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Volume 69 May, 1944
No. 818, Pages 141—168

THE ANALYST

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

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

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

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 5 p.m. on Wednesday, April 5th, 1944, in The Chemical Society's Rooms, Burlington House, London, W.1. The chair was occupied at first by Dr. J. R. Nicholls, Vice-President, and later by Mr. George Taylor, Hon. Treasurer. The following papers were presented and discussed: "The Determination of Residual Carbon Dioxide in Aerating Powder," by C. K. Boundy, A.R.I.C., and R. W. Morris, B.Sc., A.C.G.F.C., F.R.I.C.; "The Volumetric Determination of Tin in Brasses and Bronzes after Separation of Copper as Oxalate," by F. H. Edwards, B.Sc., and J. W. Gailer, B.Sc.; "The Determination of Lead as Molybdate," by H. Holness, M.Sc., A.R.I.C.

NEW MEMBERS

Honorary Member: Professor Alexander Findlay, M.A., D.Sc. (Aberdeen), Ph.D. (Leipzig), President of the Royal Institute of Chemistry.

Ordinary Members: William Miller Ord Allcroft, B.Sc. (Agric.), Ph.D.; Sidney Hartley Bales, M.Sc. (Leeds), F.R.I.C.; Frederick William Bernhardt; Ronald George Henry Baxendale Boddy, B.Sc. (Lond.), Ph.D. (Sheff.); Denis Wallace Bouchier, B.Sc. (Lond.), A.R.I.C.; Professor Henry Vincent Aird Briscoe, A.R.C.S., D.Sc. (Lond.), M.Sc. (Dunelm.), F.R.I.C.; Cecil Burkinshaw; Leslie Hugh Norman Cooper, Ph.D., D.Sc. (Wales), F.R.I.C.; (Mrs.) May Badger Craven, M.Sc.Tech. (Manc.), A.R.I.C.; Eric Eddings, M.Sc. (Lond.), A.R.C.S., D.I.C.; Harold Egan, B.Sc. (Lond.), A.R.I.C.; Arthur Clarence Francis, A.H-W.C., F.R.I.C.*; (Miss) Isabel Hodgson Hadfield, M.Sc. (Lond.); Norman George Heatley, M.A., Ph.D. (Cantab.); (Miss) Evelyn Marion Hickmans, M.Sc., Ph.D.; Frank Woolgar Jarvis, B.Sc. (Sheff.), A.R.I.C.; Frank Bentley Ridgen Marmoy, A.R.I.C.; Walter George Marskell, B.Sc. (Lond.); Frederick Alfred Mason, M.A. (Oxon.), Ph.D. (Munich), F.R.I.C.; Sydney Walter Maxted, B.Sc. (Lond.), A.R.C.S., A.R.I.C.; Roderick Everett Oakes; Leslie Charles Thomas, B.Sc.; Percy Frederick Ronald Venables, B.Sc., Ph.D. (Liv.), F.R.I.C.; Victor Charles Wallis; Edward Arthur Williams, B.Sc. (Birm.), A.R.I.C.

The Determination of Dimethylaniline in Mixtures of Aniline, Methylaniline and Dimethylaniline

BY J. HASLAM, M.Sc., F.R.I.C., AND P. F. HEARN

INTRODUCTION—Following our work on the determination of diethylaniline in ethylaniline[1] we have studied the determination of dimethylaniline in mixtures of aniline, methylaniline and dimethylaniline. Initially we attempted to apply the principle of our method,[1] *viz.*, acetylation of the primary and secondary bases, followed by distillation of the faintly alkaline soln. of the reaction product and collection of the tertiary base in excess of hydrochloric acid. After suitable dilution of the soln. of the tertiary base the colorimetric reaction with nitrous acid was applied and the proportion of dimethylaniline was deduced from corresponding colorimetric tests on dimethylaniline solns. of known concentration.

When this test was applied to known mixtures of dimethylaniline and methylaniline the results obtained were disappointing, *e.g.*,

Dimethylaniline present, % ..	1·005	2·32	4·84
,, found, % ..	0·50, 0·46	1·11, 0·98	4·44, 4·02

At this stage we suspected a certain amount of interaction between the acetic anhydride and the dimethylaniline, and known amounts of a solution of dimethylaniline in acetic

* Through the North of England Section.

anhydride were treated with water, the aqueous solns. were rendered alkaline, and the dimethylaniline was distilled into standard acid soln. The dimethylaniline in the distillate was then determined colorimetrically, but the recovery was low. It was not improved much by variation of the amount of excess alkali present during the distillation, the results obtained being of the following order: present, 0·00102 g; found, 0·00085–0·00093 g.

In view of the fact that the colour reaction of dimethylaniline with nitrous acid is extremely sensitive—a given amount of dimethylaniline producing with nitrous acid about 6 times as much colour as the same amount of diethylaniline—it was hoped that, without any distillation procedure, it might be possible to determine the dimethylaniline directly in the acetylation products of aniline, methylaniline and dimethylaniline mixtures, since the amount of substances which might interfere in the reaction of dimethylaniline with nitrous acid would be very small; thus for amounts of dimethylaniline up to *ca.* 20% in such mixtures, the final colorimetric test would be carried out on the equivalent of only *ca.* 0·01 g of the original mixture.

Accordingly, known mixtures of dimethylaniline and methylaniline were submitted to direct acetylation with a comparatively short reaction time, and the dimethylaniline in the acetylation product was determined by the nitrous acid test. The results obtained, although better than when the dimethylaniline was previously recovered by distillation of its faintly alkaline soln., were still unsatisfactory, *e.g.*,

Dimethylaniline present, % ..	1·005	2·324	4·838
,, found, % ..	0·757	1·809	4·432

There was evidence that the presence of acetic acid (from the acetic anhydride used in the test) in the final test solns. and its absence from the standards were not the cause of the discrepancies. We considered, however, that insufficient attention had been paid to the preliminary acetylation process, in which 1 g of the sample under test was treated with 5 ml of acetic anhydride, added all at once. In expts. with samples of methylaniline it was found that the temp. could rise as high as 45° C., and a study of the literature indicated that side reactions could take place between dimethylaniline and acetic anhydride on heating.

Allen[2] states "We have noticed that a small amount of tetramethyldiamino diphenylmethane is produced by the action of acetic anhydride on dimethylaniline at ordinary temperatures for a long time; this base is produced in greater quantity (10–15%) if dimethylaniline is warmed for a few hours with excess of the reagent."

Again, Reverdin and de la Harpe[3] state that dimethylaniline when mixed with acetic anhydride at ordinary temperature in a closed flask remains unchanged; if, however, a stream of air is led through the mixture then 4,4′-bis-dimethylamino-diphenylmethane may be detected after a short time. Further, on warming dimethylaniline for several hours with acetic acid in excess, some 4,4′-bis-dimethylamino-diphenylmethane is produced.[4]

We decided therefore to try to avoid any side reactions between acetic anhydride and dimethylaniline by carrying out the acetylation at a low temperature for a comparatively short time. It was noted that the colorimetric reaction between dimethylaniline and nitrous acid was almost instantaneous, so that it was possible to reduce the time of final colour development from two hr. (as in the diethylaniline test) to 5 min.

Bearing these principles in mind, a comparatively simple test was devised for the colorimetric determination of 0–20% of dimethylaniline in mixtures of aniline, methylaniline and dimethylaniline, based on the preliminary slow acetylation of 1 g of the mixture at 0° C.; the acetylation product was allowed to attain room temp. and then dissolved, and the soln. was diluted to a known vol. with water. An aliquot portion of the soln. was then treated under definite conditions, with a known amount of sodium nitrite soln., and the yellow colour obtained was measured in a Spekker absorptiometer; a calibration curve was prepared which related indicator drum reading of the Spekker absorptiometer with amount of dimethylaniline. The following results were thus obtained with known mixtures, the composition of these being unknown to the chemist making the test.

(*a*) *Mixtures of aniline and dimethylaniline*

Dimethylaniline present, %	Dimethylaniline found, %
2·71	2·73, 2·73
7·09	7·10, 7·16
11·38	11·31, 11·22
15·36	15·06, 15·03

(*b*) *Mixtures of methylaniline and dimethylaniline*

Dimethylaniline present, %	Dimethylaniline found, %
3·76	3·85, 3·86
7·53	7·37, 7·31
12·11	12·15, 11·89
20·2	20·26, 19·94

(*c*) *Mixtures of aniline, methylaniline and dimethylaniline*

	Composition of mixture, %		Dimethylaniline found, %
(1)	Aniline	47·93	
	Methylaniline ..	47·98	
	Dimethylaniline ..	4·09	4·22, 4·19
(2)	Aniline	46·05	
	Methylaniline ..	47·10	
	Dimethylaniline ..	6·85	6·69, 6·87
(3)	Aniline	44·3	
	Methylaniline ..	43·14	
	Dimethylaniline ..	12·56	12·41, 12·66
(4)	Aniline	41·99	
	Methylaniline ..	42·34	
	Dimethylaniline ..	15·67	15·42, 15·48

METHOD—Transfer by means of a Lunge Rey pipette about 1 g of the sample (accurately weighed) to a weighing bottle (60 mm × 30 mm). Cool the mixture in an ice-bath for 10 min. and add to it, while still in the bath, 2 ml of acetic anhydride, drop by drop, from a burette, stirring the mixture throughout the addition by means of a thin glass rod. The addition of the acetic anhydride should take *ca.* 5 min.

Allow the weighing bottle and contents to stand at room temp. for 30 min., then rinse the mixture into a 1-litre flask and dilute to the mark with water. Withdraw 10 ml of the dil. soln. from the flask by means of a pipette and transfer it to a 100-ml measuring flask containing 10 ml of *N* hydrochloric acid. Dilute to the mark with water and place in a thermostat at 20° C. After 10 min. add 2 ml of *N* sodium nitrite, mix and maintain at 20° C. for 5 min., and measure the absorption of the resulting yellow solution in a Spekker Photo-electric Absorptiometer using the 1-cm cell and the dark blue filters.

Prepare a calibration curve by treatment of solns. of known amounts of dimethylaniline in 100 ml of *N*/10 hydrochloric acid with 2 ml of *N* sodium nitrite, as described above. This graph is quite smooth and passes through the following points:

Dimethylaniline, g	0	0·00025	0·0005	0·00075	0·001	0·00125	0·0015	0·0020
Spekker indicator drum reading	0	0·082	0·164	0·246	0·330	0·409	0·482	0·604

Although the colour development is carried out at 20° C. for a period of 5 min. we have satisfied ourselves that: (1) slight changes in temperature have very little effect on the amount of colour produced; (2) the colour development takes place almost instantaneously, and, after full development, the colour fades very slowly indeed.

