THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, April 2nd, at the Chemical Society's Rooms, Burlington House, London, W.1. The chair was taken by the President, Mr. Lewis Eynon. The following papers were presented and discussed: "A Note on the Chapman and McFarlane Method for the Estimation of Reducing Groups in Milk Powder," by C. H. Lea, D.Sc., F.R.I.C.; "The Determination of Carotene in Dried Grass," by W. A. G. Nelson, B.Sc.,Ph.D.

NEW MEMBERS

William James Carpenter, A.R.I.C.; Henry Edward Coomber, B.Sc. (Lond.); Charles Donald Cook, A.R.I.C.; John Ruff Gwilt, A.R.I.C.; George Edmund Holmes, M.A. (Cantab.); Harold Thomas Islip, B.Sc. (Lond.), F.R.I.C.; Alexander Johnston, B.Sc. (Glas.); Henry Kenneth Lawton, B.Sc., Ph.D. (Liv.), F.R.I.C.; Leopold Ferdinand Levy, M.Sc. (Witwatersrand), Ph.D. (Lond.); Colin Marsden, B.Sc., Ph.D.; Harry Polkinhorne, B.Sc. (Lond.), A.R.I.C.; Arthur Henry Snowden, A.R.I.C., Ph.C.; John Ivor Webb, B.Sc., Ph.D. (Birm.), F.R.I.C.; William Sydney Durham Wise, B.Sc. (Bris.).

DEATH

WE regret to have to record the death of

Sura Rajagopal Naidu

SCOTTISH SECTION

THE twelfth Annual General Meeting of the Scottish Section was held in Glasgow on January 15th, 1947. The following is the list of office bearers elected for the year.

Chairman—Dr. H. Dryerre. *Vice-Chairman*—Dr. J. Sword. *Hon. Secretary and Treasurer*—R. S. Watson. *Other Members of Committee*—A. R. Jamieson, H. C. Moir, J. Sandilands, A. M. Smith, R. G. Thin and S. L. Tompsett. *Hon. Auditors*—A. R. Campbell and W. M. Cameron.

MICROCHEMISTRY GROUP

AT the Annual General Meeting of the Group, held on Friday, January 31st, 1947, at the Sir John Cass Technical Institute, London, E.C.3, Mr. Norman Strafford was elected Chairman in place of Professor H. V. A. Briscoe, who had completed his term of office, Dr. Janet Matthews was elected Vice-Chairman and Professor Briscoe, Mr. J. T. Stock and Mr. E. J. Vaughan were elected Ordinary Members of the Committee in place of Miss I. H. Hadfield, Dr. Janet Matthews and Dr. G. H. Wyatt who, having served for two years, were due to retire.

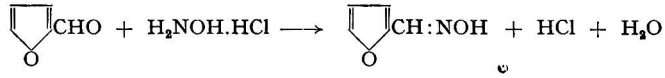
After the Annual General Meeting the following papers were read and discussed: "A Review of Electrolytic Methods of Microchemical Analysis," by A. J. Lindsey; "The Oxidation of Nitrogen during the Micro-combustion of Organic Substances," by A. E. Heron (see this Vol., p. 142); "The Micro Separation of Gums from Crystals," by A. L. Bacharach; "The Microchemical Determination of Molybdenum in Steel," by J. E. Wells and R. Pemberton (see this Vol., p. 185).

Some Analytical Methods Applicable to Furan and its Derivatives

By F. G. ANGELL

In recent years attention has been focussed on the utilisation of waste vegetable material as a potential source of commercial chemicals, notably furfural and other furan derivatives. Little information exists in the literature concerning analytical methods applicable to such compounds, and the present paper deals with quantitative methods that have been employed in the examination of a large range of furan derivatives prepared in the Research Department of Imperial Chemical Industries, Ltd. (Billingham Division), including furfural, furfuryl alcohol, tetrahydrofurfuryl alcohol, furan, sylvan, tetrahydrofuran and tetrahydrosylvan. These methods may be classified under three headings, *viz.*, Oximation, Absorptiometric and Bromination.

1. Oximation—

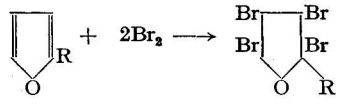


The extent of the reaction is ascertained by titration of the hydrochloric acid liberated from neutralised hydroxylamine hydrochloride and the method is in general limited to the estimation of large amounts of furfural. Various furan compounds (*e.g.*, furfuryl alcohol), when warmed with the reagent, undergo ring opening with the production of aldehydes, and this behaviour seriously limits the applicability of the method.

2. Absorptiometric—

The production of a red colour by mixing furfural and aniline acetate has been recognised for a long time as a specific qualitative test for the former compound (and thus for pentosans). By working under carefully controlled conditions on the basis of this test,[2] an absorptiometric method has been developed and is of considerable utility in the determination of subordinate amounts of furfural present as impurity in other furan compounds.

3. Bromination—



"Pyridine sulphate bromide" has been found to effect complete saturation of the ring with such compounds as furfuryl alcohol, furan, and sylvan. Little, if any, substitution occurs, for the corresponding tetrahydro compounds are practically inert towards the reagent. It is therefore of great utility in the analysis of such compounds. Certain furan derivatives, *e.g.*, furoic acid and furfural, are unaffected by this reagent although they are attacked by acidified potassium bromide - bromate solution. Furoic nitrile exhibits unique behaviour in that it is inert towards all ordinary bromination reagents.

Experimental

1. Oximation of furfural

Redistilled furfural (b.p. 162·0° to 162·2° C.) which was pale straw yellow in colour and neutral towards methyl yellow was used in investigating the method. The results in Table I, obtained by the method described below, indicated that oximation did not proceed further than 98·0 per cent. even if the sample was left in contact with the reagent for 1 hour. No increased precision resulted from the use of larger weights of sample involving correspondingly larger titrations. Examination of some solutions whose concentrations were not known to the analyst (Nos. 4 to 6 in Table I), on these lines, confirmed that the reaction was not quite stoichiometric. Owing to the intense buffering action of the excess of hydroxylamine hydrochloride, titrations with 0·1 *N* sodium hydroxide are not recommended, as the end-points so obtained are too indefinite, and in such circumstances larger quantities of sample should be used for analysis or the furfural determined by the aniline acetate method.

When applied to the determination of furfural in mixtures containing furfuryl alcohol and the like it is essential that the oximation be carried out in the cold, for on being warmed

TABLE I

Expt. No.	Wt. of sample present, mg.	0·5 *N* NaOH required, ml.	Wt. of furfural found, mg.	Percentage oximation
1	528·1	10·75	516·0	97·6
2*	630·4	12·90	619·2	98·2
3	624·8	12·80	614·4	98·3
4	492·2	10·10	484·8	98·5
5	560·9	11·45	549·6	98·0
6	422·0	8·68	416·6	98·7
			Mean	.. 98·2 ± 0·6

* 1 hr. standing.

furfuryl alcohol readily undergoes ring opening with the production of various compounds containing a carbonyl group. Although these compounds will react with hydroxylamine hydrochloride, this reaction is not suitable for the accurate determination of furfuryl alcohol, because variable quantities of dark-coloured polymerised products are often also formed.

METHOD—

Reagents—

(1) A 5 per cent. w/v solution of hydroxylamine hydrochloride in water, neutralised with 0·5 *N* sodium hydroxide to the orange tint of methyl yellow.
(2) Refined methanol (of low aldehyde content).
(3) Indicator—a 0·1 per cent. solution of methyl yellow in methanol.
(4) Sodium hydroxide solution, 0·5 *N*, free from carbon dioxide.

Procedure—Weigh 0·5 to 0·7 g. of the sample by difference, from a weighing pipette, into a 250-ml. beaker containing 15 ml. of neutralised hydroxylamine hydrochloride solution (50 per cent. excess) and 15 ml. of methanol. After half an hour titrate the liberated hydrochloric acid in good daylight with 0·5 *N* sodium hydroxide to the orange tint of the indicator, using a blank solution containing the same volume of reagents to assist in judging the end-point.

2. ABSORPTIOMETRIC DETERMINATION OF FURFURAL

Determination of subordinate quantities of furfural (about 1 mg.) have often been conducted by matching the red colour produced with aniline acetate against a series of standards diluted to a convenient volume with methanol in Nessler cylinders. Preliminary experiments indicated that in the presence of methanol the optical extinctions measured on the Spekker

TABLE II

SOLUTION CONTAINED 0·554 MG. OF FURFURAL—EXTINCTIONS IN 1-CM. CELL ($E_{1\,cm.}$) FOR HG LINE 5461A (CALOREX 503 AND ILFORD 605 FILTERS)

2·5 g. of aniline + 25 ml. of glacial acetic acid and 1 g. of $Na_2HPO_4.12H_2O$ diluted to 100 ml. with water.		2·5 g. of aniline + 10 ml. of glacial acetic acid + 1 g. of $Na_2HPO_4.12H_2O$ diluted to 100 ml. with water	
Time (mins.)	$E_{1\,cm.}$	Time (mins.)	$E_{1\,cm.}$
30	0·272	5	0·014
45	0·303	15	0·077
60	0·330	30	0·169
75	0·354	45	0·159
90	0·353	60	0·147
105	0·354		
150	0·337		

absorptiometer with an appropriate filter diminished rapidly on standing, and moreover no advantage was gained by substituting aniline hydrochloride for the acetate. Of the various methods proposed in the literature for developing this test on an absorptiometric basis the procedure of Stillings and Browning,[1] as modified by Duncan,[2] seemed the most attractive. The latter author recommended a mixture of disodium phosphate and oxalic acid as a stabiliser, but it was found that disodium phosphate alone was quite satisfactory for this purpose. Further, it seemed desirable to avoid using oxalic acid, because of the sparing solubility of

sodium hydrogen oxalate. A large volume of the mixed reagent prepared according to the directions of Duncan deposited, on standing, a considerable quantity of a crystalline precipitate, presumably this salt.

The effective time for the maximum development of the colour with aniline acetate and disodium phosphate, the period of stability, and the effect of using lesser quantities of acetic acid were next investigated. As will be seen from Table II, with less acetic acid the colour was not so fully developed and faded more rapidly, but in presence of 1 g. $Na_2HPO_4.12H_2O$ and 25 ml. of glacial acetic acid containing 2·5 g. of freshly distilled aniline the extinction was at its maximum between 75 and 105 minutes, after which time it gradually diminished.

METHOD—

Reagents—

(1) A 10 per cent. w/v solution of freshly distilled aniline in glacial acetic acid (freshly diluted each day).

(2) A 10 per cent. w/v solution of $Na_2HPO_4.12H_2O$ in water.

Procedure—Adjust the temperature of all solutions to 20 ± 0·5° C. before mixing. Measure an aliquot of the sample solution, containing 0·2 to 1·0 mg. of furfural; it should be neutral to phenolphthalein. Add 10 ml. of 10 per cent. $Na_2HPO_4.12H_2O$ solution, dilute to 70 ml. with water, and finally add 25 ml. of aniline acetate reagent and adjust the volume to 100 ml. Mix well and immediately transfer to a dark cupboard. At the same time prepare a blank solution in an identical manner. After the expiration of $1\frac{1}{4}$ hrs. measure the extinction in a 1-cm. cell for the Hg line 5461A, using Calorex 503 and Ilford 605 filters, with the blank solution in No. 2 cell. The results given in Table III were obtained in this manner; from them was derived the relation:

$E_{1\,cm.} \times 1{\cdot}50 \equiv$ mg. of furfural per 100 ml. of solutions for values of $E_{1\,cm.}$ below 0·7.

TABLE III

Wt. of furfural, mg.	$E_{1cm.}$ 5461 A	Factor
0·156	0·100	1·56
0·469	0·314	1·49
0·782	0·528	1·48
0·940	0·634	1·49
1·096	0·726	1·51
1·253	0·781	1·61
1·409	0·824	1·71

The colour is virtually specific for furfural and unaffected by acetone, furfuryl alcohol or tetrahydrofurfuryl alcohol. Table IV gives the results obtained on some typical products containing small quantities of furfural.

TABLE IV

Description of sample	Furfural % (absorptiometric)	Furfural % (by oximation)
Distillate obtained by digestion of waste vegetable material (containing ketones, etc.)	1·50	1·58
Ditto	1·48	1·54
Redistilled tetrahydrofurfuryl alcohol (containing 1·3% of furfuryl alcohol)	0·022	Detected <0·1
Ditto (containing 0·4% of furfuryl alcohol)	Not detected <0·01	—
Furfuryl alcohol: Sample A	0·66	0·64
,, B	0·69	0·61
,, C	1·10	1·11

3. BROMINATION

(1) USE OF PYRIDINE SULPHATE BROMIDE—

This reagent* has received considerable application[3,4,5,6] in the determination of the unsaturation of hydrocarbons and is stated to produce very little substitution. Thus Fitelson[7]

* For preparation of this reagent see below, p. 182.

employed it for the determination of squalene, and Wilson and Nisbet[8] applied it in the analysis of various shale oil fractions. With compounds containing a furan ring, complete saturation usually takes place in accordance with the equation given on p. 178. Further work revealed that the nature of the substituent R exerted a very marked effect on the behaviour of the reagent. Thus if R = H, CH_3 or CH_2OH saturation of the ring readily occurred, but if R = CHO, COOH or CN little or no reaction was observed. Moreover, practically no reaction occurred with the corresponding tetrahydro compounds where R = H, CH_3 or CH_2OH, thus affording strong evidence that but little substitution takes place. This reagent proved to be a powerful weapon with which to attack the problem of the analysis of furan compounds, and with its aid methods have been devised for the determination of furfuryl alcohol in admixture with furfural and tetrahydrofurfuryl alcohol and for the determination of furan and sylvan in presence of the corresponding tetrahydro compounds. Details of the method used are given later (p. 182).

(I) DETERMINATION OF FURFURYL ALCOHOL—A sample of refined furfuryl alcohol (b.p. 78° to 80° C./20 mm., D_4^{20} 1·1337, n_D^{20} 1·4870, furfural 0·24 per cent.) was analysed by the method described below and the furfuryl alcohol content calculated on the assumption that complete saturation of the ring occurred.

Time of contact with reagent	Furfuryl alcohol found, %
15 mins.	93·0
30 ,,	97·0
60 ,,	100·0, 100·0, 100·0, 100·6, 99·3

Thus quantitative bromination occurred in 1 hour, while furfural gave a "bromine value" of 30 to 35 g. of Br per 100 g. (equivalent to 10 per cent. of furfuryl alcohol) and tetrahydrofurfuryl alcohol a value of less than 1 (equivalent to <0·3 per cent. of furfuryl alcohol) under similar conditions. Mixtures of furfuryl alcohol, tetrahydrofurfuryl alcohol and furfural, whose composition was unknown to the analyst, were examined by the method detailed below, with the results given in Table V.

TABLE V

Furfural present, %	Tetrahydrofurfuryl alcohol present, %	Furfuryl alcohol present, %	Furfuryl alcohol found, %	Deviation % of mixture
0	0	100·0	98·4	−1·6
7·1	7·6	85·3	84·4	−0·9
6·0	7·3	86·7	85·9	−0·8
3·5	32·6	63·9	63·1	−0·8
31·6	40·4	28·0	28·6	+0·6
48·6	25·0	26·4	26·8	+0·4
62·2	20·9	16·9	18·7	+1·8
5·5	78·9	15·6	16·1	+0·5
68·9	19·8	11·3	11·8	+0·5
81·2	10·5	8·3	10·0	+1·7
5·4	88·9	5·7	5·9	+0·2
90·0	5·2	4·8	5·9	+1·1

Thus, provided that the furfural content of such a ternary mixture does not exceed 20 per cent., furfuryl alcohol can be determined to within ±1 per cent. of the true value and the method is obviously applicable to the determination of small amounts of furfuryl alcohol present as impurity in tetrahydrofurfuryl alcohol.

When the method was applied to the analysis of technically pure furfuryl alcohol, inconsistencies appeared in the results and were ultimately traced to the ease with which furfuryl alcohol and some other furan compounds undergo polymerisation. It was found that the furfuryl alcohol had changed after storage for several weeks. Vacuum distillation in a current of carbon dioxide yielded 89·6 per cent. of distillate (b.p. 87° C./20 mm.), which proved to be furfuryl alcohol of purity 99 per cent., 3·2 per cent. of distillate consisting of less pure furfuryl alcohol, and 5·8 per cent. of dark brown sticky residue which became a brittle solid on cooling to room temperature. The results of this experiment are summarised in Table VI.

That the low and inconsistent results were attributable to the presence of polymerised substances was finally demonstrated by addition of varying quantities of "heavy ends" to

a redistilled sample; in all instances a rapid diminution in bromine value resulted. Thus addition of an equal weight of heavy ends to a sample showing 92·0 per cent. of furfuryl

TABLE VI

Fraction No.	% of original	% Furfuryl alcohol (pyridine sulphate bromide method)
1	61·2	99·5
2	28·4	98·7
3	3·2	67·7
4 (Residue)	5·3	
Loss	1·9	

alcohol gave a result of 45·7 per cent. of furfuryl alcohol. The rapidity with which polymerisation took place is illustrated by the observation that when 10 g. of freshly distilled sample contained in a platinum dish were evaporated to dryness on a steam bath 2·1 per cent. of hard non-volatile residue remained. Therefore, all subsequent analyses by bromination methods on furan and its derivatives were conducted on freshly distilled samples.

(II) DETERMINATION OF SYLVAN AND FURAN—In an identical manner the behaviour of sylvan, tetrahydrosylvan, furan, tetrahydrofuran, furoic acid and furoic nitrile were investigated, freshly distilled material being used for the liquid substances. The results obtained are indicated in Table VII.

TABLE VII

Compound	Wt. taken, mg.	Bromine value	Apparent % of sylvan or furan
Sylvan (Theoretical bromine value 391)	190	396	101·4 sylvan
	19·9	386	98·9 ,,
	27·89	394	101·0 ,,
	24·13	392	100·6 ,,
Tetrahydrosylvan	770	12	3·1 ,,
,,	179	11·6	3·0 ,,
,,	60·5	4·6	1·2 ,,
Furan (Theoretical bromine value 470)	20·369	465	98·9 furan
	15·437	478	101·7 ,,
Tetrahydrofuran: Sample A	1780	<0·2	<0·1 ,,
,, B	444	4·7	1·0 ,,
,, C	443	5·7	1·2 ,,
,, D	443	10·0	2·1 ,,
Furoic acid	260	3·4	—
,, nitrile	300	3·9	—

The results in Table VII indicate that furan and sylvan react quantitatively with the reagent, but this exerts only a slight action on tetrahydrosylvan, possibly attributable to substitution. With tetrahydrofuran, however, independent confirmation of the presence of furan (and possibly sylvan) as impurities has been obtained from phase-rule studies of the system water, tetrahydrofuran and furan (unpublished work).[9] Thus sample A was specially purified by batch fractionation in a high efficiency column and was perfectly miscible with water in all proportions. On the other hand B, C, and D, were miscible with water only over a limited range of concentrations, and phase-rule work indicated about 2 per cent. of furan and sylvan in samples B and C and about 4 per cent. in sample D.

METHOD USED FOR THE DETERMINATION OF FURFURYL ALCOHOL, SYLVAN, AND FURAN—

Reagents—

(1) Pyridine sulphate bromide reagent (approx. 0·05 *N* with respect to bromine). Into each of 3 dry 500-ml. conical flasks measure 40 ml. of glacial acetic acid. Cool, and add to the first 16·3 ml. of pure pyridine and to the second 10·9 ml. of sulphuric acid (sp.gr. 1·84). When cold, combine these two solutions, with further cooling, and to the third flask add 3·2 ml. of bromine. Finally, add the contents of the third flask to the previous mixed solution, dilute to 2 litres with glacial acetic acid and mix thoroughly by shaking.
(2) A 10 per cent. w/v potassium iodide solution.
(3) Sodium thiosulphate solution, 0·1 *N*.

For unsaturated compounds introduce into a tared oleum bulb an appropriate weight of sample (20 to 25 mg. of furan or sylvan, 30–35 mg. of furfuryl alcohol), seal, and reweigh on a micro balance. Alternatively use larger quantities of sample, dilute to a convenient bulk with glacial acetic acid in a volumetric flask and withdraw an appropriate aliquot for analysis. With saturated compounds use any suitable larger quantity of material.

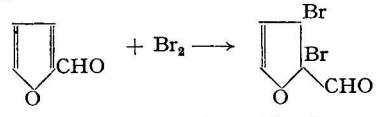
Procedure—Pipette 50 ml. of the reagent (1) into a 200-ml. stoppered "iodine" flask, introduce the sample *via* the sealed oleum bulb or otherwise, break the bulk carefully under the surface of the solution by means of a glass rod and wash down the rod with a few ml. of glacial acetic acid. Promptly replace the stopper and seal the neck of the flask with 5 ml. of potassium iodide solution. Allow to stand in the dark for 1 hour, together with a "blank" on 50 ml. of the reagent. Release the stopper carefully, allowing the potassium iodide solution to flow into the flask so that no bromine is lost. Add 10 ml. of potassium iodide solution, shake well and titrate the liberated iodine with 0·1 *N* thiosulphate, adding a little starch solution when the titrated solution becomes pale yellow. Frequently the titrated solution is pale brown at the end-point, but no difficulty is experienced in recognising the disappearance of the starch iodide colour.

If B = bromine value = g. of bromine absorbed per 100 g. of sample,

percentage of furfuryl alcohol = 100B/325
,, ,, furan = 100B/470.
,, ,, sylvan = 100B/391.

(2) USE OF ACIDIFIED POTASSIUM BROMIDE - BROMATE SOLUTION—

It has been stated by Hughes and Acree[10] that if furfural be treated with acidified bromide - bromate solution at 0° C. for 10 minutes, only one double bond is attacked, in accordance with the equation:



This procedure was investigated, both the bromide - bromate solution and the 5 per cent. hydrochloric acid used for acidification being cooled in an ice-bath at 0° C. for ½ hr. preceding the bromination tests. With the same sample of redistilled furfural (assaying 98·2 per cent. by aqueous oximation) the following results were obtained (Table VIII).

TABLE VIII

TEST SOLUTION CONTAINED 30·9 MG. OF FURFURAL

Time of bromination, mins.	Furfural found, mg.	Apparent % furfural
8	27·4	90·9
10	29·1	94·2
10	28·6	92·6
15	31·9	103·1

These results indicate that bromination for 10 minutes does not proceed beyond 94 per cent. of completion, whereas with 15 minutes reaction time bromination exceeds 100 per cent. on the basis of the above equation. Moreover, the starch iodide end-point of the subsequent titration is frequently fugitive and may well lead to even greater divergencies than result from slight variation in the time of bromination. This method does not appear to have any advantages over the oximation procedure, and in general it seems undesirable to adopt as a standard procedure any method in which the reproducibility of the results clearly depends on a careful counterbalancing of opposing sources of error.

However, acidified bromide - bromate solution, with addition of methanol as a solvent for the compound, has been applied to the determination of furoic acid alone or in admixture with tetrahydrofuroic acid. Addition of increasing quantities of methanol to the blank solution diminished its titre, but provided that at least 25 per cent. by volume of methanol was present the blank titration was unaffected. As solutions of suitable concentrations of furoic acid in 25 per cent. methanol could be readily prepared, the method was standardised on this basis. The bromination was allowed to proceed for 15 minutes at room temperature, with the usual Koppeschaar technique. Results obtained for furoic acid alone and in mixtures with tetrahydrofuroic acid whose composition was unknown to the analyst are shown

in Table IX. Tetrahydrofuroic acid alone gave a bromine value equivalent to 1 per cent. of furoic acid.

TABLE IX

Nature of sample	Furoic acid % found
Furoic acid	97·5
,, ,, ...	98·0
,, ,, ...	99·4
Mixture containing 9·8% furoic acid ..	10·2
,, ,, 48·1% ,, ,, ..	48·2
,, ,, 76·0% ,, ,, ..	77·1

Furoic nitrile was unaffected by this reagent (bromine value = 2) and towards Kaufmann's reagent (bromine + sodium bromide in methanol) it was equally inactive, yielding a bromine value of 3 to 3·5. That the impure nitrile available was an unsaturated compound was confirmed by hydrolysis with dilute sulphuric acid and extraction with ether, when an acid with m.p. 123° to 125° C. was obtained. (Furoic acid has m.p. 130° to 131° C., and tetrahydrofuroic acid is a liquid.) Thus, of all the furan derivatives investigated, furoic nitrile is unique in its inertness towards bromination agents.

SUMMARY—

Existing methods for the analytical examination of furan derivatives have been critically examined and, where necessary, improved.

1. Furfural can be determined by aqueous oximation at room temperature, although the reaction is not quite stoichiometric. Small amounts of furfural should be determined absorptiometrically, using the aniline acetate method.

2. Pyridine sulphate bromide is the most useful reagent for the determination of unsaturation in the furan series. Saturation of both double bonds occurs with furan, sylvan, and furfuryl alcohol, and substitution reactions with the corresponding tetrahydro compounds are negligible. On the other hand, furfural, furoic acid and furoic nitrile are but little attacked by this reagent.

3. Acidified potassium bromide - bromate reagent finds limited application as a brominating reagent for furoic acid, but with furfural only approximately quantitative results are obtained.

