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THE ANALYST

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Polarographic Studies: III. Determination of Vanadium

BY J. E. PAGE, B.Sc., PH.D., F.I.C., AND F. A. ROBINSON, M.Sc., F.I.C.

(*Read at the Meeting, April 7, 1943*)

WE have experienced considerable difficulty in finding a satisfactory method for determining vanadium in certain organo-vanadium preparations the chemotherapeutic properties of which we were examining[4]. In view of our ability to determine antimony polarographically in therapeutic preparations and in biological material,[1,5] we tried to devise a similar method for vanadium.

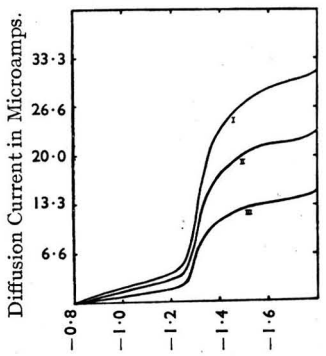
The general principles and procedures for quantitative polarography have been described in recent books and reviews,[2,3] and reference may be made to these publications for practical details. Several workers have studied the behaviour of vanadium at the dropping mercury electrode. Zeltzer[10] examined the polarograms of vanadium in various media and found that the most satisfactory polarograms are given by ammonium vanadate in ammoniacal soln. This was confirmed by Stackelberg *et al.*,[6] who showed that the wave is best defined in a supporting electrolyte composed of 0·2 *N* ammonium chloride and 6–7 *N* ammonia, the half-wave potential being about −1·32 v. *versus* the saturated calomel electrode (*vs.* the S.C.E.). They found that the diffusion current in ammoniacal soln. is directly proportional to the concn. of vanadium. The polarographic determination of vanadium in steel has also been described by Thanheiser and Willems.[8]

The method of Stackelberg *et al.* was devised for determining vanadium in steel, but we have found it equally applicable to other vanadium preparations, provided that the metal can be readily oxidised to vanadate. It is essential to destroy all organic matter before attempting to examine a sample on the polarograph, because the half-wave potential of vanadium is relatively high, and the more readily reducible matter would tend to mask the step given by vanadium. With tervalent antimony, on the other hand, it is merely necessary to dissolve the substance in *N* hydrochloric acid, as the step given by antimony appears at a much lower potential than that given by the organic constituents of the molecule.

EXPERIMENTAL.—The general polarographic procedure adopted in this investigation was similar to that described previously.[5] A Cambridge recording instrument was employed and a micro cell (capacity 3 ml) was used to hold the polarograph solns. The instrument was calibrated to read directly in microamp. by the procedure recommended by Kolthoff and Lingane.[3] The capillary used had the following characteristics: At a pressure of 41·2 cm of mercury, the drop-time (t) on open circuit in 0·1 *N* potassium chloride was 2·15 sec., weight of mercury dropping per second (m) = 1·62 mg and $m^{2/3}t^{1/6} = 1·57$. The symbols are those adopted by Kolthoff and Lingane.[3] The drop-time for the mercury cathode must be less than one drop every 2·5 sec., as at higher drop-times the diffusion-current tends to coalesce with the final ammonium step. For most other polarographic operations a drop-time of 3–6 sec. is recommended. "AnalaR" reagents were used throughout, and a special batch of "AnalaR" ammonium vanadate (B.D.H.) was used as standard.

* Through the North of England Section. † Through the Scottish Section.

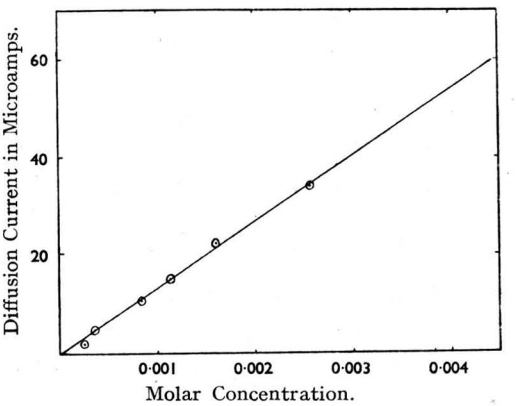
Procedure.—Dissolve a quantity of the material containing about 0·01 g of vanadium in strong sulphuric acid, oxidise with a few drops of strong nitric acid and heat until white fumes are evolved. Alternatively, fuse the organic complex with a mixture of potassium nitrate and sodium carbonate and extract with hot water. Dilute the solns. with hot water to about 30 ml, heat to boiling and pour into 20 ml of hot 10 *N* sodium hydroxide contained in a 100-ml graduated flask. The vanadium remains in solution as sodium vanadate, and any iron is pptd. as hydroxide. Cool the soln. to room temp., make up to the mark and filter through a dry filter (this is not necessary if iron is absent). Pipette 25 ml of the filtrate (*i.e.*, one quarter of the original sample) into a 50-ml graduated flask and carefully neutralise with hydrochloric acid. Add 0·5 g of ammonium chloride, cool, add 5 ml of 0·5% gelatin soln. and make up to the mark with strong (0·880) ammonia. (The gelatin is necessary to depress the maximum formed in its absence.) Allow the soln. to stand until colourless and transfer an aliquot portion to the polarograph cell. Leave the soln. for 5 min. and then record its polarogram over the range −0·8 to −1·6 v., the vanadate wave appearing at about −1·3 v.



Applied potential in volts.

I. 0·00160 *M* Sodium Vanadyl Tartrate.
II. 0·00112 *M* ,, ,, ,,
III. 0·00080 *M* ,, ,, ,,

Fig. 1.—Typical Polarograms for Sodium Vanadyl Tartrate.



Molar Concentration.

Fig. 2.—Relation between Diffusion Current and and Molar Concn. of Sodium Vanadyl Tartrate.

Some typical polarograms for sodium vanadyl tartrate are shown in Fig. 1. It will be seen from Table I and Fig. 2 that the diffusion current for vanadium in sodium vanadyl tartrate is proportional to the concn. over the range of 0·0005 to 0·005 *M*. At lower concns. it becomes increasingly difficult to measure the height of the step, *i.e.*, the diffusion current, for the step tends to coalesce with the final ammonium step. Solns. containing less than 0·001 *M* of vanadium should be avoided wherever possible. The diffusion current must always be corrected by subtracting the residual current, which becomes quite large at low concentrations.

TABLE I—VARIATION OF DIFFUSION CURRENT WITH MOLAR CONCENTRATION OF VANADIUM

Molar concentration (C)	Diffusion current (i_d) in microamp.	$\frac{i_d}{C} \times 10^{-4}$
0·00427	55·0	1·29
0·00256	34·0	1·32
0·00160	21·5	1·34
0·00112	15·2	1·36
0·000800	10·5	1·31
0·000342	4·40	1·29
0·000171	2·62	1·53

Results.—The results obtained for a variety of vanadium preparations are given in Table II, where they are compared with data obtained by a modification of Willard and Young's volumetric method[9] for the determination of vanadium in steels. This method was simplified and adapted to the special purpose of determining vanadium in organic substances by Mr. W. Solomon of the Wellcome Chemical Research Laboratories. The

max. difference between these determinations is ±4%. Polarograph curves for vanadium are reproducible to within ±2%, while complete determinations are reproducible to within ±3%.

TABLE II—COMPARISON OF POLAROGRAPH RESULTS WITH THOSE OBTAINED BY THE VOLUMETRIC METHOD

Preparation	Vanadium by polarograph %	Vanadium by volumetric method %
AnalaR ammonium vanadate	43·5	43·5
Vanadium pentoxide	56·2	56·0
Sodium vanadyl tartrate, $(VO)C_4O_6H_2Na_2.3H_2O$ ..	16·0	16·3
Sodium vanadyl *bis*catechol-3:5-disulphonate ..	8·1	7·8
Sodium vanadyl sulphosalicylate	7·3	7·6
Sodium vanadyl thiomalate	3·66	3·82

Unfortunately, this polarographic method does not provide information about the original valency state of the vanadium or the relative amounts of vanadium present in the different valency states. With antimony[5] it is a simple matter to determine on the polarograph the relative amounts of tervalent and quinquevalent metal present in a given sample.

SUMMARY.—The method of Stackelberg *et al.* for the polarographic determination of vanadium in steels was modified and adapted to the determination of vanadium in a series of organo-vanadium preparations. After destruction of the organic matter and oxidation of the vanadium to the vanadate state, the soln. was examined at the dropping mercury electrode in strong ammoniacal soln. The half-wave potential was −1·32 v. *versus* the saturated calomel electrode and the diffusion current was proportional to the concentration over the range 0·0005 to 0·005 *M*. The max. difference between the results obtained by the polarographic method and those obtained by a volumetric procedure was about ±4%.

We wish to thank Mr. W. Solomon of the Wellcome Chemical Research Laboratories for technical information, and Messrs. R. L. Barnden and B. T. Layzell for preparing the polarograph solutions.

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DISCUSSION

Dr. PAGE, replying to questions, referred to the fairly extensive literature on the polarographic determination of lead for specific details as to quantities that could be determined (*cf.* Kolthoff and Lingane[3]). It was usually necessary to destroy organic matter before examining for metals, but it was possible to determine metals of low half-wave potential, *e.g.*, antimony and copper, directly, since the steps for these metals appeared before those for the reducible organic matter. The accuracy of polarographic analysis was limited by the accuracy with which the height of the step could be measured and compared with a standard. All methods of measuring the step were somewhat arbitrary, but so long as a standard procedure (*cf.* Kolthoff and Lingane[3]) was adopted, the actual method of measuring would have little effect on the final result.

The diffusion current (*i.e.*, step height) varied with temperature, and in many determinations a thermostat had to be employed even for routine operations. They (the authors) used the instrument in a room with a steady temperature and examined a standard soln. with each batch of samples, so as to compensate for any small variation in the temperature, galvanometer sensitivity or mercury drop-time. To avoid trouble with the capillary, it was important to use clean mercury (*cf.* O. H. Müller, *Chem. Eng. News*, 1942, **20**, 1528).

The personal factor in a polarographic determination was confined to the actual measurement of the step height. Before a curve was recorded polarographically the best galvanometer sensitivity for the soln. was selected by operating the potentiometer drum manually. Methods for the polarographic determination of manganese, chromium, molybdenum and tungsten in vanadium steels had been described by Stackelberg *et al.*[6] and Thanheiser and Willems.[8]

Determination of Ascorbic Acid in Preparations containing Iron and Ammonium Citrate

By J. H. SINGER, A.I.C., and M. N. MILNER

Difficulties were experienced in determining ascorbic acid in a commercial soln. claiming to contain also iron and ammonium citrate B.P., and expts. were therefore made to investigate the effect of this substance upon the normal method for vitamin C determination, *viz.*, titration with 2 : 6-dichlorophenolindophenol. A method has been devised involving complete removal of iron from solution and subsequent titration of the vitamin C present without loss.

Ionised ferric salts, as might be supposed from their high oxidation reduction potential, immediately oxidise ascorbic acid in solution whilst being themselves reduced. On the other hand, ferrous salts cannot be reduced by ascorbic acid in solution and should not be oxidised by 2 : 6-dichlorophenolindophenol, although in practice they are oxidised to some extent in acid soln. and tend to give high results and a fading end-point when present during the determination of ascorbic acid. Small quantities of ferrous salts will also cause gradual destruction of ascorbic acid in soln.

Iron and ammonium citrate, being a non-ionised complex, might be expected to have little effect on ascorbic acid, except in so far as solns. undergo slight ionisation. Furthermore, ferrous or ferric salts in the ionised state may well be liberated during the course of the determination.

Table I

	Solution	Composition %	Ascorbic acid found %
1.	Ascorbic acid in water	0·07	0·071
2.	Iron and ammonium citrate B.P. in water	1·0	0
3.	Iron and ammonium citrate (green scales) B.P.C.	1·0	0
4.	Iron and ammonium citrate B.P.	1·0	
	Ascorbic acid	0·07	0·065
5.	Iron and ammonium citrate B.P.	1·0	
	Ascorbic acid	0·07	0·067
	Liquid glucose	9·0	
	60% alcohol	35·0	
6.	Iron and ammonium citrate (green scales) B.P.C.	1·0	
	Ascorbic acid	0·07	0·065
	Liquid glucose	9·0	
	60% alcohol	35·0	
7.	Soln. No. 5 after 3 days in laboratory		0·047
8.	Soln. No. 6 after 3 days in laboratory		0·057
9.	Commercial preparation	Less than	0·002
10.	Soln. No. 5 after 3 weeks in laboratory	,, ,,	0·002
11.	Soln. No. 6 after 3 weeks in laboratory	,, ,,	0·002

Experimentally the presence of *ca.* 1% of iron and ammonium citrate in solns. of ascorbic acid assayed by direct titration caused very low results, only *ca.* 50% of the ascorbic acid added being recovered; the end-point was almost impossible to observe owing to the colour of the soln. When hydrogen sulphide was passed through this soln. to reduce any dehydroascorbic acid present, a colloidal ppt. of ferrous sulphide insol. in acetic acid was produced and rendered titration impossible. Addition of hydrochloric acid to prevent formation of sulphide led to figures slightly higher than those obtained by direct titration but of doubtful value, since there must have been some destruction of ascorbic acid by the ferric chloride liberated, when the hydrochloric acid was added to the soln. in the first place, and subsequent reduction of this to ferrous chloride by the hydrogen sulphide in acid soln. From these results it was concluded that a satisfactory determination could only be effected by complete removal of the iron from the soln. by some procedure with no effect on ascorbic acid.

The only two methods that showed promise were pptn. of the iron as sulphide in alkaline soln., and pptn. as the complex with 8-hydroxyquinoline in slightly acid soln. Under suitable conditions pptn. of the iron as sulphide in slightly alkaline soln. can be

accomplished without loss of ascorbic acid, but, owing to the comparatively high solubility product of ferrous sulphide, traces remain in solution and give high results in the subsequent titration.

The second method, using 8-hydroxyquinoline in slightly acid soln. proved quicker and simpler, and hydrogen sulphide pptn. was therefore discarded. 8-Hydroxyquinoline has no effect on ascorbic acid in soln. and is, in fact, sometimes used as an antiseptic in parenteral vitamin C preparations; further, it will quantitatively ppt. iron from acid solns. of iron and ammonium citrate provided that the *p*H is not allowed to fall below about 2·8; it is not oxidised by 2 : 6-dichlorophenolindophenol and hence gives a perfect blank. The solns. examined in the present series of expts. were prepared to contain 1% of iron and ammonium citrate and 0·07% of ascorbic acid and the following method was finally adopted.

METHOD.—Pipette 5 ml of the sample into a 150-ml conical flask, and add 50 ml of water, 1 g of sodium acetate and 5 ml of a 2·5% soln. of 8-hydroxyquinoline in 20% acetic acid. Warm gently until the ppt. coagulates, cool, add 3 g of kieselguhr, shake, filter under slight suction through a No. 42 filter-paper, wash the flask and filter twice with 5-ml portions of water and titrate the combined filtrate and washings with 2 : 6-dichlorophenolindophenol soln. in the usual manner.

Table I shows the results thus obtained with a number of preparations made to be comparable with the commercial article under investigation.

It appears that there is some loss of ascorbic acid in solution with iron and ammonium citrate immediately after the soln. is prepared, and that this loss then continues more slowly over a period. This is borne out by the fact that immediately after preparation of the soln. its colour changes, especially with the green variety of iron and ammonium citrate, and ferrous iron can be detected in the solution with αα-dipyridyl. Probably traces of free ionised ferric ion in the iron and ammonium citrate react immediately with part of the ascorbic acid, which is destroyed, and subsequent slow destruction proceeds as further ionisation takes place.

Any commercial article, therefore, containing ascorbic acid and iron and ammonium citrate in solution is unlikely to have a life of more than a few weeks.

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Notes

USE OF AMINOSULPHONIC ACID IN THE DETERMINATION OF NITRITES

AMINOSULPHONIC acid was proposed by Baumgarten and Marggraff as a reagent for the gasometric determination of nitrite (*Ber.*, 1930, **63**, 1019). It reacts with nitrous acid thus:

$$NH_2SO_2OH + HNO_2 = N_2 + H_2SO_4 + H_2O.$$

This reaction is specific and quantitative even at dilutions of $N/100$ if the soln. be warmed.

A gasometric method is not, however, desirable where volatile substances may be present, *e.g.*, in the determination of nitrous acid produced during the nitration of volatile aromatic compounds. Further, the permanganate method (*Sutton's Volumetric Analysis*), which is usually applied when more than traces are to be determined, is subject to the objection that the control test, which must be carried out to ascertain the extent of the action of the permanganate on the organic matter after addition of urea to eliminate the nitrite, may show absorptions of permanganate relatively high in comparison with that due to the nitrite; this is obviously unsatisfactory. Permanganate, too, cannot be used if much mineral acid, together with halogens, be present.

These difficulties, due to the vigorous oxidising action of permanganate, can be overcome by employing the above reaction for estimating nitrite. Aminosulphonic acid has no general oxidising or reducing action; an $N/10$ soln. coloured with a few drops of potassium permanganate shows no decrease in colour on warming to *ca.* 60° C.

The method designed to use this reagent, and avoid the technique of gasometric analyses, is as follows:

SOLUTIONS REQUIRED.—*Standard aminosulphonic acid.*—This substance, which is now manufactured on a large scale for its fire-proofing and other properties, is available in a purified state containing not more than traces of sulphate. It occurs as small colourless crystals, readily sol. in water and yielding a soln. of good keeping properties. $N/10$ is a convenient strength (9·7 g/litre), and the soln. may be standardised against $N/10$ alkali if the aminosulphonic acid is pure, or against a nitrite soln., which, in turn, is checked against permanganate in the usual way.