We have applied the principle of the test, with appropriate dilution of the final soln. of the acetylation product, to the determination of comparatively large amounts of dimethylaniline in dimethylaniline-aniline mixtures, with the following results:

Dimethylaniline present %, 70·25 Found %, 67·43; 68·23

The test was also applied to the same mixture admixed with 4 times its weight of pure aniline, with the following results:

Dimethylaniline present %, 70·25 Found %, 70·15; 69·8; 68·08; 68·91

There are obvious limitations to the colorimetric determination of such large proportions of dimethylaniline, and, in practice, we do not use this test for amounts greater than 20%. At the lower end of the scale, however, the test may readily be extended to the determination of as little as 0·05%, by using 90 ml of the soln. of the acetylation product instead of the usual 10 ml; still further extension may be achieved by the use of the modified Spekker cells now being sold by Tintometer Limited. Attention should be drawn to the fact that dimethylaniline solns. render pipettes very greasy and for that reason frequent cleaning with a mixture of chromic and sulphuric acids is necessary.

BLUMRICH AND BANDEL METHOD—Large proportions (*ca.* 60%) of dimethylaniline may be satisfactorily determined by means of Blumrich and Bandel's method,[5] in which there is a preliminary acetylation of the secondary base followed by titration of the tertiary base with perchloric acid in glacial acetic acid either potentiometrically or with the use of cresol red as indicator. We have found that cresol red is unsatisfactory but that α-naphthol benzein gives excellent results.

We have applied this method to the determination of large proportions of dimethylaniline in dimethylaniline, methylaniline and aniline mixtures as follows:

Solutions required: (1) *α-Naphthol benzein indicator*—0·2% w/v soln. of α-naphthol benzein in glacial acetic acid. The crude material prepared by interaction of benzotrichloride and α-naphthol, followed by extraction with alkali and subsequent acidification (Doebner[6]), is very satisfactory.

(2) *N/10 Perchloric acid soln.*—Determine the strength of perchloric acid (60% w/v A.R.) by weighing out *ca.* 4 g into a conical flask, adding about 20 ml of water and titrating with sodium hydroxide, using 0·5 ml of phenolphthalein soln. (0·5% in 50% w/v alcohol) as indicator. Calculate the proportions of perchloric acid and water from this test. Next weigh out such an amount of the perchloric acid soln. as will contain 20·10 g of $HClO_4$ into a beaker and transfer it to a separating funnel (250 ml) using 50 ml of glacial acetic acid (A.R. free from easily oxidisable matter). Meanwhile weigh out redistilled acetic anhydride (AnalaR) equiv. to the water present with the perchloric acid into a conical flask, and cool it in an ice-bath. Then add the acetic acid perchloric acid soln. from the separating funnel to the acetic anhydride drop by drop, and rinse out the separating funnel with 20 ml of glacial acetic acid (A.R.). Transfer the mixture to a 2-litre flask, dilute to the mark with glacial acetic acid (A.R.) and maintain in a thermostat at 20° C.

(3) *N/10 Sodium acetate*—Weigh 1·325 g of sodium carbonate (A.R. previously heated to *ca.* 300° C. for 5 hr.) into a 250-ml beaker, cover with a clock glass, and add 50 ml of glacial acetic acid (A.R.), taking precautions against loss by spurting. Transfer the solution to a measuring flask (250 ml), dilute to the mark with glacial acetic acid (A.R.) and maintain at 20° C. in a thermostat.

Standardisation of the perchloric acid and acetic acid solution—Transfer 50 ml of the sodium acetate soln. (withdrawn by suction with the aid of a pump) to a stoppered conical flask, add 5 drops of α-naphthol benzein indicator, and titrate the soln. with the perchloric acid and glacial acetic acid soln. until the indicator changes from orange-brown to green. The end-point change is very sharp.

When they are not in use, store the *N*/10 sodium acetate and the perchloric acid and acetic acid soln. at 20° C. in a thermostat, since it is desirable to work as near as possible to 20° C. in view of the high expansion of glacial acetic acid with rise of temperature. Evidence of this may be obtained from the following density figures for glacial acetic acid; the figures for water are given for comparison.

	Glacial acetic acid	Water
Density at 20° C.	1·0497	0·99823
Density at 15° C.	1·0553	0·99913

Volumetric determination of dimethylaniline in mixtures of aniline, methylaniline and dimethylaniline—Acetylate 1 g of the mixture as described on p. 143. After 30 min. transfer the mixture to a conical flask with 50–80 ml of glacial acetic acid (A.R.), add 5 drops of α-naphthol benzein indicator and titrate with the standardised soln. of perchloric acid and acetic acid described above. 1 ml of *N*/10 perchloric acid ≡ 0·01211 g of dimethylaniline.

The following results were thus obtained.

	Composition of mixture, %			Dimethylaniline found, %
	Aniline	Methylaniline	Dimethylaniline	
(*a*)	64·79	5·09	30·12	30·06
(*b*)	53·79	5·00	41·21	41·17
(*c*)	44·22	4·97	50·81	50·56
(*d*)	20·13	20·51	59·36	59·28, 59·52, 59·44
(*e*)	59·93	29·93	10·14	10·1, 10·16, 10·3,
(*f*)	46·79	6·35	46·86	46·56, 46·61

We are indebted to Mr. N. Strafford of I.C.I. (Dyestuffs), Ltd., for details of the preparation and standardisation of the perchloric acid and acetic acid soln. and to Mr. A. H. S. Guthrie for assistance in the early part of the experimental work.

SUMMARY—A method has been worked out for the rapid colorimetric determination of dimethylaniline (up to 20%) in mixtures of aniline, methylaniline and dimethylaniline. The method is based on preliminary acetylation of the aniline and methylaniline, followed by solution of the acetylation product in water and application of the colorimetric reaction with nitrous acid to the determination of the dimethylaniline. The absorption of the solution of the yellow nitroso body produced is measured in a Spekker absorptiometer.

For the determination of larger quantities, *i.e.*, of the order of 60% of dimethylaniline in mixtures of aniline, methylaniline and dimethylaniline, use has been made of the method recently put forward by Blumrich and Bandel[5] for the titration of weak organic bases, *viz.*, titration with perchloric acid in glacial acetic acid solution. The mixture of aniline, methylaniline and dimethylaniline is first treated with acetic anhydride to acetylate the primary and secondary bases, then the acetylation product is dissolved in glacial acetic acid, and the dimethylaniline is titrated with perchloric acid in glacial acetic acid solution, α-naphthol benzein being used as indicator.

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RESEARCH DEPARTMENT
IMPERIAL CHEMICAL INDUSTRIES LTD., NORTHWICH

February, 1944

Determination of Lead as Molybdate

BY H. HOLNESS, M.Sc., A.R.I.C.

WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME

(*Read at the Meeting, April* 5, 1944)

A SURVEY of the application of Fajan's principle of adsorption indicators to the determination of lead reveals a lack of information on the *p*H value at which hydrolysis of solns. of lead nitrate begins, and nowhere are precise limits stated within which the titration must be carried out. According to Britton,[1] hydrolysis of a soln. of $Pb(NO_3)_2$ begins at approx. *p*H = 6, but no exact figure is given. A determination of this value, using the glass-calomel system, gave a figure of 5·5; hence, to avoid the formation of basic salts in lead titrations the *p*H at the end-point must fall below this figure.

The usual titrant for the determination of lead as molybdate is a soln. of ammonium paramolybdate, which when dissolved at *M*/70 strength has a *p*H of 3–4. When this soln. is titrated with an *M*/10 soln. of lead nitrate (*p*H = 4·7) the *p*H value falls to below 1·5 at the end-point, owing to formation of free nitric acid:

$$(NH_4)_6Mo_7O_{24} + 4H_2O + 7Pb(NO_3)_2 = 6NH_4NO_3 + 7PbMoO_4 + 8HNO_3.$$

While this acidity does not alter appreciably the solubility of lead molybdate,[2] it is sufficient to prevent the use of adsorption indicators. The absence of an adsorption change in this instance was noted, but not explained, by Candea and Murgulescu[3] who, using eosin as indicator, failed to observe an end-point when lead nitrate was titrated with ammonium molybdate, but obtained one when lead acetate was used. Furthermore, they found that a soln. of sodium molybdate neutralised to phenolphthalein gave a satisfactory end-point with both the nitrate and acetate.

The detrimental effect of more than small quantities of foreign ions on the determination of the end-point was shown by Raichenstein and Koroboff[4] who, using alizarin as the indicator, studied the effect of varying quantities of ammonium salts on the end-point in the titration of both lead nitrate and acetate with ammonium molybdate.

The object of the present work was to apply the principle of Fajan's method to the determination of lead in specimens of the metal and in solders. To avoid introducing unnecessary foreign ions into the titration treat the sample of metal with nitric acid, evaporate until acid fumes ceased to be evolved, take up with water and titrate with a standard soln. of magnesium molybdate. Since magnesium forms a soluble, normal molybdate[5] and is a

weaker base than sodium, no initial adjustment of the *p*H of the soln. is necessary (*p*H of *M*/10 $MgMoO_4$ = 8 approx.).

The dye Solochrome Red B (sodium salt of the *o*-carboxylic acid of benzene azo-β-naphthol-3 : 6 disulphonic acid[6]) was chosen as the indicator, since it forms a somewhat insol. compound with lead having a different colour from that of the dye. Moreover, its colour was unaffected by changing *p*H and was found to be adsorbed on the pptd. lead molybdate in presence of a slight excess of $Pb^{\cdot\cdot}$ and desorbed when $MoO_4^{\cdot}$ predominated. In hot solution the end-point was accentuated by coagulation of the ppt.

When the method was applied to the determination of lead in presence of tin it was found necessary, in order to free the tin ppt. from lead, to add an excess (1–2 ml) of the molybdate soln., boil and titrate back with a standard lead nitrate soln. When more than 70% of tin was present, it was found preferable, before titrating back, to make the soln. up to a known vol. and use an aliquot part, as the dye was permanently adsorbed on the tin ppt. and tended to confuse the end-point. In presence of antimony erratic results were obtained.