In conclusion, I wish to thank Mr. W. Hutchinson for the experimental data on the method employed for furoic acid.

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Erratum.—December issue, 1946, p. 555, line 17 from the bottom: *For* "nickel" *read* "nickelous."

The Microchemical Determination of Molybdenum in Steel

By J. E. WELLS and R. PEMBERTON

(*Read at the Annual General Meeting of the Microchemistry Group, January* 31, 1947)

In the course of recent investigations it became necessary to determine molybdenum in small quantities of steel. A review of available methods led to the conclusion that the reactions involved were not of sufficient sensitivity for the work in hand. It was known, however, that molybdenum would give a green precipitate with toluene-3:4-dithiol in acid solution,[1] and work was initiated to determine the suitability of the reaction for quantitative determinations.

Preliminary work indicated that the green molybdenum compound produced in cold hydrochloric acid solution could be extracted with amyl acetate. The amyl acetate extract could be rendered clear and free from turbidity by washing with concentrated hydrochloric acid, and the absorptiometric evaluation of its colour bore a linear relationship to the molybdenum concentration. Under these conditions, that is, in cold hydrochloric acid solution, and in absence of stannous chloride, any tungsten present reacted only slowly, the reaction rate decreasing with decreasing acid concentration.

These results suggested the possibility that under suitable conditions interference by tungsten might be eliminated, and a determination of molybdenum carried out. Investigations were, therefore, put in hand to determine these conditions.

Experimental

For the purpose of these experiments, "synthetic" steel solutions were made up from a solution of pure iron in sulphuric acid. Aliquots equivalent to four milligrams of metal were used as a test basis, and other elements added in the form of standardised solutions.

Preparation of pure iron solution—One gram of pure iron was dissolved in 18 ml. of 10 per cent. sulphuric acid. After oxidation with the minimum amount of nitric acid, the solution was boiled down almost to dryness, taken up with water, and made up to one litre in a graduated flask. Aliquots of this solution containing 4 mg. of iron were then taken per test, each containing the equivalent of 0·0012 ml. of free sulphuric acid.

Effect of Hydrochloric acid concentration on the extraction of the coloured molybdenum complex—Solutions representing 4-mg. samples of a synthetic 0·70 per cent. molybdenum steel to which the equivalent of 0·05 ml. of concentrated sulphuric acid had been added, were boiled down to fuming, and the fumed liquids were taken up in 3-ml. portions of hydrochloric acid, the concentration of which was varied from sample to sample. The solutions were then cooled, and 3 ml. of a 1 per cent. solution of toluene dithiol in amyl acetate were added to each. After standing for varying periods of time with occasional shaking, the amyl acetate layer of each was separated, washed with 3 ml. of concentrated hydrochloric acid, transferred to a 10-ml. graduated flask and made up to the calibration mark with amyl acetate. The absorption was then measured on the Spekker photo-electric absorptiometer, using Calorex heat-resisting filters H503, spectrum red filters 607, and a 1-cm. micro cell.

The results obtained, in Spekker units, were as follows:

Sp.gr. of hydrochloric acid at 15° C.	Standing time before separation (minutes)				
	5	10	20	30	40
1·021	—	—	0·090	—	0·120
1·043	—	0·130	—	—	0·190
1·051	0·295	0·330	—	—	0·350
1·061	0·530	0·560	0·560	0·560	0·560
1·075	0·555	0·560	0·560	0·555	0·560
1·082	0·560	0·560	0·565	0·560	0·560
1·097	0·565	0·560	—	0·560	0·560
1·182	0·555	0·560	0·560	0·565	0·560

These figures show uniform results for all hydrochloric acid concentrations of specific gravity between 1·075 and 1·182, and for all time intervals tested over this range of acidity. Acid of specific gravity 1·075 was selected for further work, as preliminary experiments, on

the effect of tungsten, had already indicated that this acidity would achieve the best compromise in relation to the suppression of the corresponding dithiol tungsten complex.

In such acid concentration, any tungsten present in the steel would tend to separate as tungstic acid, possibly carrying with it some molybdenum, and thus giving low results. Further experiments were, therefore, carried out in order to determine the effect of phosphoric acid on the development of the molybdenum colour. For convenience the phosphoric acid was added as "Spekker" acid (15 per cent. sulphuric acid, 15 per cent. phosphoric acid). A series of test solutions, each containing the equivalent of 4-mg. of synthetic 0·70 per cent. molybdenum steel, were prepared, and different amounts of "Spekker" acid were added to each. The solutions were evaporated to fuming, taken up in 3 ml. of hydrochloric acid of sp.gr. 1·075 and cooled; the dithiol was added, and the separations were carried out as before.

Amount of "Spekker" acid used, ml. ..	nil	0·25	0·50	0·75	1·00
"Spekker" reading	0·555	0·560	0·560	0·565	0·560

These results show that the amounts of "Spekker" acid in the range investigated have no effect on the intensity of the colour due to molybdenum. All further work was therefore carried out in the presence of 0·5 ml. of "Spekker" acid, as this amount was found effectively to prevent the hydrolysis of up to 16 per cent. of tungsten on the sample weights used.

Effect of acid concentration on the formation of the tungsten complex—Most previous work done with toluene-3:4-dithiol has been concerned chiefly with its use for the detection and estimation of tungsten[2,3] and tin.[4] It was, therefore, thought advisable at this stage of the investigation to ascertain the exact conditions necessary for suppression of the reaction with tungsten. An addition equivalent to 4 per cent. of tungsten was therefore made to a number of solution samples of synthetic 0·70 per cent. molybdenum steel, each containing the 0·50 ml. of "Spekker" acid previously decided upon. The samples were then evaporated to fuming, and taken up in 3 ml. of hydrochloric acid solution, the concentration of which was varied from test to test. After the addition of 3 ml. of dithiol solution the amyl acetate layer was separated and its absorption measured as before:

Sp.gr. of hydrochloric acid used	1·064	1·075	1·083	1·097	1·182
"Spekker" reading	0·560	0·565	0·560	0·660	0·805

These readings show that 4 per cent. of tungsten has no effect on the determination of molybdenum provided the reaction is carried out in hydrochloric acid of specific gravity between 1·064 and 1·083. This confirms that the specific gravity previously decided upon, namely, 1·075, is probably the best compromise between the conditions necessary, on the one hand for complete suppression of the tungsten colour, and on the other for complete development of the molybdenum colour.

Effect of dithiol concentrations—The above conditions were applied except for the use of dithiol solutions of differing concentrations, 3 ml. being added in each case. Extractions were made on synthetic solutions of three types of steel containing respectively:

(*a*) 0·70 per cent. of molybdenum.
(*b*) 0·70 ,, ,, ,, ,, plus 4·0 per cent. of tungsten.
(*c*) 4·0 ,, ,, ,, ,,

Sample	Concentration of dithiol solution				
	0·25%	0·50%	1·0%	2·0%	3·5%
	"Spekker" readings				
(*a*) 0·70% Mo	0·515	0·560	0·560	0·565	0·560
(*b*) 0·70% Mo + 4·0% W ..	0·520	0·565	0·565	0·570	0·615
(*c*) 4·0% Mo (0·25 cm. cell) ..	—	0·770	0·775	0·775	0·770

These figures indicate that 3 ml. of 0·50 per cent. dithiol solution would be adequate, but in order to be sure of a safe excess it was decided to use the 1 per cent. solution.

Stability of colour—The amyl acetate solution of the molybdenum complex was found, after separation, to undergo no measurable change in absorption in twenty-four hours.

Effect of temperature—Tests made on synthetic solutions containing tungsten showed that the formation of the tungsten complex is inhibited only under the conditions previously specified, in which the colour development is allowed to proceed at room temperature. For example, the following figures show for two synthetic samples one of which contained

the equivalent of 4 per cent. of tungsten, the effect on the results of heating for 15 minutes on the water bath before separation of the amyl acetate extract.

Sample contained	"Spekker" readings: Normal treatment	"Spekker" readings: Fifteen mins. on the water-bath
0·70% Mo	0·560	0·570
0·70% Mo + 4·0% W	0·560	0·700

It is clear, therefore, that the selective formation of the molybdenum complex is dependent on the use of a relatively low temperature for colour development.

Effect of other elements—The effect of other elements likely to be found in steel, in the procedure evolved, has been examined. No interference is detected when tungsten is present in amounts below 16 per cent., as shown by the following results on a synthetic 0·70 per cent. molybdenum steel containing variable amounts of tungsten:

Percentage of tungsten	0·0	2·0	4·0	8·0	16·0
"Spekker" reading ..	0·560	0·565	0·560	0·555	0·560

Cobalt, vanadium, tin, lead, niobium, tantalum, manganese, and aluminium do not interfere when present in amounts up to 10 per cent. Nickel and chromium have shown no interference below 25 per cent. Titanium has shown no interference up to 1 per cent., and selenium and silicon none up to 5 per cent.

Iron present in the ferric state is partially extracted by the amyl acetate under the conditions used, and accounts for the small "blank" observed, *i.e.*, 0·01 Spekker unit.

If 10 per cent. of copper is present, the amyl acetate layer becomes cloudy and black, but rapidly clears when washed with the concentrated hydrochloric acid, leaving a faint cloudiness which invariably gives a reading equivalent to 0·015 per cent. of molybdenum.

Zirconium, in amounts up to 10 per cent., gives a pale green colour equivalent to 0·025 per cent. of molybdenum. This colour may be due to impurities in the zirconium used.

We may, therefore, conclude that in practical steel analysis this method is specific for molybdenum.

Recommended method

Reagents—

Dithiol solution—Dissolve 0·5 g. of toluene-3:4-dithiol in 50 ml. of amyl acetate by gentle warming; the temperature should not exceed 40° C., and the solution should be prepared just before use.

"Spekker" acid—Add 150 ml. of sulphuric acid (sp.gr. 1·84) carefully to 600 ml. of water. After mixing, add 150 ml. of phosphoric acid (sp.gr. 1·75), mix, allow to cool and dilute to 1 litre.

Hydrochloric acid (sp.gr. 1·075)—Dilute 350 ml. of hydrochloric acid (sp.gr. 1·18) with water to 1 litre and adjust the specific gravity to 1·075.

Standard molybdenum solution for calibration—Molybdenum content 0·01 mg. per ml.

Procedure—

Weigh out a 4-mg. sample of the steel, dissolve it in 0·5 ml. of Spekker acid diluted with 1 ml. of water, and oxidise with nitric acid. Alternatively, an aliquot containing the equivalent of 4 mg. of the steel and 0·5 ml. of Spekker acid may be taken from a larger bulk of solution from which other samples have been taken for analysis. Evaporate the sample to fuming, take it up in 3 ml. of hydrochloric acid of sp.gr. 1·075 and cool it in a water-bath. Add 3 ml. of 1 per cent. dithiol solution and allow the test to stand for fifteen minutes with occasional shaking. Then wash the mixture into a separating funnel, using a small amount of amyl acetate; shake it, and allow the layers to separate. After removing and rejecting the aqueous layer, wash the amyl acetate layer with 3 ml. of concentrated hydrochloric acid, which removes the slight cloudiness present in it. Make up the solution to 10 ml. with amyl acetate in a graduated flask, mix, and measure its absorption on the absorptiometer, using a micro cell of suitable size, together with Calorex H 503 and Spectrum Red 607 filters. The molybdenum content is found by reference to standard calibration curves, produced by applying the above method to pure iron solutions to which known amounts of molybdenum

solution have been added. All the Spekker readings given in this paper have been obtained with a tungsten lamp in the absorptiometer, but sufficient work has been done with a mercury vapour lamp to show that similar results would be obtained, the calibration curve being similar to that obtained with a tungsten lamp.

TYPICAL RESULTS—

With this technique, a number of steels have been analysed for their molybdenum content, with the results shown in the accompanying Table. The figures given for comparison have been obtained by the normal macro thiocyanate method.

TABLE OF COMPARATIVE RESULTS

Type of steel	Molybdenum % Dithiol method	Molybdenum % Thiocyanate method
Plain carbon	0·04	0·04
,, ,, ...	0·09	0·09
,, ,, ...	0·15	0·15
Low alloy	0·06	0·06
,, ,, ...	0·24	0·22
,, ,, ...	0·24	0·24
,, ,, ...	0·30	0·31
,, ,, ...	0·32	0·34
,, ,, ...	0·51	0·52
,, ,, ...	0·68	0·68
,, ,, ...	1·71	1·68
16% Tungsten ..	0·56	0·55
High speed steel ..	4·22	4·19
13% Cr, 1·3% Nb ..	0·01	nil
18 Cr 8 Ni	1·30	1·31
,, ...	2·72	2·73
,, ...	2·40	2·42
,, ...	3·00	2·99

CONCLUSION—

The method as described above will give reasonably accurate results for the molybdenum content of a 4-mg. sample of any steel likely to be examined.

SUMMARY—

A colorimetric method for the determination of molybdenum, in milligram quantities of steel, is described. The method involves the use of toluene-3:4-dithiol in amyl acetate solution, and is virtually specific for molybdenum in steel.

We are indebted to Mr. B. Bagshawe, Assoc.Met., for his advice on this problem, and to Dr. C. Sykes, F.R.S., of the Brown - Firth Research Laboratories, for permission to publish this paper.

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A Method for the Determination of Tungsten in Steel, using Toluene-3:4-Dithiol. The Removal of Molybdenum Interference by Selective Extraction

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The traditional methods for the determination of tungsten in steel are based on hydrolytic precipitation of tungstic acid and conversion to the oxide. Such methods are reasonably satisfactory for relatively large tungsten percentages, but below about 1 per cent. of tungsten, hydrolysis from steel solutions is very uncertain and usually incomplete, and in our experience this is still true irrespective of whether precipitation aids such as cinchonine, rhodamine B, etc., are used or not. Tungsten in amounts below about 0·2 per cent. occurring as a residual from scrap contamination in non-tungsten steels is sometimes difficult to detect at all by hydrolysis methods and, in any case, the results are often erratic and usually much below the truth.

Attention was first turned to toluene-3:4-dithiol as the basis of an entirely new principle by Hamence[1] in 1940, and later by Miller and Lowe,[2] and Miller.[3,4,5] Miller's work gave a preliminary indication of the possible application of the reagent as the basis of a semi-micro method for the element in steel. More recent work on the subject is that of Vaughan and Whalley.[6] We have since made a prolonged investigation with a view to devising an accurate quantitative means of determination, of which the method given below is the outcome.

It very soon became obvious that the practical application of dithiol reagent to commercial steels was severely limited by the interference of molybdenum. Molybdenum interference is serious because (*a*) the corresponding molybdenum-dithiol reaction is one of high sensitivity, and (*b*) apart from true molybdenum steels, practically all steels are contaminated with this element as a residual impurity. There is, therefore, no useful scope for the principle of the tungsten - dithiol reaction unless account can be taken of the corresponding molybdenum effect.

Miller claims partial suppression of the molybdenum - dithiol reaction by reduction to the tervalent condition with stannous chloride. Our experience confirms her observation that whilst tervalent molybdenum may not react with dithiol there is interference due to subsequent oxidation resulting in the formation of intermediate brown complexes and, later, of the green molybdenum complex itself. The order of this interference is shown in Table I, giving typical results on steels in presence of quite small amounts of molybdenum.

TABLE I

	Steel	Mo % added	W % found
(*a*)	18 Cr 8 Ni, W- and Mo-free	nil	nil
	" "	0·05	0·04
	" "	0·25	0·21
(*b*)	18 Cr 8 Ni, low tungsten ..	nil	0·12
	" "	0·05	0·15
	" "	0·10	0·19
	" "	0·20	0·28
(*c*)	18 Cr 8 Ni, W steel ..	nil	0·78
	" " ..	0·05	0·82
	" " ..	0·10	0·83

The adverse effect of molybdenum, even in residual amounts, is therefore well marked, and it increases, although in irregular manner, with increasing amounts of molybdenum.

We attempted to remove molybdenum together with iron by ethereal extraction of 0·1-g. steel samples as ferric chloride solution containing sufficient phosphoric acid to prevent tungstic acid hydrolysis. It is known that ether extraction of molybdenum approaches 100 per cent. in presence of excess of iron, but our tests showed that the distribution ratio is reduced by the phosphate ion, necessary in this case to fix tungstic acid, with the result that considerable molybdenum is retained in the acid tungsten phase. There was also some loss

of tungsten in the ether phase, but conditions that reduce this effect to a minimum, *i.e.*, increased phosphoric acid concentration, similarly induce increased retention of molybdenum in the acid phase, hence no effective ether separation of the two elements was achieved.

A further attempt to apply the ether separation, but after conversion of molybdenum to its oxythiocyanate complex, also failed, and there was additional interference at a later stage from tin salts introduced to reduce molybdenum for the thiocyanate formation.

Later work has been concentrated on an adaptation of a preferential formation and extraction of the molybdenum - dithiol complex itself, which has been reported in a previous paper from these laboratories[7] as the basis for a microchemical method for molybdenum. Our observations are summarised as follows:

(*a*) In hydrochloric acid solutions of approximately 1·06 sp.gr. the molybdenum - dithiol complex alone is formed. If the acid has sp.gr. below about 1·05 the molybdenum complex forms only very slowly, whilst at sp.gr. 1·08 its formation is accelerated but there is also some formation of the corresponding tungsten complex, and this increases with increasing acidity and is quantitative in acid of sp.gr. 1·16.

(*b*) Below 20° C. the formation of the molybdenum complex may be retarded, and if this occurs some molybdenum may escape extraction and interfere later when the tungsten complex is formed. At temperatures exceeding 30° C. there is a gradually increasing tendency for partial simultaneous formation of the tungsten complex even in acid of sp.gr. 1·06 or thereabouts, with resultant loss of tungsten in the discarded molybdenum extract. We now develop the molybdenum complex over a period of fifteen minutes in a bath controlled within the range 20° to 25° C.

(*c*) A prompt and full development of the molybdenum complex is dependent on the use of a freshly prepared solution of the fresh reagent. This is more fully discussed in a later section of this paper.

(*d*) After the amyl acetate extract of the preferentially formed molybdenum complex has been discarded, the residual acid layer containing the tungsten can be concentrated and extracted in hydrochloric acid of sp.gr. 1·16, when further treatment with dithiol reagent on the boiling water bath results in a quantitative formation of the greenish-blue tungsten complex. This reaction is carried out in presence of stannous ion, otherwise high blanks would be obtained due to ferric chloride solubility in the amyl acetate extract of the tungsten complex.

(*e*) The tungsten - dithiol formation is much less sensitive to stale reagent solution than that of the molybdenum complex, probably owing to the influence of stannous ion in reducing oxidation products of the reagent. If the molybdenum separation is imperfect owing to stale reagent, this will be betrayed by the development of brownish tints along with the tungsten complex, which when pure and free from molybdenum should be bluish-green.

From the above observations the method described below has been derived. It is applied to 15-mg. fractions from 0·5 g. steel samples, is suitable for a wide variety of alloy steel types and moreover provides for the first time a ready means of accurately determining tungsten in low ranges, *i.e.*, from 0 to 1 per cent., with which the hydrolytic precipitation methods fail to give quantitative recovery.

Method

Reagents—

"Spekker" acid—150 ml. of sulphuric acid (sp.gr. 1·84) and 150 ml. of phosphoric acid (sp.gr. 1·75) made up to one litre with distilled water.

Hydrochloric acid (sp.gr. 1·06)—Dilute the concentrated acid with water and adjust to sp.gr. 1·06.

Hydroxylamine sulphate solution—10 per cent. solution in distilled water.

Toluene-3:4-dithiol solution—Open a sealed 1-g. phial of the reagent and dissolve in 100 ml. of amyl acetate. The solution thus prepared must be used within a few hours of preparation.

Stannous chloride solution—Dissolve 10 g. of stannous chloride in 100 ml. of concentrated hydrochloric acid.

PROCEDURE—

To 0·5 g. of the sample in a 300 ml. flask (Taylor pattern) add 30 ml. of Spekker acid and 10 ml. of concentrated hydrochloric acid. Heat until dissolved, oxidise with concentrated nitric acid and then evaporate until hydrochloric and nitric acids are expelled and the solution fumes. Extract with 100 ml. of water, boil and transfer to a 500-ml. graduated flask. Cool, dilute to the mark with water, and mix.

Pipette a 15-ml. aliquot representing 15 mg. of sample into a 50-ml. flask and evaporate to fuming. Cool, add 5 ml. of hydrochloric acid (sp.gr. 1·06) to the fumed concentrate, warm gently until all salts are in solution and then cool to room temperature. Add 5 drops of the hydroxylamine sulphate solution and 10 ml. of the toluene-3:4-dithiol reagent solution, allow to stand in a bath at 20° to 25° C. for 15 minutes and shake the solution at intervals throughout this period.

Transfer to a 25-ml. stoppered cylindrical tap funnel, rinsing 3 or 4 times with small portions of amyl acetate. Shake and allow the layers to separate. Draw off the lower acid layer containing the tungsten and reserve it in the original 50-ml. flask. Wash the remaining amyl acetate layer twice consecutively with 5-ml. portions of hydrochloric acid (sp.gr. 1·06), separating and withdrawing the acid layer each time as before. Combine the acid wash layers with the original acid layer reserved in the 50-ml. flask. Discard the washed amyl acetate layer, which contains the molybdenum. Evaporate the acid tungsten solution, carefully at first until dissolved amyl acetate is expelled, then add a few drops of concentrated nitric acid and evaporate to fuming. Add a few more drops of nitric acid during fuming to clear up any charring organic matter, rinse the flask with a minimum of water, and re-fume to expel nitric acid and water.

Add 5 ml. of the stannous chloride solution to the fumed liquid and heat on a boiling water-bath for 4 minutes. Add 10 ml. of toluene-3:4-dithiol reagent solution and continue to heat on the boiling water-bath for a further 10 minutes. Shake the solution at intervals throughout this period.

Transfer to a 25-ml. stoppered tap funnel, rinsing 3 times with 2-ml. portions of amyl acetate. Shake, separate, draw off the lower acid layer, and discard it. Add 5 ml. of concentrated hydrochloric acid to the amyl acetate layer in the separator, repeat the extraction and again discard the lower acid layer (see *Note* below).

Draw off the amyl acetate layer, containing the tungsten, into a 50-ml. graduated flask (previously rinsed free from water, first with alcohol and then with amyl acetate), make up exactly to the mark with amyl acetate and mix.

Measure the absorption of this solution in the Spekker absorptiometer, using the mercury vapour lamp, 4 cm. cells and Ilford Spectrum Red No. 608, and Calorex H.503 filters. Refer the readings to a calibration curve prepared from spectrographically pure iron to which suitable increments of standard sodium tungstate solution have been added.

Note—For steels containing appreciable cobalt further repeated washing is necessary at this stage. After the wash with 5 ml. of concentrated hydrochloric acid, give two further washes with hydrochloric acid (sp.gr. 1·06), and a final wash with concentrated acid.

RANGE—

The method as specified, *i.e.*, on 15-mg. aliquots from an initial 0·5-g. sample weight, gives a calibration range of 0 to 1 per cent. tungsten ≡ 100 drum divisions, with the 4-cm. cells. The range may be extended *pro rata* to cover higher tungsten percentages by using 2-, 1-, or 0·5-cm. cells. For the greatest accuracy in the lowest tungsten ranges, the calibration range may be reduced to 100 drum divisions ≡ 0·5 per cent. of tunsten by making the final reading in a volume of 25 ml. The reading accuracy is then of the order of ±0·005 per cent. of tungsten. With the calibration range as specified in the method, the reading accuracy is within 0·01 per cent. of tungsten over the range 0 to 1 per cent., and this fulfils all normal requirements within this range.

RESULTS—

The foregoing method was proved by tests made on austenitic 18 Cr, 8 Ni tungsten bearing steels with and without synthetic additions of molybdenum, and on synthetic preparations obtained from 18 Cr, 8 Ni steels (spectrographically free from tungsten) treated with various known amounts of molybdenum and tungsten solutions. Results are given in Table II.

TABLE II

Steel	Mo added, %	W added, %	W found, %
18 Cr 8 Ni (spectrographically free from W)	nil	nil	nil
	nil	0·50	0·50
	1·0	0·02	0·02
	2·0	0·05	0·05
	2·0	0·20	0·21
	3·0	nil	0·01
	,,	0·10	0·11
	,,	0·50	0·51
18 Cr 8 Ni steel, 0·78% W	nil	—	0·77
	,,	—	0·78
	3·0	—	0·77
	,,	—	0·78
	,,	—	0·77

These results are all accurate to a tolerance of 0·01 per cent. of tungsten, even when abnormally high percentages, *e.g.*, 3 per cent., of molybdenum are introduced.

EFFECT OF OTHER ELEMENTS—

Of the common steelmaking elements, nickel, chromium, manganese, silicon, vanadium, aluminium, titanium, niobium (columbium), and tantalum do not form coloured dithiol complexes under the test conditions and exert no adverse influence even when present in major alloying amounts.

