBIDE

Mix No.	47S/167	47S/51	47S/50
Barium carbide, per cent. ..	78·7	62·9	29·1
Barium carbonate, "	0·8	2·8	4·0
Barium oxide, " ..	8·3	20·6	52·6
Insoluble in HCl, " ..	12·2*	5·8	5·0
Free carbon, " ..	Not determined	9·0	5·5

* This figure is free carbon plus "insoluble in HCl."

TABLE II

CYANIDE CONTENT OF TYPICAL CARBIDES

Mix No. ..	47S/50	29S/110	29S/105	29S/108	29S/90	29S/68
Barium carbide, per cent.	29·1	57·0	60·1	62·8	66·3	78·7
Barium cyanide, "	0·6	0·6	0·5	0·2	0·5	0·6

APPARATUS AND METHODS

Two types of apparatus have been evolved, the first suitable for 3–5 g. samples, for which an accuracy within ±0·5 per cent. was required. With this a sample of the gas evolved on hydrolysis of the carbide could be taken for analysis. At a later stage, a miniature apparatus of similar design was built to deal with samples of 0·25–0·3 g.

ACCURATE DETERMINATION OF QUANTITY AND COMPOSITION OF GAS YIELD

The principle of the method depends upon the measurement of the pressure set up in a system of known volume when gas is generated from carbide enclosed in the system. A sketch of the apparatus, is given in Fig. 1. For a 5 g. sample placed in the large flask, A, a total volume of approximately 1000 ml. to the tap B was required; this included the volume of the barometer tube C up to a fixed mark. A 0·25 g. sample required a volume of only 200 ml. to tap B, and the large flask, A, was replaced, therefore, by a smaller one of about 125 ml. capacity. The system was evacuated and the height of mercury in C noted by means of the scale D. Between 25 and 30 ml. of brine solution were then run on to 5 g. of carbide previously placed in the flask. The flask was swirled gently, the motion being facilitated by means of the flexible glass connection, E, and when the reaction was complete the flask was allowed to cool to the temperature of the bath F, and the height of the mercury column was again noted. The difference between the initial and the final readings gave the pressure of gas in the system. After the temperature of the bath had been noted the gas pressure was corrected for the tension of aqueous vapour, and the volume of gas generated at N.T.P. calculated.

In accurate analyses, a further correction was made for the solubility of the acetylene in the brine; this is appreciable, 0·32 ml. of gas being dissolved by 1 ml. of brine at 0° C., when the partial pressure of the gas is 760 mm.

The percentage of carbide in the sample is then equal to $\frac{161{\cdot}4}{22{,}400} \times \frac{V}{5{\cdot}0} \times 100$, where V is the volume of gas at N.T.P.

If the gas was required for analysis it was drawn into the modified Toepler system G, and then transferred, after taps B and I had been closed, to the gas sampling tube H, which and been previously evacuated by the Hyvac pump. A thermos flask, J, filled with solid carbon dioxide, containing a trap for condensing water vapour, was placed before the pump.

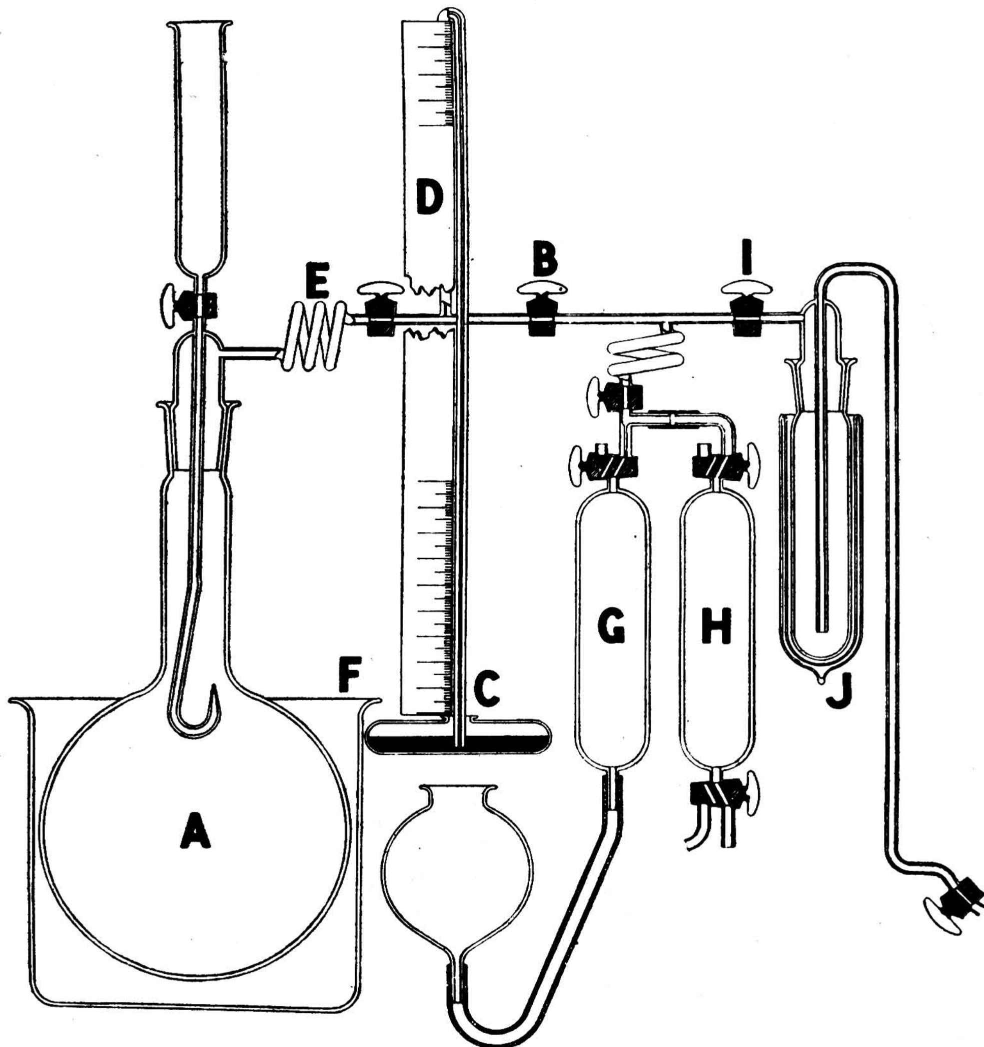


Fig. 1

DETERMINATION OF BARIUM CARBONATE, BARIUM OXIDE, FREE CARBON, TOTAL CARBON AND BARIUM CYANIDE

BARIUM CARBONATE—

The carbide was decomposed by hydrolysis in the apparatus used for the determination of the gas yield. For the carbon dioxide determination, the gaseous products were swept from the flask with carbon-dioxide-free nitrogen, and the residues transferred to a flask previously swept out with nitrogen, using carbon-dioxide-free distilled water.

With modifications to the trains purifying the carbon dioxide evolved, any of the standard gravimetric methods[1,2] for the determination of carbonates may be used. The decomposition of the carbonate was best effected by diluted hydrochloric acid (1 + 9). The purification train consisted of:

(1) A scrubber containing glass beads saturated with a solution of chromic oxide in diluted sulphuric acid (1+1) to remove moisture and sulphur dioxide.

(2) A U-tube containing silver sulphate to remove hydrogen sulphide and hydrochloric acid vapour.

(3) A calcium chloride U-tube for the final drying of the gas.

(4) A tared Sofnolite U-tube with guards of calcium chloride in each limb.

(5) A final calcium chloride guard tube.

The percentage of barium carbonate was calculated from the increase in weight of the Sofnolite tube.

BARIUM OXIDE AND TOTAL BARIUM—

The solution from the carbon dioxide determination was filtered through a tared sintered glass crucible, and the residue thoroughly washed with hot distilled water. The filtrate and washings were diluted to about 600 ml., the hydrochloric acid concentration was adjusted to about 0·5 per cent. and barium sulphate was precipitated by an excess of hot normal sulphuric acid added dropwise to the boiling solution with stirring. The precipitate was allowed to stand overnight and was filtered off on a No. 40 Whatman filter paper, washed free from chloride with hot distilled water, and then carefully ignited in a weighed crucible.

The percentage of barium oxide was calculated by deducting the equivalent of barium as carbide and carbonate from the total barium determined as above.

An error occasionally arises due to the presence of barium sulphide in the sample, but as a rule only traces of sulphide were encountered. Where considerable blackening of the silver sulphate tube in the carbon dioxide determination (indicating sulphide) was observed, it was necessary to estimate it, and to make the necessary allowance in the barium oxide computation. Some barium was also fixed as sulphate in the residue, but its amount was negligible.

FREE CARBON—

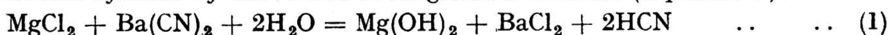
The sintered glass crucible containing the residue after the removal of soluble barium in the barium oxide determination was dried at 110° C. and reweighed to find the weight of insoluble residue. A known weight (approximately 0·2 g.) of the dried residue was weighed into a boat, this was placed in a Liebig combustion furnace, and the carbon determined in the usual way. Results were expressed as percentages of the original sample.

TOTAL CARBON—

It was occasionally desirable to make a determination of the total carbon present in the original sample. Though no information was available in the literature, it was considered that barium carbide, on combustion in an atmosphere of oxygen, would react similarly to calcium carbide, which is converted into the carbonate. Some barium carbonate was also present in the original sample. The carbonate is decomposed, however, at high temperatures in the presence of carbon,[3] and the sample was mixed, therefore, with a known weight of carbon black, whose carbon content had been accurately determined. Combustion was then carried out at a temperature of 1350° C. in the manner described by Belcher and Spooner.[4] The percentage of total carbon in the original sample was calculated from the weight of carbon dioxide obtained, after correction for the carbon as carbide, carbonate and carbon black in the mixture.

DETERMINATION OF BARIUM CYANIDE—

The method used was based on that of Feld,[5] which depends on the evolution of hydrogen cyanide from barium cyanide by the action of magnesium chloride (Equation 1).



$$MgCl_2 + Ba(CN)_2 + 2H_2O = Mg(OH)_2 + BaCl_2 + 2HCN \quad \quad (1)$$

$$2HCl + Ba(CN)_2 = BaCl_2 + 2HCN \quad \quad (2)$$

Not all the cyanide, however, was liberated by this method, and on investigation it was found that hydrochloric acid (Equation 2) was a better reagent for the purpose than magnesium chloride. The method finally adopted was as follows:

Approximately 2 g. of the finely powdered carbide were accurately weighed out and introduced into a 500 ml. measuring flask that was half full of distilled water at 40° C. After vigorous shaking for half an hour the flask was filled to the mark with water at room temperature.

Of this liquid 100 ml. were placed in a distillation apparatus similar to that used for the determination of ammonium compounds; 2–3 drops of methyl red solution were added and the apparatus was assembled. Concentrated hydrochloric acid was added until the solution was acid, and a further 2 ml. in excess. The solution was then distilled into 20 ml. of 10 per cent. potassium hydroxide solution, which, after addition of 2 ml. of 10 per cent. potassium iodide solution and 5–6 ml. of 6 *N* ammonia solution, was titrated with standard silver nitrate solution until a permanent turbidity was obtained.

DETERMINATION OF HYDROGEN SULPHIDE AND PHOSPHINE IN GAS GENERATED FROM BARIUM CARBIDE—

The objectionable impurities in the gas obtained by hydrolysis are hydrogen sulphide and phosphine. These were determined by the methods outlined in B.S. 642, and by Lunge[6] for calcium carbide. A slight modification was necessary for phosphine, the quantity present being so low that a colorimetric method had to be employed. The hydrogen sulphide and phosphine in the gas were oxidised to sulphate and phosphate by passing the gaseous products of hydrolysis of the carbide through a solution of sodium hypochlorite in the usual way. Acetylene was removed from the hypochlorite solution by boiling; the solution was then oxidised by the addition of 10 ml. of diluted nitric acid (1+1). The solution was evaporated to dryness, diluted with warm water, acidified with hydrochloric acid and filtered to remove silica. The filtrate was made up to 100 ml., 50 ml. of which were utilised for the sulphate determination, and aliquot portions, varying according to the phosphine content, used for the phosphate determination by the colorimetric method of Truog and Meyer.[7] Hardwick's note[8] on the stabilisation of the blue molybdenum colour by sodium chlorite and chlorate is of value in this determination.

SUMMARY

A method for the determination of carbide in an impure sample of barium carbide is described. The method can be applied to the analysis of other carbides which react with water or other liquid reagents. Specific methods for the determination of oxide, free carbon, total carbon, carbonate and cyanide are outlined.

Assistance in the design of the apparatus for the precise determination of carbide was given by Dr. P. J. Hardwick of the Government Chemist's Department, Clement's Inn Passage, W.C.2.

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THE BRITISH COAL UTILISATION RESEARCH ASSOCIATION
13, GROSVENOR GARDENS
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The Polarographic Estimation of Antimony in Cable Sheathing Alloy B (B.S. 801)

BY H. F. HOURIGAN

IN this alloy, which is used mainly for the sheathing of cable suspended by means of cable rings, the lead is hardened by addition of 0·8 to 0·9 per cent. of antimony. If the antimony content is much below 0·8 per cent. ring cutting may occur, owing to softness of the metal, and if it is much above 0·9 per cent. cracking and undue stiffness in handling result. The resistance of the alloy to extrusion also increases sharply as the antimony content approaches 1·0 per cent. It is therefore of the first importance that the composition of the alloy should be controlled strictly within the specification limits. Certain considerations in connection

with the manufacture and testing of such cable make it impossible to await the results of lengthy analyses, so rapid methods are essential.

Fortunately, the above requirements are met by the British Non-ferrous Research Association method of spectrographic analysis for this alloy. In this method the spectrum of a spark between electrodes of the sample alloy is photographed under controlled conditions and the intensity of blackening of specified lines of lead and antimony measured on the microphotometer. Variations of spark intensity are corrected by calculating the antimony intensity against the lead intensity, and variation from plate to plate is eliminated by including five sets of standard alloy electrodes with the samples on each plate. A combination of experimental work and statistical methods shows the probable error under the conditions of test to be 3·5 per cent. with the chance of it rising to 7·0 per cent. once in 23 times, providing the samples are photographed in triplicate. This contingency can of course be met by returning the abnormal result to the spectrographist for confirmation, but in practice this is not desirable because, although the spectrographic method lends itself admirably to furnishing large numbers of analyses with a precision that delights the heart of the busy chemist, any attempt to secure a rapid confirmation of an individual result would upset the carefully ordered scheme and lead to chaos.

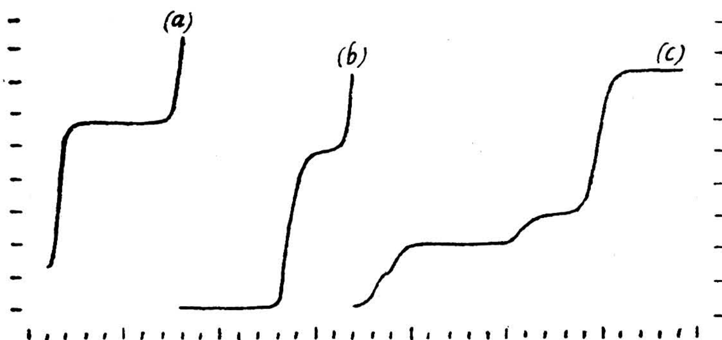

Fig. 1

Antimony in presence of 90 per cent. saturated lead chloride. The three curves show

(*a*) the whole of the bottom step,
(*b*) the antimony step for purpose of measurement,
(*c*) the general polarogram of the system to show the high lead step.

It is therefore necessary, when the spectrograph has indicated an abnormal result, to seek confirmation with a rapid chemical method such as the bromate method. But in presence of large amounts of lead chloride this method is not altogether trouble-free, and in consequence a polarographic method has been studied, with interesting results.

The normal method of getting the alloy into solution is with concentrated hydrochloric acid, the suspended antimony being dissolved by oxidation with bromine or chlorate. The half wave voltage of antimony in such a medium is approximately 0·2, and that of lead just over 0·4. It would therefore seem that under these conditions a polarographic estimation would be at once simple and obvious. This, however, was not so.

The polarogram of 1 g. of the alloy in 5 ml. of solution made up to 50 ml. with an electrolyte consisting of 450 ml. of concentrated hydrochloric acid and 550 ml. of 0·5 per cent. aqueous gelatin solution gave an excellent step with both top and bottom lines horizontal (Fig. 1), but the attempt to calibrate the instrument with a standard solution of antimony in the same electrolyte produced a step with a very poor bottom level at a sharp angle to the horizontal (Fig. 2). Trouble was also experienced in both standard and sample owing to the whole system moving towards zero voltage (Fig. 3). In fact, on one occasion the antimony step rose on the positive side of the zero. This was fortunately traced to the use of hydrogen for sweeping out the dissolved oxygen, and when nitrogen was substituted the trouble was eliminated. The poor bottom level was in its turn rectified by inclusion of a sufficient amount of lead chloride in the standard solution.