External Indicator.—Starch and potassium iodide, Griess reagent, dimethyl aniline sulphate, *m*-phenylene diamine, were all found satisfactory for indicating the presence of excess nitrite in the titration. Griess–Ilosvay reagent was the most sensitive, although somewhat slow in response. Recently, Shin (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 33) proposed the use of sulphanilamide and N (1 naphthyl)-ethylene-diamine hydrochloride instead of sulphanilic acid and α-naphthylamine, as in the Griess reagent.

Investigation of the former pair of compounds as a substitute for the Griess–Ilosvay reagent showed that improvement was effected in so far as rapidity of response was concerned, mainly in virtue of the introduction of sulphanilamide, and that a mixture of α-naphthylamine and sulphanilamide served the purpose equally well. This reagent gives a pink colour similar to that obtained with the Griess–Ilosvay reagent, but the colour develops much more rapidly to its full intensity and remains stable over a longer period. For normal uses, *e.g.*, the estimation of traces of nitrite in water, the reagent is made up as follows: *Solution A*.—0·5 g of sulphanilamide in 150 ml of 15% acetic acid. *Solution B*.—0·1 g of α-naphthylamine in 150 ml of 15% acetic acid; equal vols. are taken for use. These solns. are too weak for use as an external indicator; for this purpose 0·5 g of sulphanilamide and 0·1 g of α-naphthylamine are dissolved in 10 ml of glacial acetic acid and 10 ml of water. This strong mixed soln. keeps fairly well in a stoppered bottle in the dark.

Standard nitrite solution.—This contains 6·9 g of sodium nitrite/litre. This soln. is $N/10$ with respect to aminosulphonic acid, but $N/5$ with respect to permanganate ($HNO_2 + O = HNO_3$).

PROCEDURE.—*Nitrite in neutral or faintly acid solns*.—Pipette 25 ml of $N/10$ aminosulphonic acid into a conical flask, and add an amount of the nitrite estimated to leave approx. ¼ of the amino acid unconsumed. Add the nitrite slowly, with constant stirring, and thereafter warm the soln. to *ca.* 50° C. to complete the reaction. Cool and back-titrate the soln. with standard nitrite soln., using the modified Griess-Ilosvay reagent on a spotting plate until a permanent pink colour is obtained. Warm the soln. to 50° C. on approaching the end-point, as the reaction tends to slow up appreciably when the concn. of aminosulphonic acid becomes low. The end-point is very sensitive, and can readily be judged to within 1 or 2 drops of $N/10$ nitrite in a vol. of *ca.* 150 ml, one drop being used on the spotting plate. One ml of aminosulphonic acid ≡ 0·0047 g of HNO_2.

Nitrite in strongly acid solns., e.g., *nitrosylsulphuric acid in conc. sulphuric acid*.—Add to 25 ml of amino acid soln. in a flask provided with a glass stopper 5 to 10 g of sodium acetate and, after solution is complete, add the sample under test by dropping the weighing bottle containing it into the flask. Close the flask, shake to dislodge the stopper of the weighing bottle, and complete the titration as above. The purpose of the sodium acetate is to act as a buffer, high concns. of mineral acid decreasing the sensitivity of the indicator. A Lunge–Ray pipette may also be used to add the sample if this is done slowly, shaking after the addition of each few drops. Tests were made to ascertain whether high concns. of mineral acids affected the titration, but other than the de-sensitising of the indicator, which may be countered by buffering, no effect was apparent.

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AN IMPROVED MANGANESE SEPARATION FOR THE PHOSPHATE METHOD AND FOR GENERAL USE

HITHERTO there has been no satisfactory method of separating manganese applicable to a buffered solution of *p*H 4–5 containing ammonium salts. Under these conditions oxidation of manganese to manganese dioxide with bromine or hypochlorite is impossible, and it was for this reason that in my phosphate method[1] the use of ammonium salts was avoided until after the manganese had been separated.

Manganese can be quantitatively separated as manganese dioxide from mineral acid solns. containing ammonium salts by means of persulphate, but considerable amounts of iron, cobalt, nickel and zinc are co-pptd.[5] After these metals have been removed it often happens that a buffered soln. of *p*H 4–5 containing manganese, calcium, magnesium and ammonium salts is obtained. Thus, after removing Groups 1 and 2, the iron and aluminium group may be separated as phosphates[1] (*p*H *ca.* 3·3), acetates (*p*H 4–5·5), succinates[3] (*p*H *ca.* 4·4), or benzoates[2,4] (*p*H *ca.* 4·0). Nickel, cobalt and zinc may then be separated as sulphides at *p*H 4·5–5, leaving manganese, calcium and magnesium to be determined. From a soln. obtained by any of the above methods it has been found that manganese can be quantitatively pptd. as manganese dioxide by means of sodium periodate. The reaction is suitable for the separation of 0·5–200 mg of manganese from similar amounts of calcium and magnesium.

GENERAL PROCEDURE.—After removing metals except manganese, calcium and magnesium, boil off any hydrogen sulphide and, if necessary, adjust to *p*H 4·4–4·8 by adding acetic acid and ammonium acetate. (A greenish-blue spot test with bromocresol green indicates the correct *p*H.) Add 1 ml of *N* formic acid and then to the gently boiling soln. (usually about 300 ml) add sodium periodate soln. in increments of 1 to 4 ml, according to the amount of ppt., until the ppt. darkens. Next make a spot test for periodate with the starch iodide soln. and, if this is negative, continue additions until a positive spot test is obtained. Filter and wash the ppt. with a little water and then with hot $N/10$ nitric acid to remove small amounts of calcium and magnesium that may be co-pptd.; 100 ml of $N/10$ HNO_3 are usually ample.

The formic acid is added to overcome a tendency for a trace of manganese to be oxidised too far, giving a red soln. It slowly reduces the slight excess of added periodate. Calcium in the filtrate may be pptd. by adding ammonium oxalate and then magnesium by adding phosphate and ammonia.

PROCEDURE FOR THE PHOSPHATE METHOD.[1]—The procedure is as described originally[1] except that: (1) Use ammonium salts wherever sodium or potassium salts, except sodium nitrite, are specified. This greatly facilitates the washing of ppts. before igniting and weighing and tends to reduce adsorption. (2) After removing the zinc and preceding groups, test for manganese with periodate, as described above.

NOTE ON THE INFLUENCE OF PHOSPHATE.—The presence of a large amount of phosphate, such as frequently occurs when using the phosphate method, tends to cause the manganese dioxide ppt. to be slightly soluble in $N/10$ nitric acid. This makes re-treatment of the filtrate necessary and is most noticeable when the amount of manganese is small or when the ratio P_2O_5/MnO is greater than 5. The above periodate method is far less susceptible to phosphate than the hypochlorite method described originally and if, prior to adding the periodate, the soln. is diluted so that not more than 0·1% of P_2O_5 is present, the manganese dioxide will be completely insol. in $N/10$ nitric acid and pptn. complete.

BEHAVIOUR OF THE RARE METALS IN THE PHOSPHATE METHOD.—Some of the rare metals may be separated in Groups 1 and 2 or, alternatively, by methods described by Noyes and Bray.[6] The remaining rare metals, except vanadium and tungsten, are pptd. in the Iron Group by ammonia and would probably be pptd. with iron in the phosphate method. This is true of Ti, Zr, Th, U, Ce and Be, but the phosphates of the first three are so insoluble even in N hydrochloric acid that they cannot accompany phosphate as far as the Iron Group. Any rare metals present in the Iron Group ppt. could be detected by methods described by Noyes and Bray.[6]

Except when phosphate is present, tungstic acid is pptd. early in the analysis before the Iron Group is reached. Tungstic and vanadic acids are to a large extent co-pptd. with the Iron Group phosphates, but otherwise do not interfere with analysis by the phosphate method.

REAGENTS.—*Sodium periodate.*—Dissolve 4·0 g of the salt ($Na_2H_3IO_6$) in water containing 1·5 ml of glacial acetic acid and dilute to 200 ml. Filter. 1 ml of this soln. is sufficient for *ca.* 5 mg of MnO.

Starch iodide.—Prepare 1% starch soln. and in 100 ml thereof dissolve 10 g. of sodium bicarbonate and 1 g of potassium iodide. The bicarbonate prevents the iodate, to which the periodate is reduced, from giving the starch iodide reaction.

ANALYSIS OF MIXTURES BY THE PHOSPHATE METHOD.—In the following examples, the manganese was separated with periodate, as described above. The manganese dioxide was dissolved, converted into $Mn_2P_2O_7$ and weighed. In each expt. the Iron Group phosphates were re-pptd. and weighed as $FePO_4 + AlPO_4$. Iron in the mixture was determined volumetrically and aluminium was obtained by difference.

1. Synthetic mixture.

Taken		Found	
g		g	g
0·0529	Fe_2O_3	0·0529	0·0527
0·0522	Al_2O_3	0·0522	0·0519
0·0996	MnO	0·0994	0·0996
0·1084	CaO	0·1091	0·1093
0·1000	MgO	0·0997	0·1001
0·100	P_2O_5	—	—

2. B.C.S. basic slag "A" No. 174.

	Present	Found	
	%	%	%
Fe	8·93	8·82	8·77
"Alumina, etc."	3·91	2·80	2·78
MnO	3·27	3·30	3·38
CaO	44·73	44·56	44·61
MgO	6·85	6·90	6·88
SiO_2	16·15	16·24	16·03
P_2O_5	12·92	—	—

The soln. obtained after removing silica in the usual way was twice evaporated to a few drops in a glass beaker with perchloric acid. Without this treatment, which removes fluorides that may be present (*e.g.*, in one instance 1·2% of fluorine), the aluminium was incompletely pptd. "Alumina, etc.," by the phosphate method includes titanium but not V_2O_5 (0·8%). Both are included in the result given as 3·91%. This and the result for MnO (3·27) are not standardised.

3. B.C.S. manganese brass "B" No. 179.

	Present	Found	
	%	%	%
Fe ..	0·91	0·92	0·92
Al ..	1·62	1·65	1·66
Zn ..	33·9	33·82	33·90
Ni ..	1·01	0·98	0·98
Mn ..	1·03	1·05	1·04
Cu ..	58·8	—	—

The zinc and nickel sulphides were ignited together, converted into sulphates and weighed. Nickel in the mixed sulphates was determined with dimethyl glyoxime and zinc was obtained by difference.

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18 STRATTON DRIVE
BARKING, ESSEX

G. J. AUSTIN

January, 1943

Analytical Methods Committee

DETERMINATION OF THE CRUDE FIBRE IN NATIONAL FLOUR

RESULTS of an investigation undertaken for the Vitamin B_1 Sub-Committee of the Accessory Food Factors Committee (Lister Institute and Medical Research Council), reported by E. B. Hughes, President of the Society of Public Analysts and Other Analytical Chemists.

It was the wish of the Sub-Committee that analysts who had been participating in the trials of the methods for determination of Vitamin B_1 in national flour[1] should also submit results for the determination of crude fibre in national flour. The work was carried out under the auspices of the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists, the analysts taking part being:

F. W. F. Arnaud*; R. H. Carter, Research Association of British Flour Millers; H. E. Cox*; M. Cutting, Lister Institute of Preventive Medicine; E. Hinks*; E. B. Hughes*; G. N. Jenkins, Sir William Dunn Biochemical Laboratory, Cambridge; T. F. Macrae, R. A. F. Institute of Pathology and Tropical Medicine; E. I. McDougall, Institute of Animal Pathology, Cambridge; G. W. Monier-Williams*; J. R. Nicholls*; W. M. Seaber*; G. Taylor.*

Ordinarily the method used by analysts for the determination of crude fibre is that specified in the Fertilisers and Feeding Stuffs Regulations, 1932, S.R. & O., No. 658, and it was on the basis of this method that trials were first made. From the examination of several samples it was found that results by different analysts had not the degree of concordance desired, and further investigation was therefore necessary to establish exact conditions of procedure to ensure sufficiently close agreement between the results of different workers.

The method of the Fertilisers and Feeding Stuffs Regulations meets the requirements for materials usually examined in relation to those Regulations, but in national flour the material is in a fine state and the crude fibre content is low, generally below 1% and even as low as 0·3%.

In individual laboratories the results obtained by the exercise of the particular procedure used there in applying the standard Fertilisers and Feeding Stuffs Regulations method gave the degree of concordance required, but it was found that, to procure the same concordance in results from different laboratories, a more extensive and detailed specification of the procedure was necessary. As a result of many trials, including determinations made by analysts in each other's laboratories and examination of each one's usual procedure, precise instructions were arrived at which gave the degree of concordance required for the determination of crude fibre in national flour.

The flours examined were authentic specimens of national flour, *i.e.*, 85% extraction of the wheat grain as defined in the 2nd Memorandum on National Flour of the Medical Research Council (1941).[2] The samples were kindly supplied by the Research Association of British Flour Millers.

Method.—The method is essentially that specified in the Fertilisers and Feeding Stuffs Regulations, 1932, S.R. & O., No. 658, for the determination of the crude fibre content of Feeding Stuffs, with certain modifications found to be necessary to give sufficiently concordant results for national flour in which the crude fibre is generally below 1% and may be as low as 0·3%.

Briefly these modifications are:

(1) the flour is not subjected to the preliminary extraction with petroleum spirit;
(2) the weighed sample is made into a cream with a small proportion (20 ml) of the cold acid before adding the remainder of the acid in the boiling condition;
(3) the digestion is conducted under a reflux condenser;
(4) the fibre is weighed in a dish, not on a filter paper. The drying and weighing operations are critical, as an amount of 1 mg corresponds to 0·03% (using 3 g of flour). It is important, too, that the dish should be subjected to the same conditions before each weighing, and therefore after igniting the crude fibre in the dish the dish is moistened and dried in the same way as in the determination of the gross weight of the fibre.

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* Representing the Society of Public Analysts and Other Analytical Chemists.

RESULTS.—The results obtained by various workers in the final collaborative tests, using the recommended method, are given in Table I.

TABLE I

CRUDE FIBRE %, CALCULATED ON DRY BASIS

Analyst	Individual results	Average
A	0·87, 0·89, 0·90, 0·95	0·90
B	0·93, 0·93, 0·93, 0·95, 0·95, 0·95	0·94
C	0·83, 0·85, 0·85, 0·86, 0·87, 0·89	0·86
D	0·88, 0·90, 0·91, 0·93, 0·94	0·91
E	0·90, 0·93, 0·94, 0·94	0·93
F	0·87, 0·89	0·88
G	0·89, 0·89, 0·95	0·91

These results, on statistical analysis (made by Dr. K. Coward), show the limits of error set out in Table II.

TABLE II

LIMITS OF ERROR (as percentage of amount present) for a crude fibre value of 0·9% (dry basis)

	P = 0·95	P = 0·99
For determination in duplicate	6·1%	8·0%
,, ,, ,, triplicate ..	4·4%	5·8%
,, ,, ,, quadruplicate ..	3·8%	5·1%

METHOD FOR THE DETERMINATION OF CRUDE FIBRE IN NATIONAL FLOUR

Reagents.—(*a*) Sulphuric Acid Solution: containing 1·25 g of H_2SO_4 per 100 ml.

(*b*) Sodium Hydroxide Solution: containing 1·25 g of NaOH per 100 ml and free, or nearly so, from Na_2CO_3.

Apparatus.—(*a*) Condensers: selected to maintain constant volume of solution throughout the process of digestion.

(*b*) Digestion Flasks: 1000 ml conical flasks.

(*c*) Filter Papers: 9-cm No. 54 Whatman filter papers.

Determination.—A weighed quantity, 3 to 5 g, of the well mixed sample of flour is added slowly with gentle agitation to 20 ml of cold 1·25% sulphuric acid solution contained in a 1000 ml conical flask. 180 ml of 1·25% sulphuric acid solution measured at ordinary temperature and brought to boiling point are added to the creamed flour and the flask is briskly rotated until all the solid matter is evenly dispersed. The flask is then attached to a suitable condenser and the contents are brought to boiling on a wire gauze over a suitable burner within 1 min. of adding the boiling acid, and heating is adjusted to maintain gentle ebullition for 30 min., during which time the flask is occasionally rotated to remove particles from the sides. After 30 minutes from the beginning of boiling, the contents of the flask are poured quickly into a Buchner funnel fitted with a 9 cm No. 54 filter paper* through which boiling distilled water has just been drawn by suction. When all the acid liquid has drawn through, the flask is rinsed out three times with hot water into the funnel and the residue on the filter paper finally washed twice again with hot water. Filtration of the acid digest up to the stage of washing must be completed within 10 min.

The filter paper, with the washed residue, is now transferred to a glass funnel (conveniently of 5 in. diam.), which is placed in the neck of the rinsed digestion flask, and the residue is washed back into the flask with 200 ml of boiling 1·25% sodium hydroxide solution, this sodium hydroxide solution having been measured at ordinary temperature and brought to boiling-point. Digestion for 30 minutes is then repeated as before, the precautions given for the treatment with acid being observed.

At the end of 30 min. the flask is removed from the condenser and its contents are immediately filtered through a folded 9-cm No. 54 filter paper in an ordinary conical funnel, the filter paper having been previously wetted with hot distilled water. The fibre on the filter-paper is washed with boiling water, then with a 1% solution of hydrochloric acid and again with boiling water until free from acid, and it is then washed twice with 95% alcohol and three times with ether.