There is a small extraction of iron by the amyl acetate, which imparts a faint yellowish tinge to the ester layer in complete absence of tungsten, and in presence of very small amounts of tungsten modifies the pale bluish-green of the complex to a yellowish-green. It is, however, easy to distinguish between the pale yellow of "blank" iron and the colour introduced by as little as 0·01 per cent. of tungsten, and in any event the reading value of the yellow component is virtually eliminated by the red filters. Thus pure iron spectrographically free from tungsten gives a blank reading of the order of 0·01 Spekker unit, equivalent to about 0·01 per cent. of apparent tungsten. This is compensated by preparing the calibration from a pure iron base with suitable increments of added sodium tungstate rather than from pure sodium tungstate alone.

There is no increase of "blank" absorption from nickel or chromium, both these elements being preferentially soluble in the acid phase and rejected with it. Titanium, silicon, niobium (columbium), and tantalum may give insoluble hydrolysis products on fuming with mixed phosphoric and sulphuric acids, but there is no need to remove them. They do not occlude tungsten which is fixed as soluble phospho-tungstic acid, and any precipitate is virtually eliminated in taking the 15-ml. aliquot from the 500-ml. initial test volume. Any residual amount of these elements soluble in the test aliquot is entirely without influence. The method, therefore, provides for the first time a simple means of determining tungsten in alloy steels containing niobium (columbium), tantalum, and titanium. On a tungsten-free 0·68 per cent. niobium (columbium) steel 0·30 per cent. of tungsten was found after exactly that amount had been added as a standard solution. A sample of heat-resisting alloy (Ni 70, Cr 20, Fe 3, Ti 2·5 per cent.) gave a residual tungsten content of nil by this method and 0·21 per cent. of tungsten after addition of a further 0·20 per cent. as standard solution, whilst British Chemical Standards No. 209 (18/8 steel with 0·59 per cent. of Ti) gave 0·30 per cent. of tungsten after that amount had been added. Copper forms a black precipitate in the ester layer at the molybdenum extraction stage, but there is no interference and there is no corresponding formation at the tungsten extraction stage.

If large amounts of cobalt are present partition occurs, resulting in a partial transfer of the element to the ester layer, which becomes coloured greenish-blue by the cobalt ion. The extent of cobalt interference is indicated by the figures in Table III.

The effect is only important as it applies to cobalt alloy steels; ordinary plain and alloy steels do not contain sufficient residual cobalt to prejudice the tungsten readings. In any event the effect of as much as 10 per cent. of cobalt can be virtually eliminated by repeated acid washing of the ester phase, as described in the *Note* to the Method, *e.g.*, a plain steel treated with the equivalent of 10 per cent. of cobalt gave no measurable reading, and after addition of the equivalent of 0·5 per cent. of tungsten returned a value for this element of 0·52 per cent.

TABLE III

Steel	Cobalt added, %	Apparent tungsten, %
18 Cr 8 Ni (W nil) ..	—	nil
,, ,, ..	2·0	0·06
,, ,, ..	4·0	0·10
,, ,, ..	6·0	0·15
,, ,, ..	8·0	0·21
,, ,, ..	10·0	0·31

OBSERVATIONS ON THE METHOD OF USING DITHIOL REAGENT—

The reagent as normally supplied to us has been obtained in 1-g. sealed glass phials and occasionally in 5 g. sealed phials. It was known that its keeping properties were limited once the phials were broken, and with 5-g. lots we adopted the practice of with drawing 1-g. amounts for the preparation of solution and immediately re-sealing the tubes each time. In spite of this we experienced many inconsistencies of performance in the early stages of the investigation. It was observed that satisfactory molybdenum separations were only achieved with new solutions prepared immediately before use and the molybdenum separation became erratic when the dithiol solutions were a few hours old, and sometimes when absolutely fresh solutions were prepared from dithiol obtained from phials that had previously been opened and re-sealed. The effect was to give an incomplete conversion of molybdenum into its dithiol complex, and hence its incomplete extraction and removal, the residual molybdenum giving the characteristic brown tints at the later tungsten stage and consequent high values for tungsten.

The effect of stale reagent seemed to be confined to the molybdenum formation, as the corresponding tungsten formation was always complete even when the reagent solution was a week old. We attributed this difference in behaviour to the retarding effect of oxidising decomposition products of the reagent upon the formation of the molybdenum complex, the absence of an analogous effect at the tungsten stage being accounted for by the reducing action of stannous chloride. Stannous chloride is not a suitable reducing agent to have present at the molybdenum separation stage, as tin salts hydrolyse on fuming the extracts, with occlusion of tungsten. After trying several alternative reducing agents we obtained satisfactory results with hydroxylamine sulphate, 5 drops of a 10 per cent. aqueous solution, added immediately before the dithiol addition, being sufficient to restore maximum reagent reactivity with molybdenum, under the conditions specified. With greater amounts of hydroxylamine there is again a tendency to incomplete molybdenum extraction, possibly owing to partial reduction of molybdenum itself.

Since introducing hydroxylamine we have not experienced any difficulty in achieving complete extraction of molybdenum, but as an additional safeguard we prefer to use freshly prepared reagent solutions. Surplus reagent solution can conveniently be reserved for the tungsten extraction stage and used over a period of several days.

SUMMARY—

A method is described for the determination of tungsten in steel, using the reagent toluene-3:4-dithiol. Interference from the corresponding molybdenum - dithiol complex is prevented by forming it preferentially in cold dilute acid solution, and removing it by amyl acetate extraction. The residual acid layer is treated for formation of the tungsten - dithiol complex, which develops quantitatively in hot concentrated acid medium. The complex is extracted with amyl acetate and tungsten determined absorptiometrically. There is little or no interference from other elements and the method is applicable to practically all types of alloy steel, being particularly useful for determinations in residual and other low ranges, where the usual gravimetric methods give poor performance.

We are indebted to the directors of Messrs. Thos. Firth and John Brown, Limited, and in particular to Dr. C. Sykes, F.R.S., of the Brown - Firth Research Laboratories, for permission to publish this paper.

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THE BROWN - FIRTH RESEARCH LABORATORIES
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A Chromatographic Adsorption Method for the Estimation of the Provitamin-A Content of Foodstuffs*

By G. B. RAMASARMA, D. N. HAKIM, and S. D. RAO

A number of methods have been proposed by various authors for estimating the provitamin-A content of plant materials; these have been critically reviewed by Moon,[1] Seaber,[2] Peterson[3] and, very recently, Booth.[4] Essentially all the methods consist of four steps: (1) extraction of the pigments from the material, (2) saponification to remove oils and hydrolyse xanthophyll esters, (3) separation of the provitamins, collectively termed "carotene," from the associated pigments, and (4) estimation of the carotene by colorimetric or spectrophotometric methods of comparison. The present investigation deals mainly with the third step, which until recently consisted in the application of the Willstätter - Stoll phase partition between light petroleum and 90 per cent. methyl alcohol. This procedure is known to have some serious drawbacks; in particular, (*a*) the separation of kryptoxanthin is not sharp, the pigment being found in both the phases, and (*b*) the inactive lycopene is epiphasic and therefore contaminates carotene.

Further, recent investigations in this laboratory[5] have shown that the partition between light petroleum and methyl alcohol is unsatisfactory in that some coloured but biologically inactive degradation products also remain in the epiphasic layer and are incorrectly estimated as carotene. The presence of such carotene-like artefacts has been observed by some earlier workers also: by Kemmerer and Fraps[6] in the excreta of rats and chickens, by Wiseman and his co-workers[7] in market hays and silages, by Quackenbush *et al.*[8] in A.I.V. silage and by Whitnah *et al.*[9] in the faeces of cows. Various treatments have been suggested for the removal of these interfering pigments, *e.g.*, washing the light petroleum solution with diacetone, passing it through calcium carbonate[10] or shaking it with a small amount of specially prepared magnesium carbonate.[11] Seaber[2] proposed a chromatographic method, using Brockmann's alumina as adsorbent and light petroleum containing 3 per cent. of acetone as developer. Moore[12] reported that a column of dicalcium phosphate adsorbs all the xanthophylls and interfering pigments but allows carotene to pass down unadsorbed.

An additional drawback in the phase separation method is that no distinction is made between β-carotene and the other provitamins, which are only half as active as the former. Neither the inactive lycopene nor the "low-grade" provitamins (mostly α-carotene and kryptoxanthin) appear to be of such rare occurrence or in so negligible an amount as was hitherto supposed. This fact seems to have been realised only in the case of yellow corn and yellow maize, as can be seen from the work of Buxton,[13] Fraps and Kemmerer,[14] White *et al.*,[15] and Sadana and Bashur Ahmad,[15a] who have determined both carotene and kryptoxanthin in order to find out the provitamin-A activity. In a critical application of the chemical method for assessing the provitamin-A activity of any plant material,† it is necessary to estimate the individual provitamins and calculate the activity by using the following formula which is based on the definition of the International Unit:

$$\text{Provitamin-A activity in Internat. Units} = \frac{\mu\text{g. of } \beta\text{-carotene}}{0{\cdot}6} + \frac{\mu\text{g. of other provitamins.}}{1{\cdot}2}$$

Chromatographic adsorption, which offers the only means of separating the individual pigments, has been used extensively in qualitative and preparative work, but not for purposes

* This investigation was completed in February, 1943, but owing to unavoidable circumstances it could not be presented for publication earlier. A preliminary note, however, was published in *Current Science*, 1943, **12**, 21.

† It is to be admitted, however, that with certain foodstuffs serious discrepancies have been observed between the chemically determined activity and that obtained by biological assay. Some of these anomalies may be due to imperfections in the chemical methods already referred to, but there are other important factors influencing the biological availability—*viz.*, the extent of digestion and absorption and the presence of varying amounts of anti-oxidants (Hickman *et al.*[16]; Guggenheim[17]). It has been suggested that a relatively high figure should be fixed for the daily requirements if all the vitamin-A activity is to be derived from vegetable sources, for the activity of the provitamins is relatively low at the higher levels of intake, probably owing to inefficient absorption (Guilbert *et al.*[18]; Vitamin-A Sub-Committee of the Accessory Food Factors Committee, Lister Institute and Medical Research Council[19]). Although from a practical point of view a unit of provitamin A is not the same as a unit of vitamin A, the need for further improvements in the chemical and biological methods of assay cannot be denied.

of quantitative estimation, because considerable amounts of the pigments, varying with their adsorptive affinities, may be lost owing to incomplete elution. Thus, if a mixture of pigments is chromatographed to give a sharp separation of the constituents the loss of xanthophylls and other inactive pigments would be immaterial, but the loss of variable amounts (70 to 90 per cent.) of the provitamins would introduce a factor of uncertainty which makes the procedure unsuitable. It is obvious, therefore, that the elimination of the non-carotene pigments and the separation of the individual provitamins for quantitative estimation are not possible in one and the same chromatographic operation. It was thought, however, that a close approximation to the objectives in view might be achieved if the chromatographic adsorption were carried out in two stages; the first to estimate the "total carotene" (including lycopene, if any) and the second to estimate the relative proportions of the individual pigments in this "total carotene." Dicalcium phosphate prepared according to Moore[12] has been found to fulfil the requirements of the first stage, in which xanthophylls and artefacts should be retained completely and the hydrocarbon pigments allowed to pass down unadsorbed. In the second stage, satisfactory resolution of the pigment mixture was obtained by use of Brockmann's alumina or activated magnesia. Throughout this paper the two stages are referred to as the "first chromatography" and the "second chromatography" respectively.

EXPERIMENTAL

PREPARATION AND PURIFICATION OF THE REAGENTS—

Dicalcium phosphate—Dicalcium phosphate was prepared precisely according to Moore.[12]

Alumina—On account of the scarcity of Brockmann's alumina, it was found necessary to activate the adsorbent and use it over and over again. The used adsorbent was repeatedly washed by heating under reflux with light petroleum (b.p. 60° to 75° C.) containing 3 per cent. of alcohol, until the extract was practically colourless. The adhering solvent was removed, finally by drying at 100° C., and the alumina was heated in a nickel dish over a Meker burner (at a temperature ranging between 200° and 250° C.) for a number of hours and stirred frequently with a glass rod meanwhile. There was an initial darkening due to carbonisation of traces of organic matter, but on continued heating the adsorbent became practically white. It was allowed to cool in a desiccator over sulphuric acid and soon after transferred to glass-stoppered bottles.

Light petroleum ("petroleum ether")—As purified light petroleum was not available, ordinary automobile petrol was distilled and the fraction boiling between 60° to 75° C. was collected and purified according to Castille and Henri[20] by treatment with sulphuric acid and alkaline permanganate.

Methyl alcohol—Methyl alcohol was purified by heating under reflux with aluminium turnings (10 g. per litre) and potassium hydroxide (10 g. per litre) and then submitted to fractional distillation. In order to obtain a 92 per cent. v/v methyl alcohol for the phase separation, 90 ml. of purified alcohol were mixed with 10 ml. of water.

Ethyl alcohol free from aldehyde—This was prepared by treating rectified spirit in a manner similar to the methyl alcohol.

CHROMATOGRAPHIC TECHNIQUE—

The procedure was practically the same as that described by Moore,[12] except that smaller adsorption tubes (1·3 × 12 cm.) were employed. The chromatogram was developed under a vacuum of 40 cm. of mercury instead of the 65 cm. recommended by Moore, in order to avoid excessive loss of solvent under tropical conditions.

In order to determine the extent of the loss that may occur during the first chromatography, solutions containing 10 to 60 μg. of β-carotene were passed through identical columns of dicalcium phosphate; estimation of the carotene content of the filtrates showed that the losses of β-carotene rarely exceeded 1 per cent. This is well within the range of errors inherent in visual spectrophotometric determination. The need for care in the preparation of the phosphate has been stressed by Moore.[2,12]

Preliminary experiments showed that both xanthophylls and xanthophyll esters are retained by a column of dicalcium phosphate. Hence, in this method, both the saponification and the phase separation could normally be dispensed with, thus simplifying the procedure considerably. Nevertheless these operations have been retained in the present investigation, in order to demonstrate the existence of non-carotene pigments in the epiphase.

Some of the early trials, in which no saponification was performed, showed that the presence of oil in the extract may adversely affect the chromatographic separation. No difficulty was experienced with rich sources of carotene, *e.g.*, leafy material, the xanthophylls and artefacts from which formed sharply defined and firmly held bands on the dicalcium phosphate column, but the extracts of poor sources, such as spices, cereals, and nuts, gave rise to diffuse overlapping bands, which showed a tendency to come down on continued washing. This difficulty could be easily overcome by preliminary saponification of the light petroleum extract and removal of the soaps. That the adverse effect was due to the presence of oil was confirmed by the following experiment. Aliquots of an oil-free solution containing carotene, lycopene, xanthophylls, and artefacts were mixed with varying amounts of coconut oil and passed over identical columns of dicalcium phosphate. The pigment that flowed down unadsorbed was in each instance estimated as carotene. When the oil contents were 0, 10 and 20 mg., the carotene values were 37, 37·4, and 37·5 μg. respectively. When the oil contents were 50 and 100 mg., higher carotene values, *viz.*, 40·5 and 40·3 μg. respectively, were obtained, showing that some other pigment besides carotene was washed down. When 200 and 500 mg. of oil were used, the bands were irregular and diffuse.

These results indicate that, under the experimental conditions employed, the presence of oil to the extent of about 20 mg. in the extract does not seriously interfere with the adsorption. With larger amounts, however, saponification is necessary. Generally speaking, saponification seems to be essential when the material under test is rich in oil or poor in carotene content.

PROCEDURE—

Extraction of pigments—Preliminary treatment depended upon the nature of the material. Dried materials like cereals and pulses were ground thoroughly in a porcelain edge-runner mortar to pass an 80-mesh sieve and a sample of the powder was taken for analysis. Fresh materials such as fruits and vegetables were cut into small bits and ground with acid-washed sand; this grinding was preferably repeated after the first extraction with alcohol. The quantity of material taken for analysis was largely dependent upon its carotene content; 0·5 to 5 g. of rich sources were sufficient, while 30 to 50 g. were necessary with cereals and pulses; according to the quantities taken, the volumes of alcohol and ether used for extraction were varied.

Typical example of extraction procedure—30 g. of a powdered sample were weighed into a 500-ml. Erlenmeyer flask, 100 ml. of aldehyde-free ethyl alcohol (95 per cent.) were added and the mixture was heated over a water-bath under a reflux condenser for half an hour. The alcoholic extract was decanted, preferably through a wad of cotton wool, into a separating funnel. The residue was extracted similarly with another 100 ml. of alcohol and the extract was added to the first. The residue was further extracted by heating under reflux for 15 minutes with 100 ml. of a mixture of 2 volumes of light petroleum (b.p. 60° to 75° C.) and 1 volume of alcohol. Finally the residue was washed with 50 ml. of a similar petroleum - alcohol mixture. By this time the provitamins were completely extracted. The extracts were combined and sufficient water was added to bring the alcohol concentration to 75 to 80 per cent. The lower, aqueous alcoholic, layer was drawn off and extracted in a separating funnel with three 50 ml. lots of light petroleum (b.p. 60° to 75° C.). Further extraction was found to yield only xanthophylls.

Saponification and phase partition—The combined light petroleum extract was washed once with water and saponified by heating under reflux for 15 minutes with 25 ml. of 10 per cent. alcoholic potash solution. The chlorophyllins, soaps and excess of alcoholic potash were removed by careful washing. The light petroleum extract was then shaken with an equal volume of 92 per cent. methyl alcohol in a separating funnel and the lower, alcoholic, layer, containing xanthophylls, was drawn off. The epiphase was washed repeatedly with smaller portions of 92 per cent. methyl alcohol until the washings were practically colourless. The combined methyl alcoholic solutions were washed with a small volume of light petroleum, which was added to the epiphasic solution. The combined light petroleum solution was washed free from alcohol, dried over anhydrous sodium sulphate and made up to a suitable volume. The pigment concentration was determined by spectrophotometry and the carotene content of the material calculated. This is the value obtainable by the usual methods employing Willstätter - Stoll phase separation.

First chromatography—An aliquot of this solution (or all the solution, if the carotene content was low) was concentrated under reduced pressure in an atmosphere of nitrogen

to about 5 to 10 ml. and passed through a column of dicalcium phosphate. The xanthophylls and artefacts were held strongly at the top, while carotene passed down practically unadsorbed. Lycopene (when present) showed little separation from carotene; its presence could, however, be suspected when the top portion of the carotene band, as it approached the lower end of the tube, appeared dark red in colour. It was found convenient to collect the carotene solution in a separate test tube, which could be suspended in the flask just when the carotene reached the lower end of the column and before any pigment was washed down. The solution was made up to a suitable volume and the gross "carotene" content was estimated by spectrophotometric means.

In some experiments it was observed that a distinct, pale yellow band moved down the column soon after the carotene on continued washing of the column; this was found to be kryptoxanthin. Since, under these conditions, the xanthophylls and artefacts do not move down at all, this offers a means of separately estimating kryptoxanthin in any material.

Second chromatography—The "gross carotene" solution (filtrate from the dicalcium phosphate column) was then concentrated to a small volume under reduced pressure and in a current of nitrogen and was passed through a suitably sized column of Brockmann's alumina. The chromatogram was developed with light petroleum, which separated the constituent pigments into sharply defined bands. The bands were carefully scooped out by means of a spatula and separately eluted with light petroleum containing 3 to 5 per cent. of ethyl alcohol. The eluates were made up to suitable volumes and examined in the spectrophotometer for identification and estimation. The total pigment in the eluates did not account for all that was chromatographed. Usually the recovery was about 90 per cent., but since the three common hydrocarbon pigments—α-carotene, β-carotene and lycopene—differ but slightly in their adsorptive affinities, it was assumed that the losses were shared by them equally. The amounts of individual pigments present in the original solution were calculated accordingly.

Spectrophotometry—In order to identify and estimate the different pigment fractions, their solutions in purified light petroleum (b.p. 60° to 75° C.) were examined in a Gaertner Visual Spectrophotometer. The photometer drum readings were taken at 5-mμ. intervals over the range 430 to 550 mμ. and the extinction (E) values were read off from conversion tables (Brode[22]). The pigments were identified by their characteristic absorption maxima and the concentrations calculated on the basis of the extinction coefficients ($E^{1\%}_{1cm.}$ = 2440 at 450 mμ., 2020 at 470 mμ., and 2140 at 480 mμ.; solvent light petroleum, b.p. 60° to 75° C.) obtained for a sample of pure β-carotene isolated from Badami mango fruit.[23] The average of the values obtained for the three wavelengths was taken as the correct figure. For the sake of simplicity, all estimations were made in terms of β-carotene, irrespective of the identity of the particular fraction.

Having thus found the amounts of different provitamins present in the material, the total provitamin-A content expressed in International Units was calculated according to the formula given on p. 194.

Results for a number of food materials found by this procedure are shown in the accompanying Table. For comparison, values given in the *Health Bulletin No.* 23, Third Edition (1941), published by the Government of India, have also been included in the table; most of them were determined by De,[24] by means of a phase separation method.

Discussion

Our results clearly show that plant materials that have been dried and stored for some time contain appreciable amounts of epiphasic artefacts. These are wrongly estimated as carotene by the usual phase partition methods. By chromatography on a column of dicalcium phosphate the impurities can be removed without loss of carotene.

Sekhon[25] found that loss of carotene on dehydration of carrots was small. Analysis of a sample of dehydrated carrots (supplied by Messrs. Parry & Co.) indicated that over 20 per cent. of the epiphasic pigment consisted of destruction products showing no definite absorption spectrum. Our work shows that more reliable results will be obtained if the chromatographic method is employed for such dehydrated foods.

Another interesting fact brought out is the multiplicity of epiphasic pigments. It is generally assumed that most of the carotene of plant materials consists of β-carotene and that lycopene and kryptoxanthin are of rare occurrence. Among the materials tested, more than half the "apparent carotene" of coriander seeds and nearly 90 per cent. of that of mace

seems to be lycopene. The carotene of chillies and carrots contains nearly 25 per cent. of the α-isomer. Again, the provitamin A occurring in papaya fruit is almost entirely kryptoxanthin. The few results presented here clearly indicate the need for similar detailed analyses of the common Indian foodstuffs.

Owing to the exigencies of the war, there has been a considerable increase in the consumption of dehydrated foods, while processed and canned foods promise to come into a more extensive use than ever before. Various investigators have studied the carotene losses when foodstuffs are subjected to different cooking processes. It is highly probable that in all this work the observed provitamin-A values were vitiated by interference from non-carotene pigments. A re-investigation employing improved chromatographic methods seems to be desirable.

PROVITAMIN-A CONTENT PER 100 G. OF MATERIAL

Material	Botanical name	*Health Bulletin* No. 23 μg.(=I.U.) I	Determined by the authors: Phase partition μg. II	First chromatography μg. III	Second chromatography (calculated) μg. IV	Vitamin-A activity (calculated) I.U. V
Bengal gram	*Cicer arietinum*	316	224	162	*b*=162	270
Green gram	*Phaseolus radiatus*	158	109	26	*b*=24; ?=2	42
Red gram	*Cajanus indicus*	220	125	100	*b*=100	166
Horse gram	*Dolichos biflorus*	119	74	17	*b*=17	28
Lentil	*Lens esculenta*	450	70	25	*	42
Whole wheat	*Triticum vulgare*	108	21	6	*	10
Cholam	*Sorghum vulgare*	136	20	13	*	22
Fenugreek seeds	*Trigonella foenugraecum*	160	660	260	*b*=240; ?=20	420
Mace	*Myristica fragrans*	—	3580	2150	L=1976; *b*=174	290
Red chillies (dry)	*Capsicum annuum*	576	11200	6230	*b*=4420 *a*=1620; ?=190	8870
Pistachio nut	*Pistacia vera*	240	176	140	*b*=126; *a*=14	220
Gingelly seeds	*Sesamum indicum*	100	21	12	—	20
Mustard	*Brassica juncea*	270	178	149	*b*=141; ?=8	242
Coriander seeds	*Coriandrum sativum*	1570	230	100	L(?)=55; *b*=45	73
Mango fruit (Badami)	*Mangifera indica*	4800	—	5000	*b*=5000	8330
Papaya fruit	*Carica papaya*	2020	—	1280	*c*=1150; *b*=130	1175
Carrots (dehydrated)	*Daucus carota*	—	37800	29800	*b*=22350 *a*=7450	43460

a = α-carotene; *b* = β-carotene; L = lycopene; *c* = kryptoxanthin; ? = doubtful identity; * = not analysed, assumed to be all β-carotene.

Note:—Column I: Values reported in the *Health Bulletin No.* 23.
Column II: Values obtained by the authors, employing a similar phase-separation method. The differences between I and II are mostly due to differences inherent in the samples analysed.
Column III: Values obtained by subjecting II to chromatography over dicalcium phosphate. (II)–(III): Non-carotene pigments wrongly estimated as carotene by the phase-separation method.
Column IV: Composition of III in terms of individual pigments (calculated from the results obtained by subjecting III to chromatography over Brockmann's alumina).
Column V: Activity in International Units calculated from IV.

Finally, a word of caution is necessary with regard to the first chromatography. There are many variables in the experimental procedure—the adsorptive power and particle size of the dicalcium phosphate, the size of the column and the manner of packing, the vacuum applied during chromatography, and so forth; unless due care is taken, considerable errors may be introduced. At the outset, all these experimental conditions should be standardised to give quantitative recoveries of carotene and the details strictly adhered to subsequently. It is further recommended that every new lot of adsorbent should be tested for its proper performance before it is brought into use.