This led to the interesting conclusion that the lead step drew the antimony step towards it and caused a longer and flatter bottom step—a result which was confirmed by observing

the steps produced from a series of solutions containing increased amounts of lead chloride. Indeed, the result produced from a solution saturated with lead chloride so shortened and

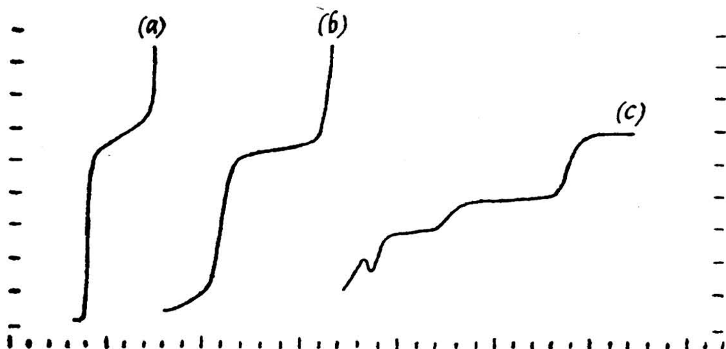

Fig. 2

Antimony with only a small amount of lead chloride (40%). The three curves show
(*a*) the whole of the bottom step,
(*b*) the antimony step for purposes of measurement,
(*c*) the general polarogram of the system to show the lower lead step.

sloped the *top* step as to affect seriously the validity of any measurement made. Some control of the lead content was obviously essential, and this was secured by making some of the electrolyte saturated in respect of lead chloride and using it to wash the filtration of the original 5 ml. solution of the alloy. Five washings were given with 5-ml. portions of saturated electrolyte and then made up to 50 ml. with the unsaturated electrolyte.

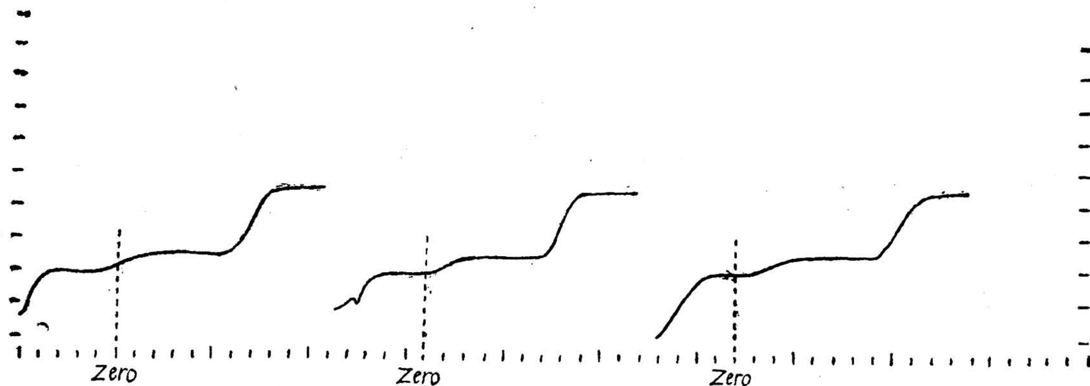

Fig. 3

Series of three general polarograms on 200 microamps. taken in quick succession to illustrate the movement of the whole system towards the positive. As the curves cannot be drawn while the gas is on, the series show the system returning to the normal.

It was now found possible to obtain rapid and accurate checks on the spectrographic method and, indeed, the method was used to confirm the accuracy of making up substandard alloys for the spectrographic work.

Bismuth and the first wave of stannic tin form an additive wave with the second step of antimony, and the method is only valid in absence of significant amounts of those metals.

Kraus and Novak, in their paper on the estimation of antimony in hard-lead[1] (*Die Chemie*, **1943**, **56**, 302), make no reference to the indeterminate first step of tin in this medium, although it is familiar enough to workers in this field (Fig. 4). This step would enhance the antimony step and is easily detected when present by the bad shape of the top as compared with the normal antimony step (Fig. 5).

These workers also claim that two steps of antimony are resolved by adding more hydrochloric acid. This is not so; actually the raising of the hydrochloric acid concentration

in their experimental series permitted the amount of dissolved lead chloride to increase, thus producing precisely the effect described earlier in this paper. The work described in this paper was carried out without knowledge of Kraus and Novak's paper, which was only

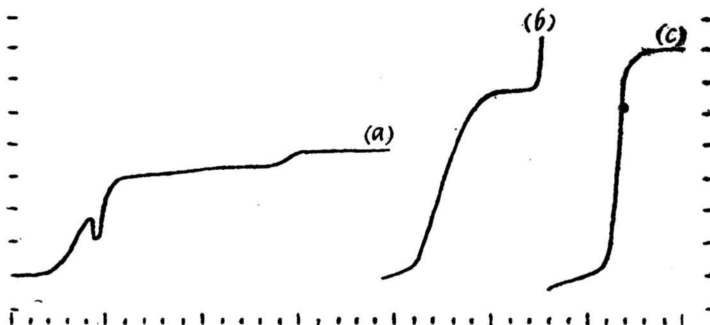

Fig. 4

Curves showing the two steps of stannic tin.

(*a*) General polarogram on 200 micro-amps.
(*b*) The first step of tin, which coincides with antimony and bismuth.
(*c*) The second tin step.

abstracted recently, and was carried out at only one concentration of acid, but with varying lead chloride concentration, and it must therefore be concluded that it is the lead chloride and not the acid concentration which is the causative factor of the movement of the second antimony step.

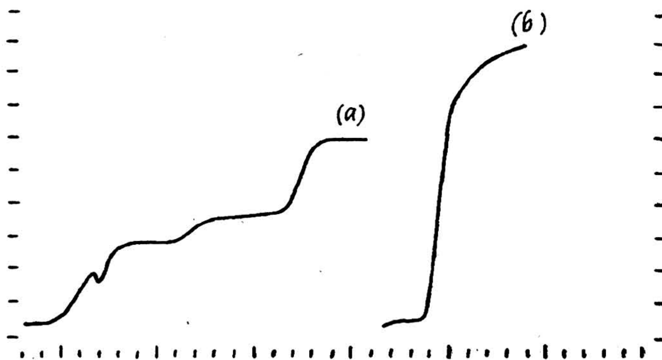

Fig. 5

If tin is present in the original alloy the antimony step is distorted and the amount over-estimated.

(*a*) General polarogram on 200 micro-amps.
(*b*) Combined antimony and tin steps.

I have to make my grateful acknowledgments to Mr. G. J. Partington for his painstaking assistance, to Mr. R. Linch for his interest, and to Sir Stanley Angwin, Engineer-in-Chief, General Post Office, for permission to publish this work.

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A New Type of Micro-Burette

By J. A. SAUNDERS

INTRODUCTION—A new type of micro-burette is described, consisting of a U-tube of precision bore tubing with limbs of different diameters and containing mercury. Water added to the wide limb from a 50-ml. burette causes delivery of the titrating solution from the narrow limb *via* a fused-on side tube ending in a downwardly pointing narrow tip which delivers by immersion. The depth of immersion is accurately controlled by means of a vertically moveable titration table. Instruments of three sizes are described having delivery capacities 0·54, 0·15 and 0·04 ml. respectively. The standard error is 0·5 per cent. of half the capacity.

DESCRIPTION

Two ten-inch lengths of Veridia (Hysil) precision bore tubing are joined to form a U-tube. Three (Pyrex) 2 mm. taps are fused to the U-tube (Fig. 1). The internal diameter of the

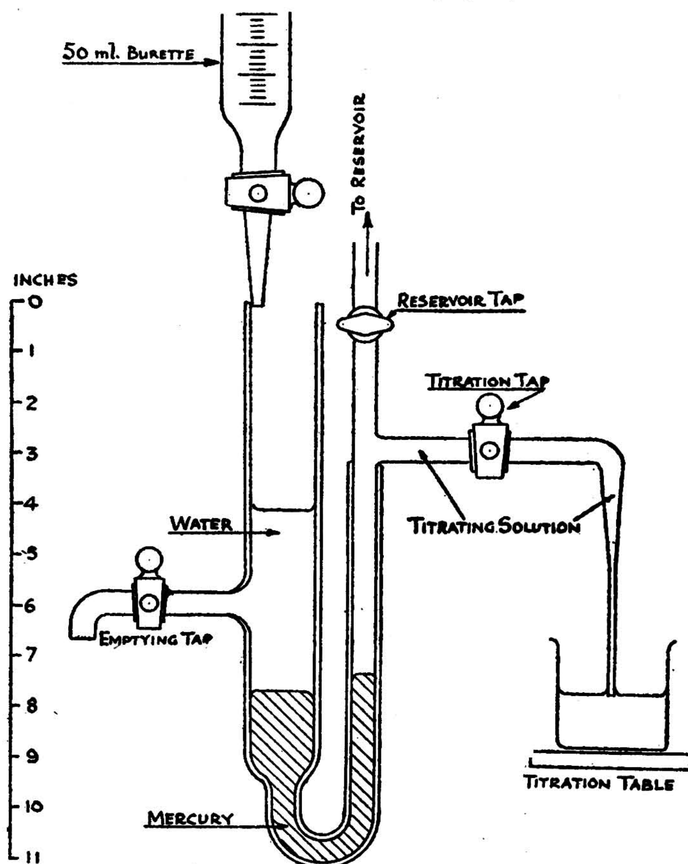

Fig. 1

large limb of the U-tube is 30 mm. (maximum tolerance ±0·01 mm.) and that of the smaller limb 12, 6 or 3 mm. (all ±0·01 mm.) respectively in the three burettes constructed; these are accordingly referred to as 30/12, 30/6 and 30/3 burettes respectively. The delivery tip is flat, with diameters: external approximately 1 mm. and internal approximately 0·5 mm. Other dimensions may be obtained from Fig. 1. A titration table capable of accurate vertical movement is an essential part of the apparatus.*

* The "large adjustible stand" supplied by C. F. Palmer (London), Ltd., was used.

THEORETICAL

If liquid 1 is added to the large limb, then liquid 3 will be delivered from the delivery tip. Provided the system is in equilibrium before and again after the addition, the relation between the volume of liquid added and the volume delivered can be derived as follows (see Fig. 2*a* and *b*, the legend of which gives the meanings of the symbols used).

In Fig. 2*a*, as the system is in equilibrium, the pressures in the two limbs of the U-tube are equal;

$$\therefore \quad (h_1 - h_2)\rho_1 + h_2\rho_2 = h_3\rho_2 + (h_5 - h_3)\rho_3 + (h_4 - h_5)\rho_4. \quad \text{(1)}$$

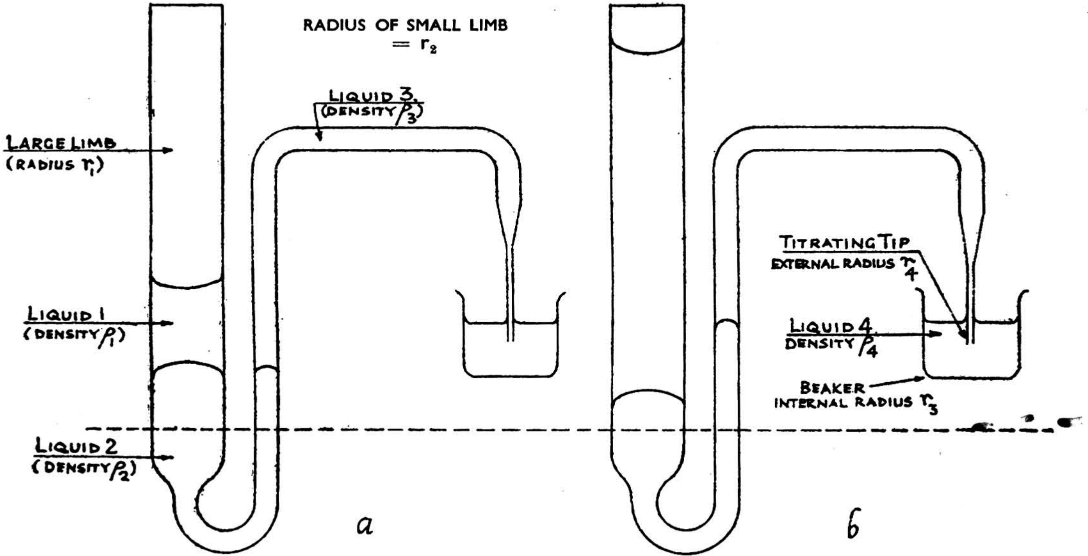

Fig. 2

Heights of menisci above base line

	Fig 2*a*.	Fig 2*b*.
Liquid 1 in large limb	h_1	h_6
„ 2 in large limb	h_2	h_7
„ 2 in small limb	h_3	h_8
„ 4 in beaker	h_4	h_9
Delivery tip	h_5	h_5

After addition of v ml. of liquid 1 to the wide limb, resulting in the delivery of x ml. of liquid 3 into the beaker, the pressures are again equal at equilibrium (Fig. 2*b*);

$$(h_6 - h_7)\rho_1 + h_7\rho_2 = h_8\rho_2 + (h_5 - h_8)\rho_3 + (h_9 - h_5)\rho_4. \quad \text{(2)}$$

It is assumed that the volume delivered is small compared with the volume of liquid 4 and that no change in ρ_4 is produced.

But
$$v = [(h_6 - h_7) - (h_1 - h_2)]\pi r_1^2 \quad \text{(3)}$$
and
$$x = (h_2 - h_7)\pi r_1^2 \quad \text{(4)}$$
$$= (h_8 - h_3)\pi r_2^2 \quad \text{(5)}$$
$$= (h_9 - h_4)(\pi r_3^2 - \pi r_4^2). \quad \text{(6)}$$

From (3),
$$h_6 - h_7 = h_1 - h_2 + \frac{v}{\pi r_1^2},$$
from (4),
$$h_7 = h_2 - \frac{x}{\pi r_1^2},$$
from (5),
$$h_8 = h_3 + \frac{x}{\pi r_2^2},$$
and from (6),
$$h_9 = h_4 + \frac{x}{\pi r_3^2 - \pi r_4^2}.$$

Substituting these values in (2),

$$\left(h_1 - h_2 + \frac{v}{\pi r_1^2}\right)\rho_1 + \left(h_2 - \frac{x}{\pi r_1^2}\right)\rho_2 = \left(h_3 + \frac{x}{\pi r_2^2}\right)\rho_2 +$$

$$\left(h_5 - h_3 - \frac{x}{\pi r_2^2}\right)\rho_3 + h_4\rho_4 + \frac{x}{\pi r_3^2 - \pi r_4^2}\rho_4 - h_5\rho_4$$

subtracting (1),

$$\frac{v\rho_1}{\pi r_1^2} - \frac{x\rho_2}{\pi r_1^2} = \frac{x\rho_2}{\pi r_2^2} - \frac{x\rho_3}{\pi r_2^2} + \frac{x\rho_4}{\pi r_3^2 - \pi r_4^2}$$

and rearranging,

$$\frac{v}{x} = \frac{\rho_2}{\rho_1}\left(1 + \frac{r_1^2}{r_2^2}\right) - \frac{\rho_3 r_1^2}{\rho_1 r_2^2} + \frac{\rho_4 r_1^2}{\rho_1(r_3^2 - r_4^2)}. \quad \ldots \quad \ldots \quad \ldots \quad (7)$$

Influence of various factors

Various possible sources of error and practical points are considered separately.

(1) *Liquid* 1—Liquid 1 should have a low vapour pressure, should be non-hygroscopic, should wet glass and should have a coefficient of thermal expansion similar to that of liquid 2 (mercury) and liquid 3 (dilute aqueous solutions). Many organic liquids fulfil the first three conditions and are suitable, provided the temperature is constant (see Results). Water has the great advantage that the density ratios are unaffected by any likely temperature change, the specific gravity of mercury (t°/t°) being 13·585 from 15° to 22° C. It has the disadvantage that wetting is apt to be imperfect, especially with a rising meniscus, and this introduces an error.

In the case of perfect wetting (angle of contact $\alpha = 0°$) the effective pressure in the limb of the U-tube is contributed by the water below the dotted line AB in Fig. 3; the pressure of the water above this line is compensated by the surface tension acting upwards, the volume of water thus held being $(2\pi r T \cos \alpha)/g\rho$ ml., where T is the surface tension and g is gravity.

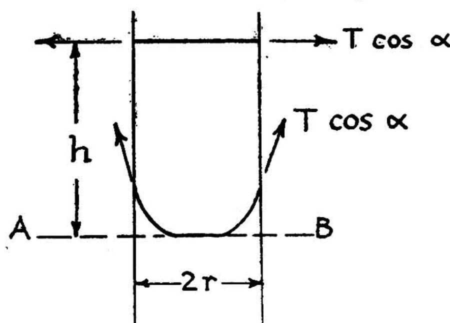

Fig. 3

If T = 73 dynes/cm. and r = 1·5 cm. this is 0·70 ml. With an addition of v ml. of water resulting in zero wetting ($\alpha_1 = 90°$), $v = (\pi r^2 h/\rho) - 0{\cdot}70$ ml., but the volume of water causing an increase in pressure is $\pi r^2 h/\rho$ ml. and therefore the volume of water needed to deliver a given volume of liquid 3 is less than the calculated amount by 0·70 ml. A solution, calculated to need 21·53 ml. of water for titration, was found to need (± standard error (n values)) 21·18 ± 0·05(6) ml. of water with bad wetting at the end of the titration, while with good wetting (using bile salt solution) 21·58 ± 0·11(5) ml. were used.