Experimental—An approx. 0·1 *M* soln. of magnesium molybdate was prepared by suspending in water in a beaker placed on a hot plate a weighed quantity of AnalaR molybdenum trioxide and adding small quantities of magnesium carbonate (heavy) until no further evolution of carbon dioxide occurred. The soln. was boiled, filtered, made up to the required volume and standardised gravimetrically (weighing as lead molybdate).

Weighed pieces of A.R. lead foil were dissolved in dil. nitric acid, and the soln. was evaporated on a low temp. hot plate (150° C.) until fumes of nitric acid ceased to be evolved. The resulting lead nitrate was dissolved in water, 1–2 ml of 0·01 *M* nitric acid were added and the soln. was titrated with the standard magnesium molybdate soln. in presence of 4–5 drops of a 0·2% aqueous soln. of Solochrome Red B until the colour changed from bluish-pink to orange-red. The end-point was improved by titrating in hot soln. and viewing either the change in the supernatant liquid from colourless to orange-red or the change on the ppt. from a bluish-pink to white. The results (Table I) show the end-point to be stoichiometric within the *p*H range 5·5–2; with a greater acidity the end-point, when obtained, was false and difficult to see.

Table I

No.	Lead taken g.	Lead found g	*p*H at end-point	Error %
1	0·9128	0·9128	2·22	0·00
2	0·8478	0·8475	2·80	−0·04
3	0·6963	0·6962	2·30	−0·01
4	0·6279	0·6267	3·00	−0·19
5	0·5227	0·5235	2·35	+0·15
6	0·4066	0·4063	3·08	−0·07
7	0·2558	0·2559	3·60	+0·04
8	0·1028	0·1026	3·85	−0·20

Lead in Presence of Tin—To explore the applicability of the method to the determination of lead in presence of tin, weighed quantities of AnalaR lead foil and AnalaR granulated tin were together treated with conc. nitric acid, the soln. was evaporated as before until nitric acid fumes ceased to be evolved, and the residue was boiled with water. Standard magnesium molybdate soln. was run in from the burette in slight excess of that required to ppt. the lead. The whole was boiled for 5–10 min., and the surplus molybdate was titrated with a standard soln. of lead nitrate in presence of 6 to 8 drops of the indicator, the end-point being determined by the adsorption of the dye on the lead molybdate. It was found that the tin ppt. strongly adsorbed the dye the whole time, thus necessitating the use of rather more of the indicator. Owing to this fact it was found advisable in presence of over 70% of tin to make up the soln., after boiling with excess molybdate, to a known volume, and to titrate an aliquot part with 0·05 *M* lead nitrate previously standardised against the molybdate soln.

Table II

No.	Lead taken g	Tin taken g	Lead found g	Error %
1	0·2861	0·7186	0·2870	+0·31
2	0·4692	0·4893	0·4714	+0·42
3	0·7653	0·2461	0·7647	−0·09
4	0·8833	0·0975	0·8820	−0·15
5	0·9046	0·1008	0·9040	−0·07

Attempts to determine lead in presence of both tin and antimony gave erratic results. It was noticed that, unlike the tin ppt., the antimony ppt. at no time adsorbed the dye. Also it was not found possible to expel the last traces of nitric acid by evaporation; when this residuum was neutralised by boiling with water containing a little solid magnesium carbonate the results obtained for lead in Standard White Metal "A" were consistently 1% low.

SUMMARY—Solutions of lead as nitrate can be determined by direct titration with solutions of magnesium molybdate using a 0·2% aqueous soln. of Solochrome Red B as an adsorption indicator. The end-point is sharp, reversible, unaffected by temp. and sensitive in a dilution of 0·005 *M*. The reaction is stoichiometric, provided that the acidity falls within a *p*H range of 2–5·5 and that few foreign ions are present. The method can be applied to the determination of lead in lead-tin alloys.

I wish to thank the Governors, the Principal and the Head of Science Department of the S.W. Essex Technical College for permission to carry out this work.

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THE SCIENCE DEPARTMENT
S.W. ESSEX TECHNICAL COLLEGE, WALTHAMSTOW, E.17

January, 1944

Trace Detection by Means of Spot Reaction: I.—Detection of Traces of Lead in Water and Fine Chemicals

BY F. FEIGL, DR.INGR., AND N. BRAILE

EVERY test involving a chemical reaction has a so-called "limit of identification." By this we mean the minimum absolute quantity (expressed in μg) that can be identified with certainty under the test conditions. When solutions are used for the test and the limit of identification and volume of the soln. are known, it is easy to calculate the degree of dilution. This is the "concentration limit." The values for limit of identification and concentration limit furnish exact data for the sensitivity of a test, and thereby provide important points of orientation for special applications, particularly in the field of microchemistry.[1]

The fundamental problem in qualitative micro analysis is the identification of small amounts of different substances. This may be irrespective of dilution, as in the identification of certain compounds after separation from other compounds, the identification of particles of solids, etc. Or the problem, often of much importance, may consist in the detection of small amounts in high dilution,—so-called "traces"; this has been termed by Emich[2] "trace detection."

For the solution of the latter problem it is not sufficient for a test to have a low limit of identification; it is essential that it should have a low concentration limit. The classical qualitative micro-analytical tests, especially crystalloscopic tests, usually have low values for the limit of identification but not for the concentration limit. Hence, as a rule, they are of no use for trace detection. Spot-test analysis is mainly concerned with tests that show low values for the limit of identification and for the concentration limit. Therefore spot-test methods may sometimes be used for trace detection.

For the detection of trace substances for which there are no tests with a sufficiently low concentration limit it is generally necessary to concentrate the soln. to an extent that will enable suitable tests to be applied. It is obvious that this procedure is tedious, is applicable only to non-volatile compounds, and requires a considerable amount of material; it may also involve some risk of contamination with other substances. In such circumstances it is sometimes possible to detect traces simply but with certainty by the use of a so-called "collector," *i.e.*, a substance capable of retaining a minimal quantity of the dissolved compound.

This fixation may be effected by adsorption or by co-precipitation of the traces not precipitable by themselves. For either purpose it would be necessary to add to the soln. an adsorbent or a precipitable compound capable of acting as a collector. After separation of the collector the traces adsorbed or co-pptd. can be identified by approved tests. Collectors may be successfully applied in spot-test analysis. A good example is the identification of traces of lead in water or in certain fine chemicals.

DETECTION OF LEAD—For the sensitive and selective detection of lead in soluble and insoluble substances Feigl *et al.*[3] recommend the formation of the insol. red rhodizonate, $2Pb(C_6O_6).Pb(OH)_2.H_2O$. The test is capable of detecting 0·1μg of lead in 1 drop of the soln., corresponding with a concentration limit of 1 in 500,000. It is therefore impossible to detect lead in 1 drop of a more dilute soln. The importance of identifying traces of lead in drinking water is widely recognised, and there is therefore a rich literature on its detection and determination in minute quantities.[4]

For the detection of lead in dilutions up to 1 in 2,000,000 Feigl and Suter[5] have devised the following procedure. A little mercuric chloride is added to 10 ml of the test soln. and hydrogen sulphide is then introduced; this ppts. mercuric sulphide together with traces of lead sulphide. The use of mercuric sulphide as a collector for minute quantities of lead was first recommended by Scott.[5] The ppt. is collected on filter-paper and calcined to convert the lead sulphide into sulphate. The residue can then be tested for lead with sodium rhodizonate.

Frequent determinations by this procedure revealed certain sources of error which, although individually negligible, are collectively of considerable importance when only traces are present. Thus, *e.g.*, loss of lead occurs during the calcination of the filter-paper, probably owing to the reducing action of the charred paper and volatilisation of the metallic lead. Also, small amounts of lead sulphate cannot be ignited without some loss.

MODIFIED METHOD—The ppt. of mercuric sulphide is formed by adding sodium mercuric sulphide, $Na_2(HgS_2)$, and ammonium chloride to the test liquid—

$$Na_2(HgS_2) + 2NH_4Cl = 2NaCl + HgS + (NH_4)_2S$$

The ppt., which acts as collector for minute quantities of lead sulphide, is filtered off on a porcelain Gooch crucible, moistened with hydrogen peroxide, dried and gently ignited. Lead sulphate may be identified by the formation of red rhodizonate of lead on spotting with sodium rhodizonate and buffer soln.

By working under the conditions described below lead may be detected in 10 ml of water at a dilution of 1 in 10 millions, or in 100 ml at a dilution of 1 in 100 millions. The test takes about 20 min.

Reagents—$Na_2(HgS_2)$ *solution*: Add 13·6 g of mercuric chloride to 60 ml of hot water, next solid sodium sulphide and then sodium sulphide soln. until the mercuric sulphide first formed has completely dissolved; make up the soln. to 100 ml.

Ammonium chloride of max. purity.

Sodium rhodizonate soln.: Dissolve 2 mg of the salt in 1 ml of water. Stored in a refrigerator the soln. is stable for 2 days.

Buffer soln.: pH = 2·79. Dissolve 1·5 g of tartaric acid and 1·9 g of sodium bitartrate in 100 ml of water.

Procedure for water—To 10 ml of the water to be tested add 0·25 ml of the sodium mercuric sulphide soln.; owing to hydrolysis partial pptn. of mercuric sulphide immediately occurs. To complete the pptn., add 0·6–1 g of pure solid ammonium chloride and heat for a short time. Collect the flocculated ppt. on a Gooch crucible and wash once with water. Then add 5 ml of hydrogen peroxide and, after 3 min., draw it off by suction. Place the crucible containing the wet ppt. on a porcelain plate and heat on an electric heating plate until the black mercuric sulphide becomes red; then ignite it gently over a burner for 1 min. After it has cooled, moisten the bottom of the crucible with 5 drops of the sodium rhodizonate soln., draw it off by suction, and decolorise the excess of the yellow reagent by spotting with 2–3 drops of the buffer soln. and then with 3 drops of water. If the water contained lead, red particles of lead rhodizonate will remain, plainly visible on the white bottom of the crucible.

The quantity of lead rhodizonate formed, and therefore the intensity of the colour, depends upon the quantity of lead in the water. Hence it is possible to compare the red residue with the residues from standard solns. of a lead salt which have been treated in the same way. By the use of standard solns. with dilutions 1 in $0{\cdot}5 \times 10^6$, 1 in 10^6, 1 in 2×10^6....

1 in 10×10^6, it is possible to estimate the lead colorimetrically, with an accuracy sufficient for many purposes.