In the second chromatography (over Brockmann's alumina) which seeks to estimate the relative proportions of the individual pigments, it is not quite correct to suppose that the losses due to incomplete elution are shared equally by the constituents. By analysis of "synthetic" mixtures, it has been found, as might be expected, that larger amounts of the more strongly adsorbed pigment are lost. The consequent errors are not high, but improvements in this direction are desirable.

Subsequent to the completion of this work (February, 1943), several groups of workers[26–30] have proposed chromatographic methods for the estimation of the true carotene content of

processed foods, feeding stuffs, and the like. It should be mentioned, however, that few of these methods, with the exception of those of Kemmerer and co-workers,[31,32] go beyond the estimation of the total carotene content. The large number of investigations of a similar nature that have been reported during recent years shows the extent to which the subject is deservedly attracting the attention of workers interested in nutrition.

SUMMARY

An improved method for the estimation of the provitamin-A content of foodstuffs has been described. It involves application of chromatographic adsorption in two stages, using suitable adsorbents: the first to estimate the total "carotene" and the second to ascertain the relative proportions of the constituent pigments. This permits the estimation of the individual provitamins and therefore a more precise estimate of the total provitamin-A activity of the material under test.

A few food materials have been analysed by this improved method and the results are presented. The results indicate that large errors might be introduced in the analysis of dried and stored materials, owing to the presence of certain modified carotenoid pigments which are devoid of any provitamin-A activity, but are estimated as carotene by the usual methods employing the Willstätter - Stoll phase separation. These impurities can be removed by chromatographic adsorption over dicalcium phosphate, the carotene recoveries being quantitative. The possible formation of epiphasic non-carotene pigments during the course of storage, cooking, canning or dehydration of foodstuffs has been discussed and the need for a thorough re-investigation by improved chromatographic methods has been stressed. In order to eliminate errors due to the presence of lycopene and the "low grade" provitamins, the second chromatography should be carried out and the individual pigments estimated.

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The Determination of Carotene in Dried Grass

By W. A. G. NELSON

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A new method for the determination of carotene in dried grass is being put forward by a Carotene Committee,* which was originally formed through the Crop Driers Association, and in 1941 proposed a tentative method of analysis.[1] This was called Method B, two other methods, A and C, having been under consideration at that time.

In order to point out the defects of this old method and show the progress that has since been made, it is necessary to give an outline of the tentative method as published in 1941.

Method B of 1941—

The sample of dried grass is ground to a fine powder with sand and extracted with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, in a Soxhlet or drip-type extractor. The extract is shaken up with a concentrated solution of potash in methyl alcohol to convert the chlorophyll present into potassium chlorophyllin, which is removed with water. The remaining extract now contains carotenoids and xanthophyll, and the xanthophyll is removed by means of a 9:1 mixture of methyl alcohol and water. The amount of carotene in the light petroleum solution thus obtained is then estimated colorimetrically.

From the work of Seaber,[2] Kon and Thompson,[3] Moore[4] and Mann,[5] it was demonstrated by chromatography that there is in grass a petrol-soluble carotenoid that does not exhibit the properties of β-carotene and has no biological activity. It was believed that this particular "carotene" forms on the average 30 per cent. of the total carotenoids in grasses and therefore analysis of a grass meal by Method B gives an untrue picture of its biological worth.

Furthermore, it was shown that it is not always possible to remove all the chlorophyll as potassium chlorophyllin, and a trace of chlorophyll in the final solution makes the colour estimation fallacious.

Any method which would give the true β-carotene content of a grass meal would, therefore, have to embody the following three important features:

(1) It must be capable of separating β-carotene completely from the non-active carotenoids of grass.

(2) It must yield for the final colorimetric estimation a solution entirely free from any forms of chlorophyll.

(3) It must be a method in which there is no likelihood of isomerisation taking place.

Points (1) and (2) have been mentioned above, and for (3) it is necessary to consider the work of Zechmeister.[6] He showed that β-carotene was capable of spontaneous isomerisation in solution and gave molecular extinction curves of the all-*trans* compound and mixtures of its stereoisomers (see Fig. 1).

Table I gives the $E^{1\%}_{1cm.}$ values of β-carotene over the wavelength range 451 to 447 mμ., calculated from the above molecular extinction curves.

Table I

Wavelength mμ.		$E^{1\%}_{1cm.}$
451·0	all *trans*	2575
450·5		2505
450·0		2435
449·5		2365
449·0		2295
448·5		2225
448·0		2155
447·5		2085
447·0	much isomerisation	2015

When a solution is being analysed colorimetrically for β-carotene the $E^{1\%}_{1cm.}$ value is usually taken to be 2500 at 450 mμ. From the figures in Table I it is obvious that, if isomerisation has taken place and the $E^{1\%}_{1cm.}$ at 450 mμ. is assumed to be 2500, inaccuracies will be incurred in determining the β-carotene present.

* Members of the Carotene Committee—Mr. R. O. Davies (*Chairman*), Dr. V. H. Booth, Dr. A. Green, Mr. J. Greenbaum, Mr. A. W. Hartley, Mr. T. Barton Mann, Dr. F. E. Moon, Dr. W. A. G. Nelson, Mr. W. M. Seaber, Mr. H. H. Ward, Dr. H. Wilkinson, Mr. R. F. Wright.

Zechmeister has postulated the possibility of the existence of twenty isomers of β-carotene within the range from all *trans* to all *cis* (see Fig. 2) and he has been able to identify several of them.

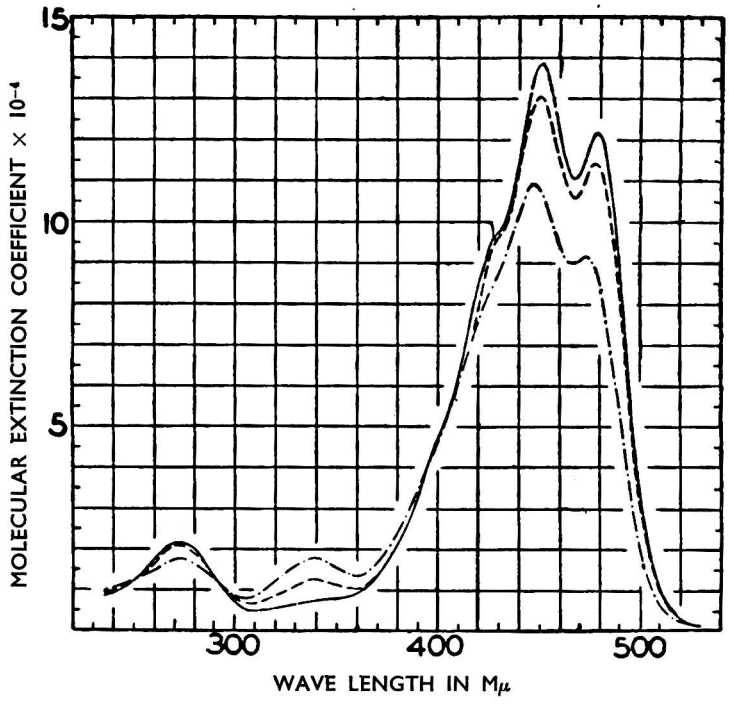

FIG. 1. Molecular extinction curves of β-carotene in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after heating under reflux in darkness for 45 min.; — . —, mixture of stereoisomers after iodine catalysis at room temperature in light. (From *J. Amer. Chem. Soc.*, 1943, **65**, 1523.

Bearing in mind the facts mentioned above, Mann, Seaber, Green and Hartley (who are members of the present Carotene Committee) collaborated with a view to finding an improved method for the determination of carotene in grasses. As a result of their investigations a chromatographic method called Method D was suggested.

METHOD D—

One g. of the sample is ground to a fine powder with sand and extracted with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, as in Method B. The mixed solvents are removed almost completely by evaporation on the water bath and the last traces are blown off with a stream of carbon dioxide to minimise oxidation. The dry residue is dissolved in light petroleum (b.p. 40° to 60° C.) and then passed through a column of bone meal (Mann's findings on the use of bone meal as an adsorbent have already been recorded[5]). The filtrate is then examined for β-carotene colorimetrically in the usual way.

The salient points emerging from the analysis of grass meal by Method D were:

(1) The filtrate from the bone meal column contained carotene which had the full biological activity of β-carotene, whilst the "carotene" fraction retained by the bone meal had no biological activity.
(2) The filtrate contained no chlorophyll.
(3) The absorption spectrum of the carotene in the filtrate coincided with that of all-*trans*-β-carotene.

It was now clear that a method had been found which gave a much more accurate determination of β-carotene in grass than the previous ones. By the combined efforts of the whole Committee, it was shown that Method D gave more reproducible results than those obtained by Method B.

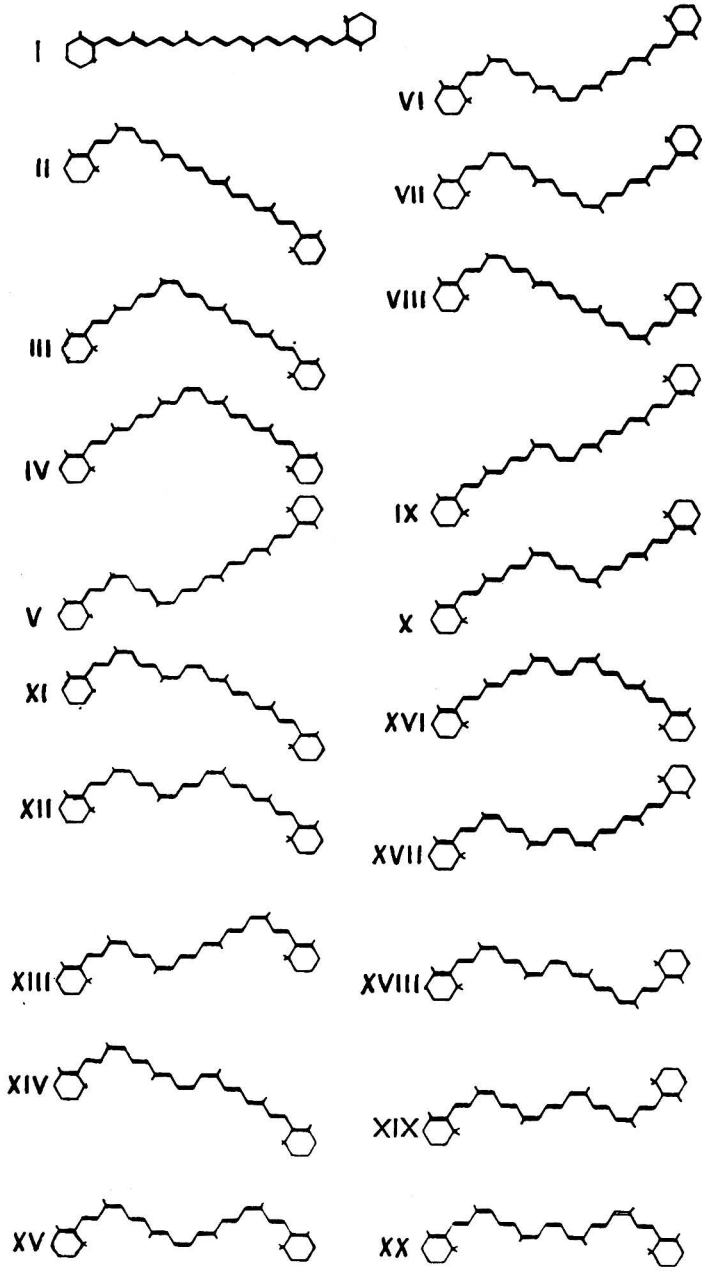

FIG. 2. Skeleton models of the twenty possible stereoisomers of β-carotene: all-*trans*-β-carotene, three mono-*cis*-β-carotenes, six di-*cis*-β-carotenes, six tri-*cis*-β carotenes, three tetra-*cis*-β-carotenes, and all-*cis*-β-carotene.

After this stage had been reached, an improvement in the method of extraction was brought about through the work of the Lever Bros. team consisting of Wilkinson, Edisbury and Gridgeman. The process of grinding with sand, followed by extraction with a 3:1 mixture of light petroleum of b.p. 40° to 60° C. and acetone, was replaced by extraction with light petroleum of b.p. 80° to 100° C. alone, without any initial grinding. This is now adopted in the method finally recommended by the Committee—Method E—which is described below.

METHOD E—NOW RECOMMENDED

Boil from 1 to 2 g. of grass meal with 50 to 60 ml. of light petroleum of b.p. 80° to 100° C. under reflux for 1 hour on a steam bath. Cool the flask and contents and filter the extract through sintered glass or any other suitable type of filter, or merely decant directly, on to a 2-inches by 1-inch column of bone meal. Rinse the flask and residue with small quantities of light petroleum (light petroleum of b.p. 40° to 60° C. may be used at this stage, for ease of subsequent removal if this happens to be desirable). Apply suction to the bone meal column, elute with light petroleum of b.p. 40° to 60° C. and concentrate if necessary. Estimate the carotene in the eluate, colorimetrically.

NOTES—

Boiling can be done in large Kjeldahl flasks, which have been found to provide sufficient condensing effect.

The bone meal should be extracted before use with a 3:1:1 mixture of petrol, acetone, and ether.

The bone meal should be of particle size passing a 120-line sieve but retained by a 200-line sieve. (Arrangements are being made for the preparation of a standard bone meal which can be supplied to anyone interested in this type of work.)

The bone meal column can be used for a large number of determinations. After repeated use it may be washed with acetone, which removes the bulk of the pigments. A further wash with light petroleum prepares the column for further tests.

The whole Committee carried out from 60 to 70 analyses on a sample of dried grass by Method D and an equal number by Method E. The average results by these two methods for all practical purposes can be considered to be identical. For this particular sample the average figures were:

By Method D: 335 mg. per kilo. By Method E: 330 mg. per kilo.

Further comparative average figures for a large number of tests were as follows:

By Method D: 129 mg. per kilo. By Method E: 135 mg. per kilo.
" " " 275 " " " " " " " 279 " " "

It has been shown that light petroleum of b.p. 80° to 100° C., without preliminary grinding of the sample with sand, is as efficient for the extraction of carotene as any other solvent or mixture of solvents. Its efficiency is only equalled by that of soaking the sample in the dark and in the cold for 48 hours with light petroleum of b.p. 40° to 60° C. This latter method, of course, is undesirable if an analysis is required quickly, as is usually the case. Previously, attempts to extract with boiling light petroleum of b.p. 40° to 60° C. had been made, but had been found to be inefficient. It seems that the light petroleum of b.p. 80° to 100° C. is much superior because of the higher temperature attained during extraction.

As mentioned previously, it was believed that about 30 per cent. of the so-called "carotene" obtained by Method B was not β-carotene. As a matter of academic interest as well as commercial importance, the Committee set out to get a more definite figure. For this purpose, 42 samples were analysed by Methods B and E, and it was found that the results by Method E were only 69 per cent. as great as those by Method B. Again this is an average figure, some meals giving, say, 60 per cent. and others 80 per cent., depending on the age of the meal and certain other factors.

From this work the following conclusions can be drawn:

(1) Light petroleum of b.p. 80° to 100° C. is an efficient solvent for the extraction of the carotenoids of grass.

(2) Bone meal is an efficient adsorbent for the separation of β-carotene from the other pigments in grass.

(3) Method E can be carried out more rapidly and easily than any other method.

REFERENCES

1. Carotene Committee of the Crop Driers Association, ANALYST, 1941, **66**, 334.
2. Seaber, W. M., *Ibid.*, 1940, **65**, 266.
3. Kon, S. K., and Thompson, S. Y., *J. Agric. Sci.*, 1940, **30**, Pt. IV, 636.
4. Moore, L. A., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 726.
5. Mann, T. Barton, ANALYST, 1944, **69**, 34.
6. Zechmeister, L., *Chem. Revs.*, 1944, **34**, No. 2, 267.

Discussion

Mr. W. M. Seaber said that in the past the estimation of carotene had been lengthy and tedious. He had been connected with this problem for a long time and had been interested to see the method develop in the direction of simplicity. He had tried the new method, not only on dried grass but also in general work, and had found it convenient and rapid. They owed a great deal to Mr. Barton Mann for bringing to light the advantages of bone meal as an adsorbent of non-carotene substances, and to the chemists at Messrs. Lever Brothers for introducing the use of light petroleum of higher boiling-point range than that previously used for the extraction. He was pleased to have taken part in the work of the Committee.

Mr. N. T. Gridgeman said that he had recently, on behalf of the Carotene Committee, statistically analysed the results of the final collaborative experiment in which methods D and E were compared. It had emerged that the reproducibility of the two methods was substantially the same (coefficient of variation = 4), and that method E yielded slightly, yet significantly, higher results (by about 3 per cent.) than method D. As in so many analytical techniques, reproducibility was found to be better within laboratories than between laboratories, and there may be good reason to believe that inter-laboratory differences (coefficient of variation = 8) were largely due to non-uniformity of instruments. Many laboratories perforce used "abridged" spectrophotometers, *e.g.*, photoelectric absorptiometers dependent upon optical filters and absorption standards. In β-carotene estimation potassium dichromate solution enjoyed an undeserved popularity as a suitable calibration medium—perhaps by false analogy with its undoubted virtues as a visual standard. The steepness of the slope of the absorption curve of potassium dichromate in the region of 450 mμ. rendered it highly unserviceable, and the position was worsened by the fact that most types of nominally identical optical filters exhibit marked variations in their transmission characteristics. He hoped that eventually a wholly satisfactory standard would be found; meantime perhaps the best expedient was to use petrol solutions of pure β-carotene.

Mr. K. A. Williams said he would like to support in the strongest possible terms Mr. Gridgeman's strictures on the use of potassium dichromate as a yellow colour standard. Whilst its use in visual colorimetry was defensible because of the limits of sensitivity of the human eye, it could not be defended for more accurate work. In spectroscopy or photo-electric measurements any standard must have an absorption curve very closely approximating to that of the test colour; it was but rarely that the curve of potassium dichromate did this.

Mr. T. Barton Mann said that from the point of view of spectroscopy one would naturally take readings at the peak of absorption. In photo-electric instruments employing filters he had found potassium dichromate relatively insensitive in so far as variations in concentration near that recommended, *i.e.*, 0·0158 per cent. (≡ 1 μg. of carotene per ml.) did not appreciably affect the graph. There was little difference between 0·0158 and 0·0164 per cent. solutions. He agreed that search should be made for an alternative standard, preferably a dye the colour of which would not be affected by change of *p*H.

Dr. C. H. Lea asked if differences in exposure to light in the course of analysis might cause a variable amount of change of the carotene.

Dr. Nelson pointed out that in method E there was no evaporation of solvent from the extract, as there was in method D.

Mr. Barton Mann said that oxidised carotene would be adsorbed on bone meal and not pass into the solution to be measured. The use of the higher-boiling light petroleum reduced risk of oxidation, because the higher temperature enabled the carotene to be extracted without previous grinding of the dried grass with sand. It would seem that the higher temperature removed the moisture of the grass meal and permitted the solvent to penetrate more readily.

Mr. Seaber remarked that light petroleum of b.p. 80° to 100° C. was apt to be rather variable in character and composition.

Mr. Williams suggested that perhaps *n*-heptane might prove more satisfactory. It had a boiling-point range of 98° to 100° C., and to ensure freedom from aromatic constituents it could be washed with sulphuric acid.

Mr. Barton Mann said that on the use of various petroleum fractions of lower or higher boiling-point ranges he would particularly like to draw the attention of analysts to a point that had arisen in the collaborative work of the Committee, *viz.*, that with change of solvent from one of lower to one of higher boiling-point there was a concomitant shift of peak absorption from a lower to a higher wavelength. For example, with light petroleum of b.p. 40° to 60° C. the E max. was 447 to 448 mμ., but with that of b.p. 80° to 100° C. it was 452 to 454 mμ. Dr. J. R. Edisbury had very kindly checked these findings and had furnished the speaker with the refractive indices of two such petroleum fractions, *viz.*, one of b.p. 40° to 60° C., 1·3656 and one of b.p. 80° to 100° C., 1·4002. These differences in absorption and refractive index were significant, perhaps not so much for users of spectrophotometers, by whom the extinction would normally be obtained at peak absorption, but certainly with colorimeters and instruments employing filters, where a change of wavelength would automatically introduce a change of colour. Pending further work along these lines it would seem advisable to adhere to the estimation "carotene in light petroleum of b.p. 80° to 100° C."

There could be no question that the method proposed did produce a solution containing the true carotene of grass meals and that intra-laboratory concordant results could be obtained. The question of inter-laboratory concordance appeared to be entirely a matter of instrument calibration, and it was to be

hoped that the Committee would tackle this problem, possibly along the lines of a master reference instrument of the photo-electric spectrophotometer type to which the owners of colorimeters could refer.

Dr. E. C. Wood asked if the accuracy of the new method had been checked by "recovery" experiments with known added amounts of pure β-carotene, and also if the method could be adapted to the analysis of substances containing vitamin A as well as carotenoids. He had read recently of an organic dyestuff, forming a stable solution, with an absorption spectrum sufficiently similar to that of carotene to make it a satisfactory standard instead of potassium dichromate. Perhaps one of the investigators could comment on this.

Mr. Barton Mann said he had no knowledge of any work on the recovery of carotene added to grass meals. The difficulty of such work would be very great. He had determined the efficacy of recovery of β-carotene from fat, using the bone meal treatment. A freshly prepared 0·03 per cent. standard, after saponification and extraction with ether, was subjected to the whole process of bone meal chromatography, and the figure returned was 0·0283 per cent. Having regard to the severity of the manipulation and the inherent errors of visual spectrophotometer estimation, one could conclude that the loss by such chromatography was nil or negligible.

With regard to vitamin A, Dr. T. W. Goodwin had found from his work with cod liver oil that vitamin A ester passes bone meal and vitamin A alcohol is adsorbed by it. If Dr. Wood had in mind the application of the method to biological material, for which saponification is usually necessary, then carotene would pass into the filtrate and vitamin A, as the alcohol after saponification, would remain adsorbed. He would refer Dr. Wood to "The separation of vitamin A from xanthophyll" (Mann, Analyst, 1943, **68**, 233); "A Chromatographic Method for Separating Free and Esterified Vitamin A" (Glover, Goodwin, and Morton, *Biochem. J.*, 1947, **41**, 94); "Relationship between Blood Vitamin A Level and Liver Stores in Rats" (*Ibid.*, 97).

Mr. Gridgeman said that the yellow substance referred to by Dr. Wood was methyl orange in aqueous solution, which had been suggested as a standard for β-carotene by R. J. Taylor in a recent paper (Analyst, 1946, **71**, 566). But it applied to β-carotene only in chloroform solution, in which the peak absorption occurred at about 460 mμ. No "control" experiments of the type Dr. Wood had in mind had been carried out, nor did he think they would be very valuable, as the separation problem was the extraction of carotene from grass cells.

In reply to a question by Mr. Bacharach, as to whether statistical analysis had isolated any inter-personal variations within laboratories, the speaker said that the inter-personal error had been studied in one laboratory and found to be negligible; but with colorimetric (in contrast with absorptiometric) methods some observational disagreements would almost certainly have to be reckoned with.

Later in the discussion Mr. Gridgeman deprecated the use of nitrogen for the removal of final traces of solvent, on the ground that it often contained some oxygen. Carbon dioxide was objectionable because of frosting of the cylinder valves. Hydrogen was always used in his laboratory and seemed to be the most suitable of the common gases for the purpose.

Notes

THE DETERMINATION OF LEAD AS IODATE IN GLASS ANALYSIS

We have recently carried out determinations of the lead content of three samples of lead glass (SiO_2 56%, PbO 30%, Al_2O_3 1%, CaO 0·5%, K_2O 7·5%, Na_2O 5%) by the gravimetric iodate method described by C. H. R. Gentry and L. G. Sherrington.[1] In every instance the lead iodate precipitate obtained showed a yellow discoloration, and a subsequent spectroscopic examination of one precipitate showed the presence of iron and titanium.

The results obtained are shown in Table I, together with results obtained in parallel determinations in which the lead was determined by separating and weighing it as sulphate.

Table I

Sample	PbO as iodate Per cent.	PbO as sulphate Per cent.	Difference Per cent.
A	29·66; 29·53	28·98; 28·94	0·6; 0·59
B	30·07; 29·98	29·48; 29·44	0·59; 0·54
C	30·42; 30·18	29·87; 29·75	0·55; 0·43

With sample A, the lead iodate precipitates, after being weighed, were dissolved through the sintered glass crucibles with warm dilute caustic soda solution, 20 ml. of hydrochloric acid were added and the solutions evaporated to small volume to eliminate iodic acid. Ten ml. of sulphuric acid were then added to each beaker and the contents evaporated to strong fuming. The determination of the lead by weighing as lead sulphate was completed in the usual manner. By this method the PbO content was found to be 29·07 and 28·94 per cent., compared with 28·98 and 28·94 per cent. found by the direct determination as sulphate.