* It is important that the filter paper used in the Buchner funnel should fit up to, but only just up to, the walls of the funnel.

The residue is then transferred with boiling water into a previously ignited dish (platinum, gold alloy or silica) and dried first on a boiling water-bath and finally in an oven at 100° C. to constant weight, the dish being cooled in an efficient desiccator for an adequate time* before each weighing. The fibre is then ignited at dull red heat, and the dish cooled, moistened with distilled water and dried at 100° C. to constant weight. The difference between the two weights gives the weight of crude fibre.

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April, 1943

Ministry of Food

STATUTORY RULES AND ORDERS†

1943—No. 701. The Edible Oils and Fats (Control of Sales) Order, 1943. Dated May 13, 1943. Price 1d.

In this Order "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 but does not include—(*a*) "butter," "cooking fats" or "margarine" (as defined in S.R. & O., 1942, No. 1474) or suet or peanut butter or peanut paste; (*b*) any aromatic oil used for flavouring purposes.

— **No. 773. The Dripping (Maximum Prices) Order, 1943. Dated May 27, 1943.** Price 1d.

"Dripping" means clear, unbleached, unadulterated fat—(*a*) produced from or by the rendering or processing of fat and bones of sheep, oxen or pigs; (*b*) of a sweet smell and untreated by any chemical process; (*c*) containing not less than 99% saponifiable matter and not more than 2% free fatty acids; but does not include imported "premier jus."

— **No. 838. The Soft Drinks Order, 1943. Dated June 11, 1943.** Price 3d.

Except under licence the manufacture or packing of any soft drink by way of trade or business is prohibited.

"Soft drink" means (i) any concentrated drink, *i.e.*, any fruit juice, squash or crush, flavoured powder or tablets, and any other product whether in liquid, semi-solid or powder form which is suitable for the preparation by dilution of a drink for human consumption; and (ii) any unconcentrated drink, *i.e.*, any fruit juice or fruit juice drink, mineral water, spa water, tonic water, ginger beer, herbal or botanic beer, non-alcoholic wine and any other drink ready for consumption without dilution; but does not include (*a*) water (otherwise than as aforesaid); (*b*) tea, coffee, cocoa, chocolate, milk or any preparation thereof; (*c*) any egg product; (*d*) any cereal product other than flavoured barley water and other than any product for the preparation of flavoured barley water; (*e*) health salts or sherbet; (*f*) meat, yeast or vegetable extracts, soup mixtures and similar products; (*g*) intoxicating liquor (as defined in the Licensing (Consolidation) Act, 1910; (*h*) any medicinal product which is not also suitable for use as a drink.

"Unfermented apple juice" means the unadulterated juice of fresh apples without the addition of any preservative, acid, sweetening or colouring matter, essence, or flavouring material and containing not more than 0·5% by weight of alcohol.

Liquid soft drinks may be sold only in containers of specified sizes and max. prices are prescribed. Their ingredients must comply with those stipulated in Schedule I of the Order. In the Table in this Schedule drinks are classified as: I, Unconcentrated and II, Concentrated, and their respective ingredients are given as follows. No other acid or sweetening matter may be used.

Section I	Min. acidity (as crystalline citric acid of 100% purity) Oz. per 10 gall.	Min. quantity of fruit juice Fl. oz. per 10 gall.	Variety of juice	Quantity of added sugar and saccharin per 10 gall.
Lemonade, Orangeade, Limeade, Ginger Ale	1½	nil		Sugar 18 ozs. and Saccharin 82 grains
Ginger Beer, Sparkling Special	¾	nil		
Fruit Cup	2½	80	Any	
Appleade	2¼	120	Apple	
Soda Water	Not less than 5 grains of sodium bicarbonate per pint.			

* For a metal dish at least 20 minutes. For a silica dish at least 45 minutes.

† A summary of some Orders. Italics signify changed wording. Obtainable from H.M. Stationery Office.

Section II	Ozs. per 10 gall.		Galls. per 10 gall.		Variety of juice	Per 10 gall.
Lemon Squash, Orange Squash	36	24	2½	2½	Lemon, Orange	
Grapefruit Squash, Lemon Barley ..	24	24	2½	1½	Grapefruit, Lemon	
Lime Juice Cordial	36		2½		Lime	
Blackcurrant Cordial, Elderberry Cordial	10	10	1	1	Blackcurrant, Elderberry	
Lemon Flavour Cordial	20		nil			Sugar 7½lbs. and Saccharin 1oz.
Lime Flavour Cordial	20		,,			
Orange Flavour Cordial	20		,,			
Blackcurrant Flavour Cordial	16		,,			
Elderberry Flavour Cordial	16		,,			
Speciality Flavour Cordial	7½		,,			
Ginger Cordial, Peppermint Cordial ..	7½	2½	,,			

Legal Notes

The Editor would be glad to receive particulars of cases with points of special legal or chemical interest.

VITAMIN B: MISLEADING LABEL

On June 10, 1943, at Upton-on-Severn Police Court, a Liverpool firm was charged under Sec. 6(1) of the Food and Drugs Act, 1938, with wilfully giving a label with a packet of Breakfast Food, calculated to mislead as to the nature, substance and quality of the food. The label included the statement "Contains Vitamin B." The defendants pleaded guilty.

The prosecution was in respect of a sample examined by the County Analyst for Worcestershire (Mr. H. E. Monk), who certified that it contained 0·25 I.U. of vitamin B_1 per gram, and added under the heading "Observations":—"the above content of vitamin B_1 is extremely low. It is about the same as that of pre-war low extraction white flour, which was always criticised as being deficient in vitamin B_1. It is one quarter that of present-day National Wheatmeal Flour (85% extraction). Six half-pound packets of this material would be necessary to supply the minimum daily needs of an adult for vitamin B_1."

In evidence Mr. Monk said that with some few exceptions all foodstuffs contained some vitamin B_1, some were good sources, others poor. Not many foodstuffs contained less vitamin B_1 than the sample examined. Oatmeal, from which breakfast porridge was made, contained about 10 times as much. Not only was the sample an extremely poor source of the vitamin, but it contained so much carbohydrate that it would be necessary for the body to obtain additional vitamin B_1 from other sources before it could utilise the carbohydrates in this preparation. So far as the body's balance sheet for vitamin B_1 was concerned, the material was not a credit but an actual debit.

For the defence it was stated that the article was prepared from wheat rusks and malt extract, and the belief that the malt extract was a rich source of vitamin B was the cause of the statement on the label. The manufacturers had since discovered that, whilst liquid malt extract was a good source of vitamin B, the same was not true of the dried extract. (In cross examination the County Analyst disagreed with this statement: he said that, allowing for the different moisture contents, there was little difference between average liquid and dry malt extracts). In spite of the County Analyst, who did not consider malt extracts a very rich source of vitamin B, they had the authority of the B.P.C. that liquid malt extract, at least, was a good source. A genuine mistake had been made, and before the prosecution took place the article had been withdrawn from sale, but sale to retailers had taken place before the wholesalers' stocks had been cleared out. There was no intention to mislead the public.

The defendants were fined £5 with £12 12s. 0d. costs.

Notes from the Reports of Public Analysts

The Editor would be glad to receive Reports containing matter of special interest.

BOROUGH OF CHELSEA: ANNUAL REPORT, 1942

Sweetened Chocolate Spread.—The sample consisted of wheat flour, ground malt and probably extracted sesame seed, with not more than 7% of dry fat-free cocoa. It was caked together so that it could not be mixed into a smooth paste, and was insufficiently sweetened. In my opinion a chocolate spread intended to be used instead of jam should contain at least 20% of cocoa.

Caraway-flavoured Powder.—Two samples consisted of 95% of wheat flour and 5% of powdered dill. The manufacturers claimed that they had used caraway seed and had added caraway oil, but that the oil had completely volatilised.

Grated Horse-radish.—A sample consisted of 50% of horse-radish and 50% of turnip.

Dessert Powder.—Three samples were reported against, as they were prepared with wheat flour. The use of the term "dessert powder" caused difficulties at first, but when the Ministry of Food directed that these preparations must contain at least 80% of starch it was possible to put a stop to this attempt to evade the Substitutes Control Order. T. McLachlan

British Pharmacopoeia, 1932. Sixth Addendum

THE British Pharmacopoeia Commission has prepared the Sixth Addendum, which has now been issued under the direction of the General Medical Council.* The new Monographs and Emendations of existing monographs became official on August 1, 1943.

In the following new monographs the characters, tests for identity and purity and dosage are given.

ACETOMENAPHTHONUM (Acetomenaphthone). ACIDUM RICINOLEICUM (Ricinoleic Acid.—Sp.gr. at 15·5°/15·5° C., 0·945–0·948; n_D^{40}, 1·462–1·468; acid val., min. 175; iodine val., 85–91. ALCOHOLIA LANAE (Wool Alcohols).—Contain not less than 28% of cholesterol; m.p., min. 54° C.; acid val., max. 3; sap. val., max. 12; acetyl val., 130–140. CHLOROXYLENOL.—M.p. 114°–115·5° C. DITHRANOL. INJECTIO PROCAINAE ET ADRENALINAE MITIS. KAOLINUM LEVE (Light Kaolin), used as substitute for bismuth carbonate. LIQUOR CHLOROXYLENOLIS (Solution of Chloroxylenol; contains also Terpineol, Alcohol, Ricinoleic Acid and Sodium Hydroxide). MENAPHTHONUM (Menaphthone; *Syn.* Menadione). NICOTINAMIDUM (Nicotinamide). RIBOFLAVINA (Riboflavine). STILBOESTROL (*Syn.* Diethylstilboestrol).† SYRUPUS FERRI PHOSPHATIS CUM STRYCHNINA (*Syn.* Easton's Syrup without Quinine). TERPINEO. UNGUENTUM ALCOHOLIUM LANAE (Ointment of Wool Alcohols). UNGUENTUM DITHRANOLIS (the ointment has a basis of soft paraffin).

Amendments have been made to the following B.P. Monographs. Acetum Scillae, Acidum Mandelicum. Atropinae Sulphas, Ergota, Ergota Praeparata, Extractum Cascarae Sagradae Siccum, Ferri Carbonas Saccharatus, Ferri Sulphas Exsiccatus, Insulinum, Ipecacuanha, Ipecacuanha Pulverata, Magnesii Trisilicas, Mepacrinae Hydrochloridum, Mepacrinae Methanosulphonas, Pamaquinum, Paraffinum Molle Album, Sulphanilamidum, Unguentum Acidi Borici, Unguentum Acidi Salicylici, Unguentum Acidi Tannici, Unguentum Aquosum, Unguentum Hamamelidis, Unguentum Hydrargyri, Unguentum Hydrargyri Ammoniati, Unguentum Hydrargyri Oleati, Unguentum Hydrargyri Subchloridi, Unguentum Sulphuris, Unguentum Zinci Oleatis, Unguentum Zinci Oxidi.

The new ointment base is a mixture of wool alcohols, hard paraffin, white or yellow soft paraffin and liquid paraffin. One hundred g will absorb 100 ml of water, forming the new Hydrous Ointment (Unguentum Aquosum).

Important changes introduced by the amendments are as follows:—*Acetum Scillae.*—To allow for the smaller extract of Indian squills, the range of sp.gr. has been extended to 1·020–1·035 (formerly 1·031–1·035). *Ergot* must now contain not less than 0·2% (formerly 0·05%) of total alkaloids (as ergotoxine) of which not less than 15% consists of water-sol. alkaloids (calc. as ergometrine). *Ipecacuanha.*—In future the total alkaloids. *Magnesii Trisilicas* must contain 66–69·5% of SiO_2. *Unguentum Acidi Borici* now contains 1% of boric acid instead of 10% and is made with Hydrous Ointment.

APPENDIX VI.—The following limits for lead are included.—Heavy Kaolin, 5 p.p.m.; Nicotinamide, 10 p.p.m.

APPENDIX VII.—The following limits for arsenic are included:—Saccharated Iron Carbonate, 10 p.p.m.; Heavy Kaolin, 2 p.p.m.; Nicotinamide, 2 p.p.m.

APPENDIX XE gives a method for determining the acetyl value of wool alcohols.

Medical Research Council

THE DETERMINATION OF BLOOD GROUPS‡

THIS Memorandum is primarily intended as a guide to the Transfusion Services, but it will also be of value to those engaged in forensic work. It opens with an outline of the principles of diagnosis of the ABO blood groups, and then describes the methods of blood group determination—(*a*) the Tube Method, (*b*) the Tile Method, using diluted blood, and (*c*) the Tile Method, using undiluted blood—and discusses their relative merits. The tube method is recommended as the most reliable, whilst the tile method with undiluted blood should not be used except by those with long experience of the technique. The technical sources of error leading to false negative conclusions in blood group determinations are (1) failure to use sera of high titre; (2) failure to use an anti-A serum capable of reacting with A_2 and A_2B cells; (3) failure to recognise the time factor; (4) the use of infected sera. False positive results may be due to (1) pseudo-agglutination or rouleaux formation; (2) cold agglutination; (3) the use of infected cell suspensions (the Thomsen phenomenon); (4) the use of infected serum. Each of these sources of error is discussed in detail.

It has recently been shown that the blood cells of about 85% of English and American human subjects contain a previously unrecognised agglutinogen, termed "Rh" from the fact that a similar agglutinogen occurs in the red blood cells of rhesus monkeys. This Rh factor is independent of the ABO group (*cf.* Boorkman, Dodd and Mollison, *Brit. Med. J.*, 1942, ii, 535, 569). Its significance and the methods for detecting Rh agglutinogens and anti-Rh agglutinins are described. Other agglutinogens, of which M and N are the best known, are referred to only briefly, because the corresponding agglutinins are of rare occurrence in human sera.

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* Published for the Council by Constable & Co., Ltd. Pp. 41. 1943. Price 5/-.

† For tests *cf.* Tusting Cocking, ANALYST, 1943, 68, 144.—EDITOR.

‡ War Memorandum No. 9, pp. 19. H.M. Stationery Office, York House, Kingsway, London, E.C.2. 1943. Price 4d. net.

Lister Institute and Medical Research Council

ESTIMATION OF VITAMIN A*

THE factor required to convert extinction coefficients into international units of vitamin A was provisionally given a value of 1600 by the second International Conference on Vitamin Standardisation. Subsequent co-operative tests on a halibut liver oil and the U.S.P. reference oil gave factors of 1570 and 1820 respectively, and it was therefore recommended that the provisional factor 1600 should be retained. A further co-operative test has now been completed on a specimen of crystalline vitamin A β-naphthoate. For the solution used, $E^{1\%}_{1cm}$ ($325m\mu$) was 0·094 and the biological results ranged from 97 to 260 I.U. per g, with a weighted mean of 166 I.U. per g. This gives a conversion factor of 1770, and the average of the three factors is 1740, which is the value now recommended for all normal vitamin A preparations, oils or concentrates. Evidence for a conversion factor of 2,000 has been obtained in the U.S.A., where a value of 3000 I.U. per g. is assigned to the U.S.P. reference oil, which is widely used as a standard instead of the β-carotene used in this country. When a direct comparison of the U.S.P. reference oil with pure β-carotene was made, the former was found to have a value of 2619 instead of 3000 I.U. per g. This discrepancy corresponds closely with the difference between the U.S. conversion factor of 2000 and the factor of 1740 now found in this country. It is therefore recommended that the U.S.P. and international units should be recognised as not being identical, the former being only about 7/8 of the latter. Although this recommendation will lead to some confusion in the literature, it is pointed out that, as the confusion already exists, it is better to recognise its existence. F. A. R.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Separation and Quantitative Estimation of Amylose and Amylopectin in Potato Starch. R. M. McCready and W. Z. Hassid. (*J. Amer. Chem. Soc.*, 1943, **65**, 1154–1157.)—Previous work has identified in starch two fractions, amylose, which can be completely hydrolysed with β-amylase to maltose and has a non-branched long chain structure, and amylopectin, which is incompletely hydrolysed and has a branched and relatively short chain structure. The present paper describes a procedure for preparing pure amylose and amylopectin. The method for estimating their relative proportions in mixtures or in unfractionated potato starch is based on the fact that pure amylose, when greatly diluted, gives a brilliant deep blue colour with iodine, whilst amylopectin gives a much less intense colour. Natural potato starch, containing about 20% of amylose, produces a colour intermediate between those of amylose and of amylopectin. Place the powdered dry sample (100 mg) in a 100-ml flask, wet it with 1 ml of ethanol and 10 ml of water and dissolve by adding 2 ml of 10% sodium hydroxide soln. and heating on a water-bath. Cool the flask and dilute the clear liquid to the mark. (Amylose and synthetic starch do not retrograde from an alkaline soln.) Transfer a 5-ml portion of the soln. to a 500-ml flask, add *ca.* 100 ml of water, acidify with 3 drops of 6 *N* hydrochloric acid, mix by shaking, add 5 ml of a 0·2% iodine soln. in 2% potassium iodide, and dilute to the mark. The colour immediately develops its full intensity and remains stable for many days. Evaluate the intensity of the colour in a photoelectric colorimeter (*e.g.*, a Klett–Summerson instrument), using the 20-mm glass cell in conjunction with the red K_{66} filter; 85% of the light transmitted through this filter has a wave-length of 640–700μm, which is near Simerl and Browning's optima for the evaluation of this blue colour (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 125). Adjust the photocolorimeter so that the blank, which has a light yellow colour due to the iodine, gives a reading of 0. A graph shows that the readings on the logarithmic scale of the Klett–Summerson instrument are directly proportional to the concn. of amylose. A criterion for the purity of the starch fractions is based on 100% hydrolysis with β-amylase to maltose. The degree of hydrolysis of unfractionated potato starch by this enzyme is 60–64%. Complete hydrolysability of amylose and of Samec and Mayer's amyloamylose coincided with a reading of 310 on the photocolorimeter. Enzymatically synthesised potato starch was 98% hydrolysed and its colour intensity corresponded to a reading of 305. The hydrolyses were carried out with β-amylase prepared from ungerminated barley, according to Hanes and Cattle (*Proc. Roy. Soc.*, 1938, [B], **125**, 387). E. M. P.