A solution of bile salt (sodium glycotaurocholate) containing 1 g. per litre, filtered if necessary, has been found perfectly satisfactory in use, provided the glass tube is clean (hot soap solution is adequate for cleaning) and the tube is kept filled and covered when not in use.

Alteration in shape of the mercury menisci would also affect the validity of the expression for v/x, but with clean mercury and clean glassware this does not occur.

Liquid 1 should be added slowly and steadily down the wall of the large limb so as to produce an even movement of the mercury in the small limb and minimise any tendency to surging. This tendency to surge is greater when r_2 is small and it is largely controlled by the internal diameter of the delivery tip, which should be about 0·3 mm. when $2r_2$ = 3·00 mm., about 0·5 mm. when 2 r_2 = 6·00 mm. and about 0·7 mm. when 2 r_2 = 12·00 mm.

(2) *Size of precision-bore tubing and errors involved*—As precision bore tubing is sold with a constant maximum tolerance (±0·01 mm.) irrespective of the actual diameter, greater accuracy will be achieved by the use of large-bore tubing. The largest size made has an internal diameter of 30 mm. and this is used for the large limb.

The size of the smaller limb governs the volume of liquid 3 delivered. Table I gives the value of v/x for different values of r_2 and also the errors involved if the maximum permitted tolerance obtains and is in opposite senses with r_1 and r_2.

TABLE I

DEPENDENCE OF v/x ON r_2 AND MAXIMUM ERRORS IN v/x DUE TO TOLERANCES IN r_1 AND r_2

System water - mercury - water; $2r^1 = 30$ mm.; $r_3 = \infty$

$2r_2$ mm.	v/x Volume to deliver 1 ml. ml.	Error for $2r_1+0{\cdot}01$ mm. and $2r_2-0{\cdot}01$ mm. ml.	Error %
30	26·17	0·013	0·05
20	41·90	0·048	0·11
12	92·24	0·189	0·20
6	328·2	1·26	0·37
3	1,272	9·44	0·74
2·5	1,826	15·73	0·82
1·5	5,048	71·3	1·41
1·0	11,340	237·5	2·09

With the burettes constructed ($2r_2 = 12$, 6 or 3 mm.) this error is negligible. It is, however, important to ensure that the menisci of water and mercury move in parts of the tube which have uniform bore and it is therefore suggested that each meniscus should be at least 1 inch distant from any point of distortion. With the dimensions shown in Fig. 1 the lowest permissible water level is 4½ inches from the top, allowing addition of 80 ml. of water. The movements produced by this addition are shown in Table II.

TABLE II

MOVEMENT OF MERCURY ON ADDITION OF 80 ml. OF WATER TO LARGE LIMB

$2r_2$ mm.	Movement in large limb mm.	Movement in small limb mm.
12	1·2	8·0
6	0·35	8·4
3	0·10	9·6

Marks on the large limb at 4½ inches and 7¾ inches from the top are useful to indicate the lowest level of water and the position of the mercury meniscus, provided they are applied without heat.

(3) *Delivery*—Delivery of liquid 3 is effected by allowing the whole system to reach equilibrium in a preliminary solution and replacing this by the solution to be titrated, the assumption being that the system is again in equilibrium on opening the titration tap (details are given later under "Technique"). This will be true provided the liquid pressure at the delivery tip is the same in the preliminary and in the titration solutions, irrespective of the actual position of the tip in the two solutions.

The simplest way to reproduce the position of the tip is to have it just touching the surface of the liquid where the surface is flat. To do this it is essential to have a table which moves smoothly and remains steady after its position has been altered.

A second essential is that the tip should touch the surface preferably where it is flat or alternatively at the same distance from the line of zero pressure.

If the tip is replaced 1 mm. below the level at which it was in the preliminary solution the "back pressure" of 1 mm. of water will have to be compensated by addition of enough water to the 30 mm. tube limb to give a pressure of 1 mm. before any liquid 3 is delivered, and the results will be high by $\pi r_1^2 \times 0{\cdot}1 = 0{\cdot}707$ ml. Accuracy of adjustment to within $\pm 0{\cdot}05$ mm. is desirable, giving accuracy of added water to within $\pm 0{\cdot}035$ ml.

(4) *Flatness of water meniscus in tubes*—Dr. K. Mitchell (Lecturer in Applied Mathematics, King's College) very kindly searched the literature and calculated the figures given in Table III. The first three diameters are from the tables of Bashforth and Adams[1] and the fourth (∞) from his own calculations. Apparently there is no theoretical relation deducible between the radius of the tube and the distance from the wall at which the surface is flat.

TABLE III

DISTANCES (mm.) FROM THE WALL OF A CIRCULAR VESSEL AT WHICH THE SURFACE IS FLAT TO WITHIN THE TOLERANCES INDICATED

Diameter of vessel, mm.	Tolerance 1 mm.	Tolerance 0·1 mm.	Tolerance 0·01 mm.
4·9	0·4	1·7	2·2
10·3	1·5	3·9	4·8
17·5	2·5	6·4	7·95
∞	2·7	9·0	15·4

An attempt was made to find the shape of the water meniscus in beakers by cementing the bottom of the beaker to a microscope slide, which was moved in a horizontal plane by means of a mechanical stage. The position was determined in two directions at right angles by the two mm. scales (with verniers) of the mechanical stage. A vertical clamped glass rod (diameter 1·15 mm.) was brought into contact with the water surface by movement of a vertical screw of pitch 5 mm. From the results the height of the meniscus was plotted against the distance from the beaker wall, across the diameter of the beaker. The results given in Table IV are from these curves. These results indicate that with vessels of diameter exceeding 30 mm. centring by eye would be satisfactory, but that with vessels of diameter less than 30 mm. some centring device would be desirable to attain the highest accuracy.

TABLE IV

SHAPE OF WATER MENISCUS

Diameter of vessel mm.	Distance (mm.) from each wall at which height of surface above centre is 1·0 mm.	0·1 mm.	0·01 mm.
35	2·2; 2	7·5; 9	13; 14
42·5	2; 2·5	7·8; 10	13; 16
49	2·2; –	7; –	—
∞*	2·7	9·0	15·4

* Dr. K. Mitchell's calculation.

An alternative method of use is possible in that a second sample may be added to the titration mixture at the end of a first titration (the titration tap being shut). If the volume of the sample be v_1 ml., then $v_1r_1^2/(r_3^2-r_4^2)$ ml. must be added before the tap is opened, and the results will be higher by the same amount than if the titration had been done by the previous technique. In a titration of 1·013 ml. samples in a beaker for which $r_3 = 21{\cdot}9$ mm. the calculated value by this method was $0{\cdot}87 + 0{\cdot}48$ ml. $= 1{\cdot}35$ ml. The value found was $1{\cdot}307 \pm 0{\cdot}03(3)$ ml. (Iodate titrated with thiosulphate, 30/12 burette.)

(5) *Size of titration vessel*—If the diameter of the titration vessel is greater than 30 mm. then the last term in the relation v/x (see equation (7), p. 530) will be less than 1 if ρ_4 is approximately 1. Values are given for various vessels and densities in Table V.

TABLE V

VALUES OF $\rho_4r_1^2/\rho_1(r_3^2-r_4^2)$ WITH DIFFERENT BEAKERS AND CONTENTS OF DIFFERENT DENSITIES

$2r_1 = 30$ mm.; $2r_4 = 1$ mm. (values in brackets are for $2r_4 = 2$ mm.); $\rho_1 = 1{\cdot}00$

Beaker .. / $2r_3$ (mm.) ..	50 ml. Tall 35	50 ml. Squat 42·5	100 ml. Squat 49	250 ml. Squat 65
$\rho_4 = 1{\cdot}00$	0·735 (0·737)	0·50 (0·50)	0·375 (0·375)	0·213 (0·213)
$\rho_4 = 1{\cdot}01$	0·74	0·50	0·38	0·22
$\rho_4 = 1{\cdot}02$	0·75	0·51	0·38	0·22
$\rho_4 = 1{\cdot}03$	0·76	0·51	0·39	0·22
$\rho_4 = 1{\cdot}04$	0·76	0·52	0·39	0·22
$\rho_4 = 1{\cdot}05$	0·77	0·52	0·39	0·22

As the values for v/x are 93, 328 and 1272 for 30/12, 30/6 and 30/3 burettes respectively, it is possible to neglect the third term (see equation (7), p. 530) without appreciable error when 30/6 or 30/3 burettes are used, provided that dilute aqueous solutions are titrated and the diameter of the titration vessel is not less than 35 mm. With the 30/12 mm. burette it is necessary to use different values of v/x for different values of r_3, although r_4 may be neglected.

(6) *Density of titrating solution* (ρ_3)—If solutions used as titrating agents are the usual dilute aqueous ones of about 0·1 *N* concentration, then ρ_3 will be less than 1·02.

The values for $\rho_3 r_1^2/\rho_1 r_2^2$ are given in Table VI for the three burettes constructed.

TABLE VI

VALUES OF $\rho_3 r_1^2/\rho_1 r_2^2$

$2r_1 = 30$ mm.; $\rho_1 = 1{\cdot}00$

	For ρ_3 =	1·00	1·01	1·02	1·03
Burette	30/12	6·25	6·31	6·375	6·44
,,	30/6	25·0	25·25	25·5	25·75
,,	30/3	100	101	102	103

TABLE VII

PERCENTAGE ERRORS INVOLVED BY ASSUMING $\rho_3/\rho_1 = 1$ AND NEGLECTING $\rho_4 r_1^2/\rho_1(r_3^2-r_4^2)$

Burette	Assuming $\rho_3/\rho_1 = 1$	Assuming $\rho_4 r_1^2/\rho_1(r_3^2-r_4^2) = 0$	Making both assumptions
30/12	+0·12	−0·81	−0·69
30/6	+0·16	−0·24	−0·08
30/3	+0·16	−0·06	+0·10

The maximum error involved by assuming $\rho_3/\rho_1 = 1$ (ρ_3 not more than 1·02) is small and positive, while the maximum error involved by neglecting the final term in the expression for v/x is small and negative. Values are given in Table VII. These assumptions can obviously be made for the two smaller burettes and v/x becomes

$$\frac{\rho_2}{\rho_1}\left(1 + \frac{r_1^2}{r_2^2}\right) - \frac{r_1^2}{r_2^2}$$

with values 328·2 for burette 30/6, and 1272 for burette 30/3.

For burette 30/12 it is desirable to use

$$\frac{v}{x} = \frac{\rho_2}{\rho_1}\left(1 + \frac{r_1^2}{r_2^2}\right) - 1{\cdot}01\,\frac{r_1^2}{r_2^2} + 1{\cdot}01\,\frac{r_1^2}{r_3^2}$$

with the following values.

$2r_3$ (mm.) ..	..	35	42	49	65
v/x ..	..	93·4	93·2	93·1	92·9

TECHNIQUE

(1) Open the reservoir tap to fill the burette and then close it.

(2) Open the titration tap to remove most of the excess of the titrating solution and then close it.

(3) Move up a beaker containing a solution similar to that to be titrated until the delivery tip just touches the centre of the surface of the solution. Then open the titration tap and after 1 minute stir the contents of the beaker, close the titration tap and withdraw the beaker. Repeat this procedure if an appreciable volume of liquid 3 is delivered.

(4) Move up a beaker containing the solution to be titrated until the centre of the surface just touches the delivery tip and open the titration tap.

(5) Run water down the side of the large limb slowly (5–10 ml. per minute) while stirring liquid 4 in the beaker with a small glass rod.

(6) When approaching the end-point add water 0·1 ml. at a time, allowing ½ to 1 minute after each addition.

(7) At the end of the titration close the titration tap and withdraw the beaker.

With burette 30/12, the tip may now be re-inserted as in (3) as a preliminary to the next titration.

With burettes 30/6 and 30/3, when the term $\rho_4 r_1^2/\rho_1(r_3^2-r_4^2)$ is neglected, the burette is ready at the end of one titration for the start of the next, as in (4).

(8) Calculate the volume of liquid delivered by dividing the volume of water added by the factor v/x.

Results

The instruments have been tested by titrating with standard thiosulphate the iodine liberated from a hydrochloric acid - potassium iodide mixture by a known volume of standard iodate. The thiosulphate used was 0·1080 ± 0·0002 *N*. The iodate was diluted to the required concentration by means of pipettes and 100 ml. flasks that had been calibrated by weighing the water delivered or contained. Measured amounts of the diluted iodate were then titrated with the 0·1 *N* thiosulphate by means of the micro-burettes. Five volumes of iodate were added to 1 volume of 10 per cent. (w/v) potassium iodide solution and 1 volume of 0·5 *N* hydrochloric acid, 1 drop of a solution of soluble starch in saturated sodium chloride solution being added. The specific gravity (t°/t°) of this mixture was 1·014 and that of the thiosulphate solution was also 1·014. The diameters of titration vessels were found by measuring the alteration in height on adding a known volume of water to the 35 and 42 mm. beakers, and by measuring the 49 and 65 mm. beakers with calipers and a scale. The diameter of the delivery tip was measured by means of an eyepiece scale in a microscope.

(1) Burette 30/12—Dimensions: $r_1 = 15$ mm., $r_2 = 6$ mm., $r_3 = 21{\cdot}25$ mm. and $r_4 = 0{\cdot}95$ mm.; $\rho_3 = \rho_4 = 1{\cdot}014$.

(*a*) *Variations in liquid added* (*liquid* 1)—25·01 ml. of 0·001003 *N* iodate were titrated with 0·1080 *N* thiosulphate, using water, toluene and carbon tetrachloride as liquid 1. The calculated volume of thiosulphate was 232·3 µl.

Liquid 1	Sp.gr. 20°/20°	Ml. needed to deliver 232·3 µl. of thiosulphate: Calculated	Found
Water	1·000	21·53	21·58 ± 0·11(5)
Toluene	0·869	24·78	24·70 ± 0·07(4)
CCl_4	1·600	13·46	13·65 ± 0·11(6)

(*b*) *Using water to procure delivery of the thiosulphate, the iodate being measured from a 50 ml. burette*—

0·001003 *N* Iodate added ml.	Thiosulphate equivalent µl.	Water needed to deliver thiosulphate: Calculated ml.	Found ml.	Difference ml.
23·98	222·8	20·62	20·49	−0·13
21·17	196·7	18·22	17·83	−0·39
20·09	186·7	17·29	17·46	+0·17
18·02	167·4	15·51	15·74	+0·23
15·00	139·4	12·91	12·79	−0·12
13·11	121·8	11·28	10·99	−0·29
10·72	99·60	9·23	9·08	−0·15
1·013 (pipette)	9·41	0·87	0·803 ±0·003(3)	−0·07
				S.E. = ±0·08

The standard error of 0·08 ml. of water added is equivalent to 0·87 µl. of thiosulphate delivered.

(2) Burette 30/6—Dimensions: $r_1 = 15{\cdot}00$ mm., $r_2 = 3{\cdot}00$ mm., $r_3 = 21{\cdot}25$ mm. and $r_4 = 1{\cdot}1$ mm.; $\rho_1 = 1{\cdot}00$ and $\rho_3 = \rho_4 = 1{\cdot}014$. Iodate 0·0002511 *N*, measured by pipette.

Iodate added ml.	Equivalent of thiosulphate µl.	Water needed to deliver thiosulphate: Calculated ml.	Found ± S.E. (*n* values) ml.	Difference ml.
25·01	58·15	19·10	19·70 ± 0·05(3)	+0·60
15·025	34·94	11·47	11·60 ± 0·14(3)	+0·13
10·01	23·28	7·64	7·82 ± 0·02(3)	+0·18
5·015	11·66	3·83	4·00 ± 0·006(3)	+0·17
1·013	2·355	0·77	0·73 ± 0·06(3)	−0·04
				S.E. ±0·15

The standard error of 0·15 ml. of water added is equivalent to 0·45 µl. of thiosulphate delivered.

(3) BURETTE 30/3—Dimensions: $r_1 = 15{\cdot}00$ mm., $r_2 = 1{\cdot}50$ mm., $r_3 = 21{\cdot}35$ mm. and $r_4 = 0{\cdot}3$ mm.; $\rho_1 = 1{\cdot}00$ and $\rho_3 = \rho_4 = 1{\cdot}014$. Iodate 0·0001005 N, measured by pipette

Iodate added ml.	Equivalent of thiosulphate µl.	Water needed to deliver thiosulphate: Calculated ml.	Found ± S.E. (n values) ml.	Difference ml.
25·01	23·275	29·59	30·00 ± 0·05(3)	+0·41
10·01	9·316	11·84	12·00 ± 0·14(4)	+0·16
5·015	4·667	5·93	6·03 ± 0·10(4)	+0·10
1·013	0·943	1·20	1·20 ± 0·10(4)	nil
				S.E. ±0·13

The standard error of 0·13 ml. of water added is equivalent to 0·10 µl. of thiosulphate delivered.