Lead in fine chemicals—The method can also be used for detecting traces of lead in certain fine chemicals. From 1 to 5 g of the sample to be tested is dissolved in 10 ml of water and the procedure described above is followed. Numerous alkali salts of U.S.A. origin were tested in this way. The contents of packets labelled "Heavy metals as Pb . . . 0·0003%" and ". . . 0·0005%," respectively, gave results agreeing within 50 to 10% of the values declared. When identical quantities of the same kind of salt are thus tested it is often possible to recognise their origin by their lead content. It is noteworthy that alkali sulphates generally have a higher lead content than other alkali salts. As a rule the lead content of acetates is less than 10% of the total heavy metals declared. This also applies to Epsom salts (puriss). A direct test for lead may be applied to sulphuric acid by concentrating the sample by evaporation and treating the residue with sodium rhodizonate soln. and buffer soln.

References

1. Feigl, F., *Mikrochem.*, 1923, **1**, 4; *cf.* "*Specific and Special Reactions for Use in Qualitative Analysis*," transl. by R. E. Casper, New York, 1940, p.5.
2. Emich, F., "*Lehrbuch der Mikrochemie*," 2nd Ed., Munich, 1926, p. 5; *cf.* his "*Microchemical Laboratory Manual*," transl. by F. Schneider, New York, 1932, p. 3.
3. Feigl, F., and Suter, H. A., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 840; Feigl, F., and Braile, N., *The Chemist Analyst*, 1943, **32**, 52.
4. "*Manual of Water Quality and Treatment*," Amer. Water Works Assoc., New York, 1940, p. 38.
5. Scott, W. W., "*Standard Methods of Chemical Analysis*," 5th Ed., New York, 1939, p. 514.

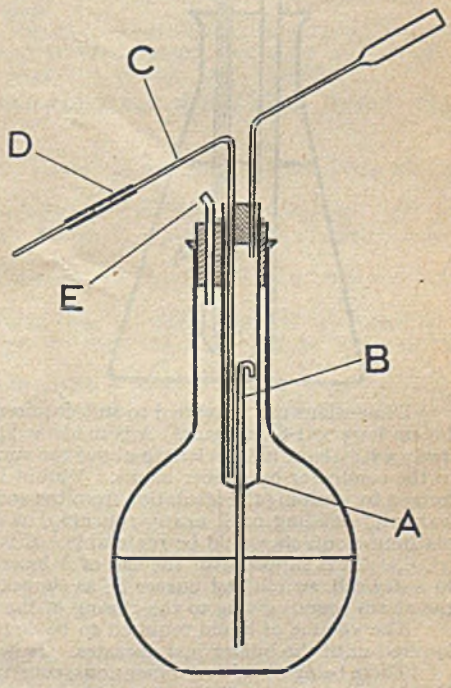
MINISTERIO DA AGRICULTURA
LABORATORIO DA PRODUCAO MINERAL, RIO DE JANEIRO

February, 1944

Notes

A WASH-BOTTLE FOR DELIVERING PRE-DETERMINED VOLUMES OF LIQUID

THE ordinary type of wash-bottle is unsuitable for washing ppts., etc. with small portions of boiling reagent. Recently we made a series of quantitative expts. on the leaching out of soluble matter from the ash of substances of vegetable origin, with the intention of developing a standard method on the semi-micro scale. For this we required a device by which small, pre-determined volumes of wash-liquid, generally at b.p., could be repeatedly delivered to the substance being examined, and the following modification of the ordinary wash-bottle was evolved.



The device comprises an inner tube A with a sealed-in siphon tube B, and carrying a stopper with a wash-bottle fitting. The delivery tube C should be made from fine tube of about 1·5 mm bore; D is a short length of cycle valve rubber. The whole unit is fitted into a flask, the stopper of which has a vent tube E.

The dimensions of the inner tube A and the height of the sealed-in siphon tube are fixed so as to deliver the largest wash-portion likely to be required. Any desired portion up to this volume can then be delivered by merely raising or lowering the tube C in the stopper. The wash-bottle is heated on a hot plate until the contents are gently boiling. The vent tube E is then closed with the forefinger and the liquid is thus caused to flow into A until the level is somewhat above the upper end of the siphon tube. After removal of the wash-bottle from the hot plate the forefinger is withdrawn, and the excess of liquid then returns through the siphon to the flask. Pressure is then applied at the mouthpiece and the contents of the inner tube A are blown from the jet, E being closed meanwhile by the forefinger. The wash-bottle is again placed on the hot-plate and the above procedure is repeated.

When using wash liquids at temps. other than b.p., A can be filled by applying gentle pressure at the vent tube E. Automatic adjustment of level and subsequent delivery are then effected as described above.

The following results were obtained with the device as applied to a wash-bottle of 300 ml capacity, the bore of A being about 13 mm and the max. delivery a little over 5 ml per sequence of operation. Distilled water was used. The only special precaution taken was to place the wash-bottle on a level

surface, so as to avoid errors due to variable tilt. When the wash-bottle is held in the hand the reproducibility is about 0·05 ml.

ml delivered at room temperature:						
Mean of 4 tests	5·00	4·01	2·99	2·01	0·98	0·50
Max. deviation from mean ..	0·02	0·02	0·01	0·01	0·01	0·01
ml delivered boiling and measured 4 sec. after delivery:						
Mean of 4 tests	4·99	4·00	3·01	1·99	0·99	0·51
Max. deviation from mean ..	0·02	0·01	0·01	0·01	0·02	0·02

As a refinement, graduation lines may be etched on the inner tube A to assist in fixing the position of the tube C for delivering various volumes.

A suggested further use for this device is as an automatic pipette for delivering very small volumes.

We wish to thank Mr. J. Dimmick, B.Sc., A.R.I.C., Acting Principal, for his interest and encouragement.

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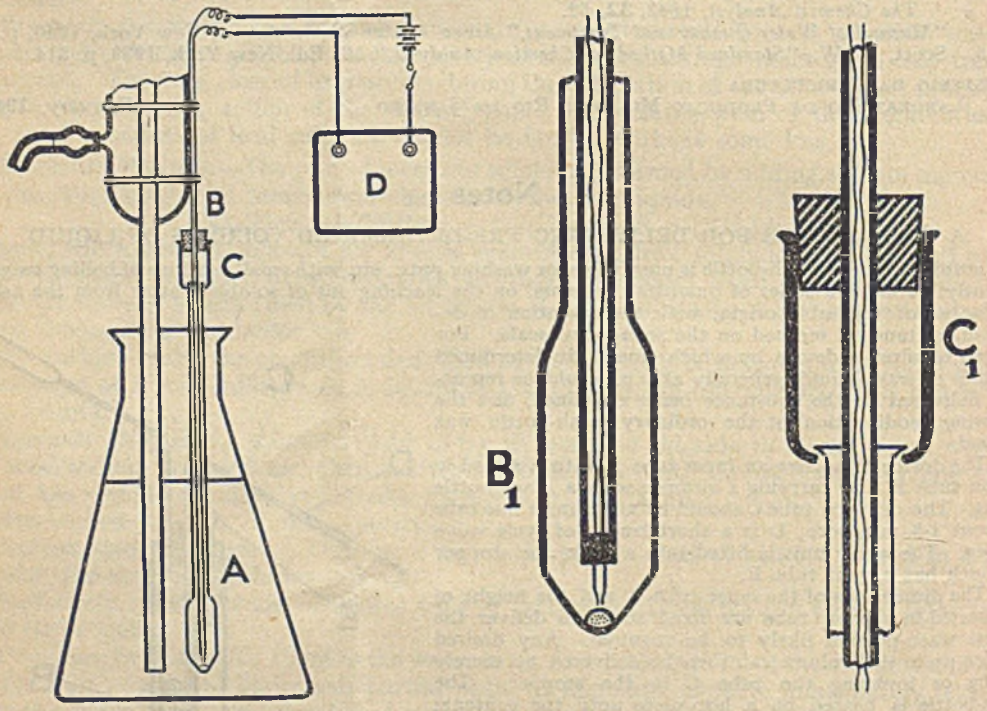
AN ELECTRICAL INDICATOR FOR COLLECTING A CONSTANT VOLUME OF DISTILLATE

Routine distillations involving appreciable quantities of liquid, such as Kjeldahl nitrogen estimations, determination of sulphur dioxide, etc., are frequently left unattended, with the risk of over-distilling. The usual remedy is distilling for a standard time, as indicated by a clockwork alarm. This is sufficient when the volume of distillate does not require very close control. Recently we made a series of distillation determinations having a large blank correction. Hence a closer control of the vol. of distillate was required. To avoid continuous attention, the indicator described below was devised.



Dimensions can be varied to suit requirements. As used, float A was 12 cm overall with bulb approx. 2·5 cm long × 1·3 cm diam. A is made as light as possible and contains a bead of mercury, so as to float freely with about half its length above the surface. Guide B slips easily into the stem of A. It is attached to the condenser by rubber bands. Within B pass the 26 S.W.G. copper leads to the contacts, which are formed by scraping the insulation from the ends of the leads, filing to chisel-edges, cementing in with sealing-wax, and bending until nearly touching, as shown enlarged at B_1. For use over long periods, sealed-in platinum contacts would be preferable. C is a piece of 1-cm bore tubing closed slightly at the lower end (*cf.* C_1). It is slipped over the end of A before the flange of the latter is formed. The leads are connected to a dry cell, switch and buzzer D, as shown. As soon as sufficient liquid has distilled to cause A to rise, the alarm sounds owing to the closing of the circuit by the mercury bead.

The volume of liquid required to be distilled is first placed in the receiver and the indicator is slowly lowered until the buzzer just operates. It is then secured by the rubber bands, as shown.

There being no critical dimensions, construction is easy. Only a very small bead of mercury is required. No alteration to the distillation apparatus is necessary, the float merely being supported to dip into the receiver to the required depth. The rod-like shape facilitates rinsing on removal. The indicator was consistently found to operate when a depth of distillate had collected differing by not more than a few mm from the predetermined amount. Thus practically a constant vol. of distillate could be collected.

Other devices may be substituted for, or added to, the alarm. Thus, if electrical heating is used, the hot-plate may be switched off by use of a thermal or other relay.

Owing to the sensitivity of the float-system it seems from preliminary experiments that it could be used, on the hydrometer principle, to follow increases in density during distillations, such as solvent-recovery.

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

August, 1943

Ministry of Food

STATUTORY RULES AND ORDERS*

1943—**No. 1766. The Cheese (Control and Maximum Prices) Order, 1943. Dated December 29, 1943.** Price 1d.