The yellow coloration of the original lead iodate precipitates, together with the spectrographic evidence, suggested that iron might be present as an impurity. To explore further the influence of small amounts of iron on the determination of lead as iodate a series of standards was prepared containing known added quantities of iron. The iron was added in the form of ferric nitrate to a solution of pure lead nitrate of which the PbO content was approximately that of a 1-g. sample of the glasses under examination. The lead content was determined gravimetrically as iodate, as before. In Table II are given the results, together with the lead content determined as lead sulphate. Assuming that the iron is completely co-precipitated with the lead as ferric iodate, the presence of 0·07 per cent. of iron (expressed as Fe_2O_3) in a glass should theoretically give rise to a positive error of 0·20 per cent. ($Fe(IO_3)_3 \times 0{\cdot}1374 = Fe_2O_3$; $Pb(IO_3)_2 \times 0{\cdot}4005 = PbO$.) The actual difference found was 0·25 per cent.

TABLE II

PbO as sulphate Per cent.	Fe_2O_3 added Per cent.	PbO as iodate Per cent.	Difference Per cent.
29·80	0·0	29·91	0·11
	0·07	30·17	0·37
	0·14	30·42	0·62
	0·28	30·92	1·12

The iodate method has also been tried on glasses containing both barium and strontium in addition to lead; the results are shown in Table III.

TABLE III

Sample	PbO as iodate Per cent.	PbO after conversion of iodate to sulphate Per cent.
A	19·4; 19·4	18·5; 18·5
B	21·2; 21·2	19·6; 19·7

These precipitates also were badly discoloured and obviously contained iron. However, the iodic acid precipitation is a valuable method for effecting a preliminary separation of lead from barium and strontium.

Gentry and Sherrington point out that ferric iron precipitates with the lead but state that "the traces of iron in lead glasses are too insignificant to interfere with the lead determination."

Our results indicate that the iodate method for the determination of lead in glass can be applied only to samples known not to contain more than very small amounts of iron; 0·07 per cent. of Fe_2O_3, for example, can give an increase of 0·25 in the percentage figure for PbO.

If the lead iodate precipitate shows a yellow discoloration it is advisable to redissolve it and to reprecipitate the lead as sulphate.

REFERENCE

1. Gentry, C. H. R., and Sherrington, L. G., ANALYST, 1946, **71**, 31.

P. M. C. PROFFITT
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January, 1947

NOTE ON THE PREPARATION OF *CYCLO*HEXANE FOR SPECTROSCOPIC WORK

IN a number of analytical processes involving the use of absorption spectroscopy *cyclo*hexane is commonly employed as a solvent. Its suitability for this purpose depends on the absence of absorption bands, and for this reason traces of benzene, from which *cyclo*hexane is prepared by hydrogenation, must be removed.

By the method of Dolin[1] it was found that a recent delivery of *cyclo*hexane contained 0·4 per cent. of benzene.

Traces of benzene are usually removed by sulphonation, a process that involves repeated shaking with oleum followed by successive washes with water, permanganate solution and water. The *cyclo*hexane is then dried over calcium chloride and redistilled.

This process of purification is time- and labour-consuming, and it has been found that the benzene can be completely removed by adsorption on passing the *cyclo*hexane through a column of silica gel. This method has the merit of simplicity, it requires no attention, and the operation is conveniently allowed to run overnight.

The apparatus consists of a glass tube approximately 1100 mm. long, with an internal diameter of 10 mm. To one end is fused the cone portion of a B14 standard joint, and a sintered glass disc is sealed into the tube at a position approximately 20 mm. from the other end. A reservoir is made from a glass tube, 300 mm. long and 45 mm. wide, tapered at the bottom and fitted with the socket portion of a B14 standard joint.

The tube is packed with powdered silica gel, of a particle size which passes a 30-mesh sieve but is retained on a 150-mesh sieve. The packing is done by small additions, after each of which the tube is gently shaken by tapping on a pad of filter papers. Approximately 100 g. of silica gel are required to pack the tube, which is then fixed in a vertical position. The cylindrical glass reservoir is attached to the top of the tube and charged with 350 to 400 ml. of *cyclo*hexane. The liquid percolates slowly through the column, the *cyclo*hexane going forward and the benzene remaining behind in accordance with their relative adsorbabilities. At the end of 2½ hours, benzene-free *cyclo*hexane may be collected from the bottom of the column at the rate of 16 ml. per hour. If the operation is continued overnight the rate of collection very slowly drops to 14 ml. per hour by the following morning.

The purified *cyclo*hexane collected during the night is tested for freedom from benzene by the formolite reaction, which consists in adding 1 drop of the sample to a mixture of 10 ml. of concentrated sulphuric acid containing 0·5 ml. of 40 per cent. formaldehyde solution. In presence of traces of benzene a yellow to red coloration at once appears. Thereafter the *cyclo*hexane coming from the column is collected in 20-ml. portions, each being tested for freedom from benzene by the formolite reaction. The first fraction to show traces of benzene indicates the "break point" of the column and the end of the run.

Using the conditions and apparatus described above, a test run was started at 12 noon, and by 4 p.m. the following day 320 ml. of benzene-free *cyclo*hexane had been collected. The result of a spectroscopic test confirmed the absence of benzene and hence the suitability of the sample. In this run the "break point" occurred after 350 ml. of *cyclo*hexane had been collected.

Silica gel is an inexpensive adsorbent and a fresh 100-g. charge has been used for each run. There would, however, appear to be no difficulty in reconditioning the silica for further use.

I wish to thank the Government Chemist for permission to publish this note.

Reference

1. Dolin, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 242.

S. A. Ashmore
February, 1946

Department of the Government Chemist
The Government Laboratory
London, W.C.2

Ministry of Food

STATUTORY RULES AND ORDERS*

1947 No. 231. The Flour Confectionery (Control and Maximum Prices) (Amendment) Order, 1947. Dated February 10, 1947. Price 1d.

This amending Order, as from February 10, 1947,

(1) *expressly excludes black puddings and white (or mealy) puddings from the definition of flour confectionery;*

(2) *provides a new method of ascertaining the maximum price of flour confectionery where some or all of the ingredients are supplied by the purchaser;*

(3) *increases the ceiling price of flour confectionery from* 1s. 6d. *to* 2s. 6d. *a lb. (where the combined fat and sugar content amounts to* 14 *per cent. or more and the combined fat, sugar and dry egg solids content amounts to* 40 *per cent. or more), while at the same time reducing the ratio of maximum price to ingredient cost where* 1s. 6d. *per lb. is exceeded;*

(4) *increases the maximum price of uncooked pastry from* 9d. *to* 1s. 0d. *a lb.*

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

1947 **No. 650. The Food Standards (Mustard) (No. 2) (Amendment) Order, 1947. Dated April 11, 1947.** Price 1d.

As from April 16, 1947, *the standard for* mustard, compound mustard and mustard condiment *is hereby reduced from* 0·35 *per cent. to* 0·28 *per cent. of allyl isothiocyanate.*

— **No. 701 The Edible Oils and Fats (Control of Sales) (Amendment No. 2) Order, 1947. Dated April 19, 1947.** Price 1d.

From April 23, 1947, *this amending Order removes the restrictions on the sale of olive oil, almond oil, peach kernel oil (or apricot oil), teaseed oil and hazelnut oil to trade users.*

It revokes the previous amending Order, S.R. & O. No. 386 *of* 1946, *and substitutes the following definition of Edible Oil for that given in Article* 1 *of the principal Order, S.R. & O. No.* 672 *of* 1944.

"Edible oil" means any oil or fat used or capable of being used for human food, or in the manufacture, preparation or treatment of human food, and includes any oil or fat used or capable of being used for the greasing of tins, trays, or other utensils used in the cooking or preparation of food for human consumption, but does not include—

(*a*) "butter," "cooking fats" or "margarine" (as defined in the Fats, Cheese and Tea (Rationing) (No. 2) Order, 1946), or suet or peanut butter or peanut paste;
(*b*) any aromatic oil used for flavouring purposes;
(*c*) petroleum or any product of petroleum, as defined in the Petroleum (No. 2) Order, 1942;
(*d*) olive oil, almond oil, peach kernel oil (or apricot oil), teaseed oil, and hazelnut oil, including any mixture of any two or more of those oils but not including any mixture of any one or more of those oils with any other oil.

— **No. 757. The Labelling of Food (Amendment) Order, 1947. Dated April 25, 1947.** Price 1d.

From May 1*st*, 1947, *this amending Order exempts the following foods from the labelling requirements of the Labelling of Food Order*, 1946, *by adding them to Table B of the First Schedule of the order*, which table specifies foods wholly exempt from Article 2 of the Order when prepacked for sale as such and partly exempt when forming an ingredient of some other food—

Fresh fruit and vegetables other than potatoes (not including fruit or vegetables which are bottled, frozen, dried or otherwise processed, but so that for this purpose cleaning or the removal of extraneous or inedible matter shall not be regarded as processing).

Single toffee apples.

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 Specifications prepared by Technical Committee FCC/4—Solvents.

CH(FCC)9402—Draft Revised B.S.573 Dibutyl Phthalate.
CH(FCC)9401—Draft Revised B.S.508 Normal Butyl Alcohol.
CH(FCC)9403—Draft Revised B.S.574 Diethyl Phthalate.
CH(FCC)9404—Draft Revised B.S.575 Carbon Tetrachloride.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Colorimetric Estimation of Proteins in Various Body Fluids. A. A. Albanese, V. Irby, and B. Saur (*J. Biol. Chem.*, 1946, **166**, 231–237)—It was found that when alkaline solutions of proteins, isolated from body fluids by treatment with trichloroacetic acid or sodium sulphate solutions of appropriate concentration, were submitted to the modified Sakaguchi reaction of Albanese and Frankston (*Ibid.*, 1945, **159**, 185) arginine values were obtained which, on multiplying by a suitable factor, resulted in protein values closely approximating to those obtained by the micro-Kjeldahl method.

Method—Total proteins of blood—Transfer 0·5 ml. of plasma to 10 ml. of 10 per cent. trichloroacetic acid in a 15-ml. conical centrifuge tube, leave for 10 min. at room temperature, and centrifuge for 10 min. at 3,000 r.p.m. Decant the supernatant liquid into a 100-ml. Kjeldahl flask and treat in the usual manner for the estimation of non-protein nitrogen by the micro-Kjeldahl method. Wash the precipitated protein by re-suspension in 5 ml. of 5 per cent. trichloroacetic acid, centrifuge, and decant the supernatant liquid. Dissolve the washed protein in 10 ml. of 10 per cent. sodium hydroxide solution and use 1-ml. aliquots for the arginine estimation.

Globulin in blood—Add 0·5 ml. of plasma to 10 ml. of 22 per cent. sodium sulphate solution in a round-bottomed centrifuge tube and incubate at 37° C. for 3 hours or more. At the end of the incubation period, add 3 ml. of ether, shake vigorously, and centrifuge for 15 min. at 3,000 r.p.m. Separate the protein that collects at the ether-water interface from both liquid layers by careful decantation, and wash the protein cake by re-suspension in 10 ml. of 22 per cent. sodium sulphate and 3 ml. of ether, centrifuge, and decant as described before. Dissolve the washed protein in 10 ml. of 10 per cent. sodium hydroxide solution, and warm the mixture to remove residual ether. Use 1-ml. aliquots for the arginine estimation. When only limited amounts of the sample are available, the estimations can be performed on 0·1 to 0·2 ml. of plasma by proportionately reducing the volume of the reagents.

Total proteins in urine and ascitic fluid—To 5 ml. in a 15-ml. centrifuge tube add 2 ml. of 30 per cent. trichloroacetic acid, leave for 10 min., and centrifuge for 10 min. at 3,000 r.p.m. Discard the supernatant liquid and re-suspend the protein in 5 ml. of 5 per cent. trichloroacetic acid and again centrifuge. Dissolve the protein in 5 ml. of 10 per cent. sodium hydroxide solution and use 1-ml. aliquots for the arginine estimation.

Globulin in urine and ascitic fluid—Incubate 10 ml. for 3 hours or more at 37° C. with 10 ml. of 44 per cent. sodium sulphate solution in a 50-ml., round-bottomed centrifuge tube. Add 3 ml. of ether, shake, centrifuge, and re-suspend the protein in 10 ml. of 22 per cent. sodium sulphate solution and 3 ml. of ether. Discard the supernatant liquid and dissolve the globulin in 10 ml. of 10 per cent. sodium hydroxide solution.

Colour development—To the 1-ml. aliquots, add 5 ml. of water and 1 ml. of a 0·1 per cent. solution of α-naphthol in 95 per cent. ethanol. After 5 min., add 1 ml. of 0·06 *N* sodium hypochlorite and, exactly 1 min. later, 2 ml. of 20 per cent. urea solution. Leave for 5 min., and evaluate the colour in a Klett-Summerson colorimeter with filter S.54. Treat a 1-ml. aliquot of a* standard containing 100 μg. of arginine per ml. and a reagent blank in the same way, and calculate the amount of arginine in the sample from the value found for the standard. Multiply the arginine values by 19·2, a factor derived from the observed mean arginine content (5·2 per cent.) of albumin and globulin of the body fluids. The albumin content is the difference between the total protein and the globulin values. F. A. R.

Colorimetric Estimation of Tocopherol. A. Emmerie (*Rec. Trav. Chim.*, 1946, **65**, 489–492)—The effect of cholesterol on the reaction between *dl*-α-tocopherol and the ferric chloride-dipyridyl reagent was investigated, as cholesterol had been alleged by other workers to interfere with the reaction. This was confirmed with a commercial sample of cholesterol, and it was shown that inhibition was much stronger in glacial acetic acid than in alcohol solution. Purified cholesterol, however, did not inhibit the reaction and the substance responsible for the inhibition could be removed from the commercial material by filtration through Floridine XS. The nature of this impurity is not known, but ergosterol was without effect. F. A. R.

Microbiological Estimation of Amino Acids. III. Methionine. C. M. Lyman, O. Moseley, B. Butler, S. Wood, and F. Hale (*J. Biol. Chem.*, 1946, **166**, 161–171)—The method uses *Leuconostoc mesenteroides* as the test organism and a medium in which most of the amino-acid nitrogen is supplied by hydrogen-peroxide-treated peptone. The results were compared with those obtained when *Streptococcus faecalis* R was used.

Method—Maintain the organism *L. mesenteroides* P–60 by weekly transfers as stabs in solid medium containing peptonised milk 1 per cent., tryptone 1 per cent., filtered tomato juice 200 ml. per litre of medium, and agar 1 per cent. Inoculate with washed cells from 18 hours-old cultures grown on a liquid medium of the same composition except for the omission of the agar.

The composition of the basal medium for the estimation of methionine by means of this organism is given in Table I.

TABLE I

MEDIUM* FOR DETERMINATION OF METHIONINE WITH *Leuconostoc mesenteroides*

H_2O_2-treated peptone	15	g.
Glucose	40	,,
Sodium acetate	24	,,
Ammonium chloride	12	,,
l(−)-Tryptophan	100	mg.
dl-Tyrosine	200	,,
l(−)-Cystine	200	,,
Adenine sulfate	20	,,
Guanine	20	,,
Uracil	20	,,
Aneurine	2	,,
Pyridoxine	4	,,
Calcium pantothenate	4	,,
Riboflavine	4	,,
Nicotinic acid	4	,,
Biotin	10	μg.
Folic acid (synthetic)	3	,,
p-Aminobenzoic acid	0·2	,,
Salt solution 1†	10	ml.
,, ,, 2‡	10	,,
,, ,, 3§	10	,,

Neutralise and dilute to 1 litre.

* Medium for 200 cultures of 10 ml. final volume (5 ml. of the above medium per culture).

† Salt solution 1, K_2HPO_4 25 g., KH_2PO_4 25 g., water 250 ml.

‡ Salt solution 2, $MgSO_4.7H_2O$ 10·0 g., NaCl 0·5 g., $MnSO_4.4H_2O$ 0·5 g., water 250 ml.

§ Salt solution 3, $FeSO_4.7H_2O$ 0·5 g., water 250 ml.

To prepare the hydrogen-peroxide-treated peptone, dissolve 50 g. of Bacto-peptone in 250 ml. of water and add 250 ml. of 2 *N* hydrochloric acid after the peptone is completely dissolved. Add 2·8 g. of 30 per cent. hydrogen peroxide, leave overnight at room temperature, and then steam for 30 min. at atmospheric pressure. Stir while hot, cool, neutralise with sodium hydroxide, and steam for one hour to destroy the excess of hydrogen peroxide. Dilute to a final volume of 1 litre.

The acid produced after 4 days' incubation is a measure of the growth of the organisms, the general procedure for carrying out the assays being the same as that used in assays with *Lactobacillus arabinosus*, except that a constant-temperature water-bath at 35° C. was used instead of an incubator, and the period of incubation was 4 days instead of 3 days.

The medium used for the estimation of methionine by means of *S. faecalis* R is given in Table II.

TABLE II

MEDIUM* FOR DETERMINATION OF METHIONINE WITH *Streptococcus faecalis* R

Glucose	40 g.
Succinic acid	20 ,,
Sodium acetate (anhydrous)	6 ,,
Adenine sulphate	10 mg.
Guanine	10 ,,
Uracil	10 ,,
Xanthine	10 ,,
Riboflavine	1 ,,
Nicotinic acid	2 ,,
Pyridoxamine	0·8 ,,
Aneurine chloride	0·4 ,,
Calcium pantothenate	0·4 ,,
Biotin	2 μg.
p-Aminobenzoic acid	2 ,,
Folic acid (synthetic)	10 ,,
Salt solution 1	10 ml.
,, ,, 2	10 ,,
,, ,, 3	10 ,,
dl-Alanine	400 mg.
l(+)-Arginine	400 ,,
dl-Aspartic acid	800 ,,
l(−)-Cystine	400 ,,
dl-Glutamic acid	800 ,,
Glycine	400 ,,
l(−)-Histidine	400 ,,
*dl-Iso*leucine	400 ,,
dl-Leucine	400 ,,
l(+)-Lysine	400 ,,
dl-Phenylalanine	400 ,,
l(−)-Proline	400 ,,
dl-Serine	400 ,,
dl-Threonine	400 ,,
l(−)-Tryptophan	200 ,,
dl-Tyrosine	400 ,,
dl-Valine	400 ,,

Add 12 gm. of sodium hydroxide pellets and finish neutralising with sodium hydroxide solution. Dilute to 1 litre.

* Medium for 200 cultures of 10 ml. final volume (5 ml. of the above medium per culture).

The assay procedure is the same as with *L. mesenteroides* except that the period of autoclaving the tubes for sterilisation is 10 min. and the incubation time is 3 days.

The results obtained by the two microbiological methods were in very close agreement with one another and with the results obtained by the method of McCarthy and Sullivan (*Ibid.*, 1941, **141**, 871) as modified by Csonka and Denton (*Ibid.*, 1946, **163**, 329). F. A. R.

Iodimetric Estimation of Glucose in Urine and Blood. E. C. Noyons (*Rec. Trav. Chim.*, 1946, **65**, 485–488)—*Method*—Prepare a protein-free filtrate of blood by the Fujita-Iwatake method, using cadmium sulphate and sodium hydroxide; the Folin-Wu method gives unsatisfactory results, apparently because it fails to remove certain interfering substances that are precipitated by means of the cadmium reagent. Add to this filtrate 20 ml. of 0·1 *N* iodine and 5 ml. of a buffer solution prepared by dissolving 3·6 g. of KH_2PO_4 in water, adding 37·5 ml. of 4 *N* sodium hydroxide, and diluting to 100 ml. After 30 min., acidify with 10 ml. of 25 per cent. hydrochloric acid and back-titrate with 0·1 *N* sodium thiosulphate. In the estimation of glucose in diabetic urine, it is advisable to treat the urine with Norit carbon (0·5 g. per 100 ml.) before addition of the iodine. The results obtained by this method agreed with those obtained by other methods, and recoveries of added glucose were satisfactory. F. A. R.

Colorimetric Estimation of Methionine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1946, **166**, 313–320)—Difficulties were encountered in applying the method of McCarthy and Sullivan (*Ibid.*, 1941, **141**, 871) as modified by Hess and Sullivan (*Ibid.*, 1943, **151**, 635) to the estimation of methionine in protein foods; a procedure was devised that overcame these difficulties. The results were in close agreement with those obtained by a microbiological method (*cf.* following abstract).

Procedure—Hydrolyse the protein either by means of acid or by digestion with papain. For the acid hydrolysis, heat 1 g. of the protein under reflux for 18 hours with 25 ml. of 20 per cent. hydrochloric acid. Concentrate the hydrolysate to about 5 ml., heat with a small quantity of Norit and filter. Wash the residue on the filter with hot water and dilute the combined filtrate and washings to 100 ml. with water. Alternatively, carry out a papain digestion as described by Horn and Jones (*Ibid.*, 1945, **157**, 153; *Abst.* ANALYST, 1945, **70**, 266) but, before the digest is made up to volume, pass nitrogen through the solution for 15 min. to remove hydrogen cyanide.

Pipette duplicate, 2-ml. samples of the hydrolysate into Evelyn colorimeter tubes and to each add 3 ml. of water and 1 ml. of 5 *N* sodium hydroxide. To one of the tubes add 0·1 ml. of 10 per cent. sodium nitroprusside solution. Leave for 10 min. with frequent shaking and then add 2 ml. of 3 per

cent. glycine solution to each tube. Leave for another 10 min. with frequent shaking and then add slowly to each tube, with constant agitation, 2 ml. of concentrated orthophosphoric acid. After 5 mins., evaluate the colour in an Evelyn colorimeter using a No. 540 filter. Use a tube containing 10 ml. of water to adjust the instrument to 100. Calculate the methionine content from the reading so obtained by reference to a calibration curve prepared as follows:

Prepare a standard methionine solution by dissolving 100 mg. of *dl*-methionine in 5 ml. of 20 per cent. hydrochloric acid, and dilute to 100 ml. with water. Into an Evelyn colorimeter tube put 2 ml. of a solution of the following composition (mg. per 100 ml.):

Alanine ..	20	Leucine ..	100
Arginine ..	48	Lysine ..	60
Aspartic acid	60	Norleucine ..	30
Cystine ..	*10	Phenylalanine	40
Glycine ..	5	Proline ..	80
Glutamic acid	210	Serine ..	60
Histidine ..	30	Threonine ..	45
Hydroxyproline	10	Tryptophan	12
*Iso*leucine ..	50	Tyrosine ..	65
		Valine ..	60

* A relatively high proportion of cystine was added, since casein contains less of this amino acid than most proteins.

together with 3 ml. of water, 1 ml. of 5 *N* sodium hydroxide and 0·1 ml. of 10 per cent. sodium nitroprusside solution. Into other colorimeter tubes put 2 ml. of the amino-acid solution, 1 ml. of the standard methionine solution containing amounts ranging from 0·1 to 1·0 mg., 2 ml. of water, 1 ml. of 5 *N* sodium hydroxide, and 0·1 ml. of 10 per cent. sodium nitroprusside solution. After 10 min., add 2 ml. of 3 per cent. glycine solution to each tube and continue the procedure as described for the protein hydrolysate. The logarithms of the readings plotted against concentrations give a straight line. Recoveries of methionine added to an acid hydrolysate of arachin ranged from 96 to 102 per cent. of the theoretical. F. A. R.

Microbiological Estimation of Methionine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1946, **166**, 321–326)—*Lactobacillus arabinosus* 17–5 was used, together with the following basal medium:

Glucose	20 g.
Sodium acetate (anhydrous) ..	12 ,,
Salts A	
K_2HPO_4	1 ,,
KH_2PO_4	1 ,,
Salts B	
$MgSO_4.7H_2O$	400 mg.
$MnSO_4.4H_2O$	20 ,,
NaCl	20 ,,
$FeSO_4.7H_2O$	20 ,,
Adenine	100 ,,
Guanine	100 ,,
Uracil	100 ,,
Aneurine chloride	2·0 mg.
Pyridoxine hydrochloride ..	0·4 ,,
Calcium pantothenate ..	0·4 ,,
Riboflavine	0·4 ,,
Nicotinic acid	0·8 ,,
p-Aminobenzoic acid	0·4 ,,
Biotin	0·01 ,,
Folic acid	30 μg.
dl-Alanine	80 mg.
l(+)-Arginine	96 ,,
dl-Aspartic acid	240 ,,
dl-Glutamic acid + H_2O ..	940 ,,
Glycine	400 ,,
l(−)-Histidine hydrochloride + H_2O	54 ,,
l(−)-Hydroxyproline	20 ,,
*dl-Iso*leucine	50 ,,
dl-Leucine	400 ,,
dl-Lysine hydrochloride ..	300 ,,
dl-Norleucine	120 ,,
dl-Phenylalanine	160 ,,
l(−)-Proline	140 ,,
dl-Serine	240 ,,
dl-Threonine	180 ,,
l(−)-Tryptophan	200 ,,
l(−)-Cystine	400 ,,
l(−)-Tyrosine	130 ,,
dl-Valine	240 ,,
Solution brought to 1000 ml. vol. *p*H 6·8	

* "Potency 5000."