COMPARISON WITH OTHER TYPES OF MICROBURETTE

Other types of microburette depend on the use of graduated narrow-bore tubing, the accuracy of measurement depending on the uniformity of bore of the tubing. Two methods of delivery are used: (1) by gravity, with a controlling tap (Bang, Conway), and (2) movement of a mercury meniscus (Rehberg). Many devices have been introduced to ensure smooth controllable movement of the mercury column in Rehberg's burette, including addition of mercury to a U-tube with limbs of different sizes.[3,4]

Table VIII gives some comparative figures for different burettes.

TABLE VIII

VARIOUS TYPES OF MICROBURETTES

Burette	Diameter of tube governing delivery mm.	Actual reading on	Vol. delivered by 1 graduation µl.	Capacity ml.
Conway*	1·13	1·13 mm. tube	1·0	0·5
Rehberg*	1·13	,,	1·0	0·1
,, (Kirk)* . .	0·5	0·5 mm. tube	0·2	(0·1)
This paper 30/12 . .	12·00	50 ml. burette	1·08	0·54
,, ,, 30/6 . .	6·00	,,	0·30	0·15
,, ,, 30/3 . .	3·00	,,	0·08	0·04

* From Conway.[2]

The author is indebted to Dr. K. Mitchell for invaluable mathematical assistance and to Professor D. Burns for his interest. A grant from King's College research fund is gratefully acknowledged.

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NEWCASTLE-ON-TYNE

June, 1946

BIOLOGICAL METHODS GROUP

AT the meeting of the Biological Methods Group on May 27, 1946 (*cf.* this Vol., p. 505), the following DISCUSSION took place after the reading of the papers by Dr. K. MATHER and Dr. G. PONTECORVO, which have already appeared in THE ANALYST (this Vol, pp. 407–411 and 411–413).

Professor BUTLER said that anything that would reduce the animal variation in biological assays, and therefore the labour and expense of the tests, would be a great boon. The practical difficulties of maintaining large stocks of animals were so great that little advance could be hoped for until the production of animals for experimental purposes was organised by some central authority. It was very difficult at present to obtain enough animals, especially rats. The method outlined by Dr. Pontecorvo for obtaining bacteria which could be used to detect a variety of different chemical substances opened up a new field for microbiological assay work, the speed and low cost of which made it very attractive. The possibility of strain selection might give us a new and important tool for investigating the mode of action of antibacterial substances.

Dr. E. R. Dawson thought that a mutant of an acid-forming organism which could be used for the assay of aneurine would be very useful indeed. The chemical (thiochrome) method gave rise to difficulties with certain foodstuffs, and the methods based on the use of either yeast or *L. fermentum* were both unsatisfactory in many ways.

Mr. S. A. Price supported Dr. Dawson's suggestion regarding the need for a reliable micro-organism for the assay of aneurin. He had been trying to use *L. fermentum*; and although the results were at first satisfactory, the organism now appeared to have acquired the ability to synthesise aneurine and was therefore useless. There was also need for an alternative to *L. helveticus* for the assay of riboflavine; perhaps a mutant of *L. arabinosus* might be produced for the purpose.

Mr. E. C. Wood wondered whether significant differences between the values found by various workers for the ratio of the potencies of vitamin D_2 and D_3 in biological assays, even though albino rats were the test organisms in each instance, might be due to genetical differences in strains, arising from adaptation to differing laboratory environments in many generations of inbreeding. Gross differences were known to occur between such different species as rats and chickens; might it not be possible, therefore, for smaller differences to occur between different strains of the same species? He also asked Dr. Mather if it was genetically sound practice, when maintaining a colony of rats for biological assays, to avoid breeding not only from rats showing disadvantageous characteristics which might be hereditary (such as cannibalism, poor fertility, or small size of litter), but also from their litter-mates as well. Some compromise was necessary in selecting one's breeders; in a laboratory in which economic considerations were important it was much better to produce per year a certain number of fairly suitable animals than a quarter of that number of very suitable animals.

Dr. F. J. Dyer asked whether colchicine was of any use in animal genetics in helping to retard mutations or in promoting homozygosity. He also enquired whether any use had been made of a radio-active isotope in culture media as a means of following its destiny in a bacterial metabolite.

In reply, Dr. Mather agreed that to establish an inbred line with good fertility and all the laboratory virtues was certainly laborious. When once such a line had been established, however, it was very economical, as the undesirable characters would reappear only very rarely by mutation. If a central authority were engaged in producing and maintaining such lines, no compromise would be necessary. Under present conditions, a policy of controlled mating, to maintain the line, between animals with good personal and family records would do something towards eliminating the unwanted characters. Animals with bad records should be used only to supply laboratory material and never for further breeding. On the question of differences in assays between strains, he pointed out that two inbred lines, although identical in one character (*e.g.*, albinism) controlled by major genes, would be expected to differ in other morphological and physiological characters, some of which might be of the utmost importance in assaying; but *within* either line there should be great uniformity for all hereditary characters if the inbreeding had been properly maintained. He added that colchicine was not likely to be useful in animal genetics, since doubling the chromosome number would upset the sex-determining mechanism found in this group.

Dr. Pontecorvo agreed with Professor Buttle that resistant strains might give much information about the mode of action of drugs. It had been shown, for instance, that strains resistant to sulphanilamide synthesised more *p*-aminobenzoic acid. If the relationship between these two substances had not been known before, a clue to the mode of action of sulphanilamide would have been obtained. On the question of aneurine assays, he said that there was no theoretical reason why a mutant of an acid-forming organism could not easily be obtained. The *Lactobacilli*, however, were the least suitable for this work, since they had very complex nutritional requirements. He believed that much work was being done at present in the United States on the use of radio-active isotopes in the manner suggested by Dr. Dyer.

Notes

A UNIVERSAL MICRO-STIRRER WITH REMOTE OPERATION

In recent experimental drop-scale titrations upon a microscope slide,[1] use of aqueous-alcoholic solutions made it desirable to replace air-jet agitation[2] by normal stirring. Although the vacuum-operated micro-stirrer previously described[3] is completely satisfactory when vertical motion is required, it will not operate in an inclined or a horizontal position. Accordingly, a micro-stirrer with a guided piston operating at 3 to 4 strokes per second has been developed. This device will work in any position, and any length of stroke from a fraction of a millimetre to about 10 mm. may be obtained. It is simple in construction and compact.

The apparatus in its simplest form is shown in Fig. 1. The stirrer head A is attached to a piston B, which is caused to move back and forth by air pulsations from the impulse-generator. The latter is merely the vacuum-operated unit referred to above.[3] Rubber tubing of 4-mm. bore connects the vent tube H of the impulse-generator to the stirrer unit and serves to transmit the pneumatic pulsations. Up to about 5 feet of tubing may be used with completely satisfactory results; use of a transmission line of 3-mm. bore glass tubing jointed with rubber enabled the stirrer to be operated some 20 feet from the impulse-generator.

The cylinder C of the stirrer is made from a 7-cm. length of 6-mm. bore glass tubing, which should be selected for circularity and uniformity of bore.[4] Flare one end of the tube slightly and constrict the other symmetrically until the bore is reduced to 4 mm. To make

the piston, close one end of a length of 3-mm. outside diameter tubing and thicken it into a ball about 7 mm. in diameter, as shown at (*a*) in Fig. 2. When the piston is cold, insert it in the cylinder until the ball, which forms the piston head, is stopped by the flared end of the cylinder. Holding the stem to prevent the piston from falling out, dip the flare and piston head into a paste of fine emery and water. Without inverting, grind the parts together by rotating the piston stem. Periodically wash out the abrasive and repeat the procedure until the piston head, which now appears as shown at (*b*) in Fig. 2, enters the cylinder and slides, without seizing, along the whole length of the latter.

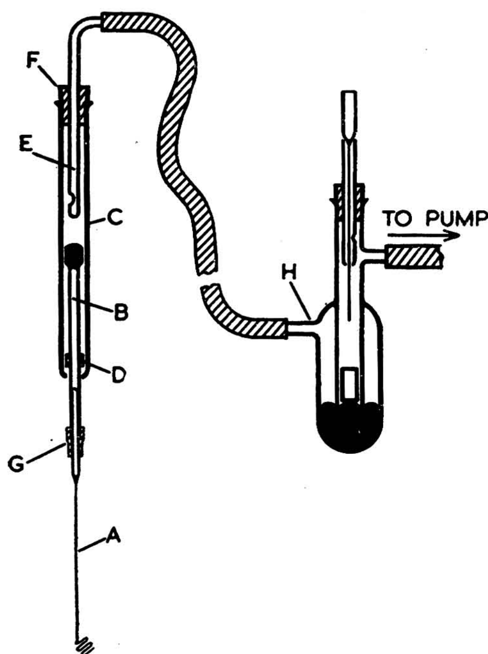

Fig 1

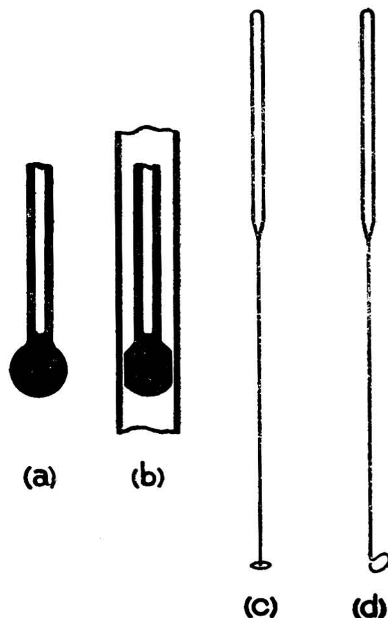

Fig. 2

Thoroughly clean and dry the parts and cut the piston stem to length. To limit the outward movement of the piston, slip on the stem a ring D cut from cycle valve rubber. Inward movement is limited by the connecting tube E, which is closed at the lower end to form a stop and has a small hole blown in the wall, as shown in Fig. 1. To adjust the stroke of the stirrer, slide the connecting tube in the rubber stopper F, which is cut from pressure tubing.

To construct the interchangeable stirrer heads, select fine glass rod which slips easily into the piston stem. Draw out and cut to leave a thread about 0·5 mm. in diameter attached to a shank about 2 cm. long. Bend the thread as shown at (*c*) or (*d*) in Fig. 2, or to other suitable form. Easy exchange of stirrer heads is permitted by using a retainer G cut from cycle valve rubber, as shown in Fig. 1.

A modification shown in Fig. 3 permits easy and accurate adjustment of the stroke whilst the stirrer is in operation. The cylinder is mounted in the straight-through portion of a T-piece, the side arm of which is connected to the impulse-generator. Rotation of the knob attached to a length of 2-BA. brass screw rod which passes through a terminal head cemented in the end of the T-piece enables the stroke to be adjusted at will. The screw rod should be a good fit in the terminal head.

Drop-scale titrations may also be carried out in the "titration boat" shown at (*a*) in Fig. 4. The stirrer unit is arranged horizontally and the shank of the "boat" is held in the piston stem. The tip of the micro-burette jet dips into the liquid in the boat; since the stroke of the stirrer may be adjusted to match the length of the "boat," efficient stirring results.

To make this attachment, blow a hole about 4 mm. in diameter in the wall of a length of 8-mm. outside diameter glass tubing as shown at (*b*) in Fig. 4. Heat carefully in the region of the hole to thicken the glass, then pull the ends of the tube apart. Allow to cool, and cut

out the narrow central portion (Fig. 4, (*c*)), which is of U-section, and form it into a spouted boat about 1 to 1·5 cm. long overall. Seal this to a drawn-down shank of glass rod suitable

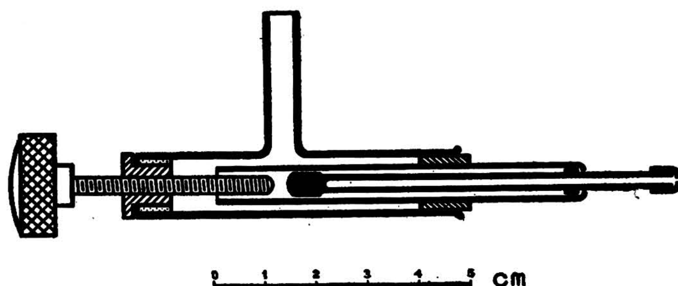

Fig. 3

for attaching to the stirrer and add the small glass counterweight to prevent the boat from inverting. In use, the course of titration is best observed by placing the eye a little below the level of the liquid in the boat. Internal reflection then intensifies the apparent depth of colour of the indicator.

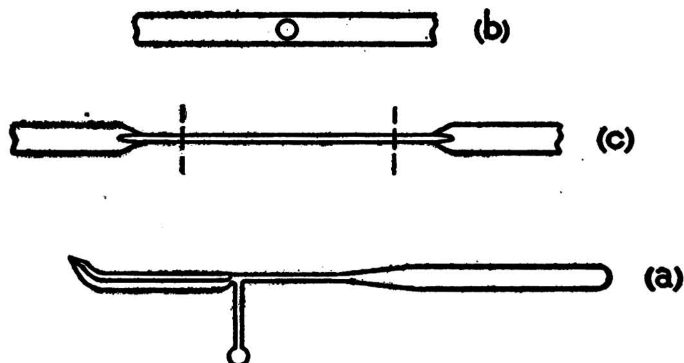

Fig. 4

Use of a platinum or silver titration boat should enable micro-potentiometric titration to be performed in a manner analogous to the "vibrating electrode" technique.[5]

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J. T. STOCK
M. A. FILL
August, 1946

A NOTE ON THE TIN ASSAY OF BRITISH CHEMICAL STANDARDS

IN a recently published paper in THE ANALYST, "The Gravimetric Determination of Tin in Alloys by the Tannin Method," by W. R. Schoeller and H. Holness,[1] it is stated that when the Bureau of Analysed Samples (Messrs. Ridsdale & Co.) Standard Alloy-Bronze "A" was assayed for tin by their method, they found only 9·70 per cent. of this element present. The certified figure given is 9.96 per cent.

The ten analyses, of which the figure 9·96 per cent. is the average, include five results exceeding 10·0 per cent. The lowest result published on the certificate is 9·70 per cent., which agrees with the figure arrived at by Schoeller and Holness. All the results on the certificate of the Bureau of Analysed Samples are averaged—without criticism.

I am in agreement with Schoeller that the certified figure for tin in the Standard Alloy-Bronze "A" is high. Using my own gravimetric method for determining tin in Bronze[2] I obtain figures between 9·70 per cent. and 9·75 per cent. in Bronze "A." Employing the method of W. C. G. Wheeler[3] I found 9·71 per cent. and 9·74 per cent. of tin from two separate determinations.

It would seem, therefore, that the tin figures given by the Bureau of Analysed Samples are in need of revision. Further analyses of the alloys should be made, especially for tin content, using some of the recently advanced methods of determination.

I wish to thank the Director of Scientific Research for granting permission to publish this Note.

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3. Wheeler, W. C. G., *Id.*, 1938, **63**, 883.

ADMIRALTY CHEMICAL DEPARTMENT
H.M. DOCKYARD, PORTSMOUTH

A. G. DUNBAR-POOLE
July **1946**

Ministry of Food

STATUTORY RULES AND ORDERS*

1946 No. 1536. Order, dated September 18, 1946, amending the Flour Order, 1945. Price 1d.

This amending Order provides for the rate of extraction of national flour to be reduced from 90% *to* 85% *and of "W" flour from over* 90% *to over* 85%, *as from September* 22, 1946. *It revokes the three amending Orders Nos.* 265, 312 *and* 662, *of* 1946.

— **No. 1545. Order, dated September 23, 1946, amending the Cheese (Control and Maximum Prices) Order, 1943.** Price 1d.

The purpose of this amending Order is (*a*) *to increase, as from September* 29, 1946, *the maximum prices of Camembert cheese imported from France;* (*b*) *to substitute, as from October* 13, 1946, *"imported blue vein cheese" for "Cheese imported from Denmark," and in consequence* (*c*) *to amend the definition of "Specified cheese," which now becomes* any description of cheese except—

(*a*) home produced blue vein cheese;
(*b*) soft cheese or curd cheese; and
(*c*) cheese made from milk other than cow's milk.

No. 1639. Order, dated October 9, 1946, amending the Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order, 1944. Price 2d.

This amending Order abolishes, from January 1*st*, 1947, *as regards chocolate and chocolate confectionery only, the exemption from the provisions of the main Order* (*S.R. & O., No.* 451, 1944; *amended by No.* 598, 1945, *and No.* 1365, 1946) *given to products containing a therapeutic dose of a medicament.* Instead, such *exemption is given—*

to products in compressed tablet form, of which each tablet contains any one or more of the following ingredients in not less quantity than that specified: phenolphthalein 0·5 *grain, santonin* 0·1 *grain, calomel* 0·05 *grain;*

and also to a number of particular named products which are set out, with names and addresses of makers, in Table A of this amending Order.

The exemption given to products which comply with recognised medical formulae or are prescribed for particular persons by medical practitioners is not affected by this Order.

Ministry of Health

ICE CREAM

CIRCULAR, No. 183/46, dated October 8, 1946, which the Ministry of Health has issued to local authorities, states that draft Ice-Cream (Heat Treatment) Regulations, 1946, which the Minister proposes to make, were published in the *London Gazette* on October 8th. The Minister has examined suggestions for cleanliness tests of ice cream, but is advised that, although bacteriological tests may usefully be employed to indicate faulty methods of manufacture and handling, no test has yet been devised of the safety of ice cream, and that there is no known test which would be sufficiently reliable for use as a statutory test of its contamination with non-pathogenic organisms. The Circular draws attention to the powers that local authorities already possess under Sections 13, 14, 16 and 17 of the Food and Drugs Act, 1938, to ensure cleanliness of conditions of manufacture and handling.