This Order revokes and replaces the Cheese (Licensing and Control) Order, 1940, the Cheese (Maximum Prices) Order, 1941, the Soft Cheese and Curd Cheese (Maximum Retail Prices) Order, 1941, and the Processed Cheese (Control and Maximum Prices) Order, 1942.

"Processed Cheese" means cheese which after manufacture has been heated and to which an emulsifying agent has been added.

"Soft Cheese" or "Curd Cheese" means cheese the moisture content of which exceeds 55%† and includes the product commonly known as "curds."

"Specified cheese" means any cheese specified in Part I of Schedule I (*viz., Wensleydale; Processed Cheese and any variety of cheese other than Wensleydale*), but does not include any blue vein, soft or curd cheese or cheese made from milk other than cows' milk.

— **No. 1768. The Animal Oils and Fats (Control and Maximum Prices) Order, 1943. Dated December 29, 1943.** Price 2d.

This Order consolidates the Animal Oils and Fats (Saponification and Splitting) Order, 1941, the Animal Oils and Fats (Provisional Control) Order, 1939, the Technical Tallows and Greases (Home Melt) (Maximum Prices) Order, 1941, and the Dripping (Maximum Prices) Order, 1943.

The quantity of free fatty acids which dripping may contain has been reduced from 2% to 1·5%.

1944—**No. 177. The Soap (Maximum Retail Prices and Conditions of Sale) Order, 1944. Dated February 22, 1944.** Price 1d.

This Order revokes and replaces the Soap (Maximum Retail Prices) Order, 1942, and its amendments.

"Abrasive soap" means a soap product in any form prepared for polishing and scouring purposes, containing not more than 6% of anhydrous soap.

"Hard soap" means soap (other than abrasive soap, dental soap (dentifrice), liquid soap, shampoo powder, shaving soap, soap flakes or chips, soap powder, powder containing 6% or less of anhydrous soap and soft soap) containing less than 70% of fatty and resin acids.

— **No. 209. Order dated February 29, 1944, amending the Soft Drinks Order, 1943.** Price 1d.

The definitions are clarified:—

(*a*) by inserting the word "solid" after the word "liquid" in par. (i) of the definition of "soft drink" in Article 1;

(*b*) by inserting the words "whether sweetened or unsweetened" (*a*) after the words "any concentrated drink" in par. (i) of the definition of "soft drink" in Article 1 and (*b*) after the words "any unconcentrated drink" in par. (ii);

(*c*) by adding the words "without any addition thereto" at the end of the definition of "Still spa water" in Article 1;

(*d*) by substituting for the words "the unadulterated juice of fresh apples," in the definition of "Unfermented apple juice" in Article 1, the words "the juice of fresh apples unadulterated and of natural strength."

The saccharin content of standard concentrated soft drinks is reduced from 1 oz. to 7/8 oz. per 10 gallons with a proviso that stocks of such drinks with the former saccharin content may be sold by the manufacturer up to April 9, 1944, and by other persons up to June 4, 1944.

— **No. 275. The Food Standards (Mustard) (No. 2) Order, 1944. Dated March 14, 1944.** Price 1d.

This Order, which should be read with the Food Standards (General Provisions) Order, 1944, replaces the Food Standards (Mustard) Order, 1944. It prescribes a single standard for mustard, compound mustard and mustard condiment, but makes it clear that the standard does not apply to: (*a*) any article not in powder form; (*b*) any article sold under the description "pickling mustard" and consisting wholly or in part of white mustard flour or brown mustard flour. As before, the standard does not apply to brown mustard flour sold under the description "brown mustard."

"Brown mustard flour" means the product obtained by grinding whole seeds (with or without their husks) of *Brassica nigra* (Linn.) Koch or *B. juncea* (Linn.) Czernj. and Cosson or a mixture of such varieties of seeds.

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

† In the previous Order it was 50%.—EDITOR.

"White mustard flour" means the product obtained by grinding whole seeds (with or without husks) of *Sinapis alba* Linn.

STANDARD—Mustard, compound mustard or mustard condiment shall be of such composition as to yield not less than 0·35% of allyl *iso*thiocyanate after maceration with water for 2 hours at 37° C. and shall consist of a blend of brown and white mustard flours with or without amylaceous flours and/or spices: provided that the proportions of amylaceous flours and spices (if any) shall not together exceed 20% by weight.

Notes from the Reports of Public Analysts

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

COUNTY OF KENT: QUARTERLY REPORTS OF THE COUNTY ANALYST, 1943

COARSE COCOA—A sample was reported as inferior owing to its very coarse condition. It is suggested that a standard of fineness for cocoa should be fixed.

DRINKING CHOCOLATE—A sample contained 14·0% of calcium carbonate. Information was laid against the vendor.

STRONG ACETIC ACID AS "VINEGAR"—A sample sold as "white vinegar" contained 21·3% of acetic acid, approximating to the strength of the B.P. acid. In my opinion no vinegar sold for consumption should contain more than 7% of acetic acid. The seller was prosecuted and fined £3 3s. and £2 2s. costs.

EXCESS OF SULPHUR DIOXIDE IN GELATIN—Two samples, containing more than 4000 p.p.m. of sulphur dioxide, were condemned as adulterated.

GLAUBER'S SALT—A sample was found to consist of dried sodium sulphate instead of the crystallised salt, so that a double dose would be taken by the consumer. Action was taken by the local authority.

SUD CAKE—Large quantities of this are made in Yorkshire from sewage sludge containing waste fibre from mills. The sludge has the grease pressed out and is generally further degreased with benzole, but may still contain 12% or more of oil. The water ranges from 10 to 20% and the ammonia from *ca.* 2 to 4·8% according to the amount of wool present. Normally there is only a trace of phosphate, whilst the potash may be about 0·3%. Mineral matter ranges from 25 to 60%. Sud cake has been sold in Kent on a 3·5% basis, but consignments have contained only 2 to 2·8%; farmers are paying about 28s. per unit of ammonia. As the demand for shoddy exceeds the supply, sud cake, despite its low manurial value and high price, finds a fairly ready market.

SOOT—Most of that offered is of good quality, but sometimes it contains a large proportion of small black ash. One sample contained only 0·2% of ammonia.

SEAWEED AS CATTLE FOOD—The sample was a dark brown powder with an odour of seaweed. It contained only 2·0% of oil, less than 5% of albuminoids, 55% of carbohydrates, and 4·4 % of salt, so that its food value was not high. In other species of seaweed the albuminoid content may reach *ca.* 30%.

FLAX STRAW—Broken flax straw contained *ca.* 0·6% of nitrogen, rather less phosphoric acid, and *ca.* 0·5% of potash. As the straw consists largely of tough fibrous matter it would be advisable to mix it with farmyard manure or to use it as an absorbent of liquid manure.

RESIDUE WASTE—This consisted of plaster, gravel and a little soot and had practically no manurial value. An "organic manure," sold at £13 per ton, contained *ca.* 90% of mineral matter (80% similar to sand), ammonia 0·2%, phosphoric acid, 0·4%, potash 0·4%, and organic matter 4 to 20%. It was practically worthless as manure.

POISONING OF DOGS WITH STRYCHNINE—In one part of the county dogs have been poisoned with strychnine, and in another area a piece of meat containing strychnine was picked up. Poisoning by strychnine had almost disappeared until war measures made it possible for certain people to obtain supplies.

F. W. F. ARNAUD

CITY OF SALFORD: ANNUAL REPORT OF THE CITY ANALYST FOR 1943

MUSTARD—Two samples contained only 0·07% of volatile oil, indicating that they consisted entirely of white mustard. The suppliers of both products were cautioned. The Food Standards (Mustard) Order, 1944, has since fixed a min. standard of 0·35% of allyl *iso*thiocyanate for mustard.

CAMPHOR AND MUSTARD OIL—The sample consisted of 5·7% of camphor dissolved in a fixed mustard oil base. The label stated "Camphor and Mustard Oil," "not to be taken," but gave no quantitative indication of the composition of the mixture. While the words on the label do not specifically "recommend the preparation as a medicine" (Sec. 11 of the Pharmacy and Medicines Act, 1941), there appears to be an indirect implication that it is intended for rubbing. The advantages of Sec. 11 of the Act with regard to the declaration of formulae will be completely nullified in respect of this and other preparations bearing similar labels and obviously intended for medicinal use unless the wording of the section is interpreted in its broadest sense as referring to all medicinal preparations.

GROUND ALMOND SUBSTITUTE—The sample contained soya flour, ground nut and partly gelatinised wheat in approx. equal proportions. Its proximate analysis gave: protein, 28·4; fat, 14·4; moisture, 9·8; mineral matter, 3·6; carbohydrates (including starch), 43·8%. The sample was sold loose and thus contravened Sec. 3 of the Food Substitutes (Control) Order, 1941, which prohibits the sale of a food substitute except in the container and description under which it was sold by the manufacturer of that food substitute. The product was withdrawn from sale.

GRAVY BROWNING OR COLOURING—The sample consisted of: brown coal-tar dye, 2·4; salt and other mineral matter, 3·0; water, etc., 94·6%. It gave a satisfactory colour to gravy, but if used to colour meat pies, or particularly meat and potato pies, the pastry and the potato had a most unappetising yellow or yellowish-green colour. A communication was made to the Ministry of Health pointing out the unsatisfactory nature of this product and suggesting that its continued manufacture and use would lead to wastage of foodstuffs.

G. H. WALKER

Government of India

SECOND TRIENNIAL REPORT OF THE BIOCHEMICAL STANDARDISATION LABORATORY

THIS Report of the Laboratory, now under the direction of Dr. B. Mukerji, covers the period from April 1, 1940, to March 31, 1943. Owing to the abnormal conditions brought about by the war, the enforcement of the Drugs Act, 1940, anticipated in the last report (ANALYST, 1942, 67, 165), could not be effected, and the projected conversion of the Laboratory into a Central Drugs Laboratory had to be postponed. In 1942 the services of the Laboratory were requisitioned for drug standardisation and testing work on behalf of the Services. This entailed a great increase in the work, and in 1942 a branch laboratory was established at Kasauli.

QUALITY AND POTENCY OF INDIAN DRUGS—Of the 3148 samples of drugs, chemicals, biological products, insecticides, disinfectants, etc., examined, 569 were not up to standard. Nearly 2500 samples of drugs were collected by inspectors from the places of manufacture and 80% of these proved to be of standard quality. This shows a definite improvement in the quality of drugs manufactured in India over the previous triennial period, when more than 50% were below standard. The figures suggest that adulteration takes place largely, if not entirely, in the course of the retail trade.