Procedure—Carry the organism on stab cultures, using a medium of the following composition: glucose 1 g., Bacto yeast extract 1 g., and agar 1·5 g. per 100 ml. Store in a refrigerator and subculture each month. Prepare the inoculum for the assay by transferring a small amount of growth from the stab culture to a centrifuge tube containing 10 ml. of a medium consisting of glucose 1 g., Bacto yeast extract 1 g., and "Difco liver infusion" 100 ml. Incubate at 35° C. for 24 hours, centrifuge, wash the cells with sterile saline, and suspend in 5 ml. of sterile saline. Use one drop of the suspension to inoculate each tube in the assay.

Hydrolyse a sample containing about 1 g. of protein by heating for 24 hours under reflux with 25 ml. of 20 per cent. hydrochloric acid. Concentrate the hydrolysate to a small volume and boil for a few minutes with a little Norit. Filter and dilute the filtrate to 100 ml. with water. Take 10-ml. aliquots of this solution for assay, adjust to *p*H 6·8, and dilute to 1 litre. Store the solutions under toluene in a refrigerator.

Put 5-ml. quantities of the basal medium into a series of tubes and to one set add sufficient of a standard solution of *l*(−)-methionine to give concentrations ranging from 0 to 20 μg. per 10 ml.; prepare each concentration in quadruplicate. To another set of tubes add 1, 2, 3, and 4 ml. of the protein hydrolysate. Dilute all the solutions to 10 ml., autoclave at 15 lbs. for 15 min., and inoculate with one drop of the *L. arabinosus* suspension. Incubate at 35 to 36° C. for 72 hours, and titrate the lactic acid with 0·1 *N* sodium hydroxide, using bromothymol blue as indicator. Calculate the methionine content of the protein hydrolysate by

reference to the standard curve. Satisfactory agreement was obtained in replicate experiments, and recoveries of methionine added to casein or gelatin hydrolysates ranged from 96 to 102 per cent.

F. A. R.

Organic

2:4-Dinitrophenylsemicarbazide. Reagent for Aldehydes and Ketones. J. L. McVeigh and J. D. Rose (*J. Chem. Soc.*, 1945, 713–714)—2:4-Dinitrophenylsemicarbazide (Kniphorst, *Rec. Trav. Chim.*, 1925, **44**, 724) gives with aldehydes and ketones characteristic derivatives which are insoluble in water and very sparingly soluble in the common organic solvents.

Preparation of 2:4-dinitrophenylsemicarbazide. To 150 g. of 50 per cent. w/w aqueous solution of hydrazine hydrate in 2 litres of boiling ethanol, add 200 g. of N-2:4-dinitrophenyl-N′-nitrourea (Reudler, *Ibid.*, 1914, **33**, 35) during 0·75 hr. Each addition causes vigorous effervescence (nitrous oxide) and, after 30 min., crystals of the semicarbazide begin to separate. Heat under refluxing conditions on the steam-bath for 1 hour, cool to 10° C., and collect the yellowish-brown crystals, wash with a little alcohol, and dry (yield, 143 g.). Recrystallise from alcohol; m.p. 195–196° C. (decomp.). The hydrochloride, from alcohol, has m.p. 202–203° C. (Found: N, 25·1. $C_7H_7O_5N_5$.HCl requires N, 25·2 per cent.)

Method of test, using benzaldehyde. To 0·31 g. of 2:4-dinitrophenylsemicarbazide in 40 ml. of boiling alcohol, add 0·15 g. of benzaldehyde and 1 drop of concentrated hydrochloric acid. Boil for 5 min., during which time yellow crystals begin to separate, and cool to 10° C. Collect the yellow needles (0·31 g.), wash with 5 ml. of alcohol, and dry; m.p. 232° C. (decomp.), unchanged by recrystallisation from chlorobenzene. (Found: N, 21·0. Calc. for $C_{14}H_{11}O_5N_5$: N, 21·3 per cent.). The 2:4-dinitrophenylsemicarbazones of the following aldehydes and ketones are given below, melting points of the derivatives being given in brackets.

Formaldehyde (207° decomp.), acetaldehyde (225° decomp.), crotonaldehyde (230° decomp.), *n*-butyraldehyde (196° decomp.), *iso*butyraldehyde (209°), furfuraldehyde (227°), benzaldehyde (232°*), *p*-dimethylaminobenzaldehyde (247° decomp.), anisaldehyde (248°), *o*-chlorobenzaldehyde (248°), cinnamaldehyde (231° decomp.), methyl ethyl ketone (235° decomp.), methyl *n*-propyl ketone (207° decomp.), methyl *iso*butyl ketone (225° decomp.), methyl *n*-hexyl ketone (192° decomp.), ethyl acetoacetate (179°), 5-diethylaminopentan-2-one (175°), di-*iso*propyl ketone (202–203°), *cyclo*hexanone (234°), benzoin (228°), chalkone (226°), benzylideneacetone (246°), acetophenone (245° decomp.), deoxybenzoin (228°).

Avoid ethers as solvents for recrystallising the semicarbazones; from dioxan, many of the substances retain solvent of crystallisation which is removed completely only on prolonged heating at 110° C. under reduced pressure.

The semicarbazones can be hydrolysed by boiling with 2 *N* sulphuric acid, and the aldehyde or ketone recovered by steam distillation. E. M. P.

Identification of Aryl Iodides. J. C. Nichol and R. B. Sandin (*J. Amer. Chem. Soc.*, 1945, **67**, 1307–1308)—Iodoso chlorides have been used for the identification of some aryl iodides. The derivatives are quickly prepared in good yield and require no purification. They can be titrated quantitatively with standard sodium thiosulphate solution after addition of an acid and potassium iodide (Sandin, *Chem. Revs.*, 1943, **32**, 257, 259). They have the disadvantages that the melting-points or decomposition points depend upon the rate of heating, and that iodoso chlorides are not stable over a period of time. However, the authors have obtained consistent "melting-points" by heating rapidly at a uniform rate, and carrying out the determinations shortly after the preparation of the derivative. The melting-point of an iodoso chloride

BENZENE DERIVATIVES

Dichloride of benzene derivative substituted as below	M.p., ° C., uncorr.		Equivalent weight		
	After 10–15 mins.	After 1 hr.	After 15 min.	After 1 hr.	Calc.
I	120–121	118–119	138	138·2	137·5
1-CH_3,2-I	91	90–91	146·5	145·9	144·5
1-CH_3,3-I[a]	104–105	99–100	145·7	145·4	144·5
1-CH_3,4-I[a]	100	96–100	146·8	153·1	144·5
1-Cl,2-I[b]	96–97	96–97	155·5	155·5	154·7
1-Cl,3-I[b]	99–100	97–98	155·3	157·3	154·7
1-Cl,4-I	115	115	156·2	156·7	154·7
1-Br,3-I	108–109	108–109	181·4	185·1	177·0
1-Br,4-I	124–125	123–124	178·9	181·3	177·0
1:4-di-I[c]	147–148	146–147	203·8	201·3	200·5
1-C_6H_5,4-I	110	108	178·8	187	175·5
1:4-di-CH_3,2-I	87–89	84	153·3	153·9	151·5

[a] An equal-part mixture melts at 80–82° C. [b] An equal-part mixture melts at 80° C.
[c] An iodoso chloride is produced instead of the expected di-iodoso chloride.

* Kniphorst (*loc. cit.*) gives 223° C.

is taken as the temperature at which the tube begins to fill with gas and liquid. All melting-points were determined with Shriner and Fuson's apparatus (*"The Systematic Identification of Organic Compounds,"* 2nd Edn., John Wiley & Sons, Inc., New York, 1940, pp. 85–87).

Preparation of iodoso chlorides—Dissolve 1 g. of solid aryl iodide, or 1 ml. of liquid compound, in 3 to 15 ml. of dry chloroform, depending on the solubility. Cool to 0° C., and pass a slow current of dry chlorine through the solution. Usually the iodoso chloride separates pure as yellow crystals. If the crystals do not separate, add 10 ml. of low-boiling ligroin. Filter by suction, wash with ligroin, and air-dry for 10 min., maintaining the suction.

Determination of equivalent weights—Place 0·5 to 1 g. of the freshly prepared and air-dried iodoso chloride in a 250–ml. Erlenmeyer flask with 50 ml. of water, 10 ml. of chloroform, 10 ml. of glacial acetic acid, and a small excess of solid potassium iodide. Titrate the liberated iodine with standard sodium thiosulphate solution.

Results are shown in the Table opposite.

Unsatisfactory results were obtained with *o*-bromoiodobenzene and 4-iodo-1:3-dimethylbenzene.

E. M. P.

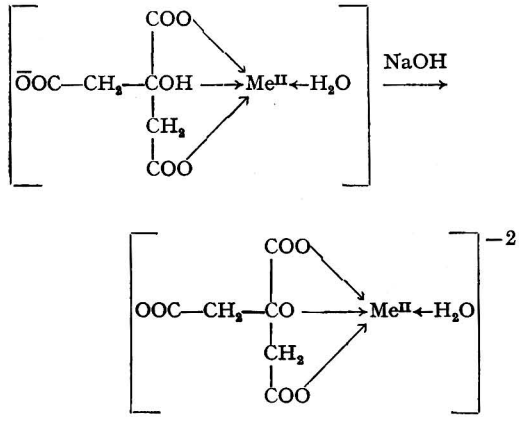
Determination of "Active" Hydrogen. E. A. Braude and E. S. Stern (*J. Chem. Soc.*, 1946, 404–406)—The use of the reaction with magnesium methyl iodide for the determination of "active" hydrogen by measurement of the methane evolved was first suggested by Tschugaeff (*Ber.*, 1902, **35**, 3912), and further developed by Hibbert and Sudborough (*J. Chem. Soc.*, 1904, **85**, 933) and by Zerewitinoff (*Ber.*, 1907, **40**, 2023; ANALYST, 1907, **32**, 299) who, owing to the high vapour pressure of diethyl ether, used di-*iso*amyl ether as the solvent. Other solvents such as anisole and pyridine have been tried, but di-*iso*amyl ether continues to be employed (*cf.* Hollyday and Cottle, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 774). The preparation of the di-*iso*amyl ether reagent is laborious owing to the difficulty of rendering the solvent completely anhydrous and free from *iso*amyl alcohol; it is relatively expensive, and is not stable for long periods unless special precautions are taken. Moreover, the apparatus is cumbersome, and difficult to clean and dry. A method has now been developed, with diethyl ether as solvent, in which these disadvantages are overcome and which requires no special apparatus other than a simple nitrometer. It is applicable, however, only to relatively non-volatile compounds with functional groups reacting completely at room temperature.

Procedure—To prepare the reagent, treat 35 g. (0·25 g.-mol.) of methyl iodide with 8 g. of magnesium in 200 ml. of sodium-dry ether and decant the liquid into a 300-ml. brown-glass, stoppered bottle in which air has been displaced by oxygen-free nitrogen. After some hours, re-decant the reagent and adjust its volume to 250 ml. The apparatus consists of an ordinary 50-ml. nitrometer filled with dry mercury, a rubber stopper carrying a small, cylindrical, graduated, and stoppered dropping funnel being fitted into the cup. Introduce about 5 ml. of dry ether above the mercury in the measuring limb of the nitrometer. Weight 50 to 200 mg. of the sample in a wide specimen tube, place this in the cup of the nitrometer and insert the stopper and dropping funnel tightly. Adjust the mercury to the zero mark with the tap of the nitrometer and that of the graduated funnel open, and then close the latter, and test the nitrometer for absence of leaks. Place 3 ml. of the reagent in the funnel and allow about 2 ml. to enter the specimen tube in the cup, drop by drop, until no further reaction occurs. Adjust the mercury level in the reservoir limb to slightly above that in the measuring limb and allow the apparatus to stand for 3 to 5 min. Finally adjust the mercury level and measure the volume of gas evolved. If the tap of the dropping funnel is greased, a blank determination must be made, and this should not exceed 2 ml. per ml. of reagent used. If draughts are excluded and room temperature is not too high, constant readings can be obtained over periods of at least 15 min. No difficulties arising from slow absorption of oxygen by the reagent have been experienced. From the nitrometer reading deduct the volume of reagent run in and the blank value (if any) to obtain the volume of methane evolved at a pressure equal to atmospheric pressure (p) less the vapour pressure (p′) of ether at the temperature of the determination. If W is the weight in grams of the sample, of molecular weight M, and T is the absolute temperature, the theoretical volume (ml.) of methane per atomic equivalent of "active" hydrogen is given by $\frac{22400\,pT}{273(p-p')} \times \frac{W}{M}$. Determinations with six standard substances, *viz.*, *cyclo*hexanol, benzyl alcohol, phenol, quinol, acetic acid, and benzoic acid gave results consistent to within ±1 or 2 parts in 100.

A. O. J.

Metallic Complexes of Tartrates and Citrates; their Structure and Behaviour in Dilute Solutions. I. Cupric and Nickelous Complexes. M. Bobtelsky and J. Jordan (*J. Amer. Chem. Soc.*, 1945, **67**, 1824–1831)—Measurements of conductivity, light absorption, and *p*H, and polarograph experiments, show that several bivalent cations, in dilute solution, form complexes with trisodium citrate and disodium tartrate, but not with free citric or tartaric acid. In this paper a study of the copper and nickel complexes is detailed. In all the complexes the molar ratio between the cation

and citrate or tartrate is 1:1. When the complexes are formed, the conductivities of the solutions are lowered and the extinctions are increased considerably. The complexes behave as if they are monobasic acids, and are neutralised by one equivalent of alkali at *p*H 6 to 8 to form more stable complexes, which are more suitable than the acidic complexes for use in retaining cations in solution during analytical procedures. When the neutralisation is carried out with sodium hydroxide, a remarkable increase in the extinctions of nickel and copper citrate solutions occurs. Addition of a second equivalent of sodium hydroxide to these solutions causes a decrease in the extinctions. The reaction caused by the addition of the first equivalent of sodium hydroxide to citrate complexes may be formulated as:



B. A.

Inorganic

Oxidation of Certain Metallic Hydroxides by a Current of Air. P. Fireman (*J. Amer. Chem. Soc.*, 1945, **67**, 1447–1449)—(*a*) *Cobaltous hydroxide*—On drawing air through a suspension of freshly precipitated cobaltous hydroxide at room temperature in the presence of varying excess amounts of sodium hydroxide, grey-brown to dark brown products are obtained. The active oxygen contents, determined by absorption in a known excess of sodium oxalate solution and back-titration with standard permanganate, vary from 1·21 to 3·62 per cent., that of Co_6O_7 being 3·44. All the products after ignition in air between 260° C. and 270° C. show an active oxygen content tending to the value of 6·64 per cent., which corresponds to cobaltosic oxide, Co_3O_4. On drying at temperatures up to about 100° C., the degree of oxygen absorption shows no tendency to become constant.

In a boiling suspension, containing a minimum amount of sodium hydroxide, the degree of oxidation in the mother liquid is much greater, the active oxygen content of the air-dried product being higher than that of the ignited product, cobaltosic oxide.

(*b*) *Manganous hydroxide*—Using a boiling suspension, and in the presence of moderate excesses of either manganous ions or sodium hydroxide, the product obtained when air is passed through is always manganosic oxide. At room temperature, the oxygen content is slightly above that required for manganosic oxide, irrespective of the presence of moderate excesses of either manganese or alkali, and of the duration of the experiments.

Solid manganosic oxide absorbs oxygen when heated to 200° C. in air.

On prolonged ignition and after absorption appears complete, the composition of manganosic oxide seems to fluctuate by 1 part in 180, probably owing to variations in atmospheric pressure.

M. E. D.

Quantitative Determination of Tungsten, Molybdenum, and Vanadium alone and in the presence of Ferric Iron, with 8-Hydroxyquinoline. R. Nierickerand W. D. Treadwell (*Helv. Chim. Acta*, 1946, **29**, 1472–1483)—The *p*H values at which quantitative precipitation of 8-hydroxyquinoline complexes occurs are for ferric iron 2·8 to 11·2, for tungstic oxide 4·95 to 5·65, for molybdenum oxide 3·70 to 7·40, and for vanadium trioxide 2·7 to 6·1. The complexes may be weighed as such, or ignited to the oxides at temperatures below 500° C., or determined titrimetrically with bromate. All the complexes can be precipitated simultaneously at *p*H 5·0 to 5·5.

Method—Adjust to *p*H 7·3 with 2 *N* sodium hydroxide a solution containing tungstate, molybdate, and vanadate ions, and heat to boiling. Add a 4 per cent. solution of 8-hydroxyquinoline in 50 per cent. alcohol to an excess of 50 to 100 per cent. Of the three metals present the molybdenum complex precipitates at this *p*H; adjust to *p*H 5·0 to 5·5 by means of 2 *N*-acetic acid and phenol red to precipitate the three compounds together. Coagulate the precipitate by heating for 5 to 10 min., and filter rapidly; use the hot filtrate to effect quantitative transfer. Wash 3 or 4 times with a small quantity of hot water. Dry to constant weight at 120° to 140° C. Ammonium ions up to a concentration representing 1 to 5 ml. of concentrated aqueous ammonia have no effect. Precipitation is quantitative in the presence of 0·01 *M* phosphoric acid after neutralisation to *p*H 5·0, but is only partial in 0·1 *M*- and is completely arrested in 1·0 *M* phosphoric acid. Results are accurate to 1 in 250 on contents lower than 20 mg., and to 1 in 1000 on 50-mg. samples.

To facilitate low-temperature conversion to oxides it is necessary to remove the 8-hydroxyquinoline by heating with (*a*) oxalic acid or (*b*) ammonia. (*a*) Treat the complex in a Gooch crucible with 5 times its weight of moist, powdered oxalic acid, cover, and heat for 1 hr. at 150° C. in a tube

evacuated by a water-pump. The oxide so obtained in the crucible can then be ignited at 480° C., at which temperature molybdenum oxide is not appreciably volatile. (*b*) Heat the complex in a stream of moist ammonia gas at 330° C. for 30 min., and then for the same time in a current of air. The oxide is ignited finally at 480° C. The conversion can be effected in a sloping tube closed with asbestos and heated in an air-bath at 330° C. Ammonia is drawn from a cylinder and moistened by passing through a wash-bottle containing concentrated aqueous ammonia heated to 80° C.; otherwise the conversion takes considerably longer. The efficiency of the conversion is good, especially for larger quantities.

Bromometric titration of the 8-hydroxyquinoline complexes of tungstic oxide, molybdenum oxide, vanadium trioxide, and ferric iron—The iron and vanadium complexes dissolve in dilute, warm phosphoric or hydrochloric acid, also slowly in cold, 2 *N* hydrochloric acid. The molybdenum and tungsten complexes are soluble only in warm concentrated phosphoric acid. Dissolve by pouring small quantities of pure, hot phosphoric acid through the filter, and dilute the solution so that it is 1·0 *M* with respect to phosphoric acid. Add 5 ml. of diluted hydrochloric acid (1+1), 1 g. of potassium bromide, and titrate with 0·1 *N* potassium bromate, using bright platinum wire to measure the potential. The end-potential occurs at eH = 1·1 volt, but the potential at the beginning of the titration depends largely on the cation present. Vanadium shows a break at about 0·2 v., tungsten about 0·65 v., molybdenum about 0·35 v., and pure solutions of 8-hydroxyquinoline about 0·95 v. In 4 *M* phosphoric acid, 8-hydroxyquinoline can also be sharply titrated, but the increased acidity decreases the break in the curve.

Results show an accuracy of 1 in 600 on contents greater than 20 mg., and are slightly less accurate for contents down to 1·5 mg.

A visual determination may be made by adding a small excess of bromate and titrating back iodimetrically.

Weighing the oxides and 8-hydroxyquinolinates in conjunction with the bromometric titration—The ignited oxides dissolve readily in 2 *N* sodium hydroxide, molybdenum trioxide also in concentrated aqueous ammonia, but iron requires the addition of phosphoric and perhaps of hydrochloric acid. From the solutions so obtained the complexes can be again precipitated, dissolved in phosphoric acid, and titrated bromometrically. Hence indirect determinations of tungsten-molybdenum, tungsten-vanadium, and molybdenum-vanadium mixtures can be made, the weights of combined oxides and of combined hydroxyquinolinates, or the titration of the combined complexes and the weight of the combined oxides being utilised in the calculations. Results are accurate to 1 part in 250 on the figures given for tungstate-molybdate and vanadate-molybdate mixtures.

Direct determinations—Vanadium with tungsten and molybdenum—Precipitate and weigh the complexes, leach out the vanadium compound with 2 *N* hydrochloric acid, and wash the residue with a small quantity of water. Determine vanadium in the filtrate bromometrically, and molybdenum and tungsten in the residue by an indirect method.

Molybdenum and tungsten—Precipitate the molybdenum derivative in weakly alkaline solution, filter, add more reagent to the filtrate, acidify with 2 *N* acetic acid, and boil to precipitate the tungsten complex.

Tungsten and iron—Add 4 g. of Rochelle salt to the weakly alkaline tungstate solution containing some iron and treat with the reagent. The iron complex is precipitated, but the tungsten remains in solution even on acidification with acetic acid.

To permit determination of tungsten as well as of iron, the tartrate must first be replaced by a weaker complex-forming reagent such as glycerol. Treat the solution in 0·01 *M* phosphoric acid with 0·5 to 2 ml. of glycerol, and add sodium hydroxide solution to weak alkalinity. Add a 50- to 100-per cent. excess of the reagent, and boil to coagulate the precipitated iron complex, which can be determined either gravimetrically or with bromate. On adding more reagent to the filtrate, acidifying with acetic acid and boiling, the tungsten complex forms. Too large an excess of glycerol gives low results for iron, some of which remains for precipitation with the tungsten, but it can be extracted with cold 2 *N* hydrochloric acid.

Iron can also be removed from the combined and weighed complexes by extraction with cold, 2 *N* hydrochloric acid. Warm acid attacks the tungsten complex also, and *N* acid is inefficient, so careful control is necessary for good results.

Vanadium and ferric iron, and *molybdenum and ferric iron* may both be separated by the same method.

Since the complexes of divalent nickel, cobalt, and manganese, and of tervalent chromium are all more soluble than that of ferric iron, these may all be the more easily separated from tungsten, molybdenum, and vanadium.

Reduction by cadmium—By passage of a solution of sodium tungstate in *M* phosphoric acid through a cadmium reductor, the tungsten is reduced to the quinquevalent state, but when a solution of the tungsten 8-hydroxyquinolate in *M* phosphoric acid is similarly treated no reduction occurs. In a *M* phosphoric acid solution of the molybdenum complex, the molybdenum is reduced to the tervalent condition. M. E. D.

Extraction of Vanadium from Aqueous Solutions by *Iso*propyl Ether. J. J. Lingane and L. Meites, jun. (*J. Amer. Chem. Soc.*, 1946, **68**, 2443–2447)—Although ether extraction has been recommended for the separation of iron from vanadium, few quantitative data on the behaviour of vanadium in this extraction are available. A study has now been made of the extraction of vanadium from aqueous solutions by *iso*propyl ether, and from the results obtained the optimum conditions for the separation of vanadium from ferric iron are defined.

No detectable amount of vanadium is extracted by peroxide-free *iso*propyl ether from solutions in diluted hydrochloric acid of quadrivalent vanadium, provided the duration of the extraction is short. If peroxide is present, or is formed during the extraction by the action of light on the ether, a vanadic acid is formed and this passes into the ether phase. The percentage of vanadium extracted from solutions in diluted hydrochloric acid, originally containing the vanadium in the quinquevalent state, is initially large, but decreases as the vanadate is reduced by chloride ion. For solutions of both quinquevalent and quadrivalent vanadium in the presence of hydrochloric acid, and for quinquevalent vanadium in the presence of sulphuric acid, the percentages of vanadium extracted at equilibrium, in diffuse daylight, rise with acid concentration from zero per cent. for 4 *M* acid to values greater than 5 per cent. at higher acid concentrations. The optimum conditions for the separation of vanadium from ferric iron by extraction are: the vanadium must be present in the quadrivalent state; the *iso*propyl ether must be peroxide-free; the extraction must not be prolonged for more than 10 min. in the presence of light; the concentration of hydrochloric acid should be between 7 and 8 molar, since the distribution coefficient of ferric chloride has then the most favourable value. A very sharp separation is obtained when these conditions are fulfilled.

The *iso*propyl ether can be freed from peroxide by distillation from dilute, aqueous potassium permanganate, and should be used at once. The quadrivalency of the vanadium can be ensured by evaporation of the iron-vanadium solution to dryness with concentrated hydrochloric acid. B. A.

Behaviour of Uranyl Solutions in a Mercury Reductor. E. R. Caley and L. B. Rogers (*J. Amer. Chem. Soc.*, 1946, **68**, 2202–2204)—If a sufficiently high concentration of hydrochloric acid is present in the solution, the uranyl ion is reduced by the mercury reductor to the quadrivalent state. For a solution of uranium in 7 to 9 *N* hydrochloric acid reduction is rapid, but is incomplete by from 4 to 7 parts per thousand of uranium, depending on experimental conditions. The reduced solution is sufficiently stable, for analytical purposes, towards oxidation by air. A volumetric determination of uranium, in which the uranium was reduced in the mercury reductor, gives accurate results provided that a positive correction of 5 parts per thousand is applied to the final titration. Reduction in 18*N* sulphuric acid, or in uranyl acetate solutions containing sodium chloride, is slight.