* Obtainable from H.M. Stationery Office. Italics signify changed wording.

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT FOR 1945

The Director, Mr. F. Hirst, reports that although the station is still short of its normal peace-time scientific staff it has reached the end of the war without any serious curtailment of its activities and with every prospect that normal working conditions will be speedily restored. The investigations undertaken during the period covered by this report included the following.

Hydrogen swells—The phosphorus and copper contents of the steel baseplate influence the rate of corrosion of lacquered cans, but not of plain cans. Hot-rolled plate generally corrodes faster than cold-reduced plate, but if these two types of plate are coupled in the same can it is better to have the cold-reduced plate in the ends and the hot-rolled plate in the body than *vice-versa*. Blackplate can be used for the ends of cans of certain vegetable packs if suitable precautions are taken. Electrolytic tinplate, carrying 8 oz. of tin per basis box, is not very satisfactory with plums, even if the cans are lacquered on both sides of the plate. The weight of tin coating, and possibly also its method of application, influences the rate of corrosion. Thiourea added to the lining compound of cans inhibits the corrosion of the end seams and thus reduces the rate of formation of hydrogen swells in most canned fruits, but not in canned rhubarb, where corrosion is accelerated. The post-lacquering of cans by spraying or flushing is the most promising method of reducing losses from hydrogen swells and is strongly recommended.

Fruits—All fruits contain varying quantities of natural accelerators and inhibitors of corrosion. Methods are suggested for reducing the corrosive properties of canned prunes. Spray residues containing elemental sulphur or polysulphides, act as very strong accelerators in canned acid fruits. The effect of sulphur dioxide in sugars used for making the syrups for canned fruits is apparently negligible up to a maximum concentration of 20 p.p.m. on the raw sugar. Ponceau 2R, used as a colouring matter, does not accelerate corrosion. The results of tests on other possible accelerators—including thiodiglycol, a break-down product of mustard gas—are described.

Vegetables—Canned vegetables, beetroot, stringless and runner beans, and carrots in lacquered cans may give trouble, but vegetables in plain cans are practically free from hydrogen swells.

Leaks—Slight leaks in a very small percentage of fruit cans may possibly be responsible for some of the earliest hydrogen swells noted in any season. This point is being investigated further.

Internal corrosion of cans, Progress Report III—Studies of the peculiarities met with in the corrosion of cans made from two different types of tinplate (see reports of 1943 and 1944; by D. Dickinson) have been continued and an explanation of the phenomena associated with their corrosion is now offered. Assuming that the side seam is predominantly cathodic and the end seam predominantly anodic, and using the knowledge that hot-rolled plate is normally anodic to cold-reduced plate the effect of corrosion in a "mixed" can is explained as follows. In cans made entirely of hot-rolled plate, the net rate of corrosion is controlled by anodic polarisation of the end seam and the extent to which local action can proceed on the side seam. The same conditions apply to cans made entirely from cold-reduced plate, but the rate of corrosion is less because this type of steel is more resistant to attack. When the side seam is of cold-reduced plate and the end seam of hot-rolled plate the rate of corrosion is almost entirely governed by the rate of diffusion of the anodic products out of the end seam; local action on the cathodic side seam will be comparatively slight. Consequently, the over-all rate of corrosion of a can so constituted will be less than that of a can made entirely from hot-rolled plate. When the side seam is of hot-rolled plate and the end seam of cold-reduced plate, the cathodic metal is in the anodic position and *vice-versa*. The experimental results showed that this combination intensified the dissolution of the side seam and it appears as if the difference of potential between the two steels tended to inhibit local action on the side seam. The over-all rate of corrosion is, therefore, governed by the rate of diffusion of cathodic products out of the end seam. It is not difficult to see that this process may well be very slow. Experiments designed to check this theory and to include the effect of thiourea in the lining compound of fruit cans, led to the conclusion that the end seam is normally anodic to the side seam and that cold-reduced plate is cathodic to hot-rolled plate. This has been substantiated by measurements of the potential difference between the side and end seams of some of the "mixed" cans used in the experiments. While the cans were still sealed and actively corroding it was found that a difference of the order of 0·01 millivolt prevailed, the end seam being the anode. These findings are in conformity with a previous report to the effect that post lacquering of the side seam greatly reduces the rate of corrosion. When this is done both cathodic and anodic reactions are thrown on to the end seam and the over-all rate of hydrogen formation is reduced. It is also shown that the function of thiourea is inhibition of the anodic reaction.

Corrosivities of varieties of the plum—The effect of variety on corrosivity is most evident when the fruits are under-ripe; and in practice, especially when the fruits are not graded for ripeness, there is likely to be little apparent difference between the keeping qualities of different varieties when they are packed in lacquered cans. Practical canning trials on a sufficient scale remain, however, the only completely satisfactory test.

Effect of dithiocarbamate spray residues on canned blackcurrants—Experiments with ferric dimethyldithiocarbamate as a spray for the control of blackcurrant leaf spot disease are described. They were designed to check those described in the report of 1944, in which it was shown (by Marsh and Dickinson) that the disease fungus, *Pseudopeziza ribis*, was adequately controlled by the dithiocarbamate spray; and to check the effect of spray residues on the canned fruit. The results are summarised as follows. (1) The 1945 spraying trials confirm the findings of the previous year. (A supplementary test showed that tetramethylthiuramdisulphide was not effective against the disease.) (2) The currants treated with ferric dimethyldithiocarbamate carried about 45 p.p.m. of spray residue irrespective of whether the fruit had been sprayed immediately before picking or had stood three weeks—protected or unprotected from the rain—between spraying and picking. The spray residue was thus very stable and adherent to the fruit.

(3) Whole unstrigged fruit showed about 15 p.p.m. of the dithiocarbamate spray residue after canning and storage, but pulped fruit contained only slight traces. (4) The dithiocarbamate spray residues appeared to have no effect on the production of ascorbic acid in the plant, or on its retention during subsequent canning or pulping. The extent of the interference with the determination of ascorbic acid was negligible. (5) The dithiocarbamate spray residues appeared to accelerate corrosion very slightly in cans stored at 95° F. A sulphide film was formed on the inner surface of the cans, and the canned treated currants contained more iron and less tin than the controls. (6) The colour of the whole currants containing dithiocarbamate spray residues was paler than the controls and had little of the blue tint associated with dissolved tin. (7) The flavour of the whole currants containing dithiocarbamate spray residues was unsatisfactory, even in the cans stored at normal temperatures. The worst samples were considered uneatable. The conclusions to be drawn from the canning trials are that ferric dimethyldithiocarbamate spray residues are not harmful to the colour, flavour, ascorbic acid content or shelf-life of canned blackcurrant pulp, but may seriously affect the flavour of canned whole currants in syrup. As boiling the fruit causes ferric dimethyldithiocarbamate to decompose rapidly, it seems probable that this spray could be safely used for currants which will subsequently be stewed, or made into jam or pulp. It cannot be recommended for fruit required for canning as dessert currants in syrup.

ANALYTICAL METHODS—*Ferric dimethyldithiocarbamate*—When heated in acid solution, this substance decomposes into carbon disulphide, dimethylamine, and the ferric salt of the particular acid. A method of determination has been worked out and is described fully elsewhere (ANALYST, **1946**, **71**, 327). *Ascorbic acid*—The standard method of titration of the extract in 5 per cent. metaphosphoric acid over chloroform with 2:6-dichlorophenolindophenol was used in all determinations. Potentiometric check titrations showed that the dye suffered no interference from ferric dimethyldithiocarbamate.

ESTIMATION OF MATURITY OF CANNED GREEN PEAS—This investigation was designed to find laboratory methods to replace subjective tests for maturity. It continues work started in 1944. The tests used were: (1) *Maturity rating*—Based on prominence of the radicles, and texture as determined by the mouth. (2) *Burst skins*—Mature peas split to a width of 1/16 inch. (3) *Turbidity*—The peas were drained, weighed and returned with the brine to the cans. A sample of the brine was poured into a 100 ml. cylinder and allowed to settle for 15 min. The transmission of light through a 1 cm. cell was then compared with that of distilled water, in a Spekker photoelectric absorptiometer set at 1·0 for distilled water. The reading of the brine sample multiplied by a hundred was used as an empirical measure of the relative transmission; the higher the turbidity the lower the reading. (4) *Crushing tests*—(A) The average weight required to crush single cotyledons to one quarter of their original diameter, and (B) the percentage of peas crushed by a greater weight than 2 lb. (5) *Brine flotation tests*—The brines contained 10, 11, 12, 13, 14 and 15 g. of sodium chloride per 100 g. of solution. (6) *Alcohol-insoluble solids*—Each sample of peas was drained, rinsed in water, drained again for two minutes and minced. Ten grams of the minced sample were boiled in 150 ml. of 80 per cent. alcohol and the residue was filtered through a tared paper, washed with a further 100 ml. of alcohol, dried in a steam oven for 4 hours at 98–100° C. and weighed. The tests described form only a preliminary survey of the possible grounds on which standards for maturity might be based. More work is required before limits can be set for the maturity as measured by the most suitable alternative tests. The results recorded in the paper suggested that a test based on the prominence of radicles and the texture in the mouth might give a useful rating for maturity that could be generally employed, but that methods based on physical and chemical tests—particularly the alcohol-insoluble solids content and the crushing test—might be used where objective standards are required.

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 OSC/1—Tallows.

- CH (OSC) 6679—Draft for Home Produced Technical Bone Grease and Draft for Home Produced Technical Tallow.

Draft Specifications prepared by Technical Committee OSC/12—Vegetable Oils.

- CH (OSC) 7122—Draft for Castor Oil ("Firsts" Quality) (Revision of B.S. 650)
- CH (OSC) 7123—Draft for Refined Cotton Seed Oils (Revision of B.S. 655)
- CH (OSC) 7124—Draft for Crude Soya Bean Oil (Revision of B.S. 653)
- CH (OSC) 7125—Draft for Crude Palm Kernel Oil (Revision of B.S. 652)
- CH (OSC) 7126—Draft for Crude Rape Seed Oil (Revision of B.S. 631)
- CH (OSC) 7127—Draft for Crude Sesame Oil (Revision of B.S. 656)
- CH (OSC) 7128—Draft for Crude Ground Nut Oil (Revision of B.S. 629)
- CH (OSC) 7129—Draft for Edible Olive Oil (Revision of B.S. 630)
- CH (OSC) 7130—Draft for Crude Maize Oil (Revision of B.S. 651)
- CH (OSC) 7277—Draft for Perilla Oil (Revision of B.S. 654)
- CH (OSC) 7278—Draft for Raw Linseed Oil for General Purposes (Revision of B.S. 632)

Draft Specifications prepared by Technical Committee FCC/4—Solvents.

- CH (FCC) 7320—Draft Revised B.S. 506 Methyl Alcohol (Methanol)
- CH (FCC) 7321—Draft Revised B.S. 663 Ethyl Lactate
- CH (FCC) 7322—Draft Revised B.S. 552 Amyl Acetate

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Nicotine in the Air. W. E. McCormick and M. Smith (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 508–512)—Nicotine forms a coloured compound with cyanogen bromide in presence of an aromatic amine (Konig, *J. prakt. Chem.*, 1904, **69**, 105). This reaction, as used by Markwood (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 427; 1940, **22**, 792; ANALYST, 1939, **64**, 611; 1941, **66**, 21) for determination of nicotine in tobacco, appeared to afford a satisfactory method for determination of nicotine in air in order to investigate existing or potential health hazards in industries where workmen are exposed to nicotine vapour or tobacco dust. In either form the nicotine can be collected by the impinger described by Bloomfield and Dallavalle (*U.S. Public Health Bull.*, 1935, **217**).

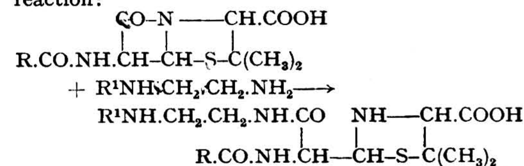
Draw the contaminated air through the impinger, containing water, at the rate of 1 cub. ft. per min. until the total amount of nicotine collected is at least 50 μg. If protected from light the samples will keep without loss of nicotine for several days. Dilute the contents of the impinger with water to 100 ml. and, after thorough shaking, transfer a 10- to 11-ml. aliquot to a graduated 15-ml. centrifuge tube. Note the exact vol. of the liquid, add 0·1 ml. of 30 per cent. sodium hydroxide soln., stopper the tube and shake it occasionally during 10 to 15 min. The alkali releases nicotine from tobacco dust, and if free nicotine is being determined this step may be omitted. The soln., however, should be carefully neutralised and centrifuged as described (*infra*). Add 2 drops of a 0·04 per cent. alcoholic soln. of phenolphthalein and neutralise the soln. carefully with acetic acid to the disappearance of the pink colour, approaching the end-point with diluted acetic acid (1 in 20) and completing the neutralisation with a 1 to 2000 dilution from a fine-bore pipette. Read the vol. to 0·1 ml. Centrifuge the tube at 3000 r.p.m. for 20 min. or until all the suspended dust is deposited at the bottom of the tube. Pipette exactly 10 ml. of the liquid into a colorimeter tube, add 2 ml. of alcoholic potassium acetate soln. (2 g. in 100 ml. of redistilled alcohol, stored in the refrigerator to ensure its being at a constant low temp. before use) and 4 ml. of β-naphthylamine reagent (0·6 g. in 100 ml. of redistilled alcohol also stored in the refrigerator). Shake the tube, measure the transmission (to compensate for colour due to tobacco) and add 1 ml. of the cyanogen bromide reagent (6 g. of Eastman No. 919 reagent grade cyanogen bromide in 100 ml. of 95 per cent. redistilled alcohol), taking due precautions against the toxicity and volatility of the reagent. Shake the tube and place it in the dark for 30 min. at 22° to 28° C., after which time interference from nornicotine is small. To minimise temperature variations and to reduce the effect of heat of dilution all the reagents should be cooled to some standard low temp., *e.g.*, 5° C., and used at that temp. Measure the light transmission in a photoelectric colorimeter or spectrophotometer at 490 mμ and determine the nicotine content by referring the reading to a previously constructed calibration curve. Obtain the 100 per cent. transmission reading with a blank tube containing 10 ml. of water and the reagents. Calculate the amount of nicotine in the impinger from the formula $\gamma_T = \gamma V_2/V_1$, where γ_T is the μg. of nicotine in the impinger, γ the μg. of nicotine in the 10-ml. aliquot as read from the curve, V_1 (ml.) the vol. of aliquot used and V_2 (ml.) the vol. of the aliquot after the final neutralisation. If the instrument has a cell too small to accommodate the abovementioned amounts of sample and reagents the liquid can be mixed and developed in suitable volumetric flasks and transferred into the cell for transmission measurements.

To prepare the calibration curve add to four colorimeter tubes 0·25, 0·5, 1·0 and 2·0 ml. of standard nicotine soln. made by diluting 10 ml. of a stock soln. containing 1 mg. of nicotine per ml. to 500 ml. Add enough water with its *p*H adjusted as described (*supra*) to make the total vol. 10 ml. and develop the colour as already described. Plot the transmission readings as logarithmic ordinates against nicotine content on semi-logarithmic paper. A straight line should result.

Insecticides used on tobacco plants, *viz.*, lead arsenate, calcium arsenate, cryolite, lime-sulphur, Paris green and Bordeaux mixture, when added to known amounts of nicotine, had no significant effect on the transmission readings. Under the conditions described interference from nornicotine is reduced to a minimum. A. O. J.

Sanitary Code for [Paper and Paperboard] Food Wrappings. J. R. Sanborn (*Pulp and Paper Mag. Canada*, 1946, **47**, May, 83–85)—A guide to points to be observed in improving the sanitary standard of food wrap manufacture, *viz.*: Elimination from paper and adhesives, during manufacture, of poisonous and deleterious substances (metals, toxic chemicals, insects, and slime spots and associated objectionable odours); use of uncontaminated stock (*e.g.*, chemical or mechanical wood pulp, and clean broke); adequate treatment for water-resistance; protection of the finished product from contamination; plant sanitation; personnel hygiene. Bacteriological standards proposed are: not more than 250 colonies per g. of disintegrated paper or board stock; below 25 colonies per quart of waterproof container capacity on the inside (rinse test); below 1 colony per 10 ml. of non-waterproof container capacity on the inside (rinse test); below 10 colonies per whole milk bottle closure (contact test); absence of coliform bacteria. Methods of determining compliance with bacteriological standards are at present based on one of the following criteria: (1) No action taken if 3 out of 4 standard plate counts on consecutive samples are satisfactory. (2) Numerical tabulations showing the percentage of counts which are above and below the specified upper limit. (3) The average standard plate count is taken to mean the average of the logs of the last 4 consecutive counts in a given inspection period; this method avoids penalties for an occasional high count which may be difficult to account for. J. G.