ANALYSIS OF DRUGS BY BIO-ASSAY METHODS—Approved methods of the B.P., or in "*Biological Standardisation*," by J. H. Burn, have been used. For testing organo-metallic compounds a combination of the B.P. and U.S.P. methods has been adopted. In each instance chemical assays of arsenic and antimony contents were made side by side with toxicity determinations. For the evaluation of coal-tar disinfectants, several brands of which are now made in India, simultaneous chemical and bacteriological (B.S.I. Standard Rideal–Walker technique) tests have been made.

ANALYSIS OF DRUGS BY PHARMACOGNOSTIC AND CHEMICAL METHODS—The standard methods of the B.P., B.P.C., U.S.P., and New Formulary have invariably been used for the older drugs, whilst for new drugs and chemicals recourse has been had to the methods described in the "Extra Pharmacopoeia" and in "New and Non-Official Remedies." Crude vegetable drugs purchased in the open market are often grossly adulterated and of poor quality. Gum benzoin (Loban) and other varieties of gums and resins were found to be either manufactured artificially or of poor quality. Stearoptenes, *e.g.*, camphor, menthol, etc., showed lower m.p., lower optical rotation and more non-volatile residue than genuine samples. Of the organic preparations, citric acid often contained traces of tartaric acid, and coal-tar derivatives were frequently below standard. Oils commonly adulterated were castor, arachis, sesame and olive. The olive oil sold in India is usually mixed with cottonseed oil and has a high acid value. Cod-liver oil is often rancid, with high acid value and low vitamin A content.

COMMERCIAL TESTING OF DRUGS—Towards the end of 1941 the Laboratory's technical service was made available to the public on payment of approved fees. Commercial firms are entitled to submit samples for test and to obtain a certificate or test report on their products, provided that they give an undertaking in writing that the reports issued will not be used for advertisement purposes. Since the initiation of the scheme nearly 100 samples from all parts of India have been tested.

The following problems *inter alia* have been investigated.

STANDARDISATION: *Digitalis*—The "cat method" was originally recommended by the Laboratory as suitable for India, and this has been confirmed after re-investigation. This conclusion finds corroboration in the fact that the U.S.P. XII, 1943, has adopted this method in preference to the "frog method" of previous Pharmacopoeias.

Thyroid Gland—Chemical and biological assay of thyroids from Indian cattle indicate that their thyroxin-iodine contents is in no way poorer than that of foreign glands from animals maintained in a better state of nutrition.

Insulin—The problem of the sensitivity of Indian rabbits has been studied and a dose-response curve has been worked out.

Ergot—Owing to the acute shortage of ergot in India its production under laboratory conditions is under investigation in collaboration with a mycologist. For the first time in India ergot has been cultivated by artificial inoculation of rye glumes with a culture of the fungus. This ergot has given satisfactory results in chemical and biological tests.

Vitamin-containing Preparations—Rats kept on a rachitogenic diet away from light are now being used for the assessment of vitamin D in fish liver oils. Satisfactory results were obtained in correlating the values for vitamin B_1 given by the thiochrome method with those given by biological assay. When vitamin B_1 was present in small quantities (less than 25 units per tablet) the thiochrome method did not give accurate results.

Coal-tar Disinfectants—Preliminary investigation of disinfectants manufactured from Indian tar indicate that that tar contains more of the *o*-cresol fraction and, unless specially treated, would not yield as high a Rideal–Walker value as imported tars, at the same distillation range.

SEARCH FOR A QUININE SUBSTITUTE—In view of the world shortage of quinine, the indigenous materia medica of India has been searched for a substitute. Two reputed anti-malarial remedies, *Alstonia scholaris* and *Cesalpina bonducella*, were found of little value in monkey and fowl malaria. Reports received from China indicated that certain varieties of *Fraxinus chinensis* and *F. malacophylla*, yield an alkaloid similar to quinine and are clinically useful in malaria. Similar species of *Fraxinus* occur in India, and investigation of their clinical utility is in progress.

CHLORINATED ETHYLENE COMPOUNDS AS ANAESTHETICS—Some of these compounds have high analgesic properties with the advantage of high b.p. over the commonly used volatile anaesthetics. Expts. have proved that purified and stabilised trichloroethylene may be safely used in particular types of surgical operations.

CANADA BALSAM SUBSTITUTE—An indigenous mounting medium for microscopical work to replace

imported Canada balsam has been developed. It consists of: rosin, 2 g; dammar (from species of *Shorea, Hopea* and *Balanocarpus*), 3 g; xylene or benzene, 7 ml. It has good sealing and drying properties.

Coal-tar Food Colour—A dyestuff, benzene-azo-β-naphthol, frequently used in India to colour vegetable ghee (hydrogenated fat) was found to be definitely toxic to experimental animals on prolonged feeding. The haphazard use of colouring agents which have not been submitted to thorough toxicological examination should not be permitted in food products.

The British Pharmaceutical Codex, 1934

SIXTH SUPPLEMENT*

This Supplement contains additions and amendments to the monographs in Part I and Part II with amendments to formulae in Part III. The additions to Part I consist of new monographs on substances contained in the Sixth Addendum to the B.P., but not previously described in the British Pharmaceutical Codex, and monographs on surgical catgut. The amendments include alterations in the standards for substances which are now included in the B.P., but previously standardised in the Codex, together with alterations and additions to preparations at the ends of the monographs, so as to bring the Codex monographs into line with pharmacopoeial requirements and to record changes made in the preparations of the Formulary.

The additions and amendments to Part II are necessitated by the scarcity of rubber and silk. Alternative specifications are included for Battiste and Jaconet and a monograph for Oiled Artificial Silk (waterproofed rayon) to replace oiled natural silk. The limits of boric acid in boric lint, gauze and wool have been widened.

In Part III the amendments include changes in the formulae for ointments to permit the use of yellow soft paraffin in place of the white variety and the use of hydrous ointment of the Sixth Addendum to the B.P. in making certain ointments made with a paraffin basis and to continue a paraffin basis in ointments in which hydrous ointment is unsuitable. Other amendments include changes in the formulae for several preparations containing alcohol.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Methods for Determination of Solubility of Dried Whole Egg. J. R. Hawthorne (*J. Soc. Chem. Ind.*, 1944, 63, 6–8)—The solubility of dried egg may be determined by either (1) physical methods which measure the percentage of total solids dissolved by water or other solvent under specified conditions, or (2) chemical methods in which the solubility of a particular constituent is determined. Thistle, Pearce and Gibbons, who have published methods for determination of solubility of dried whole egg in water and in 10% potassium chloride solution (*Canad. J. Res.*, 1943, **21**, D, 1), state that solubility methods based on nitrogen determinations operate at high and narrow ranges; also that the results are less useful as a measure of quality than are those of total solid methods, *e.g.*, the "water value" and the "potassium chloride value." The Low Temperature Research Station method determines in the egg the nitrogen soluble in 10% potassium chloride soln. and expresses it as percentage of the total nitrogen. By this method, solubilities in potassium chloride soln. have ranged from 98 to 42% for commercial dried egg samples; and after prolonged storage of an experimental sample a value of 17% was obtained. While too laborious for use as a rapid routine method, the "potassium chloride soluble nitrogen" is used as L.T.R.S. reference method for checking the accuracy of other methods. The Haenni method, which shows good correlation with the L.T.R.S. method, is as follows. Add 5 ml of 5% sodium chloride soln. to 1 g of egg powder in a test-tube, close the tube with a rubber stopper and, after shaking gently for *ca.* 1 min., set aside for 15 min.; then invert 10 times. Again invert 10 times, 30 min. after the initial shaking. Insert a 2-mm glass tube, the top closed by the finger, 5 min. later under the surface of the liquid; rotate it sharply, open the top momentarily and close, remove from the soln. and wipe the outside. Transfer a drop to a refractometer and read the refractive index. The Haenni val., or "solubility index by refractometer," is (n_D^{25} of soln. $-$ n_D^{25} of solvent) $\times$ 1000. This result eliminates any absolute error of the refractometer and minimises errors due to temp. variations (for routine work the measurement at room temp., *ca.* 20° C, is accurate enough). The method is rapid and is considered the best for routine determinations. Other methods described are the centrifuged heat-coagulable solubility index and Esbach's sedimentation solubility index (Stuart, Grewe and Dicks, *U.S. Egg and Poultry Mag.*, 1942, 48, 498). The solubility is usually a measure of the damage the egg has suffered during drying or storage. Some highly soluble samples have an unpleasant acid flavour, but this is due to other factors, *e.g.*, bacterial spoilage of the pulp.

E. B. D.

Determination of Glycerol and 2,3-Butylene Glycol [in Wine] in presence of Invert Sugar. G. Hoepe (*Helv. Chim. Acta*, 1943, 26, 1931–1939)—Since it is possible to determine each constituent of a mixture of glycerol, ethylene glycol and 1,2-propylene glycol by the periodate method of Hoepe and Treadwell (*Helv. Chim. Acta*, 1942, 25, 353; Analyst, 1942, 67, 272), the application of the method to determination of glycerol and 2,3-butylene glycol in wine, after removal of invert sugar, was studied. Preliminary expts. showed

* The Pharmaceutical Press, 17, Bloomsbury Square, London, W.C.1. Price 2s. 6d., postage 2d. (pre-paid).

that separation from invert sugar could be effected by treatment with alkaline lead acetate with subsequent steam distillation. Oxidation of the distillate by potassium periodate converted glycerol into formic acid and formaldehyde, and butylene glycol into acetaldehyde. Titration of the formic acid indicated the amount of glycerol originally present, and determination of the total aldehyde radical and the portion of it due to formaldehyde gave, by difference, the portion due to acetaldehyde derived from 2,3-butylene glycol originally present. *Method*—Heat 10 ml of wine with 10 g of lead acetate and 10 g of solid sodium hydroxide, add 100 ml of alcohol and remove the ppt. by filtration through a glass filter. Concentrate the filtrate *in vacuo* to 5 ml and add 10 ml of water. Distil the liquid from an oil-bath at 140° C. (the b.p. of 90% glycerol) and pass in steam superheated to 320° C. Neutralise the distillate, if necessary, with sodium hydroxide soln. and shake for 3 hr. in a mechanical shaker with excess of potassium periodate. Filter off the excess of potassium periodate, washing it with a little water, and titrate an aliquot portion of the filtrate with 0·1 *N* sodium hydroxide, using methyl red indicator. (Each ml ≡ 9·2 mg of glycerol.) To determine the total aldehyde radical treat another aliquot portion with 50 ml of sodium sulphite soln. (125 g per litre), add thymolphthalein and titrate the sodium hydroxide formed with 0·1 *N* hydrochloric acid, correcting the result by means of a blank titration of the sodium sulphite soln. (Each ml ≡ 2·9 mg of CHO.) To determine formaldehyde, treat a third aliquot portion, as a blank expt., with 0·5 ml of 50% nitric acid and 30 ml of 0·1 *N* silver nitrate, filter off the ppt. of silver iodate and periodate, wash it with a little very dil. nitric acid and titrate the residual silver with ammonium thiocyanate in presence of ferric alum. Treat a fourth aliquot portion with 30 ml of 0·1 *N* potassium cyanide, 0·5 ml of 50% nitric acid and 30 ml of 0·1 *N* silver nitrate. Filter and wash the ppt. as in the blank expt. and titrate the residual silver in the same manner. (Each ml of 0·1 *N* potassium cyanide ≡ 3·0 mg of formaldehyde or 2·9 mg of CHO.) Deduct the aldehyde radical due to formaldehyde from the total aldehyde radical and calculate the residual aldehyde radical into acetaldehyde and finally into 2,3-butylene glycol. (1 g of CHO ≡ 1·517 g of acetaldehyde or 1·550 g of butylene glycol.) A. O. J.