Microscopical Method for the Detection of Insect Excreta in Flour and Meal. K. L. Harris and R. T. Elliott. (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 257–259.)—It is not always practicable to obtain a sharp separation of flour from insect excreta by flotation methods. The following method renders the flour particles almost transparent and the insect excreta relatively opaque, especially in the field of a binocular microscope. Weigh 0·2 g of flour on a tared glass disc of 7–7·5 cm diam., add clove oil and spread the mixture into a thin uniform layer, using enough oil to clear the flour and present a smooth surface. Place a wire grid over the disc and examine the preparation with a Greenough-type binocular microscope at ×25–30. Another procedure is to weigh a larger portion of the sample, float off most of the flour with a mixture of chloroform and ether or of chloroform and toluene of sp.gr. 1·40, rinse the residue on to a smooth ruled filter-paper in a Hirsch funnel, transfer the paper to a Petri dish, flood with clove oil and examine with a dark background and intense reflected light. The pellets of excreta stand out as whitish or opaque masses. The phosphates and salts found in self-raising flours remain opaque and may bear a superficial resemblance to insect excreta, and some fragments of cereal may not be completely cleared. Mineral salts will be either crystalline or angular with flat or irregular cleavage sides Internally

* Report of the Vitamin A Sub-Committee on Accessory Food Factors, appointed by the Lister Institute and Medical Research Council (*Secretary*, E. M. Hume). (*Nature*, 1943, **151**, 535–536.)

the excreta show a heterogeneous laminated appearance, whilst the salts are homogeneous. A. O. J.

False Positive Phosphatase Tests. E. Kaplan. (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 259–263.)—The possibility of false positive results with phosphatase tests on pasteurised milk, due to bacteria capable of hydrolysing the substrate, has been suggested by various workers, some of whom point out that results with samples of milk having microscopical counts > 8 million per ml or standard plate counts > 2 million per ml should be interpreted with caution and that milk containing bacteria in numbers sufficient to cause bacterial taint or a clot on boiling should be excluded from the test. The usual un-incubated control tests guard against errors due to deterioration of reagents and presence of interfering substances, but do not indicate the presence of thermostable substances of bacterial origin showing phosphatase activity. To recognise a false reaction, *i.e.*, one not attributable to imperfect pasteurisation, the following procedure is applied to samples that have shown a positive reaction in the ordinary procedure. Make the usual reagent control test and the un-incubated control test to eliminate preformed phenolic compounds and other interfering substances of this type. Re-pasteurise 5 ml of the sample in a test-tube at *ca.* 145° F. for 30 min. in a water-bath, using an accurate thermometer, constantly immersed, as stirring rod. Make the phosphatase test, as well as an un-incubated control test, on the re-pasteurised sample. If the original reaction was not due to imperfect pasteurisation, there will be no significant lowering of the colour reading after re-pasteurisation. Of 4500 commercially pasteurised samples, *ca.* 1% gave positive results and, of these, 21 were judged to be "false positive" reactions. The false reaction was probably due to a hitherto unidentified thermophilic bacillus of the genus *Lactobacillus* isolated from a suspected sample of commercially pasteurised milk. The morphological, biochemical and cultural characteristics of this organism (*Lactobacillus enzymothermophilus*), as well as its ability to produce a thermostable phosphatase enzyme, have been described by Buck (*Amer. J. Pub. Health*, 1942, **32**, 1224). Low-count certified raw milk was inoculated with this organism and maintained at 142°–143° F. for 5½ hrs., samples being removed periodically for examination for phosphatase, *p*H determination and clot tests. It was found that growth of the organism resulted in the production of a thermostable enzyme with phosphatase activity. Heating for a further 30 min. at 180° F. had little effect on the phosphatase activity, which, however, was destroyed when the milk was heated for several min. in a boiling water-bath. The phosphatase tests were made by the Gilcreas and Davis procedure (25th Ann. Rept. Internat. Assoc. Milk Sanitarians, 1936, 15) and by the Scharer procedure (*J. Dairy Sci.*, 1938, **21** 21). A. O. J.

Detecting Adulteration of Ethylvanillin with Vanillin. H. Nechamkin. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 268–269.)—The two compounds, together with coumarin, are widely used in the manufacture of imitation vanilla flavours. In the U.S.A. the compound used must be declared on the label, and, if ethylvanillin is used, Federal Specification EE–E–911a requires that at least 0·33 g shall be present in 100 ml of the flavouring substance. It would thus be profitable to substitute the cheaper vanillin for some of the ethylvanillin. To detect the adulteration, extract the ethylvanillin-vanillin fraction with alcohol-free ether and, if necessary, purify the extract (*Methods of Analysis of the A.O.A.C.*, 1930, XXV, 5(a)). Shake 25 mg of the residue, dried over sulphuric acid, with 3–4 ml of 95% alcohol, add 1 drop of 2% alcoholic potassium hydroxide soln. containing excess of ammonium thiocyanate and 1 drop of a mixture of 1 ml of 7% aq. crystalline copper sulphate soln., 4 ml of 3% alcoholic ferric chloride (dodecahydrate) soln. and 5 ml of 95% alcohol and shake gently for 1 min. In presence of vanillin a red colour forms and fades only slightly during several hrs. If only ethylvanillin is present, a reddish colour will fade very rapidly to a yellow colour. Make a control expt. with 25 mg of ethylvanillin. For quantitative purposes compare the colour of the unknown soln. with that of known mixtures of vanillin and ethylvanillin in a colorimeter. The method will detect 1 mg of vanillin, and coumarin does not interfere. Fresh reagents should be prepared every 2 days and the details of the procedure should be followed closely.

A. O. J.

Tobaccos Classified according to the Nature of their Alkaloids. L. N. Markwood and W. F. Barthel. (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 280–283.)—Tobacco is usually graded commercially according to its value as smoking material, but, since its alkaloids are important insecticides, a classification based upon the nature of these is of interest. Recently some strains of tobacco have been found to contain nornicotine as the principal alkaloid, with nicotine in relatively small proportion, and, since these strains are potential sources of nornicotine, a method for recognising them is desirable. Digest 2 g of powdered tobacco with 6 ml of (9 + 1) sulphuric acid in a moderately cool water-bath until the tobacco has completely disintegrated (2–3 min.). Stir-in 50 ml of water, without cooling, and heat the mixture on a steam-bath for 30 min. Cool to room temp., filter and wash the insoluble residue with water until the filtrate is colourless or does not give a reaction with silicotungstic acid. The vol. of the filtrate and washings should be kept below 100 ml. Make the combined filtrate and washings strongly alkaline with solid sodium hydroxide and exhaust the liquid with 30-ml portions of benzene. Dry the slightly yellow clear benzene extract with sodium hydroxide, if necessary, and extract the alkaloids with a slight excess of 0·1 *N* hydrochloric acid (10 ml). Wash the benzene layer twice with 5–10 ml of water and add the washings to the acid extract, add 0·1 *N* sodium hydroxide until the liquid is just neutral to phenolphthalein and filter if a turbidity develops. Add enough sat. picric acid soln. to ppt. all the alkaloids, boil the liquid to dissolve the ppt. and allow it to cool slowly. Collect and wash the crystals with dil. picric acid and finally with water and dry. Determine the m.p. in a capillary tube, keeping the rate of rise of temp. near the m.p. at 0·5° per min. Nicotine picrate crystallises sharply in long needles and melts at 223°–224° C. (corr.) with decomposition. Nornicotine picrate has a colloidal appearance as it begins to separate from soln., and the crystals are short, coarse, often irregular, slightly curved needles, clumping together in T and Y shapes and melting to a clear stable liquid at 191°–192° C. Picrate from the nicotine type of tobacco usually has a fairly sharp m.p., *i.e.*, within 3° C.; picrate from the mixed and nornicotine types of tobacco is characterised by a

much wider range, which may reach 20° C. About 90 samples were examined by this method and nearly all belonged to the nicotine type (m.p. of mixed picrates, 217·5–224° C.). These included samples from *Nicotiana rustica*. Samples of the mixed type were special tobaccos cultivated as low-nicotine types; their alkaloidal picrates had m.p. 192°–215° C. The 2 samples of the nornicotine type (m.p. 178°–190·5° C.) are of special interest because this alkaloid is still rare; Robinson's Maryland Medium Broad Leaf had been previously investigated, but Flue-cured Cash (Moss 1937) from Virginia is reported for the first time as a potential source of nornicotine. A. O. J.

Quantitative Determination of Nicotine and Nornicotine in Mixtures. L. N. Markwood. (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 283–289.)—The method consists in (1) treatment of the mixture with nitrous acid whereby the nornicotine is converted into nitroso-nornicotine, whilst the nicotine is unaffected and can be separated by steam distillation; (2) methylation of another portion of the sample with formaldehyde and formic acid, whereby the nornicotine is converted into nicotine and the treatment is then as for nicotine alone, the nornicotine being found by difference. Treat the aq. alkaloidal soln., diluted to *ca.* 15 ml, in a 300-ml Kjeldahl flask with 2 ml of 30% acetic acid and 10 ml of freshly prepared 5% sodium nitrite soln., and leave the mixture at room temp. for 15–20 min. Make slightly alkaline to phenolphthalein first with 30% and then with 5% sodium hydroxide soln., rinse down the neck of the flask, add 2% acetic acid, drop by drop, until the pink colour is just discharged, remove acid vapours by aspirating air through the space above the liquid, add 10 ml of *p*H 10 buffer soln. and steam distil the liquid at low vol. (*ca.* 15 ml) until all the nicotine has passed over. Determine the nicotine in the distillate by pptn. with silicotungstic acid or by titration with 0·05 *N* hydrochloric acid to methyl red indicator. By titrating each 75-ml fraction of the distillate, the course of distillation may be followed. To determine nornicotine, gently boil a mixture of 5 ml of aq. alkaloidal soln., 0·1 ml of formic acid (87%) and 5 ml of formaldehyde (37%) with a fragment of pumice under reflux for 15 min. in a 300-ml Kjeldahl flask. Cool, add 10 ml of 30% sodium hydroxide soln. and, after 15 min., steam distil the liquid. Treat the distillate as described for nicotine. The difference in the titrations corresponds with the amount of nornicotine originally present. Each ml of 0·05 *N* hydrochloric acid ≡ 0·0074 g of nornicotine or 0·0081 g of nicotine. Commercial formalin may be used for methylation, provided that it yields a satisfactorily low blank value when distilled from alkali. A. O. J.

Determination of Iodine in Tetraiodophenolphthalein. S. Weiner, B. E. Leach and M. J. Bratz. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 373–374.)—*Adsorption Indicator Method.*—This is almost identical with that of the U.S.P. XII (1942, p. 251), but starch-iodide is used as an adsorption indicator (*cf.* Butler and Burdette, *Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 237); it has the advantage that sharp end-points are obtained at *p*H 0·5, so that adjustment of the *p*H to 4·5–7·5 is unnecessary. Dissolve 0·15–0·20 g of the sample or its sodium salt (accurately weighed) in 10 ml of 5% sodium hydroxide soln. in a 500-ml conical flask on the water-bath, add 25 ml of sat. potassium permanganate soln., digest for 45 min. on the bath (to convert the iodine into iodate), cool, add 75 ml of water and 10 ml of sulphuric acid (1:1), and run 3·0 *M* sodium bisulphite from a burette, with swirling, until the soln. is colourless; this reduces the iodate and excess of permanganate; 10–18 ml are usually required, but the necessary vol. should be added as rapidly as possible to avoid loss of iodine. Next add dil. potassium permanganate soln., drop by drop, until the soln. is permanently faint yellow, and then 10 ml of 1·0 *M* ammonium carbonate and 4 drops of dil. starch soln. Titrate with 0·1 *N* silver nitrate until the last trace of blue, green or grey just disappears, leaving the soln. and the ppt. a clear canary-yellow colour; if the ppt. coagulates, watch for the colour change; on rare occasions it fails to occur, and the end-point may then be uncertain. Close agreement (eosin as indicator) was obtained for *ca.* 0·15–0·25 g of the sample containing *ca.* 51–56% of iodide; there was also satisfactory concordance with the theoretical value in the analysis of potassium iodide or iodate, alone or in presence of an equal wt. of phenolphthalein. *Reduction Method* (after Clark and Jones, *J. Assoc. Off. Agric. Chem.*, 1942, **25**, 755).—Dissolve 0·15 g of the sample in 15 ml of 5% sodium hydroxide soln. in a 500-ml conical flask on the water-bath, add 25 ml of sat. potassium permanganate soln., digest for 45 min. on the bath, and add at once 10 ml of glacial acetic acid and then, with swirling, 5% sodium nitrite soln. from a burette dropwise until the red colour is replaced by the brown shade of the manganese dioxide. Add 5 ml of conc. sulphuric acid slowly, and then again 5% sodium nitrite soln., drop by drop, until the soln. is clear and colourless (warming if necessary); destroy the excess of nitrite by addition of 10 ml of 5% sulphamic acid, cool, add 10 ml of 33% sodium hydroxide soln.; cool again, add 10 ml of 1·0 *M* potassium iodide, and titrate the iodine liberated from the iodate with 0·1 *N* sodium thiosulphate, using starch or 5 ml of carbon tetrachloride as indicator. Nitrates will not oxidise the iodides rapidly enough to interfere if the final *p*H exceeds 2 and the soln. is cool. The data tabulated are concordant and agree closely with the results obtained with pure *o*-iodobenzoic acid; it was, however, necessary to heat the acid under reflux with the potassium permanganate soln. for 8 hr. over a free flame. Mixtures of phenolphthalein with potassium iodide or iodate (*cf. supra*) also gave good results, but it was then necessary to use 45 ml of the permanganate soln. and to digest the mixture for 1 hr. J. G.

Biochemical

Partition Chromatography in the Study of Protein Constituents. A. H. Gordon, A. J. P. Martin and R. L. M. Synge. (*Biochem. J.*, 1943, **37**, 79–86.)—The method previously described (*Biochem. J.*, 1941, **35** 1358; ANALYST, 1942, **67**, 210) has been improved and applied to the isolation and identification of amino acids and peptides in protein hydrolysates. The silica gel used in the column is now prepared as follows. Dilute commercial water-glass to 3 vols. with water containing a little methyl orange, add 10 *N* hydrochloric acid with vigorous stirring until the reaction is permanently acid to thymol blue, leave for 3 hrs., filter and wash with about 2 litres of water per 250 g of dry gel. Suspend the gel in 0·2 *N* hydrochloric acid and "age" for two days at room temp. Filter, wash with 5 litres of water per 250 g of dry gel until the washings are free from methyl orange,

and dry at 100° C. in an air-oven. Although methyl orange is partly extracted from the aqueous phase by the developing solvent, it is the most satisfactory indicator so far discovered, with the exception of pelargonin chloride. It gives rise to artifacts but not in sufficient quantities to interfere with the analysis. Three different solvent mixtures were investigated. Chloroform—*n*-butanol—water and propanol—cyclohexane—water proved the most useful; as they give different results it is advantageous to use them in succession. In general, acetamino acids with longer and less polar side chains move fastest down the column, although there are exceptions to this rule; also, the less polar solvent, cyclohexane, discriminates better between these compounds than does the more polar solvent, chloroform. Acetyldipeptides travel at rates intermediate between those of the acetyl derivatives of their constituent amino acids. It is now possible to determine the following amino acids in protein hydrolysates: phenylalanine, leucine+*iso*leucine, valine, methionine, proline, alanine, tyrosine, tryptophan and, in some instances, glycine. Sometimes it is preferable to reverse the order in which the chromatograms are used, and to adopt other solvent mixtures. Evaporate the hydrolysate, corresponding to 25 mg of protein, to dryness *in vacuo*, add 2 ml of 2 *N* sodium hydroxide and then 0·4 ml of 6 *N* sodium hydroxide, cool to 0° C. and immediately add 0·2 ml of acetic anhydride, followed at 2-min. intervals by four 2-ml portions of 2 *N* sodium hydroxide and four 0·2-ml portions of acetic anhydride, added alternately. See that the soln. remains alkaline throughout. Leave for 10 min. and then make acid to thymol blue by addition of 10 *N* sulphuric acid. Extract with five 50-ml portions of 17% butanol—chloroform, filter the extracts into a flask and remove the solvent *in vacuo*. Transfer the extracted acetamino acids by means of ethanol to a small flask and again remove the solvent *in vacuo*. Saturate 3 g of silica gel, prepared as above, with methyl orange soln. and suspend in 3% butanol-chloroform. Pour the suspension into a chromatogram tube with an internal diam. of 1 cm, and extract the acetylated mixture with five 1-ml portions of the hot solvent mixture, running each of the extracts in turn on to the column. Finally, fill the chromatogram tube with 3% butanol-chloroform. Collect separately the bands corresponding to (1) phenylalanine, (2) leucine+*iso*leucine, and (3) proline+valine+methionine. Allow the surface of the solvent to sink until it is level with the top of the silica gel, and then add a soln., in about 1 ml of 17% butanol-chloroform, of the residue of the acetylated amino acids which was insoluble in the 3% solvent mixture. Develop the column with 17% butanol-chloroform, and collect (4) the tyrosine band, and (5) the alanine band. Remove the solvent from each of the 5 fractions and analyse fractions (1) and (2) on columns containing 2 g of silica, using 5% propanol—cyclohexane instead of butanol—chloroform. Similarly, refractionate fraction (3) on a column containing 3 g of silica; this gives three bands corresponding to valine, methionine and proline. Refractionate fractions (4) and (5) on columns containing 2 g of silica and develop with 30% propanol—cyclohexane. Titrate each of the 7 final fractions with 0·001 *N* baryta, using bromthymol blue as internal indicator. (1 ml ≡ 0·14 mg of N for all amino acids except tryptophan for which 1 ml ≡ 0·27 mg of N.) Recoveries of the above 7 aminoacids from a synthetic mixture containing 19 amino acids ranged between 92 and 105%. Wool and gelatin hydrolysates were analysed by the method and gave results in close agreement with values previously recorded. F. A. R.