Colorimetric Method for the Estimation of Penicillin. J. V. Scudi (*J. Biol. Chem.*, 1946, **164**, 183–194)—The method is based on the following reaction:



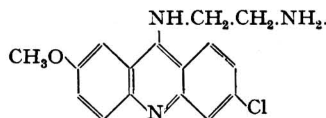
where R^1 represents a chromogenic group. The most suitable reagent was found to be the azo dye N-(1-naphthyl-4-azobenzene)-ethylenediamine. This gives a 90% yield of amide after reaction for 18 hours; the excess reagent can readily be separated from the reaction medium.

Preparation of reagent—To prepare the dye, add dropwise with stirring a solution of sodium nitrite (8 g. in 125 ml.) to a cold solution (0 to 5° C.) of 12·5 g. of aniline hydrochloride in 625 ml. of water containing 16 ml. of conc. hydrochloric acid. After 15 mins. at 0 to 5° C., decompose the excess nitrite with solid ammonium sulphamate. Add the cold diazonium salt solution with stirring to a solution of N-(1-naphthyl)-ethylenediamine dihydrochloride (25 g.) in 1,250 ml. of cold water containing 40 ml. of conc. hydrochloric acid. Leave for 10–15 mins. at room temperature and then for 30 mins. at 50 to 60° C. Cool and add strong sodium hydroxide solution until the colour changes from deep purple to orange (*p*H above 9·6), filter off the product and dry. The yield should be about 29 g. The product is a mixture which can be separated by heating the dry powder with 2·5 litres of petroleum spirit b.pt. 85° to 100° C. (Skellysolve C) under reflux and removing the insoluble residue. On cooling, the filtrate yields a crystalline product, m.pt. 105 to 107° C. This on recrystallisation from Skellysolve C (10 g. per litre) gives a product of m.pt. 107 to 108° C.

Method—Dissolve a weighed amount of the sample of penicillin to be tested in water, taking precautions to keep the sample dry during the weighing. Cool the solution to 0 to 5° C. and add 5 ml., containing 20–120 μg. of penicillin, to 5 ml. of a glycine buffer of *p*H 2·0 (add about 540 ml. of 0·2 *N* hydrochloric acid to 600 ml. of 0·2 *M* glycine in 0·2 *M* sodium chloride) and 25 ml. of pure chloroform. Shake vigorously for 2 mins., allow to separate and transfer the chloroform extract to a cooled 25 ml. glass-stoppered graduated cylinder. Add 3 g. of anhydrous sodium sulphate, and add 20 ml. of the extract to 15 ml. of benzene containing 10 mg. of the dye. Immediately add 5 ml. of a solution of 5 ml. of glacial acetic acid in 1 litre of benzene, and leave at room temperature in a closed container for 3 hours ($\pm$3 mins.). Shake vigorously for 10 secs. with 25 ml. of 0·05 *N* sodium hydroxide, in a 125 ml. separating funnel and allow to separate. Draw off the lower layer slowly, leaving 0·2–0·5 ml. in the funnel and add 25 ml. of chloroform to the sodium hydroxide solution. Shake for 10 secs., and again allow to separate. Remove all but 0·2–0·3 ml. of the chloroform layer, and add 1 ml. of conc. hydrochloric acid and 15 ml. of a mixture (1:4) of butanol and benzene. Shake for 10 secs. to transfer the red condensation product to the solvent phase, and to 10 ml. of the latter add 2 ml. of a mixture (1:19) of conc. hydrochloric acid and absolute ethanol. Evaluate the colour in a photo-electric colorimeter equipped with a No. 540 filter. Care must be taken in removing the excess reagent as, owing to the intensity of the colour, traces retained by the stopcocks and stoppers of the separating funnel can give rise to appreciable errors. Moderate variations of time and temperature do not significantly affect the results, but the agglomeration of condensation products at interfaces may give rise to serious errors unless care is taken to avoid loss of this material. Calibration curves were prepared, using the sodium salt of penicillin II; with 6 standard solutions the average deviation from the best straight line was $\pm$1·5%. The sensitivity was not critical, the method giving equally good results with amounts ranging from 10 to 100 μg. The inactivation product of penicillin did not react with the coloured amine, but interference from certain other types of substances may be anticipated, *e.g.*, ether-soluble keto-acids, lactones, azlactones (oxazolones or dihydro-oxazoles) and esters. Nevertheless, when applied to a large number of commercial samples of penicillin, the method gave results in good agreement with those obtained microbiologically, the correlation coefficient being +0·964. Interfering substances were encountered with low potency samples and broths. To estimate the penicillin content of broths, the latter must first be filtered through a layer of filter aid (0·5 g. per 50 ml.) and then one portion of the filtrate assayed as described above and another portion assayed after inactivation by standing with an equal volume of 0·4 *N* sodium hydroxide for 1–2 hours. Recoveries of penicillin II added to broths averaged 98·7% of the theoretical.

F. A. R.

Rapid Micro Method for the Fluorometric Determination of Penicillin. J. V. Scudi and V. C. Jelinek (*J. Biol. Chem.*, 1946, **164**, 195–201)—The method previously described (*cf.* preceding abstract) is limited in sensitivity, and to extend its range of application the strongly fluorescent compound, 7-methoxy-2-chloro-5-β-aminoethylamino-acridine



was used in place of the amino azo compound. This acridine derivative condenses with penicillin at a more rapid rate than does the azo dye, so that the condensation time can be reduced to one hour.

Preparation of reagent—Warm 5 g. of halocrin (2:5-dichloro-7-methoxy-acridine) for 20 mins. on a steam-bath with 100 ml. of anhydrous ethylene diamine until the solid is dissolved (10–15 mins.). Boil for 3 mins., and then distil under reduced pressure to recover unchanged ethylene diamine (about 60 ml.). Add 250 ml. of cold water to the cooled residue and dissolve the precipitate in 500 ml. of cold 0·1 *N* hydrochloric acid. Filter and add 30% sodium hydroxide solution to the filtrate until maximum precipitation occurs. Recrystallise the dried product (5·4 g.) from 3 litres of petroleum spirit, b.pt. 80° to 100° C. (Skellysolve C). This should give 4·5 g. of pure compounds, m.pt. 141–142° C.

Method—To 5 ml. of benzene containing 10 mg. of the aminoacridine, add in succession 2 ml. of reagent grade acetone, 10 ml. of a chloroform extract of penicillin and 5 ml. of a solution of glacial acetic acid (10 ml.) in benzene (1 litre). Leave the mixture in a glass container at room temperature in absence of light for 1 hour $\pm$ 5 mins., and shake vigorously for 10 secs. with 10 ml. of 0·5 *N* sodium hydroxide. Allow to separate and remove the solvent layer. Shake the alkaline solution vigorously for 5 secs. with two 5 ml. portions of chloroform and discard the chloroform extracts. Acidify the alkaline solution with 1 ml. of glacial acetic acid and extract the condensation product from the reaction mixture by shaking vigorously for 30 secs. with 15 ml. of a mixture (1:2) of butanol and benzene. Discard the aqueous layer and shake the solvent layer for 30 secs. with 10 ml. of 5% acetic acid. Discard the aqueous layer and add 50 ml. of chloroform and

15 ml. of 0·5 N sodium hydroxide. Shake for 30 secs., discard the solvent layer and add 1 ml. of conc. hydrochloric acid to the alkaline solution. Evaluate the fluorescence of the aqueous solution in a Pfaltz and Bauer fluorophotometer equipped with a No. 5113 Corning glass filter, 2 mm. thick, in the path of the incident beam and a No. 3385 Corning glass filter, 2 mm. thick, between the sample and the photocell. As a preliminary, adjust the instrument to a constant light intensity using a solution of 0·8 μg. of the aminoacridine in 16 ml. of 4 N hydrochloric acid, arbitrarily adjusting the fluorescence of the standard solution to 60% of the galvanometer scale. To eliminate the influence of variations in the electric current use 2 balanced cuvettes, one for the standard and one for the unknown solution. Subtract from the final readings the values obtained in blank determinations and calculate the results from calibration curves prepared with pure penicillin. The average deviation from the mean in 24 analyses of standard solutions containing 0·0625–0·625 μg. of penicillin per ml. was $\pm$10%. To prepare the chloroform solution of penicillin shake 8 ml. of an aqueous solution containing 0·0625–0·625 μg. of penicillin per ml. with 12 ml. of pure chloroform and 2 ml. of a Sørensen glycine buffer of *p*H 2·0. Allow the phases to separate and run the chloroform layer into a chilled glass-stoppered graduated cylinder and dry rapidly with 1–2 g of anhydrous sodium sulphate. Where more dilute solutions have to be examined, one of three modifications of this method may be employed. In the first of these, which is preferred, extract 80 ml., as described above, using a larger volume of chloroform, *e.g.*, 60 to 120 ml., extract the chloroform solution at 0 to 5° C. with 8 ml. of a Sørensen phosphate buffer solution of *p*H 7·0, discard the chloroform layer, and then extract the aqueous solution with 12 ml. of fresh chloroform after addition of 2 ml. of 0·4 N hydrochloric acid. Alternatively, extract 80 ml. of the penicillin solution with 12 ml. of chloroform: this procedure gives satisfactory results with solutions containing 0·0125–0·05 μg. per ml., but not with more dilute solutions. Thirdly, extract 80 ml. of the penicillin solution with chloroform and evaporate the *thoroughly dried* chloroform extract to a smaller volume in a current of dry air at 0 to 5° C.; this last modification is not recommended for routine use. In assaying urine, dilute the filtered sample 50-fold and extract 8 ml. by the standard procedure. In assaying whole blood or plasma, first prepare a protein-free filtrate, *e.g.*, by means of the Haden modification of the Folin-Wu method (*J. Biol. Chem.*, 1923, **56**, 469) and then acidify 8 ml. of the centrifugate to *p*H 2·0 by addition of 2 ml. of 0·1N hydrochloric acid instead of the glycine buffer.

F. A. R.

Organic

Qualitative Test for Carbohydrate Material. R. Dreywood (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 499)—Although there are a large number of specific tests for certain types of saccharides there are very few general tests for carbohydrate materials. The well-known Molisch test with α-naphthol is generally applicable to soluble carbohydrates.

Anthrone, which is used for the determination of glycerol (Schutz, *Papier-Fabr.* (*Tech. Tl.*), 1938, **36**, 55) gives a green colour with cellulose, and further expts. showed that a positive test was obtained with all of a group of 18 carbohydrate materials examined, including several cellulose derivatives. Furfural is the only non-carbohydrate compound hitherto encountered that gives a green colour with anthrone, but the colour is rapidly obscured by a brown ppt. and when the sample is diluted with 50 per cent. sulphuric acid or glacial acetic acid a heavy brown ppt. forms. Carbohydrates, on the other hand, may be diluted to any extent with these reagents and the green colour persists even at extreme dilutions. Negative tests were obtained with all of a large group of non-carbohydrate materials examined, including a variety of non-cellulose synthetic resins, organic acids, aldehydes, phenols, fats, terpenes, alkaloids and proteins.

To about 1 mg. of the material to be tested in a test tube add 1 ml. of water and 2 ml. of a 0·2 per cent. soln. of anthrone in conc. sulphuric acid. The final sulphuric acid concn. in the test soln. should always be greater than 50 per cent. to prevent precipitation of anthrone and the heat produced by dilution of the acid is a necessary part of the test. In presence of carbohydrate material a clear green colour will appear and rapidly increase in intensity until a dark blue-green soln. results. The test soln. can for comparison be diluted with glacial acetic acid or 50 per cent. sulphuric acid. In absence of carbohydrate, but in presence of other organic compounds, a brown colour may be produced by action of the acid upon other organic compounds. Anthrone can be prepared according to the directions given by Schutz (*loc. cit.*) or by the procedure given in *Organic Syntheses*, Collective Vol. I, p. 60 (New York, John Wiley & Sons, 1941). Care should be taken during the preparation to avoid contamination with carbohydrate material (*e.g.*, filter paper pulp) which would produce a green colour in a blank test with the reagent. The appearance and deepening of the colour appears to be as rapid with polysaccharides as with equal amounts of monosaccharides, thus suggesting that hydrolysis may not be a necessary part of the reaction. Even the most insoluble synthetic cellulose resins will answer to the test, thus affording a means of classifying these resins into a cellulose and non-cellulose group. Many plastic moulding compositions, however, contain wood flour, which would give a positive reaction. An attempt is being made to apply the test quantitatively to the determination of small amounts of cellulose in solution. The test is extremely sensitive and proved to be 10 to 40 times as sensitive as iodine for detection of starch. About 1 part of starch in 900,000 parts of water can be detected.

A. O. J.

Detection of Elemental Sulphur in Gasoline by the Sommer Test. G. E. Mapstone (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 498–499)—Sommer (*Id.*, 1940, **12**, 368; ANALYST, 1940, **65**, 532) describes a colour test for free sulphur in pyridine soln. in which the addition of a small amount of an alkaline soln. precipitates the sulphur in the form of a blue colloidal soln. The reaction was obtained with as little as 2 p.p.m. of sulphur, but hydrocarbons reduced the sensitivity of the test. In presence of pyridine Doctor soln. gave a visible reaction with 10 p.p.m. of free sulphur.

Sommer's test was applied to the detection of free sulphur in gasoline by mixing the sample with pyridine and adding sodium hydroxide soln. Three phases were formed, *viz.*, an upper gasoline phase, a middle pyridine phase and a lower aqueous phase. The colour appeared in the middle pyridine phase, occasionally in the lower aqueous phase but never in the upper gasoline phase. The inverse

Doctor test with butyl mercaptan is customarily used in plant testing but is sometimes insufficiently sensitive. The alternative mercury test is considered too sensitive for plant control. The conditions for the maximum sensitivity of the Sommer test were determined and the sensitivities of the various tests were compared.

Commercial *iso*-octane (sp.gr., 0·691), freed from traces of sulphur by treatment with mercury and filtered before use, was used as gasoline and referred to as such. Standard sulphur soln. was prepared by dissolving flowers of sulphur in 1 ml. of hot pyridine, allowing almost all the solvent to evaporate and adding enough gasoline to form a soln. containing 0·01 per cent. of sulphur by wt. Other concns. were obtained by diluting this soln. with gasoline. A pure commercial grade of pyridine containing about 5 per cent. of α-picoline was used untreated, since it was not discoloured by treatment with mercury. The butyl mercaptan soln. contained 1 ml. of *n*-butyl mercaptan in 1400 ml. of gasoline and was shaken with mercury and filtered before use. Doctor soln. was prepared by shaking 10 per cent. sodium hydroxide soln. with excess of litharge for 15 min. and decanting the clear liquid. Sommer used 2 *N* sodium hydroxide and sat. sodium bicarbonate soln. and obtained greater sensitivity with the latter, but in presence of gasoline sodium hydroxide provided the greater sensitivity. Addition of sodium hydroxide soln. to the pyridine - gasoline mixture gave a colour several times more intense than that formed by addition of pyridine to the gasoline - soda mixture. Highly aromatic gasoline was not available, so mixtures of motor benzene and commercial *iso*-octane were used. It was found that the proportion of the middle pyridine phase decreased as the benzene content of the blend increased, but the sensitivity of the test appeared unchanged. Sixty per cent. of benzene in the blend was the maximum that could be tested without changing the conditions of the test. Increasing the amounts of pyridine and sodium hydroxide or decreasing the sodium hydroxide concn. enabled the test to work satisfactorily with the higher benzene blends. Blends containing more than 80 per cent. of benzene did not give three phases in the test, but a blue colour was produced in the pyridine - gasoline phase and the sensitivity of the test was much reduced. With the benzene blends the blue was less stable to air and was destroyed by shaking for 1 minute.

In order to compare the sensitivity of the different tests available, the following tests were made. *Inverse Doctor Test*—The sulphur-containing gasoline (5 ml.) was shaken with 5 ml. of butyl mercaptan soln. and 5 ml. of Doctor soln. In the absence of free sulphur the lead butyl mercaptide coloured the gasoline phase yellow and some yellow mercaptide collected at the interface. In presence of free sulphur the colour deepened through orange to dark brown or black according to the concn. Owing to the colour of the lead mercaptide it was necessary to use a blank test for comparison with borderline samples. *Modified Inverse Doctor Test*—The gasoline (5 ml.) was mixed with 5 ml. of pyridine and 5 ml. of butyl mercaptan soln. before addition of 5 ml. of Doctor soln. Addition of pyridine prevented the colour due to formation of lead mercaptide. Three liquid phases were formed and, in presence of sufficient free sulphur, the lead sulphide formed collected mainly at the interfaces, thus making the test easier to observe than the unmodified test. *Sommer Test*—The gasoline (5 ml.) was mixed with 3 ml. of pyridine and then shaken with 0·5 ml. of 2 *N* sodium hydroxide soln. In presence of free sulphur a sky-blue colour was formed in the middle phase. With long shaking the colour disappeared owing to oxidation of the sulphur to thiosulphate. *Mercury Test*—The gasoline was shaken with a little mercury. In presence of free sulphur black mercury sulphide was formed and the suspension coloured the gasoline grey.

Attempts to use purified pyridine homologues (boiling range, 120° to 250° C.) isolated from the cracked pressure distillate, in place of pyridine, failed owing to the higher miscibility of these bases with the gasoline and failure of the mixture to separate into three phases upon addition of water. This is probably the desensitising action of hydrocarbons on the test observed by Sommer.