Determination of Nicotine and Nornicotine in Tobaccos. C. V. Bowen and W. F. Barthel (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 740–741)—In the method of Markwood (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 283; ANALYST, 1943, **68**, 283) for determination of nicotine and nornicotine present together in solution, no procedure is given for obtaining the alkaloids from tobacco or for separating them from interfering substances. In the method here described, since nornicotine is difficult to remove completely from alkaline plant material by solvent extraction, steam distillation is used and the assumption is made that anabasine and other secondary-amine alkaloids volatile in steam (with the exception of nornicotine) are absent. Steam distil 2·5 g of powdered tobacco with a small piece of paraffin wax, 10 ml of 30% sodium hydroxide soln. and 10 g of sodium chloride into 3 ml of dil. hydrochloric acid (1 + 4) until a few drops of the distillate fail to answer to a test with silicotungstic acid soln. Reduce the vol. of the distillate by boiling and finally adjust it to 25 ml. Ppt. nicotine and nornicotine from an aliquot portion (10 ml) with 12% silicotungstic acid ("*Methods of Analysis of the A.O.A.C.*," 1940, 64), and collect, ignite and weigh the ppt. in a Gooch crucible (residue A). Neutralise another 10-ml portion of the distillate to phenolphthalein, add 2 ml of 30% acetic acid and 0·5 g of sodium nitrite (Markwood, *loc. cit.*) and, after 20 min., make the soln. alkaline with sodium hydroxide and steam distil into 3 ml of hydrochloric acid. The distillate contains the nicotine, which is collected, ignited and weighed as before (residue B). The wt. of nicotine in the aliquot portion taken is the wt. of residue B × 0·1140; the corresponding wt. of nornicotine is the difference between residue A and residue B × 0·1042. The results of analyses of several samples of tobacco (*Nicotiana tabacum*), *N. rustica* and commercial nicotine sulphate showed an unexpectedly widespread occurrence of nornicotine and that in its presence the accepted method of analysis of the A.O.A.C. cannot be depended upon to give reliable results. An improved steam distillation apparatus (Bowen and Barthel, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 596) is recommended to reduce the time of distillation. A. O. J.

Semi-permanent Colour Standards for Micro-estimation of Sulphonamides. S. W. Lee and N. B. Hannay (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 763)—As a supplement to the method for sulphonamide determination previously reported (Lee, Hannay and Hand, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 403) a simple procedure has been devised, for which colour standards prepared from easily procurable water-sol. dyes are used. The method is accurate to blood levels of 15 mg per 100 ml (for sulphathiazole) and the error was found to be ±0·5 mg per 100 ml to levels of *ca.* 6 mg per 100 ml and ±1 mg at higher levels to 15 mg per 100 ml. The vol. of blood needed (0·3 ml) may be taken from the finger tip if the hand is vigorously washed to remove traces of the drug. Flat-bottomed 10-ml vials (18 × 46 mm), calibrated for the purpose are preferable to centrifuge or test tubes. Standards of known concn. may be made from any of the sulphonamides and the values for the others obtained by using molecular wt. corrections. The values for sulphathiazole may be multiplied by 0·68 to convert them into values for sulphanilamide, whilst those for sulphapyridine are the same as for sulphathiazole. The reagents and procedure are the same as for the parent method (*loc. cit.*), but only the blood and precipitating soln. vols. need accurate measurement. The nitrite and dye solns. (2 drops or 0·1 ml) are added from a dropper, and comparisons with standards are made visually in vials of uniform shape. Intensities of transmitted light are best compared if the absorbing substance is held 1 to 3 cm from the eye. To prepare semi-permanent standards, dissolve 0·1 g of acid fuchsin in 250 ml of water and dilute 8-fold with water to produce a soln. containing 0·00005 g per ml. Dissolve 0·1 g of methyl violet in 250 ml of water and dilute 2 ml to 250 ml (0·0000032 g per ml). To prepare standards corresponding with the following concns. of sulphathiazole in mg per 100 ml, add to 0·9 ml of fuchsin soln. and 9·1 ml of methyl violet soln. the no. of ml of water shown by the figure in brackets—2, (36·0); 3, (25·3); 4, (19·0); 5, (14·7); 6, (11·2); 7, (8·8); 8, (7·0); 9, (5·4); 10, (3·9); 11, (2·9); 12, (2·1). Several combinations of red and violet water-sol. dyes gave shades almost identical with that of the

azo-colour formed in the reaction. The dye combination is bleached by sunlight but is stable in the dark for *ca.* 1 month; the stronger solns. of the dyes are more stable. A. O. J.

Permanent Fading of Alkaline Phenolphthalein Solutions. M. H. Hubacher (*J. Amer. Chem. Soc.*, 1943, **65**, 2097–2098)—Permanent fading of phenolphthalein is due to air oxidation, the products being 39–45% of 2-(4′-hydroxybenzoyl)-benzoic acid, a small quantity of phthalic acid and a brown semi-solid mass the composition of which is not yet known. This type of fading occurs also with *o*-cresolphthalein, which yields 2-(3′-methyl-4′-hydroxybenzoyl)–benzoic acid and phthalic acid. 3.4-Dihydroxydiphenylphthalide is oxidised to phenol and phthalic acid. On oxidation with hydrogen peroxide, phenolphthalein and *o*-cresolphthalein yield, in addition to phthalic acid, hydroquinone and *o*-toluhydroquinone, respectively, this being another example of a reaction described by Dakin (*Amer. Chem. J.*, 1909, **42**, 477, and "*Organic Syntheses*," Coll. Vol. I, 1941, 141). These two types of fading should be taken into consideration in the colorimetric estimation of phenolphthalein; otherwise the results will be low.

E. M. P.

Biochemical

Estimation of Tocopherol in Serum, Milk and Animal and Plant Fat. M. Kofler (*Helv. Chim. Acta*, 1943, **26**, 2166–2176)—The tocopherol contents of different sera were measured by the method of Furter and Meyer, by oxidation with ferric chloride soln., fluorimetrically and by the phenazine method described below. It was found that the first two methods gave much higher results than the fluorimetric and phenazine methods; as these high values are due to interfering impurities, the other two methods are to be preferred. Evaporate a light petroleum extract of tocopherol to dryness and dissolve the residue in 10 ml of abs. alcohol. Add 2 ml of conc. nitric acid, heat to boiling over a small flame and immerse the flask for 5 min. in a boiling water-bath. Transfer the soln. to a 50–100 ml separating funnel, and rinse the flask with 2 ml of abs. alcohol and 15 ml of light petroleum, adding the solns. to the contents of ths funnel. Add 15 ml of water, shake and allow to separate. Transfer the aqueous phase to a second separating funnel and re-extract with 15 ml of light petroleum. Wash the light petroleum extract in the first separating funnel with water and re-extract the washings by means of the light petroleum in the second funnel. Evaporate the combined petroleum solns. in a 50-ml conical flask to dryness, and to the residue add 5 ml of a 1% soln. of *o*-phenylenediamine in glacial acetic acid and leave the flask for 1 hr. on a boiling water-bath. Cool, pour the soln. into a dry 50-ml separating funnel, rinse the flask with 5 ml of glacial acetic acid and then add 15 ml of light petroleum and 15 ml of water. The light petroleum should be saturated with water before use. Allow the layers to separate, transfer the aqueous phase to a second separating fuunel, and re-extract with 15 ml of light petroleum. Wash the extract successively with water, dil. hydrochloric acid, dil. sodium hydroxide soln. and again with water, and extract each washing in turn with the light petroleum in the second funnel. It is important that during the condensation with *o*-phenylenediamine there should be free access of air to the solns. Purify the light petroleum extract chromatographically by running it through a column of activated aluminium oxide, 8–10 cm × 1 cm, first saturating the column with a few ml of light petroleum. In ultra-violet light a yellowish-green fluorescent ring is observed in the column; on development with 25–50 ml of a mixture of benzene and light petroleum (1 : 4), this ring broadens and descends down the column. Develop, elute the tocopherol-phenazine derivative with 30–50 ml of benzene-petroleum (1 : 1), remove the solvent *in vacuo* from the eluate, and dissolve the residue in 1 ml of butyl alcohol by gently warming. Add 9 ml of methyl alcohol and transfer the soln. to a test-tube; if a turbidity develops, add more butyl alcohol. Dilute a standard soln. of tocopherol so as to obtain a range of concns. from 10 to 150μg per 10 ml. Oxidise each of these solns. with 2 ml of nitric acid, condense with *o*-phenylenediamine, chromatograph, and dissolve in a mixture of butyl and methyl alcohols (1 : 9) as described above. Transfer the solns. to test-tubes and compare the fluorescence of the unknown soln. visually with those of the standards in the light of a quartz lamp. The nature of the preliminary extraction varies with the nature of the material under examination. With human serum, for example, mix 5 ml (or with animal serum 10–20 ml) with 2·5 ml of 60% potassium hydroxide soln. and 7·5 ml of 95% alcohol. Shake with two 50-ml portions of light petroleum, wash the petroleum extract successively with 20 ml of 50% alcohol, dil. hydrochloric acid and water, extracting each washing in turn twice with light petroleum. Alternatively, the serum may be saponified. Heat a mixture of 5 ml with 2·5 ml of 60% potassium hydroxide soln. and 20 ml of 95% alcohol under reflux for 1 hr. in a stream of pure nitrogen, cool, transfer the soln. to a separating funnel, rinse out the flask with 5 ml of alcohol, and add 50 ml of light petroleum of low b.p. and 25 ml of water. Shake vigorously and re-extract the aqueous phase with 50 ml of light petroleum ether. Wash the petroleum extract as before, re-extracting the washings with more light petroleum. The saponification of milk and animal and plant fat is carried out in a similar way. The light petroleum extracts thus obtained are then evaporated, oxidised and treated with *o*-phenylenediamine as described above. F. A. R.