Amino Acid Composition of Gramicidin. A. H. Gordon, A. J. P. Martin and R. L. M. Synge. (*Biochem. J.*, 1943, **37**, 86–92.) Gramicidin was investigaged by a modification of the method described in the preceding abstract. The gramicidin was hydrolysed and acetylated, and the acetylated mixture was dissolved in propanol—cyclohexane. The column was developed first with 5% and then with 30% propanol-cyclohexane. The first and second fractions were refractionated on butanol-chloroform columns, yielding the acetyl derivatives of (*a*) leucine and (*b*) tryptophan and valine. The third and fourth fractions consisted of substantially pure acetylalanine and acetylglycine, and did not require further fractionation.

F. A. R.

Partial Hydrolysis of Cow-hide Gelatin. A. H. Gordon, A. J. P. Martin and R. L. M. Synge. (*Biochem. J.*, 1943, **37**, 92–102.)—The monoamino acids in gelatin hydrolysate were determined by the usual method, except that the series of refractionations with propanol—cyclohexane was omitted; 1%, followed by 17%, butanol—chloroform was used for developing the columns. The amino-acid composition of gelatin thus obtained agreed fairly well with the values previously recorded. The effect of partial hydrolysis was next studied, the protein being allowed to stand in 10 *N* hydrochloric acid at 37° C. The products obtained after 4 and 19 days' standing showed 1/3rd and 2/3rds conversion into free amino acids respectively. These partial hydrolysates were separated by electrodialysis into basic and neutral fractions. The basic fractions obtained from the 4- and 19-day hydrolysates were completely hydrolysed and analysed for monoamino acids by the chromatographic procedure. Assuming that these monoamino acids were all linked as dipeptides with basic amino acids, the yields from the 19-day hydrolysate account almost quantitatively for the residues present in peptide linkage. It was also observed that the amount of phenylalanine, leucine, valine and methionine residues bound in basic peptides after 4 days' hydrolysis was about the same as after 19 days' hydrolysis. Preliminary expts. with synthetic acetyl dipeptides showed that these could be separated readily by partition chromatography, travelling at rates intermediate between those of the acetyl derivatives of their constituent amino acids. The neutral fraction obtained from electrodialysis was therefore acetylated, and the acetylated mixtures from both the 4- and 19-day hydrolysates were fractionated on an ethylacetate—water column. Each fraction was then refractionated on a second column, which was developed first with 1% and then with 17% butanol-chloroform. The material from the 19-day hydrolysis was a less complicated mixture than that from the 4-day hydrolysis and was accordingly fractionated in greater detail. Each of the final fractions was titrated with baryta soln. to obtain an indication of the number of equivalents of acetylpeptide and/or amino-acid present, each was then hydrolysed with acid, and the monoamino-acids were determined as described above. The results showed that the 19-day hydrolysate contained the tripeptide proline-alanine-glycine and the dipeptides proline-alanine, proline-glycine and proline-hydroxyproline. It was found impossible

to recrystallise and identify the various acetyl dipeptides, but some indication of the manner in which the amino acids were linked together was obtained by partial hydrolysis. Thus acetyl glycylleucine on partial hydrolysis should yield acetylglycine but not acetylleucine, whereas acetyl leucylglycine should yield acetylleucine and not acetylglycine. In this way it was shown that the 19-day hydrolysate probably contained glycylproline, and the conclusion will be checked when further material is available. The acetoamino acids ultimately obtained were, however, isolated and characterised; all were obtained as the *l*(+) isomers. F. A. R.

Application of the Spekker Photoelectric Absorptiometer to the Determination of Vitamin C. A. Mc.M. Taylor. (*Biochem. J.*, 1943, **37**, 54–58.)—Decolorisation of 2 : 6-dichlorophenolindophenol by ascorbic acid, glutathione and cysteine takes place at different rates, the oxidation of ascorbic acid being much more rapid than that of the other two substances. Ascorbic acid can be estimated in their presence by plotting the residual colour against time and extrapolating back to zero time. Ascertain the approx. endpoint by a preliminary titration and prepare a range of indophenol solns. of such vols. that, on adding a measured vol. of test soln. to each, at least 2 of the mixtures will contain a deficiency and at least 5 an excess of dye, the vol. of indophenol reagent being increased by 0·2 ml each time. The dye soln. contains 0·25 mg per ml and is stabilised by addition of 10% of Clark's phosphate buffer soln. of *p*H 7·0. Prepare a blank by adding 5 ml of test soln. rapidly to 4 ml of water in the 1-cm. cell of a Spekker absorptiometer and adjust the instrument to give a reading of 1·000 with the No. 6 light-blue filter in position. Next add 4 ml of test soln. rapidly to the series of dye solns., and set a stop-watch going. Take a reading as quickly as possible, noting the time, and then at intervals second and third readings. Plot the Spekker reading against time for each mixture and extrapolate back to zero time. Then plot the extrapolated initial reading against the amount of indophenol soln. added for the whole series of mixtures examined. This curve comprises two parts—a horizontal line corresponding to the colour of the mixtures in which all the indophenol has been decolorised by the ascorbic acid present, and the other inclined steeply corresponding to the progressively increasing colour of the excess of indophenol. The point of intersection between these two parts corresponds with the end-point of the titration. It was shown that direct visual assessment in this way gives sufficiently accurate results. Mixtures of synthetic ascorbic acid with varying amounts of cysteine and glutathione were tested by this method and, even in presence of 65 mg per 100 ml of either of the two substances, the results were identical with those for the ascorbic acid content of similar solns. without added cysteine or glutathione. F. A. R.

Determination of Carotene in Vegetable Oils without Saponification. E. Bickoff and K. T. Williams. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 266–268.)—The more rapid methods for the determination of carotene in oils depend upon measurement of the light transmission of a soln. of the oil in a suitable solvent and may be subject to error when coloured products result from storage of the carotene soln. It was found that aluminium oxide (<80-mesh, activated specially for chromatographic analysis; Aluminium Ore Co., East St. Louis, Ill.) can be used for separating carotene from other chromogens in oil. Light petroleum containing 2% of acetone removes carotene quantitatively from the aluminium oxide column and permits its separation from coloured oxidation products of carotene, xanthophylls and chlorophylls. *Method.*—Pack Tswett tubes (internal diam., 11 mm) with 12 g of aluminium oxide, applying gentle suction and tamping with a flattened glass rod until columns uniformly of *ca.* 10 cm height are obtained. Cover each column with *ca.* 1 g of anhydrous sodium sulphate. Run a 10-ml aliquot portion of a light petroleum (b.p. 30°–60° C.) soln. containing 0·1 g of oil per ml on to the column. After all the soln. has passed into the aluminium oxide, develop the column and simultaneously elute the carotene with the required amount of light petroleum containing 2% of acetone. Application of pressure or suction is not necessary. Since the commercial oxide varies somewhat in activity, the vol. of eluent needed must be determined for each batch. Run a 10-ml aliquot portion of a light petroleum soln. of crystalline carotene, containing 0·1 g of oil per ml into the column. Prepare several columns, determine the min. vol. of 2% acetone in light petroleum that will remove all the carotene, and use this vol. in the analysis. Dilute the carotene eluate to 100 ml and determine the carotene content colorimetrically in an Evelyn photoelectric colorimeter with a 440$m\mu$ filter. This measures β-carotene together with any α- or neo-β-carotene that may be present, and the two last-mentioned substances are more readily eluted from the column than is β-carotene. Methods are available for the individual estimation of these components (Shrewsbury *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 253; Zscheile and Beadle, *id.*, 1942, **14**, 633; ANALYST, 1942, **67**, 400). The method may be used to separate carotene from other pigments in light petroleum soln. free from oil, but more eluent is needed than when oil is present. Expts. with known amounts of carotene and comparisons with other methods showed this method to be accurate. Satisfactory results with solns. of carotene in alfalfa, cottonseed, refined corn, refined soya, raw coconut and raw rice bran oils indicated that the method is probably applicable to other vegetable oils. A. O. J.

Ascorbic Acid in Dehydrated Foods. L. W. Mapson. (*Nature*, 1943, **152**, 13–14.)—There is strong evidence that titration of ascorbic acid in common foodstuffs with 2,6-dichlorophenolindophenol soln. is reliable if carried out rapidly and in acid soln. (Harris, and others, *Biochem. J.*, 1933, **27**, 303; ANALYST, 1942, **67**, 302), and usually provision can be made for interference by other reducing substances present (Mapson, *id.*, **29**, 305). However, certain dehydrated foods which have undergone heat-treatment have been found to contain reductones and allied compounds, which resemble ascorbic acid in chemical structure, in the variation of their stability with the *p*H value, in the catalysis of their oxidation by copper and enzymes, and in the current-voltage curves (polarographs) of their solns. Lugg (*id.*, 1943, **68**, 25) has shown that formaldehyde may be used to differentiate certain reducing substances from ascorbic acid, and this is the basis of the method now proposed:—Extract the sample in the dark with sufficient 5% metaphosphoric acid by the method of Harris and Olliver (*Lancet*, 1943, i, 454) so as to obtain a soln. containing or equiv. to 0·04–0·10 mg of ascorbic acid/ml. If the sample is too poor in

ascorbic acid to enable this to be done, use the min. vol. of acid consistent with adequate extraction. If a sulphite, sulphide or sulphydryl compound is suspected, adjust an aliquot portion to *p*H 0·6 by means of 50% sulphuric acid, then add sufficient formaldehyde to produce a concn. of 4%, and, after 8 min. at 20° C., titrate against the indophenol reagent. The above substances are thus removed by condensation with the formaldehyde, so that this titration (*A*, *vide infra*) measures ascorbic acid plus reductones; in absence of reductones omit the addition of formaldehyde and titrate the extract (*p*H 1·2) directly. Adjust a second aliquot portion of extract to *p*H 2·0 by means of sodium citrate soln., add sufficient formaldehyde to produce a concn. of 8%, and allow the mixture to stand at 20° C. Withdraw samples at intervals of 5–10 min. up to 90 min., at once adjust the *p*H of each to 0·6 by addition of 50% sulphuric acid (to stop the reaction), and titrate with indophenol reagent within 5 min. of taking the sample; the acid need not be added if the titration can be carried out immediately after withdrawing the sample. In this reaction the ascorbic acid is completely removed by condensation, whilst the reductones are condensed only slowly. Plot the titrations (expressed as ascorbic acid) as ordinates against the times elapsing between the beginning of the reaction and of the titration; *A* (*supra*) is the initial value, corresponding with zero time. Extrapolate the line joining the points on the later (flat) part of the curve back to the ordinate. If it cuts this at *X*, then (*A*—*X*) corresponds with the ascorbic acid content of the extract. With fresh vegetable and animal tissues and some typical cooked foods, jams, canned foods, etc., the results agree well with those obtained by the direct indophenol method, but with certain heat-treated dehydrated foods the new method gives the lower result. In particular, if dried cabbage, for which concordant results are obtained by the two methods and by the biological assay, is stored at above 25° C., there is a gradual production of substances which behave as reductones. Scorching produced during drying has similar results (*cf.* following abstr.). J. G.

Apparent Vitamin C in Certain Foodstuffs. F. Wokes, J. G. Organ, J. Duncan and F. C. Jacoby. (*Nature*, 1942, **152**, 14–15.)—Certain substances in heated or fermented foodstuffs have properties very similar to those of ascorbic acid, but are without biological activity (*cf.* Mapson, preceding abstr.). Thus, when germinated grain was heated with water containing 1–2% of conc. hydrochloric acid there was evidence of the formation of compounds which were rapidly destroyed by oxidases from cabbage or tomato skins, by shaking with charcoal in air, or by low concns. of hydrogen peroxide ($1 : 10^8$) or of percarbonate ($1 : 10^6$); concns. of 200–300 mg/100 g are recorded. The absorption maxima of 256–264 and 275–280$m\mu$ suggest the presence of reductic acid (*cf.* Reichstein and Oppenauer, *Helv. Chim. Acta*, 1933, **16**, 988), but attempts at extracting it with ethanol, methanol or ether have failed. These substances are named "apparent vitamin C" to distinguish them from reductones (*cf.* Mapson, *loc. cit.*), since, unlike the latter, they are unstable in metaphosphoric acid soln.; at *p*H 4–5 formaldehyde reacts with them only to the extent of 5–10%, whilst it destroys all the ascorbic acid present. Consequently they may be estimated by titrating potentiometrically with the indophenol reagent (*cf.* Harris, Mapson and Wang, ANALYST, 1942, **67**, 304), then destroying the ascorbic acid by the action of 6% formaldehyde for 6 min. at 20° C. and *p*H 4–5, and again titrating. The difference between the results of the titrations, corrected for the small amounts of interfering substances which have also condensed with the formaldehyde, corresponds with the true vitamin C content. The extracts in metaphosphoric acid should be stabilised with nitrogen as soon as they are prepared. Expts. under widely different conditions gave duplicate values agreeing to within the experimental error of the potentiometric method (which is preferred for coloured solns.). Apparent vitamin C has not yet been found in fresh fruits and vegetables, although Hochberg, Melnick and Oser (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 182) consider that such a substance gradually develops in dried tomatoes and potatoes, and may contain dehydroascorbic acid. Their method of estimating true vitamin C in presence of these substances (photometric determination of the rate of decolorisation of the indophenol reagent) is considered less reliable than that described above. J. G.

Vitamin P Activity of some British Fruits and Vegetables. A. L. Bacharach and M. E. Coates. (*J. Soc. Chem. Ind.*, 1943, **62**, 85–87.)—The method previously described (*Biochem. J.*, 1942, **36**, 407, *cf. Abst.*, ANALYST, 1942, **67**, 368; *id.*, 1942, **67**, 313) has been used to determine the vitamin P contents of a series of fruits and vegetables, the results being expressed in terms of a standard preparation of which 1 mg contains 1 "provisional unit" (P.U.). Hesperidin contains 100 P.U. per g. The results in P.U. per 100 g were as follows: apple, 60; beetroot, 15; blackberry, 60; blackcurrant, 500; cabbage, 60 (Apr.), 100 (Oct.); carrot, 10 (Apr.), 40 (Aug.); cauliflower, 40; cherry, black 60, white 50; dandelion, 30; dock, 20; lentils, 0; lettuce, 80 (May), 100 (Sept.); parsley, 130; parsnip, 40; pea, dried 40, germinated 80; maple pea, dried 100, germinated 10; plum, 50; potato, old 25 (Apr.), 40 (July); rose hips, 600, 400; rowan berry, 300; rhubarb, 20; spinach, 130 (May), 130 (Sept.); swede, 20; tomato, 70 (July), 60 (Aug.); turnip, 20 (June), 30 (July); walnut, 100; watercress, 10 (Apr.), 70 (Oct.). Eleven of the samples were tested for vitamin C, as well as for vitamin P, activity, but there was no correlation between these two values. Furthermore, whereas peas showed a 15-fold increase in vitamin C content after germination, the vitamin P activity was only doubled. This is evidence against Szent-Gyorgyi's suggestion that ascorbic acid and a "flavone" type of vitamin P are linked together in one common catalytic system for hydrogen transfer. The results also suggest that there is no association between vitamin P activity and plant pigments. F. A. R.

Agricultural

Determination of *p*-Dichlorobenzene in Soil. R. D. Chisholm and L. Koblitsky. (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 273–277.)—A method authorised by the U.S. Dept. of Agriculture (B.E.P.Q., 499) for the treatment of nursery stock to kill the Japanese beetle requires the use of *p*-dichlorobenzene mixed with soil. The plant balls are placed in this mixture and kept within a specified temp. range for several days, after which the mixture of soil and insecticide is discarded and fresh mixtures are made for further treatment. A suitable method for determining residual *p*-dichlorobenzene would enable the original concn. of insecticide in the soil to be restored and avoid waste of soil and high labour

costs, and the following procedure is suggested. The apparatus consists of a round-bottomed litre boiling flask with a short ring neck closed by a rubber stopper equipped with two glass tubes, one (with its lower orifice covered with cheese cloth) reaching from the bottom of the flask to a steam generator and the other from the inner surface of the stopper to a vertical 300-mm Liebig condenser leading into a 500-ml separating funnel containing 25 ml of refined kerosene of n_D^{25} 1·4330. The flask is immersed in an oil-bath at *ca.* 110° C. By means of a thin steel tube (1 in. diam.) transfer a representative number of cores of soil to a graduated cylinder and gently compress the mass until a vol. of 500 ml is obtained. Transfer the sample to the flask and steam distil until *ca.* 300 ml of distillate have been obtained. If crystals form in the condenser, transfer them to the separating funnel by interrupting the flow of cooling water. Dissolve the *p*-dichlorobenzene in the kerosene, remove the aq. layer, filter the kerosene soln. and determine its n_D^{25}. Expts. showed that, with concns. of *p*-dichlorobenzene ranging from 0·3 g to 3·0 g in 500 ml of soil, the results could be xepressed by the formula $10^4n = 33{\cdot}33c$, where n is the change in refractive index and c the wt. in g of *p*-dichlorobenzene in the 25 ml of kerosene. The departure from linearity of the curve representing the results of expts. from which soil had been omitted was so slight that the calibration curve could be represented by a straight line, and a determination of the line of best fit by the method of least squares gave the following relationship: $10^4n = 29{\cdot}37c + 2{\cdot}80 \pm 1{\cdot}4$, or, since concn. in nursery practice is expressed in lb. per cb. yard, $Y = 1148n - 0{\cdot}32 \pm 0{\cdot}16$, where Y is the concn. of insecticide in lb. per cb. yard. The n_D of each batch of kerosene used should be determined, and control expts. are necessary with any given soil without inclusion of *p*-dichlorobenzene, to compensate for deviations in n_D caused by the volatile substances in some soils. The method is probably applicable to the determination of other volatile substances, *e.g.*, naphthalene and β-naphthol.