The foregoing expts. showed that the mercury test is by far the most sensitive test, but it is too sensitive for plant control. The inverted Doctor test is positive to 15 p.p.m. of sulphur but the colour of the lead mercaptide tends to make detection difficult near the limiting concn., and in presence of pyridine the modified test is easier to observe and a little more sensitive. The Sommer test is sensitive to about 3 p.p.m. of sulphur, being about five times as sensitive as the inverted Doctor test and yet not too sensitive for plant control.

If the gasoline being tested contains a high proportion of aromatic compounds it is advisable to determine the optimum proportions of reagents for the test before using it in routine plant control.

A. O. J.

Molecular Weight and Mercaptan Content of Mixtures of Primary Mercaptans. Gravimetric Determination. H. A. Laitinen, A. S. O'Brien and J. S. Nelson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 471–472)—A method was required for determining the average molecular weight of the mercaptan fraction of commercial mixtures containing alkyl disulphides, alkyl halides, alcohols, etc., rather than that of the whole mixture as would be determined by cryoscopic measurements. From the weight of silver mercaptide formed from a known number of molecular equivalents of mercaptan, the molecular weight of the mercaptan fraction can be calculated.

Dissolve 0·3 to 0·5 g. of mercaptan in 400 ml. of absolute ethanol in a 600-ml. beaker, add 6 ml. of conc. ammonia and titrate with 0·1 *N* silver nitrate to an amperometric endpoint (Kolthoff and Harris, *Ind. Eng. Chem., Anal. Ed.*, **1946**, **18**, 161), adding the reagent drop by drop with effective stirring. If more than 0·1 ml. of reagent is added in excess, it is advisable to titrate back with a very dilute soln. of mercaptan in alcohol. After allowing the ppt. to settle, pour the clear liquid through a tared sintered glass crucible without transferring any ppt. Pour the suspension of the ppt. into centrifuge tubes and centrifuge at high speed for 3 min. Wash the ppt. quantitatively into the tubes with absolute ethanol, loosening it with the aid of a rubber-tipped glass rod. Stir the ppt. in the tubes and centrifuge again. Repeat the operation of adding ethanol stirring and centrifuging until the ppt. has been washed three times. Transfer the ppt. into the crucible with the minimum quantity of ethanol and allow it to drain. Dry the ppt. for 2 hr. in a vacuum oven at 60° to 70° C. and weigh. The ppt. decomposes at 100° C. but at temperatures up to 70° C. no change in weight after prolonged heating was observed, although the colour darkened to a deep orange. Mercaptides containing excess of

silver nitrate were less stable than carefully washed samples. If M is the average mol. wt. of mercaptans in the mixture, W the wt. of silver mercaptide, A the amount (ml.) of silver nitrate solution of normality N used and S the wt. of sample, $M = (1000W/AN) - 106\cdot9$ and the percentage of mercaptan in the sample is ANM/10S.

The procedure described is intended for primary mercaptans. With tertiary mercaptan mixtures two difficulties are encountered. The silver mercaptides of mixed tertiary mercaptans are soluble in alcohol, but are pptd. by addition of enough water after addition of the silver nitrate to make the alcoholic content of the final soln. 75 to 80 per cent. However, the mercaptides are liquid at room temp. and filtering and washing operations are difficult. If the titration is carried out at 0° C. to −15° C., the mercaptides are pptd. as solids which can be filtered and washed with 70 per cent. alcohol without centrifuging. Unfortunately, even the low temperature technique does not permit filtration without centrifuging when applied to primary mercaptans. The accuracy of the modified method for tertiary mercaptans was not established, although the accuracy was comparable with that of the method for primary compounds. From the results of the primary method it is concluded that the method yields the molecular weight correct to within 1 or 2 units (0·5 to 1 per cent.), and duplicate determinations are reproducible to ±1 unit in the molecular weight. A. O. J.

Determination of Ethylene Chlorohydrin. K. Uhrig (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 469)—The refractometric methods of Berry (*J. Soc. Chem. Ind.*, 1919, **38**, 145T; ANALYST, 1919, **44**, 305) and Gomberg (*J. Amer. Chem. Soc.*, 1919, **41**, 1414) for determination of ethylene chlorohydrin in binary mixtures are convenient but not specific. The colorimetric method of Sapadinsky (*Z. anal. Chem.*, 1928, **74**, 273; ANALYST, 1928, **53**, 556) and a method mentioned by Berry (*loc.cit.*), which involved heating the sample with silver nitrate in nitric acid soln. under reflux, were found unreliable. Miller and Denny (*Contrib. Boyce Thompson Inst.*, 1936, **8**, 121) hydrolysed the chlorohydrin with barium hydroxide and determined the chloride ion in the resulting barium chloride as an indication of the chlorohydrin concn. in the sample. This suggested that ethylene chlorohydrin could easily be hydrolysed with alkali hydroxide and the chloride ion of the alkali chloride determined, and this proved a successful and reliable method.

Dilute 3 to 5 g. of the sample to 100 ml. with water and take aliquots containing not more than 350 mg. of ethylene chlorohydrin. Heat the aliquots under reflux with 50 ml. of 5 per cent. potassium hydroxide soln. for 1 hr., cool to room temp., add 10 ml. of conc. nitric acid, 50 ml. of 0·1 N silver nitrate, 2 ml. of ferric alum indicator (3 g. of ferric ammonium sulphate, 10 ml. of water and 2 ml. of nitric acid) and titrate the residual silver nitrate with 0·1 N ammonium thiocyanate. Correct the result by a blank titration of 50 ml. of the potassium hydroxide soln. omitting the refluxing. Each ml. of 0·1 N silver nitrate ≡ 8·05 mg. of ethylene chlorohydrin. If the sample should be dissolved in a non-aqueous water-insoluble solvent, the sample weight should be such that not more than 350 mg. of the chlorohydrin are actually present and the entire sample should be heated under reflux. This amount of ethylene chlorohydrin is the maximum that can safely be determined with 50 ml. of 0·1 N silver nitrate.

Solvents and compounds containing loosely combined halogens interfere with the determination. If these compounds are present, extract the chlorohydrin with two equal volumes of water and determine it in each extract. If X_1 is the amount found in the first extract and X_2 that found in the second the amount present in the original sample is given by $X_1^2/(X_1-X_2)$. Some chlorinated solvents (*e.g.*, chloroform) are appreciably soluble in water and since these are usually highly chlorinated a relatively low solubility may introduce considerable error. In such instances distillation is probably the best means of separating the chlorohydrin from interfering substances. Distillation is also the most convenient method of separation if the chlorohydrin has to be determined in presence of inorganic chlorides. Care must be taken that sufficient water is added so that the constant-boiling mixture (42·5 per cent. of ethylene chlorohydrin, 57·5 per cent. of water, b.pt. 96° C. at 760 mm.) will carry over the chlorohydrin with the first portions of the distillate. Results show the accuracy of the method to be satisfactory. A. O. J.

Determination of Acetone, an Ultra-violet Spectrophotometric Method. G. L. Barthauer, F. V. Jones and A. V. Metler (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 354–355)—In connection with the production of propylene by the catalytic dehydration of *iso*propyl alcohol it was necessary to develop a rapid and accurate method for measuring acetone produced as a by-product of the main reaction. Mono-olefines of low molecular weight, di-*iso*propyl ether and unchanged *iso*propyl alcohol were also present in considerable amounts. For rapidity and accuracy a photometric method based on the absorption of ultra-violet light by acetone seemed the most promising method. Hartley (*J. Chem. Soc.*, 1881, **39**, 153) has reported the complete absence of ultra-violet bands (above the Schumann region) for *iso*propyl alcohol. Bielecki and Henri (*Compt. rend.*, 1912, **155**, 456) detected a very slight continuous absorption for *n*-propyl alcohol, but since subsequent work by Massol and Faucon (*Bull. Soc. Chim.*, 1912, **11**, 931) showed that the degree of absorption of a secondary alcohol is lower than that of the corresponding primary alcohol this absorption could apparently be disregarded for analytical purposes. Carr and Walker (*J. Chem. Phys.*, 1936, **4**, 751) in their investigation of the ultra-violet spectra of mono-olefines of low molecular weight found that the absorption is insignificant above 260 mμ, a wavelength well below the absorption maximum of acetone. No data on the absorption of di-*iso*propyl ether were found in the literature, but the absence of significant bands was proved experimentally.

The reagents used were acetone (C.P.); di-*iso*propyl ether (Eastman Kodak No. 1193), purified by successive fractionation and chemical treatment until only a faint trace of acetone remained; *iso*propyl alcohol, purified in the same way; 1-octene (Connecticut Hard Rubber Co.) of 99 per cent. purity, the remainder consisting of close boiling isomers; 2:2:4-trimethylpentane (*iso*-octane, Rohm and Haas) carefully purified before use by means of silica gel (Graff *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 556). All spectral measurements were made with a Beckman DUV spectrophotometer equipped with a hydrogen discharge source, the wavelength scale being calibrated with a mercury vapour lamp to within ±0·1 mμ immediately before use. Matched quartz cells (10 mm.) were

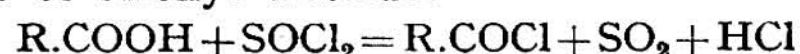
used to contain the sample and the 2:2:4-trimethylpentane reference soln. A band of width 0·5 mμ was used throughout the entire wavelength range. Acetone showed a broad band centred at about 280 mμ. A calibration curve prepared at this wavelength by plotting the optical densities against corresponding concentrations of acetone in 2:2:4-trimethylpentane showed no significant deviation from Beer's law. The molecular extinction coefficient k of each compound was calculated from the foregoing data by means of the modified form of the Beer-Lambert equation, *viz.*, $I_x = I_o 10^{-klc}$ where l is the cell-length (cm.), c the molar concn. and I_o and I_x the incident and emergent light intensities respectively. A series of synthetic samples containing varying amounts of the pure components was analysed for the acetone content, each sample being accurately diluted with 2:2:4-trimethylpentane until the optical density fell within the range of the calibration curve. By reference to the curve and application of the appropriate dilution factor the acetone content of the original sample was easily calculated. The rate of change of the extinction coefficient of acetone is small at its absorption maximum. This would suggest the ready application of filter photometers for transmittancy measurements despite the relatively broad wave bands of such instruments. A. O. J.

Colorimetric Determination of Fatty Acids and Esters. U. T. Hill (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 317–319)—Existing methods for the estimation of fatty acids and their esters are tedious and often inaccurate when interfering substances are present. This investigation was undertaken to develop a rapid and accurate method for the determination of small amounts of palm oil, cottonseed oil, dibutyl sebacate and lanolin applied to tin plate and other metallic surfaces for rust prevention and lubrication.

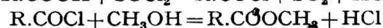
When an ester is warmed in an alkaline medium with hydroxylamine, hydroxamic acid is formed according to the reaction

$$R.CO.OR + NH_2OH = R.CO(NHOH) + ROH$$

(Feigl, "*Laboratory Manual of Spot Tests,*" 1943, pp. 186–187, New York Academic Press). Ferric iron forms a bright red or lavender complex with hydroxamic acid in acid media and this complex is readily soluble in aqueous ethanol, methanol or *iso*propanol; it is least stable in the last solvent. The stability decreases with increasing water content, about 5% of water being the optimum amount for the three alcohols. The ferric hydroxamate complex gains about 0·1% transmittancy per min. for the first 20 min. but becomes relatively stable after this period, the rate being then less than 0·05% per min. A rise in temperature produces a gain of 0·2% transmittancy per °C. at room temp. Addition of a small amount of sodium carbonate increases the colour stability. Feigl (*op. cit.*) uses ferric chloride and hydrochloric acid to form ferric hydroxamate. When ferric perchlorate and perchloric acid are substituted for these the intensity of colour of the complex follows Beer's law. Fatty acids do not form ferric hydroxamate directly but are first quantitatively methylated in anhydrous ethereal soln. either by means of thionyl chloride:

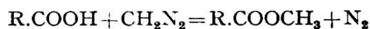


$$R.COOH + SOCl_2 = R.COCl + SO_2 + HCl$$



$$R.COCl + CH_3OH = R.COOCH_3 + HCl$$

or diazomethane:



$$R.COOH + CH_2N_2 = R.COOCH_3 + N_2$$

and the esters are determined.

Reagent—To prepare ferric perchlorate soln. dissolve 0·4 g. of iron or the equiv. amount of ferric chloride in 5 ml. of conc. hydrochloric acid, add 5 ml. of 70% perchloric acid, evaporate almost to dryness and dilute to 100 ml. with water. To 10 ml. of this soln. add 5 ml. of 70% perchloric acid and dilute to a litre with 95% ethanol. Prepare weekly.

Method—Prepare an absolute ethereal soln. containing 0·05 to 0·1 mg. of the esters in a wide-mouthed 250-ml. Erlenmeyer flask. Add from a pipette 0·3 ml. of a 2·5% soln. of sodium hydroxide in 95% ethanol saturated with sodium carbonate and prepared fresh daily and 0·3 ml. of a 2·5% soln. of hydroxylamine in 95% ethanol also prepared daily. Evaporate to dryness on a water-bath at 60° to 70° C. and heat for 5 sec. longer. Add 10 ml. of the ferric perchlorate soln. and allow the mixture to stand for a few min. until all salts have dissolved. Prepare a blank in the same manner. Adjust the temp. to 27° C. and compare the transmittancy of the sample with that of the blank in a spectrophotometer, using a 1·0 to 1·5-cm. cell at 520 mμ. Obtain the amount of ester from a previously prepared curve showing the relation between ester concn. and transmittancy. With fatty acids prepare an ethereal soln. containing 0·05 to 1 mg. of the acid in 10 ml., add about 3 ml. of thionyl chloride and evaporate almost to dryness. Add a few drops of absolute methanol and heat for a short time. Remove the excess of reagents by blowing a gentle current of air into the warm beaker. Add about 20 ml. of anhydrous ether and complete the determination as for esters.

Comparison of wavelength with transmittancy against a blank was made with a Coleman Model 11 spectrophotometer having a 35-mμ band. Maximum absorption is at 520 mμ. In adapting the method to the Cenco-Sheard-Sanford photelometer, a 515-mμ filter was used with a 1-cm. cell. Technical *iso*propyl ether, which may be used instead of ethyl ether, was found to contain esters but was readily purified by saponification with glycerin and potassium hydroxide and subsequent distillation. Esterification of fatty acids may be carried out by adding excess of diazomethane in ether and boiling off the excess. Diazomethane may be prepared by the method of Staudinger and Kupfer (*Ber.*, 1912, **45**, 505).

The method has been applied in the determination of cottonseed oil on electrolytic tin plate, palm oil on hot-dip tin plate and lanolin-base oil on black plate, and it may be applied to lubricating oils containing fatty esters without preliminary separation. Sulphurised sperm oil was determined in a heavy mineral oil by employing a suitable blank. Preliminary work has been done on the determination of hydroxyl groups, the esterification being effected by means of acetyl chloride in ethereal soln. After removal of the ether the excess of acetyl chloride is destroyed with water and the dried esters are determined as described. Probably the method can be applied to the determination of more volatile esters by the use of *iso*propyl ether and a suitable reflux condenser. Comparison of the results of the colorimetric method with those of a gravimetric method established the accuracy of the method described. A. O. J.

Application of the Silica Gel Partition Chromatogram to the Estimation of Volatile Fatty Acids. S. R. Elsden (*Biochem. J.*, 1946, **40**, 252–256)—A silica gel column, 40 cm. in length, was prepared by grinding 3 g. of finely-powdered

silica gel, made according to the method of Gordon *et al.* (*Biochem. J.*, 1943, **37**, 79; ANALYST, 1943, **68**, 283), with 1·8 ml. of a 0·2% bromocresol green solution containing 17·4 ml. of 0·1 *N* sodium hydroxide per 100 ml. until a homogeneous, finely divided, light blue powder was obtained, and then adding in small amounts at a time with intermittent grinding 30 ml. of a water-saturated 1% solution of butanol in chloroform and pouring the slurry into a chromatogram tube. Air bubbles were removed from the tube, care being taken to prevent the surface from becoming dry. One ml. of 0·03 *N* *n*-valeric acid in 1% chloroformic butanol solution was run through the column and the column then washed with the solvent. This checked the evenness of packing of the column and neutralised any local concentration of alkali.

When a mixture of fatty acids dissolved in chloroform was run on to such a column formic acid remained fixed at the top, whilst the other acids passed down the column as separate yellow bands, moving at different rates; *n*-valeric acid moved fastest and acetic acid slowest. The leading edge of each band was sharp, but the rear edge diffuse. Propionic, *n*-butyric and *n*-valeric acids were washed through the column with 1% chloroformic butanol, but acetic acid required 5% and formic acid 20% chloroformic butanol. In practice, however, solutions of butanol in chloroform stronger than 1% cannot be used, as they leach out the indicator.