The reductors used in the investigation were of the type described by McCay (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 1). The general procedure was as follows. A 25-ml. portion of standard uranium acetate solution was pipetted into a reductor containing 250 g. of distilled mercury. A measured volume of acid, usually hydrochloric, was added, and the water phase diluted to 100 ml. The air above the liquid was displaced by carbon dioxide or nitrogen, and the reductor shaken at a known rate for a given time; in 7 to 9 *N* hydrochloric acid, reduction reaches a maximum in a few minutes. The reduced solution was filtered through a folded filter, the flask and filter being washed with air-free distilled water, and the filtrate and washings being collected in a measured volume of 0·1 *N* potassium dichromate. The excess of potassium dichromate solution was determined by back-titration with standardised ferrous sulphate solution, a platinum-tungsten pair of electrodes and a vacuum tube voltmeter being used to determine the end-point. B. A.

Fractional Separation of Hafnium and Zirconium by Means of Triethylphosphate. H. H. Willard and H. Freund (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 195–197)—This paper describes an extension of previous work on separations in which the precipitating ion was not added as such, but was formed slowly by hydrolysis in solution (Willard, *et al.*, *Ibid.*, 1937, **9**, 357; *J. Amer. Chem. Soc.*, 1937, **59**, 1190, 1197). By this method of precipitation a precipitate that is normally gelatinous may be obtained in a granular form that can easily be filtered and washed. Larsen *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 512) have described a fractional separation of zirconium and hafnium in which the metal phosphates are precipitated with phosphoric acid under conditions so controlled that the gelatinous nature of the precipitate is overcome. The hydrolysis of triethyl phosphate in sulphuric acid solution is now employed successfully in this separation, the ester hydrolysing in a step-wise manner and precipitating zirconyl or hafnyl ethyl acid phosphate. If sufficient ester is added to precipitate only a fraction of the hafnium and zirconium, the hafnium concentrates in the precipitate.

The hafnium and zirconium salts were obtained from cyrtolite, the mineral being treated with concentrated sulphuric acid by the method of Larsen. To follow the separation two analyses were required, one for combined hafnia and zirconia and

the other for the hafnia present in the combined oxides. Phosphate precipitates were decomposed by fusion with a mixture of sodium hydroxide and sodium peroxide and the hydroxides dissolved in sulphuric acid. Usually the combined oxides were first determined and then the oxides returned to solution by a potassium bisulphate fusion. The hafnium in this solution was determined by the following method. By a double precipitation with ammonia, followed by solution in 10 ml. of diluted hydrochloric acid (1+1), the hafnium and zirconium were transferred to hydrochloric acid solution. The liquid was boiled with 5 ml. of 30 per cent. hydrogen peroxide to destroy organic matter from the filter paper, and diluted to 200 ml. for precipitation of the selenites with 50 ml. of 20 per cent. selenious acid solution. The analysis was completed by the method of Schumb and Pittman (*Ibid.*, 1942, **14**, 512), in which the hafnium and zirconium are converted first to selenites and then to oxides. To remove silica from the combined oxides so obtained, 10 drops of concentrated sulphuric acid and 0·5 ml. of hydrofluoric acid were added and evaporated cautiously from the oxides. The sulphates were ignited to the oxides carefully and the ignition was completed at 950° C.

In the fractionation experiments, sufficient triethyl phosphate was added to solutions containing 0·1 g. mol. of combined oxides per litre of 6 *N* sulphuric acid to precipitate the desired fraction of the oxides as ethyl phosphates. A molar ratio of ester to oxides of slightly greater than 2 to 1 was required, to allow for side reactions. The mixture was boiled for 20 hours, cooled, filtered, and the precipitate washed with 2 per cent. sulphuric acid solution. Graphs are given showing the relation between initial mole fraction of hafnium, mole fraction of precipitate, and mole fraction of hafnium precipitated. In a series of fractionations for which data are given, 215 g. of combined oxides containing 16·0 weight per cent. of hafnia were enriched in five steps to 7·16 g. of combined oxides containing 91·1 weight per cent. of hafnia. B. A.

Analytical Chemistry of the Rare Earths. Active-oxygen Determination. G. L. Barthauer and D. W. Pearce (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 479–480)—Evaluation of the rare-earth metal content of a mixture of rare-earth oxides would be greatly simplified if a method were available by which the "active oxygen," the oxygen present in excess of that in the sesquioxide, could be determined. Such a method would also be useful in studying the composition of rare-earth oxides. The method of Bunsen for the determination of cations in higher valency states cannot be used in the presence of cerium dioxide, as there is only partial reaction between this compound and hydrochloric acid. Several modifications of the Bunsen method have now been tested, and a procedure using potassium iodide has been found to give satisfactory results for the amounts of active oxygen in pure cerium dioxide and in cerium dioxide lanthanum sesquioxide mixtures.

Method—The apparatus consists of a 125-ml. Kjeldahl flask fitted by a ground-glass joint to a water-cooled reflux condenser, the upper end of which is connected, by similar means, to a tube bent to a semi-circle and terminating in a Bunsen valve.

Procedure—Place a weighed sample of the oxide in the Kjeldahl flask, and add 20 ml. of 10 per cent. potassium iodide solution and 10 ml. of 12 *N* hydrochloric acid. Attach the condenser and Bunsen valve immediately, and immerse the valve in 80 ml. of a 4 per cent. potassium iodide solution, contained in a beaker. Heat the flask gently until the oxide has dissolved, cool, remove the vapour trap and, using the vapour trap solution, wash any iodine from the tube of the condenser into the flask. Transfer the solution to a 500-ml. Erlenmeyer flask, dilute to 350 ml., and titrate with 0·1 *N* sodium thiosulphate, starch being used as indicator. Perform a blank determination, using the same volumes of solutions and the same times of heating. The titration required for the blank should not exceed 0·5 ml.

An application of the method is illustrated by observations made on the ignition products of pure praseodymium oxalate. The composition of the oxide obtained by heating this compound in air at 955 ± 10° C. closely approximates to Pr_6O_{11}. B. A.

Agricultural

Determination of Quartz Content of Clay Fraction of Soils. L. de Leenheer and G. Waegemans (*Bull. Soc. Chim. Belg.*, 1945, **54**, 384)—The determination of the quartz content of clay fractions is important because the quartz forms an inert fraction which has no useful function as a soil colloid. Of the three most recent chemical methods of determination, that of Trostel and Wynne (*J. Amer. Ceram. Soc.*, 1940, **23**, 18) offers several advantages. In this method the clay fraction (0·5 g.) is fused with potassium pyrosulphate (10 to 15 g.) for 45 min. at 1000° C. A single fusion is sufficient, and the method is suitable for series determinations. According to the original authors, no correction need be applied to the results. Fusion may be performed in a quartz crucible.

The method has now been tested on fractions of pure, powdered quartz having definite particle size. The melt was dissolved in hot water, to which were added 12 g. of sodium hydroxide, and filtered through a filter crucible (porosity less than 2 μ. for finer fractions). The residue was washed with hot water, and then with dilute hydrochloric acid. The filtrate was found to contain appreciable

quantities of dissolved silica, which was determined. The results show the claim of the original authors, that no correction is necessary, to be incorrect. The corrections required are as follows:

Fraction	Correction factor
below 2 μ.	1·064
2 to 5 μ.	1·044
5 to 10 μ.	1·039
10 to 20 μ.	1·029
20 to 50 μ.	1·027
50 to 100 μ.	1·022

When applied to the fractions below 2 μ., derived from a number of minerals, the following results were obtained:

Mineral	Quartz content Percentage
Montmorillonite (Geisenheim)	4·00
Muscovite (unknown origin)	3·08
Halloysite (Indianite)	1·85
Halloysite (Leoben)	3·53
Nontronite (Bavarian)	2·41
Kaolin (Meissen)	2·20

G. M.

Identification and Estimation of the Montmorillonite Group of Minerals, with Special Reference to Soil Clays. D. M. C. MacEwan (*J. Soc. Chem. Ind.*, 1946, **65**, 298–304)—This paper describes the detection and estimation of montmorillonite (implying the whole montmorillonite group) by an X-ray diffraction method based on the formation of a montmorillonite-glycerol complex.

When the water-montmorillonite complex is employed as a means of detection of montmorillonite by X-ray diffraction, use is made of the 15 A basal reflection, which corresponds to two layers of water molecules between the structural sheets. In order to make this reflection as sharp and as strong as possible, careful control of the state of the base saturation and the hydration is necessary. Under the most favourable conditions, less than 5 per cent. of montmorillonite cannot be detected by this method, and there is liable to be ambiguity in identification because chlorites and vermiculites give reflections in the region of 14 to 15 A.

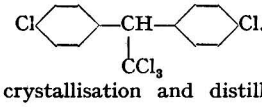
The glycerol-montmorillonite complex, which is formed by addition of glycerol to the clay (not necessarily free from water), has the following advantages over the water-montmorillonite complex: —(1) Intense first-order basal reflection at 17·7 A. This reflection, and the higher order basal reflections, do not interfere with reflections from other likely minerals. (2) Since glycerol is miscible with water, it is easy to change from a water system to a glycerol system. (3) Spacings and intensities of the glycerol-montmorillonite basal reflections are unaffected by wide variation in the water content. (4) The complex is extremely stable, even in an evacuated camera.

A list of basal reflections and intensities due to the glycerol-montmorillonite group is reproduced, together with other reflections likely to occur on soil clay photographs.

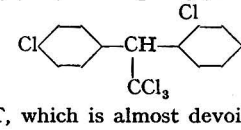
A discussion is given on the possibility of other minerals giving the 17·7 A reflection, and includes the question of distinction between montmorillonite and hydrous micas. Experience so far shows that all minerals giving the 17·7 A reflection when treated with glycerol, may be provisionally assigned to the montmorillonite class and those giving a 10 A reflection, to the mica class.

For detecting small quantities of montmorillonite, the oriented aggregate technique is used, the specimen being made by evaporating a suspension to dryness. An evacuated camera having an efficient collimating system should be used. By this method, 1 per cent. of montmorillonite can be detected, without ambiguity, in mixtures containing any other clay minerals. The extension of the method to rough quantitative analysis of montmorillonite clays is also described. E. G. S.

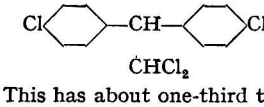
Chemical Investigations of the Insecticide DDT and its Analogues. Part I. Reactions of DDT and Associated Compounds. J. Forrest, O. Stephenson, and W. A. Waters (*J. Chem. Soc.*, 1946, 333–339)—Pure DDT prepared by Zeidler's method (*Ber.*, 1874, **7**, 1181) by condensing chlorobenzene with chloral or chloral hydrate in concentrated sulphuric acid crystallises from solvents in long, white needles with m.p. 108° to 109° C., but the uncrystallised technical product prepared by the same process on the large scale is a white powder with a lower setting point and inferior physiological potency. The most potent insecticide in crude DDT is 1:1:1-trichloro-2:2-di-(4-chlorophenyl) ethane



By careful crystallisation and distillation under reduced pressure of the residues in the mother liquor from the crystallisation of crude DDT from alcohol, there was obtained 1:1:1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane,



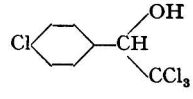
or *iso*-DDT, which is almost devoid of insecticidal activity. Technical DDT normally contains over 90 per cent. of these isomers in the approximate ratio of 4 of pure DDT to 1 of *iso*-DDT. A common impurity in technical DDT is 1,1-dichloro-2:2-di-(4-chlorophenyl)ethane



or DDD. This has about one-third the insecticidal activity of true DDT, and can be prepared by condensing chlorobenzene with either dichloroacetal or

dichloroacetaldehyde; it is evidently formed from these impurities in the technical chloral used in the manufacture of DDT. It has the effect of lowering the setting point of DDT to a value not commensurate with the loss of physiological activity.

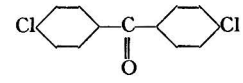
It is characteristic of DDT and the above-mentioned associated compounds that, by treatment with alcoholic alkali, they lose one molecule only of hydrochloric acid, yielding very stable ethylenes, which are resistant to further alkaline hydrolysis. Thus extraction with benzene or petroleum, hydrolysis of the washed, salt-free extract with alkali, and subsequent estimation of chloride can be made the standard method of analysis of DDT for detecting gross impurities, as well as for its estimation in insecticidal sprays, or powders, or upon fabric. This method, however, does not differentiate between pure DDT and the physiologically inert *iso*-DDT. Neither can it be used as a sensitive test for the presence of DDD since the hydrolysable chlorine content of DDD (11·1 per cent.) is only slightly more than that of DDT (10·0 per cent.). Estimation of the total chlorine (44·3 per cent. for DDD and 50·1 per cent. for DDT) is more indicative of this contamination, though it is impracticable as a rapid routine method. High hydrolysable chlorine figures (over 10·5 per cent.) indicate appreciable contamination with the half-condensation product 1:1:1-trichloro-2-hydroxy-2-(4-chlorophenyl)ethane



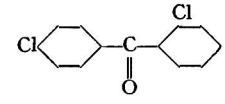
because this on boiling neutralises nearly four equivalents of alkali, liberating three equivalents of chloride per gram-molecule. Crude DDT may contain about 3 per cent. of an oily substance consisting mainly of this impurity.

A study of the products of oxidation of the substituted ethylenes formed by hydrolysis of DDT led to the following general procedure for the analysis of technical DDT.

Procedure—Dissolve 25 g. in 200 ml. of warm alcohol and heat under reflux for 0·5 hr. with 8 g. of sodium hydroxide dissolved in 50 ml. of water. Remove the excess of alcohol on the water-bath, dilute the residue with water, and acidify with hydrochloric acid. When cold, decant the aqueous layer from the gummy residue, extract it with chloroform, and evaporate the extract to dryness. Dissolve the brown gum, together with the residue from the chloroform extract, in 75 ml. of hot, glacial acetic acid and add gradually a saturated solution of 50 g. of chromic acid. Reflux the mixture for 4 hr., dilute while still hot with 500 ml. of water, and allow to cool. Extract the cold liquid with chloroform, and wash the extract four times with small amounts of diluted aqueous ammonia, and finally with water. Evaporate the alkaline aqueous layer to small bulk, filter, acidify, and collect and weigh the precipitate (Y) in a Gooch crucible. Dry the washed chloroform extract over calcium chloride and collect the ketone mixture (X) quantitatively by evaporation of the filtered solution. Heat the residue above its m.p. to remove traces of chloroform, cool, and weigh. Determine the setting point of the mixture, which consists of the dichlorobenzophenones



and



The precipitate (Y) consists of *p*-chlorobenzoic acid derived from the half-condensation products. A wide range of samples of technical DDT yielded ketone mixtures (X) of setting point 132° ± 2° C. corresponding to a content of 26 per cent. of the 4-2′-isomer. The composition of the ketone mixture may be determined by means of a curve showing the relation between the setting point of a mixture of the two ketones and the proportion in the mixture of one of them. Most technical samples of DDT have setting points above 75° C., and the figures calculated from the analytical results fall between the following results found with two samples:—Setting point, 91°, 80° C.; DDT per cent., 76, 65; hydrolysable chlorine per cent., 10·2, 10·6; total chlorine per cent., 49·2, 48·9; DDT compounds per cent. from weight of ketones, 88, 85; setting point of ketones, 133°, 134° C.; half-condensation products per cent., 3·2, 2·6; probable per cent. of DDD, 2, 16; maximum *iso*-DDT + DDD, 29, 25.

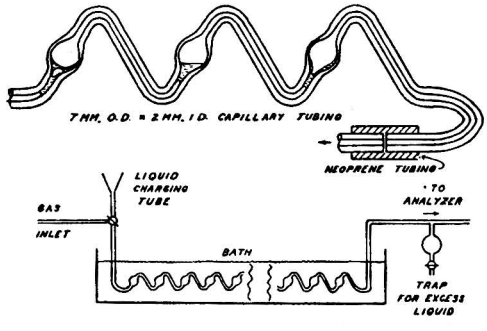
Other reactions of DDT and its analogues have been studied. When DDT, *iso*-DDT, and DDD are nitrated, the nitro-groups enter the aromatic nuclei in the 3 and 5 positions. The reduction of DDT affords an example of a reversed benzil-benzilic acid transformation, DDD and 4:4′-dichlorostilbene being produced. A. O. J.

Gas Analysis

Identification of Sulphur Compounds in Gas Mixtures. C. S. Oldach and E. Field (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 669–672)—A method of identification and determination of organic sulphur compounds at a concentration of a few parts per million is based on solubility differences in inert solvents, combined with a highly sensitive analytical method (*Ibid.*, 1946, **18**, 665, 668). The method can also be applied to any class of compounds for which á sensitive analytical method is available.

From Raoult's Law it can be deduced that $n=P/p_\pi$, where P is the total gas pressure, p_π is the vapour pressure of the pure solute at temperature T, and n is the minimum quantity of gas required to saturate one g.-mol. of liquid with the solute. Experimental determination of n reveals the solubility or vapour pressure of the solute, which usually will identify the organic sulphur compound. The relationship between the two phases holds both when a liquid is being saturated by absorption of a component from a gas, or a gas is being saturated by stripping a compound from a liquid. To determine the minimum quantity of gas saturating a given volume of liquid, an efficient saturator, such as the one described, is essential.

Method. Apparatus—A suitable saturator is constructed from twenty 60-cm. lengths of 2 × 7 mm. capillary tubing containing 200 enlargements as shown in the figure, the sections abutting in Neoprene joints. White oil, the inert solvent, is introduced through the liquid charging tube and distributed by passing hydrogen, thus obtaining 200 pools of solvent. The whole is immersed in a thermostat bath giving a temperature regulation to ± 0·15° C.; any liquid carried out of the saturator is caught in a trap. The exit gases may be analysed by conversion to hydrogen sulphide over heated alumina, followed by spectrophotometric determination (*loc. cit.*).



Absorption run—Distribute a known volume of sulphur-free solvent through the saturator, and adjust to a known temperature. Remove hydrogen sulphide from the sample gas by means of a Thylox unit, and sample the gas by filling a stainless steel cylinder under pressure. Pass the hydrogen-sulphide-free gas into the apparatus at a rate of 0·005 cu.ft. per min, and analyse the emergent gas at frequent intervals (*loc. cit.*, p. 668). Plot the sulphur contents found against the volume of treated gas. A break in the curve indicates absorption of a new sulphur compound; the volume at which it occurs serves to identify the compound, and the magnitude of the break shows its concentration.

Calculation of the "break volume"—For carbon disulphide: $P = 1$ atmosphere, p_π is 0·308 atmospheres at 14° C., thus $n = 3{\cdot}25$ g.-mols. of gas to saturate 1 g.-mol. of oil with carbon disulphide. Using 48 ml. of solvent oil of density 0·77 and average molecular weight 154, a total of 0·000529 pound-mols. of oil, the amount of gas required is $3{\cdot}25 \times 0{\cdot}000529 = 0{\cdot}00172$ pound-mol. Under wet meter conditions this gives a calculated break volume of $0{\cdot}00172 \times 359 \times \frac{295}{273} \times \frac{760}{741} + 0{\cdot}02$ cu.ft. of free space in the absorber = 0·70 cu.ft. The amount of carbon disulphide is thus shown by the magnitude of the break at 0·70 cu.ft., at standard temperature and pressure. The similarity of the vapour pressures of hydrogen sulphide and carbon disulphide makes their distinction difficult, thus hydrogen sulphide is determined separately by absorption in cadmium acetate, acidification with acetic acid and iodimetric titration (Shaw, *Ibid.*, 1940, **12**, 668). For the determination of small concentrations of gases less volatile than carbon disulphide a stripping run is necessary, the precision being better than ± 0·1 grain per 100 cu.ft. when the concentration of sulphur in the gas drops below 1 grain per 100 cu.ft.

Stripping run—Saturate a 100-ml sample of the solvent with the gas by bubbling it through at 70 cu.ft. per hr. for 2 hr. at −5° C. in a flask fitted with a sintered-glass sparger. Transfer about 50 ml. to the saturator and distribute by means of a stream of hydrogen, avoiding all contact with air. Strip then by passing hydrogen through at 0·007 cu.ft. per min. at a temperature of about 20° C.; analyse the gas and plot a graph as before. A temperature correction must be made to allow for th e difference in temperature between saturating and stripping, and is given by the ratio of the vapour pressures at the saturating and stripping temperatures. The more volatile constituents are removed before accurate analyses can be made, but accurate results are obtained for the less volatile constituents. A complete analysis usually requires both an absorption and a stripping run.

Sources of error—The accuracy of the method has not been tested on known samples, but the results obtained agree with previous experience of industrial gases. Certain compounds are unstable to white oil, oxygen, and metal walls, so determinations should be made rapidly. Any inert solvent that is not highly volatile or too viscous may be used. In the presence of a high concentration of a compound, the solvent will increase in volume and corrections must then be applied for variations in the solubility. The accuracy of the stripping run is increased by saturating at a temperature below that used in the stripping. Adsorption of sulphur by alumina during the catalytic reduction of organic sulphur compounds to hydrogen sulphide is minimised by using only 1 ml. of the catalyst.

Sulphur content - gas volume curves obtained in identifying and determining the sulphur compounds present in coke-oven gas are presented. M. E. D.

Microchemical

Combined Micro-gravimetric and Titrimetric Micro-determination of Thallium in Toxicological Material. J. F. Reith and K. W. Gerritsma (*Rec. Trav. Chim.*, 1946, **65**, 770–780)—*Preparation of the material*—For urine, evaporate 500 ml. acidified to Congo red paper to about 70 ml., add 15 ml. of concentrated hydrochloric acid and small amounts of potassium chlorate to the warmed liquid until it is of a clear yellow colour and smells distinctly of chlorine. Cool, filter, and collect the filtrate in a 100-ml. cylinder and rinse the filter with a little water. For faeces, rub the material with water to a thin paste and take a known amount of the homogeneous preparation. Grind viscera to a homogeneous paste in a meat grinder. Use a maximum amount of 60 g. of faeces of normal consistency and 60 g. of viscera for the determination. To the material in a tall, 300-ml. beaker add an equal weight of 4 *N* hydrochloric acid, 10 mg. of manganese (*e.g.*, 10 ml. of 0·1 *N* potassium permanganate) as catalyst, and about 1 g. of potassium chlorate. Heat to the boiling-point and, as the liberated chlorine is consumed, add 0·5- or 1-g. portions of potassium chlorate until the liquid has a clear yellow colour, with a heavy sediment of chlorinated fats, and no longer becomes brown after being heated for a further 30 min. on the water-bath. Filter the liquid, which must retain an odour of chlorine, heat the insoluble portion with a little 4 *N* hydrochloric acid and potassium chlorate, pour the liquid through the filter and rinse with a little water. For hair and nails, destroy the organic matter with nitric acid and a little sulphuric acid, dilute with water, increase the hydrochloric acid concentration to 2 *N* and introduce a little free chlorine by means of a hot solution of potassium chlorate in 4 *N* hydrochloric acid.

Extraction—Transfer the clear liquid to a separating funnel and confirm the presence of free chlorine by means of starch-iodide paper. Shake vigorously with an equal volume of ether, separate the aqueous layer and transfer the ether layer, which contains thallium trichloride, to a second separating funnel. Add so much sulphur dioxide water (1 to 2 ml.) that, after vigorous shaking, the aqueous layer no longer reacts with starch-iodide paper. Adjust the volume of the aqueous layer to 5 ml., shake the mixture vigorously and separate the aqueous layer containing thallous chloride into a dish. Extract the ether layer again with about 2 ml. of water and combine this extract with the first. Shake the ether again with the prepared sample material in the first funnel, add a little potassium chlorate in hydrochloric acid, if necessary, to ensure the presence of free chlorine and shake the mixture vigorously. Transfer the ether layer to the second separating funnel, treat it with sulphur dioxide water and proceed as already described, combining the final aqueous extracts with those in the dish. Repeat the extraction procedure a third time with the same ether. Evaporate the combined aqueous extracts containing thallous chloride on the water-bath until a black syrup remains. Transfer this by means of a few drops of nitric acid to a 50 × 18 mm. Pyrex glass tube, add 0·2 ml. of concentrated sulphuric acid, and destroy the organic matter in the usual way, using the necessary small additions of nitric acid to complete the rinsing of the dish. The destruction is complete when, even after boiling, the liquid remains colourless or light yellow.

Precipitation and weighing of the thallous iodide—When the amount of thallium ranges from 0·01 to 2 mg. carry out the precipitation in Pyrex tubes of 2 ml. capacity (45 × 10 mm.). Dilute the oxidised extract with 0·8 ml. of water, cool, and filter with suction through a micro-filter of sintered glass (diameter, 9 mm.). Collect the filtrate directly in the precipitation tube and rinse several times with drops of water until the final volume is 1·8 ml. Add 0·1 ml. of freshly prepared, saturated, filtered sodium sulphite solution and mix with a glass rod. Then add 0·2 ml. of filtered, 10 per cent. potassium iodide solution and mix. An orange-yellow precipitate indicates thallium. Rinse the rod and allow the covered tube to stand for 12 to 18 hr. in the dark. Centrifuge the tube for 5 min. at 1500 r.p.m. and pour off the supernatant liquid along a glass rod. Wash the inner wall of the tube and the precipitate in the usual way with 2 ml. of 50 per cent. alcohol, stirring the precipitate with a glass rod which is then rinsed with the alcohol. Centrifuge and decant again and repeat the washing procedure with 90 per cent. alcohol. Rinse the outside of the tube with distilled water and alcohol, heat for 2 hr. at 100° to 105° C., cool, keep in the microbalance case for 5 hr., and weigh. Treat a second tube, serving as tare, in the same way with 2 *N* sulphuric acid and 50 and 90 per cent. alcohol. After removing the thallous iodide by solution in acetic acid and bromine (*infra*), rinse the tube, heat, and weigh as already described and record its weight as that of the empty tube. To convert the thallous iodide to thallium, multiply by 0·616.