A. O. J.

Nutritive Value of certain Fish Meals as Determined in Tests with Swine and Rats. E. R. Barrick, C. M. Vestal and C. L. Shrewsbury. (*J. Agr. Res.*, 1943, **66**, 125–134.)—Comparative trials of the nutritive value of several commerial fish meals were made by including them in rations of swine and rats. For swine-feeding, menhaden, sardine and herring meals were of practically equal value in a mixed protein supplement of soya bean meal (40%), meat and bone scraps (20%), fish meal (20%), cottonseed meal (10%) and alfalfa leaf meal (10%). With the sardine fish-meal ration, however, the gain in weight was slightly higher and the feed required for 100 lbs. gain slightly less, than for the others. Three feeding trials were made, a fresh supply (*a*) of each meal being required for the second or third; the resulting variations in consumption showed that, for the same brand of meal, palatability may vary considerably. Rats were fed on rations in which the protein content (*ca.* 14%) was derived entirely from the fish meal,—the other constituents being corn, cod-liver oil (1%) and mineral mixture (2%). Two vacuum-dried whitefish meals and 1 steam-dried sardine fish meal were also tested. *Ad libitum* feeding experiments indicated whitefish and, still more, sardine meal to be more palatable than menhaden or herring meal, but the nutritive value of the herring meal was comparable to that of the sardine meal. Controlled feeding trials also showed the sardine and herring meals to be more nutritive than menhaden meal. Analysis of the various feeds gave the following % results:

Ingredient	Moisture	Protein	Fat	Fibre	Ash	N-free extract
Menhaden fish meal (flame-dried) ..	5·7	66·2	4·4	0·3	17·5	5·9
	(*a*)6·4	65·8	5·3	0·3	18·3	3·9
Sardine fish meal (flame-dried) ..	5·1	70·9	5·5	0·4	11·4	6·7
	(*a*)6·7	65·1	4·3	0·8	16·9	6·2
Herring fish meal (flame-dried) ..	5·1	75·1	6·6	0·4	11·1	1·7
	(*a*)8·2	69·9	9·2	0·8	11·5	0·4
Sardine fish meal (steam-dried) ..	5·5	71·0	3·4	0·6	15·7	3·8
Whitefish meal (vacuum-dried) ..	13·4	64·1	3·6	0·4	20·9	0
Whitefish meal blend (vacuum-dried)	6·5	57·2	7·5	2·2	23·4	3·2
Soya bean meal	7·7	45·4	6·8	5·6	5·0	25·0
	(*a*)8·7	46·1	5·5	6·1	4·7	28·9
Meat and bone scraps	5·0	51·0	7·6	2·4	30·1	3·9
	(*a*)5·7	49·9	6·2	2·0	34·2	2·0
Cottonseed meal	6·2	41·1	6·2	9·9	6·3	30·3
	(*a*)4·8	41·3	5·8	10·0	6·9	31·2
Alfalfa leaf meal	6·8	18·2	2·9	20·4	9·1	42·6
	(*a*)4·3	24·9	3·6	16·2	8·2	42·8

The meals marked (*a*) were used in swine feeding only. The rats also were fed with the other fish meals.

E. B. D.

Organic

Collection and Estimation of Traces of Formaldehyde in Air. F. H. Goldman and H. Yagoda. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 377–378.)—Draw the air through a "midget-impinger" (Jacobs, *"Analytical Chemistry of Industrial Poisons, Hazards and Solvents,"* 1941) containing 10 ml of 1% sodium bisulphite soln., at 1–3 litres per min.; or through a large impinger (*loc. cit.*) containing 100 ml of reagent, at 28 litres per min. Wash the whole of the soln. into a 300-ml conical flask (or take a 10-ml aliquot portion) and titrate with 0·1 *N* iodine (which need not be standardised), with 1 ml of 1% starch soln. as indicator, to a dark blue end-point. Destroy the excess of iodine by adding 1–2 drops of 0·05 *N* sodium thiosulphate, and then add 0·01 *N* iodine,

in drops, until the colour is faint blue. The excess of inorganic bisulphite is thus completely oxidised to sulphate, and the soln. is ready for the assay of the formaldehyde-bisulphite compound; if acetone is present, add 2 ml of 5% sodium bicarbonate soln. after destruction of the free inorganic sulphite (*cf infra*). Then add 25 ml of a soln. prepared by dissolving 80 g of sodium carbonate in *ca.* 500 ml of water, adding 20 ml of glacial acetic acid, and diluting to 1 litre; remove the sulphite so liberated by titration with standard 0·01 *N* iodine to a faint blue end-point. If the titration is less than 1 ml, correct it for a blank test on 10 ml of the sodium bisulphite soln. (usually not more than 0·10 ml); 1 ml of 0·01 *N* iodine ≡ 0·15 mg of formaldehyde. Determinations of known quantities of formaldehyde (0·021–5·32 mg) in soln. (standardised by Romijn's method), gave differences between the theoretical and determined vals. of +0·021 to −0·18 mg. However, it is desirable to sample a vol. of air sufficient to give at least 0·5 mg of formaldehyde for the analysis; the use of a micro-burette reading to 0·01 ml introduces a possible error of 1% in the volumetric determination of 1 mg of formaldehyde. Data for assays made in presence of 20 mg of methyl alcohol, 50 mg of acetic acid, 10 and 50 mg of bromine or 10 mg of acetone (specially treated as described, *cf. supra*) showed that these substances do not interfere. Expts. illustrating the efficiency of 1% sodium bisulphite soln. as an absorbent for formaldehyde are described. With rates of air-flow of 1–3 and 28 litres per min., *ca.* 98 and 95%, respectively, of the formaldehyde present are trapped in the first of a train of impingers containing this reagent; corresponding figures for water under the same conditions are 69–87%. The efficiency of collection increases with increase in the formaldehyde concn. Since the formaldehyde vapour is very readily absorbed by rubber tubing, any connections should have glass-to-glass contacts inside the rubber. J. G.

Mixed Unsaturated Glycerides of Liquid Fats. Low Temperature Crystallisation of Whale Oil. T. P. Hilditch and L. Maddison. (*J. Soc. Chem. Ind.*, 1942, **61**, 169–173.)—In the preliminary separation of the mixed fatty acids of whale oils, the usual lead salt and alcohol separation was preceded by crystallisation of the lithium salts from 95% acetone (*cf.* ANALYST, 1939, **64**, 640). The highly unsaturated acids of the C_{20} and C_{22} series, remaining in solution as sol. lithium salts, were converted into methyl esters and fractionally distilled, whilst acids from the insol. lithium salts were separated by the lead salt and alcohol method. The component glycerides in an Antarctic whale oil have been studied by crystallisation of the oil, from acetone containing solid carbon dioxide at −10, −20, and −30° C. (*cf.* ANALYST, 1940, **65**, 656), into four fractions and determination of the component acids present in each fraction. The large number of unsat. acids of differing degrees of unsaturation makes close estimation of individual glycerides impossible, but the chief types were: "oleo"-saturated-unsaturated (C_{14}, C_{16}, C_{20}, C_{22}) glycerides, 66; "oleo"-di-unsaturated (C_{14}, C_{16}, C_{20}, C_{22}) glycerides, 12; "oleo"-myristopalmitins, 8; saturated-di-"oleins," 6; tri-unsaturated (C_{14}, C_{16}, C_{20}, C_{22}) glycerides, 4% (mol.). One unsat. C_{18} group per triglyceride mol. occurred in 80% of the triglycerides present in the oil, and two of these groups were present in a further 8% of the oil. About half the oil contained no acid above the C_{18} series and about one-third contained one group per triglyceride mol. of the highly unsat. C_{20} or C_{22} acids. It is suggested that polymerisation or oxidation of these highly unsaturated acids during low temp. crystallisation could be minimised by reversing the procedure, *i.e.*, by operating first at −40° C., subsequently recrystallising the separated glycerides from acetone at −40° C., and determining the component acids in the two −40° C. filtrates as rapidly as possible. The insol. glycerides should be crystallised successively from acetone at −20° C. and −10° C. E. B. D.

Detection of Acid or Basic Substances in Damaged Fabrics. C. Whitworth and D. W. Poxon. (*Nature*, 1943, **151**, 198–199.)—Feigl and Da Silva's method for detecting acid and basic substances in materials normally regarded as insol. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 317, 519) is applied to damaged fabrics. Thus, if a soln. of a nickel salt is incompletely pptd. with dimethyl glyoxime and filtered, the filtrate produces a pink colour with cotton or woollen fabrics which have been damaged by sodium hydroxide. A sat. soln. of silver chromate in 6 *N* ammonia gives a dark red-brown colour on many fabrics damaged by acids. Cotton damaged with hydrochloric acid, however, gives a poor reaction; wool damaged by this acid gives a white spot inside a dark ring. With coloured materials the ppt. is not always easily visible to the naked eye, but it can readily be seen under the microscope. J. G.

Determination of Monoalkyl Ethers of Ethylene Glycol. H. W. Werner and J. L. Mitchell. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 375–376.)—The principle of the method is oxidation with potassium dichromate soln. by an adaptation of Muehlberger's modification of the Nicloux method (McNally, "*Toxicology*," 1937, p. 648), which was evolved for ethyl alcohol. With the glycol ethers, however, the most consistent results were obtained when 1–3 ml of an aq. soln. of the sample was added to a cooled mixture of 5 ml of 0·33 *N* potassium dichromate and a vol. of conc. sulphuric acid equal to the combined vols. of acid and sample; with the propyl derivative the proportion of acid should be increased from 50 to 55%. The oxidation is best carried out in 25 × 200-mm test-tubes in a boiling water-bath (90–100° C.); after the specified period (*vide infra*), cool the tubes, dilute their contents to *ca.* 300 ml in 500-ml wide-mouthed conical flasks, and titrate with 0·05 *N* sodium thiosulphate, with starch sol. (added near the end of the titration) as indicator. Curves relating the time of heating with the extent of the oxidation show that, under the above conditions, the reactions are stoichiometric for times of 15, 15, 240 and 120 min. for the methyl, ethyl, *n*-propyl and *n*-butyl compounds, respectively. Theoretical factors based on reactions requiring 16, 14, 20 and 26 equivalents of oxygen, respectively, are thence derived, *viz.*, 1·584, 2·141, 1·736 and 1·514, respectively. From a consideration of the vol. of reagent used in excess of that corresponding with the 10 oxygen-equivalents required for the complete oxidation of the glycol portion of the mol. to carbon dioxide and water, it appears that the above radicals are oxidised as follows: methyl, to carbon dioxide and water; ethyl, to water and acetic acid; *n*-propyl and *n*-butyl to carbon dioxide, water and acetic acid. Other sets of conditions are indicated which enable less accurate results to be obtained in shorter times; *e.g.*, with an oxidising mixture containing 55% of acid a reaction requiring 20·4 equivalents for the *n*-propyl derivative occurs within 60 min.

Recoveries recorded for *ca.* 1–7·5 mg of the sample are: 96·3–100% for 50% acid, and 97·4–104·6 for 55% acid; in general, the former results are not significantly affected by the wt. of sample taken, but the latter are low for large, and high for small samples. Vapours are treated similarly, the heating stage being carried out in stoppered round-bottomed flasks with 5 ml of the potassium dichromate soln. and 5 ml (6 ml for the *n*-propyl derivative) of the acid. Determinations on known mixtures containing air with *ca.* 0·9–21 mg of the vapours per litre gave results differing from the true values by −10·4 to +9·9%; the larger deviations, as compared with the results for aq. solns., are attributed to the difficulty of preparing standard vapour mixtures, rather than to any increased error of the method (*cf.* Elkins, Storlazzi and Hammond, *J. Ind. Hyg. Toxicology*, 1942, **22**, 229). J. G.

Determination of Carbonyl and Carboxyl Groups in Chromic Anhydride Oxystarch and Oxycellulose by Means of Hydroxylamine. E. K. Gladding and C. B. Purves. (*Paper Trade J.*, 1943, **116**, Apr. 8; *T.A.P.P.I.* Sect., 150–155.)—The method is based on formation of oximes between hydroxylamine hydrochloride and the carbonyl groups of reducing sugars, the course of the reaction being followed by titrating the resulting hydrochloric acid with standard alkali (*cf.* Bryant and Smith, *J. Amer. Chem. Soc.*, 1935, **57**, 57). Dissolve 10 g of pure hydroxylamine hydrochloride (recrystallised, if necessary, by cooling a hot, very conc. aqueous soln.) in water, adjust the *p*H to *ca.* 5 with 0·5 *N* sodium hydroxide (required, *ca.* 48 ml), and dilute the soln. to 200 ml. Add 50-ml aliquot portions of the fresh reagent to weighed quantities (*x* g) of the sample in 125-ml glass-stoppered, conical flasks; choose quantities such that not more than 60% (≡ 0·0036 g-mol.) of the available reagent is utilised, because the consumption of a larger proportion brings the final *p*H of the soln. to below 3·5, and oxime formation is then incomplete. After 1·5 hr. (or 10–18 hr., *vide infra*) at room temp., add 6 drops of a standard 0·1% soln. of bromophenol blue in alcohol, and titrate with 0·5 *N* sodium hydroxide. If the titration is *ca.* 6·5 ml, the titration-error is *ca.* 0·1 ml. A blank determination should be made simultaneously, and, if the difference between the blank and the experimental titrations is *y* ml of blank and the experimental titrations is *y* ml of *N* alkali, the carbonyl content in g-mol. per g is $y/1000x$. Tests with known quantities of various sugars gave results ranging from 95 to 103% of the theoretical value, but it was found necessary to vary the reaction times (*vide supra*) on account of the variation in the speeds of reaction of the different sugars. Thus "fast" carbonyls (1·5 hr.) were mannose, galactose, xylose and arabinose; "slow" carbonyls (10–18 hr.) were glucose, fructose, lactose and cellobiose. To follow the course of the reaction (*e.g.*, with "slow" carbonyls), the procedure is modified as follows. Dissolve 2·5 g of hydroxylamine hydrochloride in 40 ml of water, add the indicator, and adjust the *p*H to 3·5 with the 0·5 *N* alkali. Dissolve the sample in this soln. in a dry flask, note the time, and after *ca.* 1 min. titrate back to the end-point, at intervals, with the 0·5 *N* alkali. If the quantities available for either method necessitate the use of 0·1 *N* alkali, the titration should be carried out electrometrically to *p*H 3·2; duplicates agreed within 0·05–0·10 ml. If carboxyl groups are present, they are determined by forming the calcium salt of the sample as follows. Place 1·0–1·5 g in 60 ml of 0·5 *N* calcium acetate soln., in a stoppered flask; after 1·5 hr. at room temp. filter (using a dry Buchner filter and flask), and titrate a 50-ml aliquot part of the filtrate to phenolphthalein with 0·1 *N* alkali. After allowance for any blank on the calcium acetate, the titration corresponds with 5/6 of the carboxyl content of the sample (*cf.* Yackel and Kenyon, *id.*, 1942, **64**, 121). Duplicates agreed to within 0·1 ml on a total titration of 2–5 ml. Wash the calcium salt well on the funnel, and determine its carbonyl content as described. The methods were used to account quantitatively for the oxidant consumed in the preparation of oxycelluloses and oxystarch, and to learn something of their structure. All the groups in such compounds produced by periodate were of the "fast" type, although the reactions were not of the first order. The results for oxy-compounds produced by means of chromic anhydride are also correlated with the resulting swelling effect and the effect of the colloidal surface of the original cellulose or starch. J. G.

New Microchemical Stain for Cellulose. E. E. Post and J. D. Laudermilk. (*Stain Technol.*, 1942, **17**, 21; *Tech. Bull. Paper Makers' Assoc.*, 1943, **20**, 6.)—Apply to the specimen 2–3 drops of a 2% soln. of iodine, in 5% potassium iodide soln., diluted with 9 vol. of water and containing 0·28% of glycerin. Blot with filter-paper, and dry as completely as possible. Apply 1 drop of a sat. soln. of lithium chloride in water, and cover the specimen. After 5 min. cellulose gives a light blue colour. The stain is suitable for plant histology. J. G.