To estimate volatile fatty acids, first separate these from other acidic substances by steam distillation and then destroy the formic acid (which can readily be estimated by any of the known methods) in the distillate by adding 2 ml. of 10 *N* sulphuric acid, 50 g. of magnesium sulphate and 0·2–0·3 g. of mercuric oxide and again steam-distil (*cf.* Friedemann, *J. Biol. Chem.*, 1938, **123**, 161). Next transfer the acids to as small a volume of chloroform as possible by means of the following procedure. Add a drop of phenol red, make the solution alkaline with a slight excess of 0·1 *N* sodium hydroxide and evaporate to 0·1–0·2 ml. Cool and add 3–4 g. of anhydrous potassium bisulphate to form a fairly dry, pink powder. Extract this with six 4-ml. portions of dry 5% chloroformic butanol and dilute the combined extracts to 25 ml. Estimate the total volatile acids in a portion of the extract by addition of 20 ml. of water and titration with 0·01 *N* sodium hydroxide, using phenol red as indicator. Transfer another portion of the solution to a column prepared as described above and develop the chromatogram with 1% chloroformic butanol. Collect each fraction separately and titrate with 0·01 *N* sodium hydroxide. Acetic acid must be estimated by difference as, although it can be eluted by means of 5% chloroformic butanol, so much indicator is leached out that the end-point of the titration is obscured. With a synthetic mixture of butyric, propionic and acetic acids the method gave almost theoretical results. If, after each experiment, acetic acid is removed with 5% chloroformic butanol, a column can be used up to six times without loss of efficiency. F. A. R.

Physical Methods, Apparatus, etc.

Systematic Polarographic Metal Analysis. Determination of Tin, Lead, Nickel and Zinc in Copper-Base Alloys. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 429–32)—Interference of copper is prevented by its removal by electrolysis with a platinum cathode at automatically controlled potential (Lingane, *Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 332, 640). Antimony and bismuth are removed with the copper. Lead and tin may then be determined in separate portions of the soln., lead in a supporting soln. of sodium hydroxide and tin in one containing ammonium chloride and hydrochloric acid. Tin forms two waves in the latter medium; measurement of the second wave is recommended. The lead present contributes to the height of this wave and must be allowed for. Iron is in the ferrous state and does not interfere. Tin and lead, in addition to copper, antimony and bismuth, are removed from a second sample by controlled potential electrolysis. Nickel and zinc may then be determined simultaneously in an ammoniacal medium.

All potentials are with reference to the saturated calomel electrode. An 18G lead wire anode may be used in the polarography of nickel and zinc. It is wrapped in a helix upon the dropping electrode capillary and has a potential in the medium used of about −0·68 volt. A silver wire anode may be used in the determination of lead and tin. This has a potential of about −0·2 volt in the sodium hydroxide medium and of about −0·1 volt in the acid ammonium chloride medium. Such anodes and the use, where possible, of sulphite to remove dissolved oxygen, are well suited to rapid routine analysis. Accurate determination of the four metals takes about 2½ hr.

Method—(*a*) *Lead and tin*—Dissolve 0·5 to 1 g. of the sample in a warm mixture of 6 ml. of 12 *N* hydrochloric acid, 1 ml. of conc. nitric acid and 4 ml. of water. Dilute to about 50 ml., and boil briefly to remove oxides of nitrogen and chlorine. Add 2 g. of hydrazine dihydrochloride, dilute to about 200 ml. and electrolyse with the cathode potential maintained at −0·35 volt until the current decreases and has remained constant for 5 to 10 min. Remove the beaker without breaking the circuit and rapidly wash the electrodes with a minimal amount of water. Cool the soln. and washings and make up to 250 ml., when the soln. should be 0·4 *N* in hydrochloric acid. To a 50-ml. aliquot add 24 ml. of 5 *N* sodium hydroxide and 2·5 ml. of 0·2 per cent. gelatin soln. and dilute to 100 ml. Transfer a portion to a polarographic cell maintained at 25·0 ± 0·2° C., remove dissolved air by passage of nitrogen or hydrogen and polarograph. (Deoxygenation by addition of sulphite is not efficient in sodium hydroxide soln. containing lead.) Measure the diffusion current i_d of lead (half wave potential −0·76 volt), the drop time t at the potential of measurement of i_d and the mass of mercury flowing per sec., m. Compute the concentration C of *lead* from $C = i_d/3{\cdot}40m^{2/3}t^{1/6}$.

To a second 50-ml. aliquot add 21 g. of ammonium chloride, 6·6 ml. of 12 *N* hydrochloric acid, 2·5 ml. of 0·2 per cent. gelatin soln. and water to about 90 ml. Dissolve by shaking and make up to 100 ml. after warming to room temp. Polarograph after removal of dissolved air as above and measure the diffusion current of the second tin wave (half wave potential −0·52 volt) and also m and t. Deduct the contribution of lead to the current (the diffusion current constant of lead is 1·036 times greater in the ammonium chloride—hydrochloric acid medium than in the sodium hydroxide medium) and compute the concentration of *tin* from $C = i_d/3.49m^{2/3}t^{1/6}$.

(*b*) *Nickel and Zinc.* Dissolve 0·5 to 1 g. of the sample and prepare for electrolysis as above. Electrolyse first at −0·35 volt; when most of the copper has been deposited increase the potential to −0·70 volt to deposit tin and lead. Continue

for about 10 min. after the current decreases to a constant value and then dilute the soln. to 250 ml.

To a 50-ml. aliquot add 8 ml. of 15 *N* ammonia, 1 to 1·5 g. of anhydrous sodium sulphite and 2·5 ml. of 0·2 per cent. gelatin soln. and dilute to 100 ml. Polarograph a portion and measure the respective diffusion currents of nickel (half wave potential −1·06 volt) and of zinc (half wave potential −1·33 volt). Measure the appropriate values of *m* and *t* and compute the concentrations from $C = i_d / x m^{2/3} t^{1/6}$, where *x* is 3·54 for *nickel* and 3·78 for *zinc*. If the amount of nickel is small compared with that of zinc, record the nickel wave at increased sensitivity.

A final current increase at about −1·5 volt, due to reduction of ferrous iron, is usual with samples containing much iron. This interferes with the full development of the zinc wave. To eliminate this interference, add to the electrolysed soln. (or an aliquot) 5 ml. of conc. nitric acid, evaporate to dryness, take up in dil. hydrochloric acid and proceed as above. J. T. S.

Reviews

*p*H AND PLANTS. AN INTRODUCTION FOR BEGINNERS. By JAMES SMALL, D.Sc., Ph.C., etc. First Edition. Pp. 216. London: Baillière, Tindall & Cox. 1946. Price 12s. 6d.

Except for some eleven pages in the first chapter which treat briefly and in outline with methods for the determination of *p*H values, this book of 216 pages deals with theoretical and practical considerations of the relationship of *p*H to plants. Throughout the book Professor Small refers to hydrogen-ion activity only in terms of *p*H, hoping by this means to avoid the "mental gymnastics and discipline" required for the continual conversion of the opposing terms of *p*H and hydrogen-ion concentration. Although this undoubtedly assists in a quicker understanding, it does not avoid the necessity of maintaining a mental appreciation of acidity and alkalinity as a background to the ever-changing *p*H figures.

Chapter I deals with the *p*H scale and methods for *p*H determination. In assessing the relative values of different methods, it is to be noted that the hydrogen electrode may give false readings with many plant fluids, as the passing of hydrogen gas through such fluids results in liberation of carbon dioxide, thus affecting the *p*H. Also, while of necessity only indicator methods are generally available for testing sections or cells of plants, single indicator methods are not as reliable as the range indicator method where several indicators are used and suitable precautions taken.

Chapter II is a brief exposition of the action and distribution of buffers. It clearly indicates the author's view of the superior value of buffer-index curves as compared with titration curves.

The effect of change of carbon dioxide content on the *p*H value of plant sap and tissue has not hitherto been sufficiently appreciated. A general resumé of such effects is given in Chapter III. The potato, a fairly thoroughly investigated plant, serves well to illustrate the changes of *p*H following on changes of content of carbon dioxide, during growth, cultivation, storage and utilisation.

Chapters IV to XII present the relationship of *p*H to plant sap, cell walls, protoplast, plant enzymes, aquatic life, soil and plants, plant pathology, succulents and cytoplasmic life. This is the story of a new approach to the understanding of plant life, based on a conspectus of a vast mass of experimental data—some of it admittedly of doubtful validity, but all tending in the same direction—of the greatest interest to every chemist. The conception of plant metabolism in terms of *p*H and buffer complexes marches with the chemist's theory of fertiliser balance. The former dominates plant growth but is itself dependent on the availability of nutrients, which again may be determined not only by a sufficient supply, but also by a differential E.M.F. set up by a variation in the *p*H of the fluids on each side of a cell membrane. To quote the author: "*p*H is a factor which is nearly as widely important as temperature in many biological and industrial processes." Changes in the *p*H of plant sap may be due to changes of buffering compounds as the result of metabolic processes. The gradual rise of *p*H during the ripening of fruits affects the cell walls in respect of the pectin matrix and its gelation value, and this has important applications in the manufacture of jams and jellies and the canning of fruits and vegetables. The activity of plant enzymes is strongly affected, or even controlled, by the *p*H of the medium. This is, of course, well known, but the author adds interest to this chapter by a full and detailed account of the empirical practice in coffee growing in tropical countries of arranging the plantations so that the trees are so shaded that they do not receive full insolation during more than a few hours each day, a practice which is justified scientifically by considerations of stomatal movements conditioned

by the factors of buffers, carbon dioxide effects, cell-sap reactions and *p*H optima for enzymic hydrolysis and synthesis.

Perhaps Dr. Small's work is of the greatest interest to soil chemists. Many new and arresting views are put forward. Lime-induced chlorosis in the presence of a theoretical sufficiency of iron in the soil is discussed at length, and the suggestion is made that the chlorosis may be due to progressive precipitation of iron in tissues of relatively high *p*H. It would further appear that the high *p*H is due to increased absorption of calcium by the plant, and that it may be reduced by fertilising with potash at the time of liming. Be this as it may, it is a provoking thought that "non-availability" of iron in the soil may be only one of the chlorosis-causative factors, with the *p*H value of the plant juices as an equally important factor. Again, investigations of the *p*H of the capillary layer of the soil solution in contact with the roots of plants point to the possibility that the normal natural healthy range for all absorbing roots falls within the relatively narrow limits of *p*H 5·6–4·0, whatever the soil *p*H may be. Yet again, following on a grouping of plants based on their normal habitats, with "acidiphilous" and "alkaliphilous" as the terminology of the extremes, it is propounded that acidiphilous species have a dominant organic-acid metabolism producing an oxalate or oxalate - malate buffer system associated with a lack or minor development of the citrate and phosphate buffer systems, whereas alkaliphilous species possess a phosphate system with little or no organic-acid - salt system, the consequence being that normal metabolism is injured by passage into the plant respectively of alkaline or acid medium.

In dealing with the control of soil *p*H, the author suggests that the soil chemist should no longer be content with the vague terms "moderate," "slight" and "strong," but should obtain comparable quantitative values for buffer capacities of soils. The soil chemist might doubt, however, whether the means would justify the end; the results of field treatment based on investigational work in the laboratory frequently diverge so seriously from the expected as to indicate that the above-mentioned vague terms are still sufficiently quantitative for the present time. A more fully detailed outline of the proposed method would, however, be of additional value in the next edition.

Scattered throughout the book are many reference tables, as, for example, optimal *p*H values for gelling points, plant enzymes, amylases, crop plants, bacteria and fungi. The relationship of *p*H and [H˙], and the "common sense of dissociation curves" are dealt with in two appendixes. Finally, there is an extensive bibliography covering thirteen pages.

This book serves such a real purpose in its presentation of a modern conception of plant life that the limitation suggested by its sub-title, "an Introduction for Beginners," seems not only unwarranted but also somewhat misleading. GEORGE TAYLOR

ENZYMES AND THEIR ROLE IN WHEAT TECHNOLOGY. Issued by the American Association of Cereal Chemists: Monograph Series, No. 1. Edited by J. A. ANDERSON, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg. Pp. ix+371. New York: Interscience Publishers Inc. 1946. Price $4.50.

This is the first of a series of monographs on cereal chemistry which it is the intention of the American Association of Cereal Chemists to publish. At one time it was not difficult to write a broad survey of the chemistry appertaining to cereals and to cover the subject in one volume but, although such books still exist to-day and indeed are of considerable value in giving a general picture, the tendency has been to specialise still further even in what might at one time have been regarded as a specialised branch of chemistry. The American Association of Cereal Chemists is to-day a strong and important organisation with membership in most civilised countries in the world. It has already published its own comprehensive methods of analyses, although these are in many respects similar to those found in the A.O.A.C. Now that there is need, especially for research workers, to possess an intimate knowledge of all the important work in comparatively restricted fields, the American Association of Cereal Chemists has decided to cover the wide field of cereal chemistry, which begins with the plant and proceeds *via* milling and baking to the finished bread, cake, biscuit and the like, with a series of monographs.

The volume under review is the first of these and it sets a high standard for the rest. It has eleven sections, or chapters, each written by an acknowledged authority on the particular subject. There are, excluding the editor, no less than fifteen contributors. As the Preface states, the subject of enzymology was chosen for the first monograph in view

of the important part it plays in wheat technology and the rapid increase in knowledge in this subject in recent years with which those working in the field should be conversant.

The general arrangement is interesting. Besides a survey of the chemistry of enzymes, there are twin chapters on each sub-division, such as on the amylases, on the esterases, on the proteases, on the oxidases, and so forth. In the first of each pair is given a broad review of existing knowledge, covering the function of the particular group of enzymes, both of plant and of animal origin. The second of the pair is in each case reserved especially for the influence the particular class of enzyme has on wheat, milling and baking. This arrangement is good, although some overlapping must occur. With such an arrangement much depends on editorship and this has been in the capable hands of Dr. J. A. Anderson of Winnipeg, the head of the laboratory of the Board of Grain Commissioners, Canada.

The monograph is attractively printed and errors are few. References are copiously given—in one chapter alone there are close on three hundred. Some idea of the thoroughness of the monograph may be gathered from the fact that over one thousand workers in the field of enzymology are cited and as each person has published on the average about four or five papers on the subject, it can be appreciated that the field has been well covered.

The modern cereal chemist is expected to possess a very wide range of knowledge, or at least to know where to find such knowledge and to be able to read it up intelligently. It has become increasingly difficult to do this and only by the compilation of books of this kind is it now possible. All cereal chemists and those interested generally in biochemistry will welcome this book, which can only be described as first class. Naturally, being written on the other side of the Atlantic, there is a preponderance of references to American workers, but the book cannot be criticised as being unbalanced. Chemists will be grateful to the Association of American Cereal Chemists and will look forward impatiently to the other members of the series.

D. W. KENT-JONES

THE VITAMINS IN MEDICINE. By FRANKLIN BICKNELL and FREDERICK PRESCOTT. 2nd Edition. Pp. xii+916, with 208 illustrations in text. London: William Heinemann Medical Books, Ltd. 1946. Price 50s.

It is not necessary to review at length a second edition of this standard work. Nothing said about it when discussing the first edition (ANALYST, 1943, **68**, 295) needs to be withdrawn, for it remains probably the only, and certainly the standard, reference book in English for all those concerned with the vitamins in medicine. The emphasis is, naturally, on physiology, pathology and nutrition, but due attention is paid to the structure and biochemistry of the various chemical substances involved.

Since the first edition these have been increased in number by the addition of folic acid, which was not mentioned in 1942 and of which we have by no means heard the last, if only because of its certain but incompletely understood connection with pernicious and other forms of macrocytic anaemia.

The book bears the date June, 1945, and this is probably why it contains no reference to the "splitting" of vitamin B_6 into pyridoxine, pyridoxamine and pyridoxal, or the connection between the last and certain enzymes involved in the metabolism of amino acids. So thorough, however, has been the authors' revision of the material that appeared in the first edition as to make it certain that all further necessary revisions will, in due course, be made in the third edition. May the dueness of the course not be long.

A. L. BACHARACH

QUALITATIVE ORGANIC MICROANALYSIS. By FRANK SCHNEIDER, Ph.D. Pp. iv+218. 1946. New York: John Wiley & Sons, Inc., $3.50. London: Chapman & Hall, Ltd.; 21s.

It is indeed difficult for the reader to believe that "Although the great value of (qualitative) organic analysis as a method of instruction in organic chemistry has long been realised, many colleges and universities (in the United States) have hesitated to offer such courses because of the expense and space required." The author hopes that by using micro methods the great economy of space and equipment ensuing will enable such institutions to include courses in organic analysis in their curricula. No such justification for this excellent book is required.

The first half of the work provides detailed descriptions of the many techniques which have proved useful for the isolation, purification and determination of the physical constants

of micro quantities of organic compounds. The descriptions are themselves extremely detailed and the lavish provision of excellent line drawings (usually to scale) renders the procedures easily comprehensible. Adequate literature references are given.

The remaining chapters consist of an adaptation of a standard scheme of organic analysis (Mulliken and Huntress) for use on the micro scale. The procedures are lucidly described and almost all of the simple apparatus involved, except possibly the hand centrifuge, is already to be found or can simply be made in most laboratories.

This comprehensive book is indeed a notable addition to the literature, providing as it does a unique collection of information previously scattered through many journals and text books. It should be widely appreciated by, and will be of great utility to, all organic chemists who, although normally engaged upon operations on the macro scale, inevitably find it necessary, upon occasions, to struggle with only a few milligrams of material.

E. R. H. Jones

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