Titration of thallous iodide—Treat amounts of thallium ranging from 10 to 250 μg. as follows. Place in the dry tube containing the precipitate, which must be free from alcohol, 0·1 ml. of glacial acetic acid and, from a thin pipette, 1 drop of bromine. Shake momentarily every 5 min. until no solid particles remain visible and then allow to stand for 15 min. Rinse the contents of the tube

with water into a 25-ml. Erlenmeyer flask using not more than 2 ml. of water. Heat the flask carefully until the liquid is light yellow and allow it to cool. Add 2 *M* sodium formate until the solution is colourless and then an excess of 0·2 ml. Mix carefully, allowing the liquid to moisten the walls, and set the tube aside for 5 min. Add successively 2 ml. of 30 per cent. sodium chloride solution, 1 drop of 10 per cent. potassium iodide solution, 0·2 ml. of 4 *N* sulphuric acid and 5 drops of 0·2 per cent. starch solution. Titrate with 0·01 *N* sodium thiosulphate, each ml. of which is equivalent to 255·5 μg. of thallium. With amounts of thallium ranging from 250 to 5000 μg., proceed in the same way with suitable increases in the volumes of the reagents used. A blank titration should always be performed and, as a check on all the reagents, a blank analysis should be made on 100 ml. of distilled water. Since thallium salts are rarely pure, the thallium content of thallium solutions used as standards should be determined by titration in the manner described after treatment with acetic acid and bromine. Lead, mercury, copper, arsenic, antimony, bismuth, and iron do not interfere with the method. A. O. J.

Thiocyanate Complex as a Means of Extracting Cobalt before its Micro-determination by Other Methods. N. S. Bayliss and R. W. Pickering (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 446–448)—The complex thiocyanate of cobalt and ammonia is soluble in organic solvents, and can be used for the colorimetric determination of small amounts of cobalt, but not for the very small amounts present in biological samples. Iron, nickel, copper, and zinc form similar complexes; that of iron is relatively insoluble in ethereal solvents, and the others do not interfere with the final determination of cobalt by the nitroso-R-salt method.

Procedure—Dissolve a sample containing 10 to 20 μg. of cobalt in enough hydrochloric acid to furnish an excess of 5 ml. of 6 *M* acid. Add 20 ml. of 60 per cent. ammonium thiocyanate solution, previously purified by extraction with a 0·1 per cent. solution of dithizone in chloroform and washing with chloroform and amyl alcohol. Then add *M* ammonium citrate until the red colour due to ferric thiocyanate just disappears. Dilute with water to 50 ml., and saturate with 4 ml. of ether. Extract the aqueous solution with three 20-ml. portions of a mixture (35+65) of amyl alcohol and ether to remove the cobalt from the aqueous phase as $(NH_4)_2Co(CNS)_4$, the iron not being extracted. Shake the combined extracts with two 20-ml. portions of 2 *M* aqueous ammonia to return the cobalt to the aqueous phase. Evaporate the ammonia extracts to dryness, and then evaporate again to dryness with 20 ml. of 6 *M* nitric acid to decompose the thiocyanate. Neutralise the residue with 2 *M* aqueous ammonia and evaporate to dryness. Determine cobalt in the residue by the method of Marston and Dewey (*Australian J. Exptl. Biol. Med. Sci.*, 1940, **18**, 343), by adding 5 ml. of water, 5 ml. of *M* ammonium citrate, and 0·5 ml. of a 1 per cent. solution of nitroso-R salt. Heat the solution on the water-bath for 5 min., add 5 ml. of concentrated nitric acid, and heat 5 min. longer. Cool, add 2 ml. of a saturated solution of bromine in water, and boil to expel the excess of bromine. Determine the cobalt content by comparisons with standards of known content prepared from standard cobalt sulphate solution and ammonium sulphate.

The optimum solvent concentration was found experimentally to be a mixture (35+65) of amyl alcohol and ether, the extraction of cobalt being about 85 per cent. on a single treatment of the aqueous phase at a *p*H of approximately 3·4. Efficient extraction requires a minimum thiocyanate concentration of 20 per cent. in the aqueous phase, half that value giving an efficiency too low for analytical use.

Since biological samples always contain an iron content large relatively to that of cobalt, the iron complex must be suppressed when determining cobalt. It is found that ammonium citrate destroys the ferric thiocyanate coloration at a *p*H value close to that at which maximum extraction of cobalt occurs; iron thus forms a useful indicator which does not interfere with the final colour comparison, as do many organic indicators.

Thiocyanate is conveniently removed by 6 *M* nitric acid, stronger acid resulting in a reaction that is too violent; incomplete removal causes a violent reaction during the nitration of the excess of nitroso-R salt. M. E. D.

Physical Methods, Apparatus, etc.

Particle Size by Spectral Transmission. E. D. Bailey (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 365–370)—A method is described for determining two-parameter size distributions from transmission measurements on suspensions in the visible and near infra-red, based on an empirical relationship between scattering, particle size, refractive index, and wavelength.

Suspensions are prepared by means of a pestle and mortar, and the concentration is adjusted to give about 20 per cent. transmission with blue light. Transmissions are measured at 14 points over a wavelength range of 0·4–2·0 μ., a monochromator and photo-cell or vacuum thermopile being used. The results are plotted and compared with a standard family of curves computed from the distribution law of Lansing and Kraemer (*J. Amer. Chem. Soc.*, 1935, **57**, 1369). The experimental curves

are evaluated in terms of r_o, the radius value of the maximum of the distribution, B, the non-uniformity coefficient which defines the spread of the curve, and a percentage expressing the extent to which the selected distribution law is obeyed. The method of analysis is rapid, requiring only 2 hours, but is limited to non-absorbing materials and to particle sizes for which optimum scattering occurs in the wavelength ranged used ($r_o <$ about 1 μ.).

The results are in fairly good agreement with those obtained with the ultracentrifuge.

Comparisons of particle-size distributions in samples of anatase, bentonite, rutile, and zinc oxide are given. B. A. S.

Internal Standard Method of Spectrographic Analysis as applied to the Determination of Lead in High-Purity Zinc. L. Griffith and J. N. Kirkbride (*J. Soc. Chem. Ind.*, 1946, **65**, 39–48)—An investigation has been made into the fundamental features of the internal standard method of spectrographic analysis, the controlled direct current arc-method for the determination of lead in high-purity zinc with bismuth as the internal standard being used as the example. The working conditions for this investigation were standardised, and, using the average deviation as a criterion, a precision of 2 per cent. over the lead range 0·025–0·003 per cent. was reached.

The principal error arises from the failure of the Eastman "33" plates used to obey the reciprocity law. Reduction of this error can only be achieved by using plates having little reciprocity failure, since the use of the direct-current graphite-arc precludes utilisation of a relative intensity calibration. Allied to this, is the error due to the intermittency effect, which can be reduced by speeding up the stepped sector.

The second error is the Eberhard effect, which occurs when there is a large range of density over a small distance of plate; this gives rise to local differences in concentration of the developer, and produces an erroneous density - intensity curve. The best solution to this problem seems to be increased agitation of the plate during development.

Bismuth is not an ideal internal standard since the lead line 2833·07 A and the bismuth line 2897·98 A are not a perfectly homologous pair. Although this constitutes the third source of error, it is doubtful whether any improvement is possible.

The error involved by the use of the direct current arc is certainly less than the errors already enumerated. D. A. P.

Comparison of Optical and Electron Microscopy of Pigments. D. L. Tilleard and N. D. P. Smith (*J. Soc. Chem. Ind.*, 1946, **65**, 261–264)—The high resolving power of the electron microscope enables problems relating particle size and shape to pigment colour, wetting, and other characteristics to be investigated. The small numerical aperture (0·001 radian) employed and the resultant depth of focus (10 μ. as opposed to about 0·4 μ. with N.A. 1·25) enables useful stereoscopic micrographs to be obtained.

Most pigments can be dispersed in a mixture of linseed stand oil and white spirit by grinding on a glass plate with a spatula. A drop of this dispersion is placed on a supporting film prepared by allowing a drop of a 2 per cent. solution of nitrocellulose (high nitrogen content; medium viscosity) in amyl acetate to fall on the surface of clean water. When the spirit has evaporated, selected portions of the film are attached to supporting screens and the excess oily matter is removed with white spirit. *Comparison of optical and electron micrographs*—The electron micrographs of various pigments frequently show particles whose size is below optical resolution. The detail of the shape of many particles in the region of uncertain optical resolution (between 0·5 and 0·25 μ.) is disclosed. In the electron micrograph some particles of red earth pigment appear grey, indicating that they are extremely thin; others suggest a hexagonal shape similar to that observed in micrographs of clay. The micrograph of "ferrite" yellow shows rod-shaped particles, 0·03 to 0·1 μ. wide and about 0·5 μ. long, whereas another synthetic iron oxide pigment, Turkey Red, shows rounded particles less than 0·1 μ. diameter. The varying sizes and shapes of zinc oxide pigments to be seen in the electron micrographs reproduced may be related to methods of manufacture; the electron microscope reveals the regular crystalline shape of the particles of all the zinc oxides examined. Differences in samples of lead chrome pigments are also seen, and may be related to the lead sulphate content of the pigment, although the tendency to change from monoclinic towards orthorhombic habit with an increase in lead sulphate content cannot be deduced from the micrographs. When interpreting electron micrographs it is necessary to remember that the particles seen may be only the smallest in the pigment; particles commensurate with the size of the whole field of the microscope may be neglected; and that the quantity of material in the whole field is very small and, except in special circumstances, quantitative conclusions are not justified. G. A. B.

Oxide Films Formed on Alloys at Moderate Temperatures. Electron Diffraction and Electron Microscope Study. E. A. Gulbranson, R. T. Phelps, and J. W. Hickman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 640–652)—This paper describes the study of oxide films by reflection and transmission methods of electron diffraction, in addition to electron microscope studies. The alloys studied were those consisting principally of iron, cobalt, nickel, and chromium, and are typical commercial protective, refractory, magnetic, and sealing

alloys. A miscellaneous group of experimental alloys has also been included.

Electron diffraction results—(1) Iron and chromium ions diffused more readily to the surface of the oxide film than the other metal ions. (2) Stratification of oxides occurred even for films 100 to 300 A thick. (3) Chromium sesquioxide was always observed in the oxide films on the protective or refractory alloys examined; where stratification occurred, chromium sesquioxide appeared to be in contact with the substrate. (4) Nickel monoxide was never observed on the surface of the film, but sometimes occurred in the body of the film; this may indicate a low diffusion rate for the nickel ion. (5) Solid solution of two simple oxides to give the spinel-type structure may occur. (6) Except for iron and chromium, metals constituting not more than 5 per cent. of the alloy did not occur as simple oxides on the outer surface, but appeared to form spinels in the body of the film. (7) Simple oxides of cobalt, manganese, silicon, vanadium, and tungsten were not observed.

Electron microscope results—(1) The thin oxide films consisted of a continuous film of oxide crystals 100 to 1600 A in size. (2) The oxide films were not of uniform density and the crystals had irregular shapes. (3) Longer oxidation time increased average crystal size. (4) Crystals on non-protective alloys such as mild steel, at 250° C., were of the same size as those found on protective and refractory alloys at 600° C. (5) At a given temperature, a correlation may exist between crystal-size of the oxide and the protective quality of film. (6) Thickness of boundary zone between crystals in the mosaic structure was of the order of 50 A.

Electron diffraction data, and numerous electron micrographs of oxide films on stainless steels, nichrome, cobalt-iron, chrome-iron, silicon-iron, manganese-iron, Iconel, and mild steel are reproduced. E. G. S.

Determination of Extinction Corrections in Infra-red Analysis of Gaseous Hydrocarbon Mixtures. J. H. Lee (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 659–661)—The usual procedure in the infra-red analysis of gaseous hydrocarbon mixtures involves the calculation of various corrections to be applied to the observed extinction coefficients of the mixtures. The sources of error for which corrections must be applied are, energy scattered within the instrument, the structure of the absorption band, and inter-molecular action. By use of the relation, $E_c = E_o(1+\alpha E_o)$, where E_c and E_o are, respectively, the corrected and the observed values of log. I_o/I, and α is a constant for the particular instrument, gas, and absorption band concerned, one method of correction, allowing for the errors caused by the several factors, can be applied. When molecular interaction has an appreciable effect on absorption, addition of a diluting agent such as air is desirable if the proposed method of correction is to be used. Details of the method for calculating corrections to be applied directly to observed extinctions are given. B. A.

Preparation of Powdered Materials for Electron Microscopy. M. C. Schuster and E. F. Fullam (*Ind. Eng. Chem. Anal. Ed.*, 1946, **18**, 653–657)—The aggregates in powdered materials must be adequately dispersed to furnish electron micrographs suitable for statistical particle-size distribution analysis. Specimens are mounted on thin (10 to 30 mμ.), electron-transparent, resin films which are in turn supported on 0·3-cm. diameter metal screens having perforations of 20 to 100 μ. Deflocculation may often be brought about by choice of a mounting medium with suitable wetting properties. A common method of film formation is to drop a small amount of resin solution on water and allowing it to spread and dry. Screens may be placed on it and picked up from beneath with an annular tool. The thickness of the film is determined by the concentration and spreading coefficient of the solution and the volatility of the solvent. A purified nitrocellulose dissolved in amyl acetate is in general use. Mercury is, theoretically, an excellent substrate, but the manipulative difficulties are considerable. Tougher films are formed by Formvar 15/95, Grade E, a polyvinyl formal polymer. A solution (0·2 to 0·4 per cent. by weight) in ethylene dichloride, which should be stored in a brown bottle, is spread evenly on to a microscope slide and held vertically in an atmosphere of the solvent to dry. The film is scored round the edge with a needle and floated on water by inserting the slide obliquely. Breathing on the dry slide and application of additional coats of solution round the edge facilitate stripping. Films showing interference colours are too thick.

Powders that can be dispersed in a volatile liquid may be mounted by placing a drop of suspension on a filmed screen and allowing it to dry. An adaptation of the turpentine dispersion method in optical microscopy has been devised. The pigment in a drop of turpentine is rubbed out with a glass rod on a microscope slide until dry. The dispersion is flooded with resin solution and floated on to water. Screens are placed on areas selected under an optical microscope. This method is most suitable for uniform powders 0·5 μ. and greater. Many excellent dispersions may be prepared by working a pigment suspended in a viscous resin solution on a plate glass table with a stiff, stainless steel spatula. The proportion of specimen to resin for a suitable concentration of particles in the field depends on particle size and can only be found by experience. Substances too soft to rub out mechanically may be made brittle by freezing with solid carbon dioxide.

Dispersion by supersonic and other vibratory methods has not been entirely satisfactory. A small pile of dry powder placed on a filmed slide may often be dispersed by passing a spark from a Tesla coil across the under side of the slide. Mechanical mills for dispersion have the disadvantage of requiring a large sample. The resin containing a dispersed material is made into a thin film by methods similar to those already described for clear films. Alternatively, the suspension may be evaporated to the consistency of thick cream and drawn down on a slide with a spatula by a series of short strokes. Samples prepared in this way exhibit fewer holes in the film. Large-size powders (1 to 10 μ.) may be mounted, using dibutyl phthalate or a high-boiling hydrocarbon as suspending medium and drawing down as before on a film-covered slide.

Electron micrographs of different types of mounts showing the particle size and shape of iron oxide, antimony oxide, chromium oxide, zinc oxide, calcium carbonate, and ultramarine are reproduced.

TABLE I. RESINS AND SOLVENTS

Resin	Recommended solvents: For dispersion[a]	For mounts cast on glass[b]	For mounts spread on water[c]	For mounts drawn down on glass[c]
Nitrocellulose (Parlodion)	Cellosolve acetate	Methyl acetate Ethyl acetate	Methyl acetate Ethyl acetate Amyl acetate Cellosolve acetate Octyl acetate	Ethyl acetate Amyl acetate
Formvar 15/95	Dioxan plus a few drops of dimethyl dioxan Nitroethane	Ethylene dichloride Nitroethane	Dichloroethylene Propylene dichloride Ethylene dichloride plus 0·1 per cent. of oleic acid Nitroethane 1 : 1 Dichloro-2-nitroethane	Dioxan Nitroethane
Polystyrene	Xylene	Ethylene dichloride Toluene	Benzene Toluene Xylene	Benzene Toluene
Methyl methacrylate or cellulose acetate	Methyl amyl ketone Methyl ethyl ketone plus 10 per cent. of *iso*phorone	Acetone	Methyl ethyl ketone Methyl amyl ketone *Iso*phorone	Methyl ethyl ketone

[a] 10 to 20 per cent. solutions, approximately. [b] 0·1 to 0·5 per cent. solutions, approximately.
[c] No fixed concentration; suspension allowed to dry to suitable consistency.

G. A. B.

Reviews

PENICILLIN: ITS PROPERTIES, USES AND PREPARATIONS. Published by direction of the Council of The Pharmaceutical Society of Great Britain. Pp. viii + 199 + xv. London: Pharmaceutical Press. 1946. Price 10*s.* 6*d.*

The publication simultaneously in *Nature* and *Science*, at the end of 1945, of what was then suggested as, and is still held to be, the chemical formula of penicillin, cleared away much of the mystery from its behaviour, which had previously appeared somewhat freakish. A book such as the one before me, wherein pharmacists in the first instance, but medical men and chemists as well, are given a full, straightforward, yet not over-simplified account of the penicillins, their properties and uses, could hardly have been written before the disclosure, not previously allowed for reasons of national security, of their chemical structure, on which their chemical properties depend and can be to a considerable extent explained. But we are no nearer with the penicillins than with most other drugs to understanding how biological behaviour is determined by chemical composition.

It appears certain that the biggest foe to penicillin as an entity—and, what is more to the practical point, to its anti-bacterial activity— is water, for the irreversible hydrolytic breakdown of the –CO–NH– link is, besides naturally being accelerated by heat, catalysed by

hydrogen and hydroxyl ions and also by the bacterial enzyme penicillinase. The other weak spot in the molecule, the bridge between S and CH, is open to attack by different agents, particularly the ions of heavy metals.

The chemist who reads from, or even browses in, this excellent survey of knowledge to the end of the summer of 1946 will see it largely as a series of logical conclusions drawn from these simple facts about the constitution of the penicillin molecule. On the other hand, he will appreciate how much there is yet to be discovered about the exact *modus operandi* of the drug's action on the bacterial cell, about what it is that causes some bacteria to be extremely sensitive to this attack and others quite indifferent and about the small but interesting question of why penicillin K, with its seven-membered straight hydrocarbon side chain, should be apparently more easily destroyed than the other three, for, superficially, examination of the four formulae would point to it as likely to be the stablest.

Although those parts of the penicillin story of most interest to the chemist are rightly treated in a somewhat condensed manner, because this book was primarily designed by the Pharmaceutical Society for its own members, not only can these portions be read by chemists with interest and profit, but the rest of the book also is packed with information that must often be of use both to pharmacists, and to those in the sister professions of medicine and chemistry.

The book is on the whole accurate and clearly written, though the writing bears signs, if not of a dual author, at least of an author who has at some times been in much more of a hurry than at others. A number of minor criticisms could well be made. One can question the desirability of using the word glycerine (*e.g.*, on p. 33) when describing the effects of the chemical substance glycerol; again, in referring to the effect of heavy metals on penicillin, it is not made sufficiently clear that the metals themselves probably have no destructive action, but only their ions in solution. The accuracy of writing, to which I have referred above, is hardly maintained on page 55, where it is said, of a full bacteriological investigation, "it should not be awaited before administration is commenced."

In general, however, it must be agreed that the author (or group of authors) has very successfully dealt with the greatest difficulty that arises over the writing of books like this. They tend to go out of date so rapidly that, even in normal times, a second edition appears to be needed immediately the first has been issued. In present circumstances things are often much worse, though this particular book seems almost to have created a record in the post-war world by the speed of its publication, judging by the information and references that are to be found in a volume bearing the issuing date of October, 1946. All the same we have already to modify some of the statements in the book, both on the experimental and on the manufacturing side. It is, for example, no longer true that the half million unit size of penicillin must consist of material that has been freeze-dried in bulk, for the technical problems involved in drying that amount *in situ* have now been surmounted. Nevertheless, in spite of any advances made since the manuscript of this book went to press, it remains one of the most concise and precise accessible sources of information about one of the most striking and useful drugs ever made available to doctor and patient. A. L. BACHARACH

GENERAL CHEMISTRY. By E. P. SCHOCH, W. A. FELSING, and G. W. WATT. International Chemical Series. Second Edition. Pp. xiii + 540. London: McGraw-Hill Publishing Co., Ltd. 1946. Price 20s.

This is a first year's text-book of general and theoretical, inorganic and organic chemistry that essays a mean path, in its method of treatment, between the modern and more mathematical outlook and the older descriptive chemistry. In this regard, the authors' prefatory hope "that a satisfactory degree of modernisation has been achieved without sacrifice of an essentially conservative approach," has been realised.

The treatment of the subject matter is superficial, but complete within the range of an introductory course. The sections on the periodic table, atomic structure and nuclear fission are particularly clear. In selecting examples for instructional purposes, the authors have not hestitated to draw upon the most recent advances in theoretical and practical chemistry. Amongst these are to be found the structural formula for *l*-ascorbic acid, a table of vitamins that includes biotin and inositol, a picture of a reverberatory tin smelting furnace erected during the recent war and of the new 60-inch cyclotron at the University of California; also chapters on synthetic rubber, nuclear reactions and artificial radioactive isotopes. Modernisation such as this should help to keep present in a student's mind the ever expanding nature

of chemical science and go far to prevent that impression of an already complete and static subject so often created, in the not very distant past, by a similar class of book.

In the more descriptive parts of the book are to be found chapters on metals and alloys, iron and steel, acids, bases and salts; but there is no systematic description of the elements and their compounds.

The text shows some evidence of multiple authorship in its phraseology and orthography; as an instance of the latter, "coworkers" and "overall" (as a variant of total) sometimes carry their meanings more clearly and easily to the mind by appearing in full dress, complete with hyphens, as "co-workers" and "over-all."

With the exception of recognised American variations in spelling and syntax, the writing is, for the most part, in clear closely-knit English; as is but to be expected of a book that carries as its motto a quotation from such a master of scientific prose as Thomas Henry Huxley. It may be added that there is distinct evidence of the authors' having kept this exemplar before them in much of their own writing.

This book is produced in complete conformity with the established standards of its printers and publishers. F. L. OKELL

SUPPLY OF LABORATORY APPARATUS AND CHEMICALS

THE Council of the Society will be pleased if any member who is experiencing difficulty in obtaining delivery of LABORATORY APPARATUS will send *full details* of such difficulties to the Honorary Secretary, Mr. K. A. Williams, 6, Milner Street, London, S.W.3, in order that *specific instances* may be brought to the notice of the Controlling Authority.

The following communication has been received from the Chemical Council regarding supplies of LABORATORY CHEMICALS—

> Cases of difficulty in obtaining supplies of laboratory chemicals have been brought to the notice of the Chemical Council, who have discussed the question with officials of the Board of Trade. Some of the delays are admittedly due to transport difficulties and shortage of containers, aggravated by the fact that users often do not return bottles, etc., promptly. The Chemical Council has been assured that the Board of Trade will be ready to take up any questions of genuine shortage which are reported with full particulars. Communications should be addressed to R.M.2T, Board of Trade, Raw Materials Department, Horseferry House, Horseferry Road, London, S.W.1.

REPRINTS

THE following cloth-bound reprints of papers read at meetings of the Physical Methods Group, from THE ANALYST, are now obtainable by application to W. HEFFER & SONS LTD., CAMBRIDGE, price 2/6 each.

SYMPOSIUM ON POLAROGRAPHY (from ANALYST, 1946, **71**, 49–65).

SYMPOSIUM ON CHROMATOGRAPHY (from ANALYST, 1946, **71**, 251–267).

SYMPOSIUM ON SPECTROSCOPIC ANALYSIS (from ANALYST, 1946, **71**, 348–376) with Lecture by Dr. H. W. THOMPSON, F.R.S., on "The Use of Infra-red Spectra for Analysis."

THE ANALYTICAL METHODS COMMITTEE REPORT ON THE MICROBIOLOGICAL ASSAY OF RIBOFLAVINE AND NICOTINIC ACID

is now obtainable in the form of paper-covered reprints; price, to members 1/6, to non-members 2/-. Applications, with remittance, should be sent to Mr. J. H. LANE, Editor, THE ANALYST, 7–8, Idol Lane, London, E.C.3.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.