Inorganic

Organic Sulphur Compounds as Reagents for Metals. A. K. Majumdar. (*J. Indian Chem. Soc.*, 1942, **19**, 396.)—Known reactions of dimercaptothiobiazole (I) and phenyldithiobiazolonthiol (II) are briefly summarised. (I) gives a red ppt. with bismuth, a black ppt. with mercury and white to yellow ppts. with other metals of the sulphide group. (II) gives yellow ppts. with gold, silver, mercury, lead, platinum, arsenic and antimony, white ppts. with cadmium and zinc, a brown ppt. with copper, and red ppts. with tin, bismuth and palladium. Dubsky (*Z. anal. Chem.*, 1934, **96**, 267, 412) used both reagents for the detection of traces of bismuth, and found the sensitivity 1 part in 28,000 parts of soln., the lower identification limit being 1·2μg. Rây and Gupta (*J. Indian Chem. Soc.*, 1935, **12**, 308) found with (I) that the sensitivity was 1 part in 1,600,000 parts if a nitric acid (not a hydrochloric acid) soln. of bismuth was used. For a colorimetric determination the red ppts. of the bismuth compounds given by both reagents may be peptised with gum acacia. S. G. C.

Substituted Dithiocarbamic Acid Reagents for Copper. E. Geiger and H. G. Müller. (*Helv. chim. Acta*, 1943, **26**, 996–1002.)—Callan and Henderson (ANALYST, 1939, **54**, 650) used N,N-diethyldithiocarbamic acid and Harry (ANALYST, 1931, **56**, 736) used piperidine-N-dithioformic acid as reagents for copper. The copper compounds are only slightly soluble in water and may require extraction with solvents to produce coloured solns. for colorimetry. Carbon tetrachloride is the best solvent for the purpose. Alternatively, various other dithiocarbamic acid derivatives were tried with a view to obtaining coloured copper compounds more soluble in water. The cupric salt of N,N-dihydroxyethyldithiocarbamic acid has a

solubility in water of 20 times, and in ethyl alcohol 10 times, that of N,N-diethyldithiocarbamic acid, *viz.*, 200 and 10,000μg of copper respectively in 100 ml. The reagent is employed in alcoholic soln. The cupric compound has an intense yellowish-brown colour; it is insoluble in carbon tetrachloride. Copper must be in the cupric condition, as the cuprous compound is much less strongly coloured. The cupric compound is formed quantitatively in the *p*H range 1·4 to 9·0. Sodium, potassium and ammonium salts in the amount of 1 g to 100μg of copper in 100 ml did not affect the intensity of colour. No interference was produced by the following ions (1000μg in 100 ml):—aluminium, arsenic, barium, calcium, cadmium, chromium, mercuric, manganese, lead, strontium, titanic, zirconium and stannic. Small amounts of ferric, silver, cobalt and nickel ions interfered. This could be overcome as follows:—ferric ion, add sodium hexametaphosphate; silver, add sodium thiosulphate; nickel and cobalt, add potassium cyanide, acidify to *p*H 4 to decompose the cupricyanide ion, and add the reagent. To prepare the reagent, add 1·0 g of carbon disulphide to 10 g of diethanolamine in 85 ml of methyl alcohol and dilute to 100 ml with methyl alcohol. The reagent keeps well in a stoppered bottle. For a test for copper, 3 drops of the reagent are sufficient; not more than 100μg of copper should be present in 100 ml. S. G. C.

Colorimetric Determinations by means of 8-Hydroxyquinoline. T. Moeller. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 346–349.)—The following metals can be determined, like indium (ANALYST, 1943, **68**, 263) by agitation of their aqueous solns. with a soln. of 8-hydroxyquinoline in chloroform:—ferric iron, aluminium, bismuth, nickel, cobalt and copper. Absorption maxima and optimum *p*H ranges for complete extraction are: Iron, 470 and 570mμ; *p*H 1·9–3·0. Aluminium: 395mμ; *p*H 4·3–4·6. Bismuth: 395mμ, *p*H 4·0–5·2; Cobalt: 420mμ; *p*H 6·8 or higher. Nickel: 395mμ; *p*H 6·7 or higher. Copper: 410mμ; *p*H 2·7–7·0. For amounts up to 1 mg of metal in the aqueous soln. treated, the chloroform solns. show little or no deviation from Beer's law at the above wavelengths. Under proper *p*H control (consult original) it should be possible to determine iron in presence of bismuth, copper or nickel, and copper in presence of cobalt, cadmium or zinc.

W. R. S.

Colorimetric Cobalt Determination. L. G. Overholser and J. H. Yoe. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 310–313.)—The reagent is an aqueous 0·05% soln. of the sodium salt of *o*-nitroso-resorcinol; the buffer soln. (*p*H 6·0) contains 363 ml of 0·5*M* sodium hydroxide, and 500 ml of 0·4 *M* potassium biphthalate, solns. diluted to 1 litre. Transfer the test soln. to a 100-ml flask, add 25 ml of buffer soln. and 5 ml of reagent, and adjust the volume. For visual comparison use Nessler tubes and a series of standards (0, 0·05, . . . 0·25 mg of cobalt in 100 ml). Spectrophotometric comparison is more sensitive, the limit of detection being 1 : 50,000,000. The reagent is highly coloured and absorbs strongly in the same spectral range as the cobalt compound (420–450mμ). Beer's law proved valid. The cobalt in the soln. must not exceed 0·26 mg; larger amounts do not deepen the colour, because the amount of reagent cannot be increased without serious loss of sensitivity. Other metals interfere more or less, especially iron. The nickel compound is less stable; hence, with the limited amount of reagent added, digestion for some hours on a steam-bath will cause gradual formation of the cobalt complex. Visual comparison can be applied with a standard series containing appropriate amounts of nickel. For spectrophotometric data the original should be consulted. W. R. S.

Spot Test for Chromium. W. O. Philbrook. (*Metal Progress*, 1942, **42**, 1035–1037.)—For an approx. determination of chromium in steel samples place 1 drop of "dissolving acid" (250 ml of water, 200 ml of conc. nitric acid and 50 ml of 85% phosphoric acid) on the clean steel surface. After 30 sec., or when the initial effervescence has ceased, touch the spot with the flat, ground-off end of a glass rod (3 mm in diam.) and mix the liquid adhering to it with 1 drop of sodium hypobromite soln.. (400 ml of water, 20 g of sodium hydroxide and 2·5 ml of bromine) in the depression of a white spot plate. Add 1 drop of dil. (1 : 5) sulphuric acid and stir until the brown ppt. of ferric hydroxide has dissolved. Add 1 drop of phenol soln. (5 g of phenol in 50 ml of acetic acid) to destroy excess of bromine, and then 1 drop of indicator soln. (0·1 g of diphenylcarbazide in 5 ml of acetic acid diluted to 50 ml with alcohol). Compare the red colour with those given under the same conditions by steels containing known amounts of chromium. If care is taken to secure approx. the same amount of the sample on the end of the glass rod the method may be used for solns. containing chromium.

S. G. C.

Colorimetric Determination of Small Amounts of Molybdenum in Ores. F. S. Grimaldi and R. C. Wells. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 315–316.)—*Scheelite.*—Fuse 0·5 g with 2·5 g of sodium carbonate and *ca.* 0·05 g of nitrate in platinum, extract with water, add paper pulp, filter and wash with warm 1% sodium carbonate soln. Just acidify with hydrochloric acid, and reduce any chromate with a drop of sulphurous acid soln. Evaporate to 25 ml on the water-bath, neutralise with 10% sodium hydroxide soln. and add 10 ml in excess. Digest hot for 10 min. Filter hot, wash with dil. sodium hydroxide soln., barely acidify with hydrochloric acid (phenolphthalein as indicator) and transfer to a 100-ml flask. *Molybdenite, wulfenite, and siliceous ores.*—Attack 0·5 g with nitric acid, evaporate with 2 ml of sulphuric acid until fumes are evolved, cool, dilute, add 10% sodium hydroxide soln. (10 ml excess), filter, digest hot, and proceed as for scheelite. *Reagents.*—10% stannous chloride soln. in 1 : 1 hydrochloric acid; 10% ammonium thiocyanate soln.; ammonium citrate; acetone; molybdenum soln. (dissolve 0·1 g of pure molybdenum trioxide in a little sodium hydroxide soln., neutralise to litmus with hydrochloric acid and dilute to 1 litre; renew every month.) *Determination.*—To an aliquot portion (15 ml) add 1·5 g of ammonium citrate and stir until it dissolves, then add 5 ml of thiocyanate soln. and 25 ml of acetone, cool and add exactly 7 ml of stannous chloride soln., drop by drop, while stirring. The measurement should be accurate, as it governs the acidity. The molybdenum compound is amber-yellow. Prepare a standard with 15 ml of water and the same quantities of reagents, and titrate with molybdenum soln. until the tints match. The citrate prevents interference of tungsten; acetone has a stabilising effect. Iron interferes and must be absent. Phosphate with tungstate causes gradual fading after about 10 min.; if the colour is matched at once interference is negligible. Vanadium interferes

by colouring the reduced soln.; 0·002 g in the final soln. is permissible. Arsenic and antimony do not interfere. W. R. S.

Determination of Calcium by Precipitation with Picrolonic Acid and Polarographic Measurement of the Residual Picrolonic Acid. G. Cohn and I. M. Kolthoff. (*J. Biol. Chem.*, 1943, **147**, 705–719.)—Picrolonic acid has been used for estimating calcium, the pptd. calcium picrolonate, $Ca(C_{10}H_7O_5N_3)_2.2H_2O$, being determined directly by weighing, by a colorimetric method or by combustion or indirectly by titrating the excess picrolonic acid with methylene blue. In the present method the calcium is pptd. with a relatively slight excess of picrolonic acid, which is then determined polarographically without filtering. In potassium or lithium chloride soln., picrolonic acid gives current-voltage curves which exhibit maxima, but these can be suppressed with gelatin or thymol, or, better, with camphor. The curves then show one wave between −1·0 and −1·3 volt and another at a potential more negative than −1·4 v. In acid solns. gelatin was a better suppressor than camphor, and with gelatin in an acetate buffer soln. of *p*H 3·8 two waves were obtained, the second being a well-defined band between −1·0 and −1·2 v., the height of which at −1·0 v. was proportional to the concn. In absence of gelatin the height of the wave at −1·2 v. was proportional to the concn. and was unaffected by changes in the concn. of acetate buffer or of indifferent electrolyte. These conditions were therefore selected for the polarographic measurement. Calcium picrolonate crystallises only slowly and, as most alkali metal picrolonates are not very sol., errors may be introduced when the concn. of alkali metal ions is large. Lithium picrolonate is relatively sol. and lithium salts were therefore chosen to buffer the soln., the following mixture being used:—*M* lithium chloride, *M* acetic acid, 0·125 *M* lithium acetate. This was diluted 10-fold in the actual reaction mixture. Calcium picrolonate was shown to have a min. solubility when the excess picrolonic acid was equal to or larger than $1·5 \times 10^{-3}$ *M*. To obtain satisfactory results it was necessary to allow the samples to stand overnight, the best results being obtained in the ice-chest with calcium concns. of $1–5 \times 10^{-3}$ *M*, and at room temp. with higher concns. Reproducible results were obtained with picrolonic acid that had been recrystallised with 33% acetic acid, but with material recrystallised from 95% ethyl alcohol it was necessary to filter off the pptd. calcium picrolonate before measuring the polarogram. Alkali metals and magnesium interfere when present in high concns., and sulphate and phosphate result in co-pptn. of the corresponding calcium salts. Potassium and ammonium interfere even more than magnesium. The method recommended is as follows:—Add to the sample sufficient of the lithium chloride and acetate buffer soln. to become diluted 10-fold in the final mixture. Add a standard 0·01 *M* picrolonic acid soln. to 5–10 ml of the unknown so that the concn. of picrolonic acid after completed pptn. becomes at least 0·001 *M*. The amount added should not exceed the 4-fold molar concn. of the calcium. Leave the mixture overnight in an ice-box if the calcium concn. is not greater than 5×10^{-3} *M*, or at room temp. if the concn. is in excess of this value. Without filtering, estimate the concn. of picrolonic acid polarographically at a cathode potential of 1·1 v. versus the saturated calomel electrode, or, when a mercury pool anode is used, at an applied potential of −1·2 v. F. A. R.

Volumetric Determination of Bromide in Brines. H. H. Willard and A. H. A. Heyn. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 321–322.)—Neutralise the test soln. (25–50 ml; max. bromine content 13 mg) with acetic acid or sodium hydroxide and add 5 g of pure sodium chloride unless already present. When it has dissolved add 5–10 ml of hypochlorite soln. (7 g of sodium hydroxide in 150 ml of water; saturate with chlorine, pass air for 10 min., add 1·8 g of sodium hydroxide and dilute to 200 ml). The bromide is oxidised to bromate. Adjust the *p*H to 6·0–6·5 by means of a glass electrode or by adding 0·1 g of zinc acetate in 5 ml of water, then sodium hydroxide soln. until a ppt. appears, and lastly just enough acetic acid to dissolve the zinc hydroxide. Rinse down the sides of the beaker, boil and add 2·5 g of sodium formate. Rinse down again, boil for a few sec., cool, dilute to 150 ml and add 1 g of potassium iodide; if this liberates iodine, the assay must be repeated. Add 8 ml of strong hydrochloric acid diluted to 30 ml, and 2 drops of 0·25 *M* ammonium molybdate catalyst. Titrate at once with 0·01 *N* thiosulphate standardised against iodate; 1 ml ≡ 0·13319 mg of Br. Deduct the blank result. Iodide and oxidising agents interfere. W. R. S.

Colorimetric Determination of Hydrogen Peroxide. G. M. Eisenberg. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 327–238.)—A method for determining 0·2–3·0 mg of hydrogen peroxide per 100 ml is described. The sample is treated with titanic sulphate reagent and the colour intensity is measured in a photoelectric colorimeter. The solns. conform to Beer's law. For working details the original should be consulted. W. R. S.

Microchemical

Micro-determination of Pyruvic Acid and of Glucose. E. Haag and C. Dalphin. (*Helv. Chim. Acta*, 1943, **26**, 246–250.)—Wieland's method (*Annalen*, 1924, **436**, 229), depending on the oxidation of pyruvate to oxalic acid by means of iodine is inaccurate unless a large excess of iodine is used. The relationship between the amount of pyruvate present and that estimated when the reaction is allowed to proceed for 90 min., is shown graphically. It may be defined by the expression $y = p.x^{-1/3} + c$, where x = mg of pyruvic acid present, y = % of pyruvic acid estimated, $p = 144·9$ and $c = -42·3$. With a large excess of iodine, 1 ml of *N*/100 iodine ≡ 0·1467 mg of pyruvic acid. The determination of glucose after Kolthoff (*Die Masse Analyse*, 1931, **2**, 485) depends on a similar oxidation. For a mixture of pyruvate and glucose the oxidations may be considered complete if an excess of at least 18 ml of *N*/100 iodine was present throughout the reaction. Thus by iodimetric determination a figure for the sum of pyruvate and glucose can be obtained. The glucose may be determined separately and the amount of pyruvate found by difference. J. W. M.

Micro-determination of Chlorine and Bromine in Organic Compounds with Simultaneous Determination of Hydrogen. H. Gysel. (*Helv. Chim. Acta*, 1941, **34**, 128–134E.)—Decompose the substance in a current of oxygen with platinum contacts, as in the Pregl method, pass the combustion products through an absorption tube of the Pregl type containing fine-grained calcium chloride for the absorption of water, absorb the halogen products in a soln. of alkali and peroxide, and titrate the soln. by Volhard's method. The

determination is complete in 30–40 min. (or 40–45 min. if hydrogen is also to be determined) and is suitable for the analysis of air. The ordinary type of combustion tube is used, heated electrically. To prevent condensation in the capillary, it may be warmed by a wire connected to the heater; two platinum contacts are used. The water absorption tube filling lasts for about 50 analyses. The halogen absorption soln., of which 3 ml are used, consists of 0·02 *N* potassium hydroxide mixed with perhydrol in the ratio of 10 : 1. Micro-burettes graduated in 1/100 ml are employed. Very accurate results are obtained. J. W. M.

Physical Methods, Apparatus, etc.

Identification of Carbon Black by Surface Area Measurements. F. H. Amon, W. R. Smith and F. S. Thornhill. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 256–258.)—Parkinson (*Trans. Inst. Rubber Ind.*, 1940, **16**, 94) has suggested that the particle size of lamp-black recovered from a tyre-tread by means of nitric acid is the same as that of the supply originally used. The authors have found (*Ind. Eng. Chem.*, 1941, **33**, 1303) that the low-temp. nitrogen absorption method of Emmett and De Witt (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 28) for the determination of the surface-area of finely-divided substances is a comparatively rapid and accurate method of measuring the total surface of commercial carbon blacks. Since the surface areas of most of the standard carbon blacks have been determined (*loc. cit.*) a method for the identification of the carbon black present in an unknown rubber stock can be evolved, so long as it can be established that the carbon black can be recovered from the stock with its surface-area unchanged. Tests were made on these lines with various grades of carbon black in Buna-S and in rubber, and the following extraction procedure was found to be satisfactory:—Digest 0·75–1 g of vulcanised stock at 60–70° C. for 1 hr., with 15 ml of conc. nitric acid; add 35 ml more of the acid, and digest for a further 2 hr. In order to avoid contamination of the lamp-black for the subsequent surface-area determinations, filter the soln. on a sintered crucible (Grade FS–20–10υ) instead of on an asbestos Gooch crucible, wash the residue according to the standard method (Oldham and Harrison, *ibid.*, 1937, **9**, 278), dry it at 100° C. for 2 hr. and weigh it. Determine the surface area and ash content of the recovered black, using separate portions, and correct the former for the latter if necessary; in the examples studied, however, the ash-content was only *ca.* 0·4% by wt. of the whole rubber stock, so that the correction was only 1–2 sq. m. per g, a value too small to cause any confusion in the identification of the black. The experimental data indicate that carbon black undergoes no appreciable change in surface area during incorporation with, or vulcanisation of, a rubber stock and, with one exception, the surface areas are substantially the same before addition to the stock and after removal. With this exception ("Cabot," grade 4) a 25% increase in surface area was recorded; this cannot be explained, but it is nevertheless insufficient to confuse the identification. Surface areas recorded (8 samples) range from 26 to 330 sq.m. per g. The cresol method for the separation of the carbon black (Roberts, *Rubber Chem. Tech.*, 1941, **14**, 241) also gave satisfactory results, except with the grades of finer particle-size, for which low surface area values were obtained; it is probable that with this method impurities are retained by the carbon and block up the interstices of the black which would normally be accessible to the nitrogen, and the method is therefore not recommended where the nature of the black in the rubber stock is unknown. The cresol method was also unsatisfactory with the Buna-S stock, and even with the nitric acid a somewhat longer digestion time than that specified above was required. If blends of blacks are present in the rubber stock some secondary method of identification may be required, *e.g.*, non-impingement types of black are readily identified microscopically, and the known surface areas of these materials and the total carbon content of the stock then usually suffice to enable the blend to be identified. Although the unchanged surface area of the black does not necessarily mean a complete lack of surface reaction during curing, it supports the physical concept of carbon black reinforcement, and the theory that the chief rôle of the black is to orient the chains of rubber molecules, and so to alter the extent and the type of the rubber-sulphur bands normally formed in non-reinforced rubber stocks. J. G.

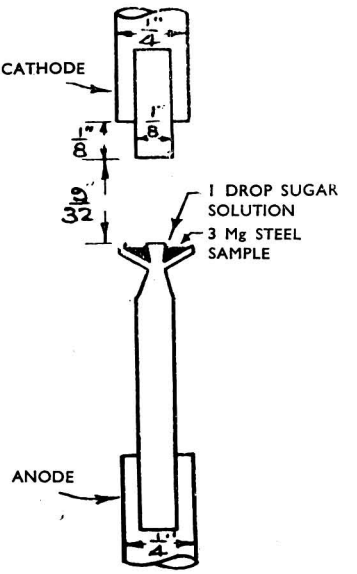
Differentiation of Bleached Pulps in Ultra-Violet Light. Agahd. (*Woch. Papierfabr.*, 1942, **73**, 170–171; *Tech. Bull. Paper Makers' Assoc.*, 1943, **20**, 6.)—Add 30 ml of a 0·05% soln. of Rhodamine B extra, with stirring, to 90 ml of a 0·05% soln. of Brilliant Dianil Green G. When bleached soda or sulphite pulps are moistened with this reagent, they both appear reddish-violet in ordinary light; in filtered ultra-violet light, however, they are blue and rose-coloured, respectively. J. G.

Swelling-Staining Reagent for Studying Fibre Structure. W. M. Harlow. (*Paper Trade J.*, 1943, **116**, Apr. 15; *T.A.P.P.I. Sect.*, 170.)—Treatment of cellulose fibres with certain quaternary ammonium hydroxides produces localised increases in diam. ("ballooning") and X-ray patterns which differ from those of the untreated fibres (*cf.* Sisson and Saner, *J. Phys. Chem.*, 1939, **43**, 687). This phenomenon, together with the use of a stain to bring out the fine detail of the swollen fibres, is the basis of the present test. Dissolve 20 mg of ruthenium red in 20 ml of a 50% aqueous soln. of trimethylbenzyl ammonium hydroxide, filter through glass wool if solution is incomplete, and place 1 drop of the reagent on the fibres, which are prepared for microscopical examination in the usual way. The use of a green filter enhances the contrast produced by the stain, which has a special affinity for the least swollen portion of the fibres, The reagent should be made up in small amounts, as it fades after standing for a few hours. J. G.

Resistance of Sized Papers and Paperboard to Water at Elevated Temperatures. P. W. Godwise. (*Paper Trade J.*, 1943, **116**, Mar. 4; *T.A.P.P.I. Sect.*, 90–93.)—For many purposes it is important to know the resistance of paper to penetration by hot aqueous solns. (*e.g.*, when warm adhesives are used, or for containers which are to be filled with warm liquids), and the following test is therefore recommended:—Cut a sample 4 × 4 in., turn up 0·5 in. of each side, and secure the adjacent corners with paper clips so as to form a "tray." Place this gently on the surface of hot distilled water (*vide infra*), avoiding trapped air-bubbles, and note the time after which 90% of the area has darkened in colour owing to complete wetting; this procedure avoids the effects of pinholes or other imperfections.

Bring the water to the b.p., and then maintain it near this temp. (*i.e.*, at not below 206° F.) before applying the paper, and test each side of the sample separately. Curves relating temp. and penetration time are shown for a wrapping paper, a waste paperboard, a newsboard and a pulpboard (thicknesses, 0·0065–0·063 in.). They all show a rapid but decreasing fall in penetration time (originally ranging from 466 sec. to over 24 hr.) with rise in temp. between 72° and *ca.* 130° F., followed by a slower decrease, and approx. const. values (5–49 sec.) are attained at 170°–190° F. and over. Since the method places the penetration-times of these samples, and of different samples of the same type, in the same order whatever the temp., it may also be used to accelerate the cold-water test on samples having high penetration times; moreover, the cold-water values may be obtained by extrapolation from those given by the hot water method. Limitations of the method arise from the fact that some samples resist the penetration even of boiling water for more than 24 hr.; it seems that the rate of evaporation from the top side may then be greater than the passage of water through the paper by capillary action. Ordinary hard sizing corresponds with a penetration time (by the hot test) of *ca.* 1800 sec., and consequently tests for longer periods are seldom necessary. The above conclusions were reached with papers and boards sized with rosin and alum in the usual way, and there is no evidence that they will necessarily apply to the other methods of sizing. The penetration time is not necessarily proportional to the amount of rosin and/or alum used. J. G.

Quantitative Spectrographic Analysis of Stainless Steels. M. F. Hasler, C. E. Harvey and H. W. Dietert. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 102–107.)—The method developed enables stainless steels to be analysed for chromium up to 28% and for nickel up to 20%, with results accurate to ±2% of the quantity being measured. At the same time satisfactory silicon, manganese, niobium, molybdenum and titanium determinations can be made. Preliminary work with a spark method showed that for alloys such as stainless steel, where the impurities occurred as high percentages, the surface on which the spark played could not be expected to be representative in composition and uniform in physical form. Tests confirmed that the spark method could not be relied upon to give uniform results. A special arc method was therefore devised which is claimed not to suffer from the usual disadvantages of arc techniques. Sufficient of the sample is prepared in the form of fine filings to give a representative 3-mg portion containing about 1500 individual particles. The lower electrode (positive), in which the sample is to be placed, is machined so as to have an annular crater with a centre post of carbon left in position. The 3-mg portion is cemented in place with a drop of sugar soln. which has been preceded by a drop of alcohol to wet the carbon. The upper electrode is a normal cylindrical carbon rod. Upon arcing, the discharge starts at the centre post above the sample and holds to it for about 30 sec. This heats the sample sufficiently by conduction, so that it melts first and then evaporates up into the arc. This melting gives reproducible conditions independent of the previous history and form of the sample. The arcing proceeds smoothly, as the molten metal stays spread out in a thin layer, there being no tendency to form a single large globule, which usually causes arc wandering and instability of the discharge. The centre post burns away smoothly, vaporising much of the metal and subsequently the conical platform that originally carried the sample.



Special Carbon Electrodes turned from Rod Stock

When nothing remains but the stub, the whole of the sample has been evaporated. This arcing to completion ensures max. accuracy for the determination of refractory substances used as stabilisers, *e.g.*, compounds of titanium and niobium, as well of the more volatile metals. The remainder of the investigation follows conventional lines. Working curves were prepared for the various constituent elements, using iron as the internal standard. A correction is made when interpreting the results to allow for the iron content of the sample under test, since, in stainless steels, this may vary from 55% to 90%. B. S. C.

Spectrographic Limit of Identification of Potassium. D. P. Norman and W. W. A. Johnson. (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 152–153.)—Steadman, Hodge and Horn have reported (*J. Biol. Chem.*, 1941, **140**, 71) that, using the potassium lines 4044·1A and 4047·2A, they could detect 1 to 5μg of potassium in the direct current arc. The present investigation showed that the spectroscopic sensitivity of potassium, as determined by these lines, varied by factors of several hundredfold, and that the sensitivity was not as high as reported. The more sensitive lines of potassium at 7664·9A and 7699·0A are rarely used, since specially sensitised plates are required to photograph them, but the present tests show the very great advantage of using these near infra-red lines. The spectrograms were taken in the first order of a 3-metre grating spectrograph having a dispersion of 5·6A per mm. The 4000 doublet was photographed on Eastman 33 plates and the 7600A doublet on Eastman Spectroscopic I-N plates. The sample was packed in a 5 mm by 4 mm crater in a 6-mm diam. pure graphite electrode used in the lower position as cathode, the upper positive electrode being pointed. The arc was operated with a 3-mm gap, 40 to 50 volts across the gap, current 15 amp. The limits of detection for six

potassium salts and for four compounds containing potassium are as follows:

Sample	Limit of detection (μg) 4044/4047	7665/7699
KCl	120	0·6
KOH	180	0·08
K_3PO_4	95	0·08
$KMnO_4$ ..	320	0·2
$K_2Cr_2O_7$ ..	240	0·8
$KC_2H_5SO_3$ ..	200	0·2
Na_2CO_3 ..	10	0·04
$Na_2C_2O_4$ ..	16	0·04
V_2O_5	40	0·04
Glass	20	0·06

The big variation in the sensitivity, using the 4000A doublet, can be attributed primarily to uncontrollable changes in the excitation conditions produced by variations in the constituents being volatilised. This is particularly noticeable with the two samples whose major constituent is sodium. The presence of this element reduces considerably the intensity of the strong cyanogen bands which mask the 4000A potassium doublet. The limit of detection of potassium using the 4000A doublet is fixed primarily by the intensity of the heavy cyanogen band rather than by the faintness of the potassium lines. B. S. C.

Reviews

A Handbook of Shellac Analysis. By M. Rangaswami, B.A., A.I.I.Sc., and H. K. Sen, M.A., D.I.C., D.Sc., F.N.I. Pp. xvii + 106. Namkum: Indian Lac Research Institute. 1942.

This book is one of the best things yet done by the Lac Research Institute. Naturally it is the business of the Institute to evolve standard methods of analysis and evaluation of shellac, but to secure agreement as to test details between the various trade interests and the different standardising authorities on an international scale is no small task, and, as the authors frankly admit, this desirable state has not yet been attained. In default the authors have brought together and presented in this one volume all the methods of testing, both chemical and physical in character, either employed in actual analysis or suggested for adoption. The official texts are given in full with critical notes by the authors on the significance of each test and the relative merits of different methods of making a determination of some property. The rule is one chapter to each test (except for some minor matters grouped under "Miscellaneous"), an arrangement which facilitates comparison of the different methods used and must surely lead to that goal where there will be an end to more or less private purchasing specifications for shellac or the use of tests which are regarded and kept as confidential. L. A. Jordan

National Paint Dictionary. J. R. Stewart. 2nd Edn. Pp. 142+Supplement 69; 250 Illustrations. Washington: Stewart Research Laboratory. 1942. Price $7.50.

This dictionary has been designed to give information on practically every important substance used either directly or indirectly in the manufacture of paints and on the methods and apparatus for their examination. Much of this information was previously available only in technical and trade journals, and the work will thus be of value to all analysts to whom samples of paints, inks or their constituents are submitted. Some idea of the extent of the ground covered may be gathered from the fact that the items include the characteristics not only of the ordinary oils, resins, pigments and solvents, but also of trade products such as Adm Oil (a heavy boiled linseed oil), Ti-Cal (a titanium calcium base pigment), Acra Wax, Glyceryl Monoricinoleate, Shell Dispersol and so forth. The apparatus illustrated and sometimes described includes such well-known instruments as the Tintometer (incidentally, only an early model) and the Laurie–Bailey Tester, as well as those less familiar, such as the Electronic Microscope, the Calco-Spectroscope and the Opacimeter.

The Supplement gives a series of tables of physical and chemical data of natural and synthetic resins, solvents and oils, colour identification tests, flash-points and so forth, interspersed with advertisements, which would be more fittingly placed in a separate section.

As in all technical dictionaries, one can discover that anticipated items have not been included. For instance, Saybolt data are given but there is no description of the instrument. Since the publishers invite constructive criticism, we would suggest that the value of the dictionary would be immeasurably enhanced if references were given (as is occasionally done) to the journals in which working details of apparatus and methods can be found. For example, it is difficult to understand the principle of the Johnson Nigrometer merely from the diagram of its construction, and it would be helpful to the reader puzzling over the unexplained lettering on the illustration of the Bolton and Williams Colorimeter to learn that he can find full particulars of the instrument in The Analyst. Editor

THE VITAMINS IN MEDICINE. By FRANKLIN BICKNELL, D.M., M.R.C.P., and FREDERICK PRESCOTT, M.Sc., Ph.D., A.I.C., M.R.C.S. Pp. vii + 662. London: William Heinemann (Medical Books), Ltd. 1942. Price 45s.

So far the completest volumes on the vitamins—whether on their chemistry or on their physiology and therapeutics—have been those of American and German authors, with the single exception of the classical monograph first published by the Medical Research Council in 1919 under the title of a *Report on the Present State of Knowledge Concerning Accessory Food Factors* (*Vitamines*). A second edition appeared in 1924 and the third in 1932, with a warning that it would be the last, because "the labour entailed and the sacrifices of time made by research workers in successive revisions of such a monograph are not likely to be justifiable again." The Council expressed the hope that the newly founded *Nutrition Abstracts and Reviews* would enable those interested to keep themselves up-to-date about the vitamins—as they had then come to be termed officially.

A similar argument seems to have affected publication in the U.S.A. of Sherman and Smith's *The Vitamins*, which was not revised after its 2nd edition in 1931. English-speaking workers in the field had to wait for the appearance last year of Rosenberg's *Chemistry and Physiology of the Vitamins*, and then had to pay the increased price that was bound to parallel ten years' rapid increase in knowledge, and this in spite of the fact that Rosenberg's book only covers, and only claims to cover, the chemistry and part of the physiology of the vitamins, leaving altogether on one side their dietary and therapeutic application.

It is, therefore, gratifying to find that two British authors have had the courage and the industry to tackle those aspects of the subject, and thus to supplement Rosenberg's up-to-date treatise by one of their own, equally up-to-date (references are given to many 1942 publications), equally exhaustive, equally accurate and equally useful, though perhaps to a somewhat different group of users.

Designed primarily, as its title indicates, for the physician, it abounds in information needed by all those concerned with health problems, and contains concise accounts of our more strictly chemical knowledge of the vitamins, as well as most elaborate surveys of their biochemical behaviour and physiological functions. Separate chapters are devoted to Vitamin A, the Vitamin B Complex, Vitamin B_1, Riboflavin, Nicotinic Acid, Vitamin C, Vitamin D, Vitamin E, Vitamin K and Vitamin P. There are 42 pages of (subject) index, and each chapter has its own bibliography, the shortest—on vitamin P—containing only 45 references, the longest—on vitamin C—containing 880. There are in all nearly 3200 references—some indication of the authors' unstinting labour.

This is perhaps hardly the place to discuss details of the authors' opinions, which are often more implied by their emphasis on particular points than explicitly stated. It is clear that they still hold the view, first tentatively propounded by Dr. Bicknell himself, that vitamin E may find useful application in treating various forms of muscular dystrophy. Again, they come near to accepting Scarborough's belief that the Göthlin test for capillary fragility is a measure of vitamin P deficiency, and has very little to do with uncomplicated scurvy.

As an example of the authors' method of covering the large field chosen for their labours, it may be permissible to indicate how one typical chapter, that on vitamin C, which is the longest and therefore the most fully documented, is subdivided. After giving the "history" of the vitamin, and its chemistry very briefly, its distribution in foods is presented (in tabular form); there follows a detailed discussion of its physiology and functions. Consideration is then given to human requirements of the vitamin, both in general and under various special conditions, such as infancy, childhood, old age, pregnancy and lactation. Then come descriptions of scurvy, subclinical scurvy and other conditions in which vitamin C deficiency has been observed. After a section on the laboratory methods used for detecting vitamin C deficiency there comes the final and longest section, namely, that on its clinical uses.

A closely analogous procedure is adopted in each of the other nine chapters of this exhaustive, authoritative and indispensable book, of which the technically first-class production is, in war-time, as surprising as its relatively low price. It is sure to sell out quickly, unless the publishers have been able to make very special provisions for the first printing. It is rather to be hoped, however, that they have not, for one would clearly welcome anything, such as a rapidly exhausted original edition, that encourages the authors continually to revise and bring out new editions of what is likely to become at once the standard work of reference in the subject.

A. L. BACHARACH

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, as, for example, food and drugs analysis, analysis of water (including its bacteriological examination), gas analysis, metallurgical assays, biological standardisation and micro-analysis. Papers on these and allied subjects may be submitted for presentation and publication; they may:

(1) Record the results of original investigations into known methods or improvements therein;
(2) Record proposals for new methods and the investigations on which the proposals are based;
(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.

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.

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.

To be 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 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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