Microbiological Determination of Amino Acids. I. Valine, Leucine and Isoleucine. K. A. Kuiken, W. H. Norman, C. M. Lyman, F. Hale, and L. Blotter (*J. Biol. Chem.*, 1943, **151**, 615–626)—The method depends on the use of *Lactobacillus arabinosus* 17–5 with a basal medium which includes a tomato eluate, prepared in the following way. Dilute 1350 ml of tomato juice with an equal vol. of water, centrifuge and clarify with 120 g of Filter-Cel. Adjust the filtrate to *p*H 3, add 40 g of Norit A, and shake for 30 min. Filter, wash the adsorbate with 250 ml of 50% ethyl alcohol, and elute with 200 ml of pyridine-ethyl alcohol-water mixture (1 : 2 : 1 by vol.). Heat the suspension to 60° C., shake for 15 min. and filter. Repeat the elution twice, filter the combined eluates and evaporate the filtrate nearly to dryness. Neutralise with sodium hydroxide soln. and repeat the distillation until all pyridine is removed. Heat the residue under reflux for 24 hr. with 40 ml of 8 *N* sulphuric acid and then add a hot saturated soln. of barium hydroxide until the hydrolysate is alkaline to Congo red and acid to litmus. Filter, wash the barium sulphate and adjust the filtrate to *p*H 3. Remove any

ppt. and repeat the adsorption and elution with 8 g of Norit A and 100-ml portions of eluent. Remove the pyridine-ethanol-water mixture by vacuum distillation and prepare an aq. soln. containing 5 mg of solids per ml. The basal medium has the following composition:

Glucose	40 g
Sodium acetate (anhydrous)	14·5 ,,
Adenine sulphate	10 mg
Guanine hydrochloride	10 ,,
Uracil	10 ,,
Thiamine chloride	200 μg
Pyridoxine hydrochloride	200 ,,
Calcium pantothenate	200 ,,
Biotin	0·8 ,,
Riboflavin	400 ,,
Nicotinic acid	800 ,,
p-Aminobenzoic acid	1·0 ,,
Tomato eluate	200 mg
K_2HPO_4	1 g
KH_2PO_4	1 ,,
$MgSO_4.7H_2O$	400 mg
NaCl	20 ,,
$FeSO_4.7H_2O$	20 ,,
$MnSO_4.4H_2O$	20 ,,

Adjust to *p*H 6·5 to 6·8 and dilute with water to 1 litre.

The assay media for valine, leucine and *iso*leucine are prepared by omitting the appropriate amino acid. Prepare stab cultures of *L. arabinosus* in a solid medium containing 0·8% of agar, 1% of peptonised milk, 1% of tryptophan and 200 ml of filtered tomato juice per litre. Incubate for 24 hr. at 35° C. and store in the refrigerator until required. Transfer the stab cultures to tubes containing the same medium with the agar omitted, incubate for 18–24 hr. at 35° C., centrifuge and wash once with saline. Re-suspend the washed cells in saline and use one drop of this suspension for inoculating the assay tubes. Add graded amounts, 0 to 0·1 mg with increments of 0·02 mg, of the pure amino acid soln. in duplicate to a series of tubes which are to be used as standards. Add aliquot portions of the soln. to be tested to other tubes in duplicate at three levels. Add 5 ml of the appropriate medium to all the tubes, followed by distilled water to a vol. of 10 ml. After mixing, plug with cotton-wool and autoclave for 15 min. at 15 lb. pressure. Cool and incubate at 35° C. for 72 hr. Centrifuge the tubes and titrate 5-ml aliquot portions with 0·1 *N* sodium hydroxide, using bromothymol blue as indicator. Proteins are first hydrolysed by heating under reflux for 24 hr. with 5 *N* sulphuric acid (40 ml per g) and neutralising with hot saturated barium hydroxide soln. Foodstuffs are dried *in vacuo* for 5 hr. at 95° C. and then extracted with dry ether for 16 hr.; the fat-free residue is then hydrolysed as above. F. A. R.

Microbiological Analysis of Seven Amino Acids with *Lactobacillus casei*. **S. Shankman, M. S. Dunn and L. B. Rubin** (*J. Biol. Chem.*, 1943, **151**, 511–514,)—In previous communications (*J. Biol. Chem.* 1943, **150**, 305; 477) the estimation of cystine, glutamic acid, *iso*leucine, leucine, methionine, threonine, tryptophan and valine by means of *Lactobacillus arabinosus* 17–5 was described. In the present paper the estimation of arginine, glutamic acid, leucine, phenylalanine, tryptophan, tyrosine and valine with the aid of *L. casei* (*L. helveticus*) is outlined, so that most of the "essential" amino acids can now be estimated microbiologically. Prepare stab cultures of *L. casei* on a yeast extract-dextrose-agar, incubate for 24–48 hr. at 37° C. and store in the refrigerator until required. Transfer the stab culture to the riboflavin assay medium described by Snell and Strong* (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 346) and incubate for 24 hr. at 37° C. For the assays, use the basal medium described by Hutchings and Peterson† (*Proc. Soc. Exp. Biol. Med.*, 1943, **52**, 36) with 1% instead of 2% of glucose. Adjust the basal medium and amino acid solns. to *p*H 6·8, and then to each of a series of test tubes add 5 ml of the basal medium containing all the components except the amino acid under investigation, and either 1 to 5 ml of a standard soln. of the amino acid under test, or a similar range of test substance. Add to each tube sufficient water to make the vol. in each 10 ml, plug the tubes with cotton-wool and autoclave for 15 min. at 15 lb. pressure. Cool to room temp. and inoculate each tube with 1 drop of a suspension of the organism prepared by centrifuging a 24-hr. old culture, suspending the cells in sterile saline, again centrifuging and finally suspending in 10 ml of saline. After incubating the tubes for 72 hr. at 37° C. titrate the lactic acid produced with standard alkali, using bromothymol blue as indicator. With most of the amino acids the errors did not exceed 2·5%, but with phenylalanine and leucine the errors were 5 and 10% respectively. The estimation of asparagine, cystine and serine was less satisfactory. F. A. R.

l(+)-Arginine hydrochloride	400 mg
dl-Alanine	400 ,,
dl-Aspartic acid	800 ,,
dl-Glutamic acid monohydrate	800 ,,
l(−)-Histidine monohydrochloride	400 ,,
l(+)-Lysine hydrochloride	400 ,,
dl-Phenylalanine	400 ,,
l(−)-Proline	400 ,,
dl-Serine	400 ,,
l(−)-Tryptophan	400 ,,
dl-Methionine	400 ,,
dl-Threonine	400 ,,
dl-Tyrosine	400 ,,
dl-Valine	400 ,,
dl-Leucine	400 ,,
dl-*Iso*leucine	400 ,,
l(−)-Cystine	400 ,,

Estimation of Mepacrine in Biological Fluids and Tissues. **B. B. Brodie and S. Undenfriend** (*J. Biol. Chem.*, 1943, **151**, 299–317)—A double-

* This consists of photolysed, sodium hydroxide-treated peptone 0·5, glucose 1, sodium acetate 0·6, and cystine 0·01%, together with riboflavin-free yeast supplement equiv. to 0·1% of yeast extract and 5 ml per litre of soln. A containing K_2HPO_4 10% and KH_2PO_4 10% and 5 ml per litre of soln. B containing $MgSO_4.7H_2O$ 4, NaCl 0·2, $FeSO_4.7H_2O$ 0·2 and $MnSO_4.4H_2O$ 0·2%. For making up cultures this assay medium is supplemented with 0·5–1·0μg of riboflavin per 10 ml.

† This consists of glucose 2·0, sodium acetate 0·6, acid hydrolysed casein 0·5, tryptophan 0·01, and cystine 0·01%; adenine 2·0, riboflavin 0·02, calcium pantothenate 0·02, nicotinic acid 0·02, pyridoxine 0·02, biotin (free acid) 0·00001 and eluate factor conc. 0·001 mg/100 ml; together with the following salts: K_2HPO_4 0·5, KH_2PO_4 0·5, $MgSO_4.7H_2O$ 0·2, NaCl 0·01, $FeSO_4.7H_2O$ 0·01 and $MnSO_4.4H_2O$ 0·01 g per litre.

extraction procedure is recommended for the estimation of mepacrine in plasma, whole blood, tissue and urine. It gives recoveries averaging 98% with as little as 0·1μg. A single-extraction method is also described which, though less sensitive, is rapid and simple to operate. Add 3 ml of 0·2 *M* Na_2HPO_4 and 30 ml of ethylene dichloride to 1–10 ml of biological material. Shake vigorously for 5 min., decant into a 50-ml centrifuge tube and centrifuge for 10 min. Remove the supernatant layer, break up any gel that has formed and re-centrifuge. Transfer the ethylene dichloride to the original vessel, add an equal vol. of 10% sodium hydroxide soln. and shake for 3 min. Remove most of the aqueous layer, transfer the remainder of the liquid to a narrow test-tube and centrifuge for 1 min. Remove the aqueous layer, wash the ethylene dichloride soln. with a little water and again remove the aqueous layer. Transfer exactly 20 ml of the ethylene dichloride soln. to a glass-stoppered bottle with 1 ml of water and 10 ml of 85% lactic acid, and shake vigorously for 5 min. Transfer to a centrifuge tube and centrifuge for 1 min. Measure the fluorescence of 8 ml of the aqueous phase and compare with that of a standard prepared by adding 1 ml of a soln. containing 0·5μg of mepacrine to 10 ml of 85–90% lactic acid. Use a mixture of 10 ml of lactic acid and 1 ml of water for the blank setting of the instrument. Use a 2-mm No. 5113 Corning glass filter to isolate the activating light and a Corning No. 3385 filter to limit the transmission of the resulting fluorescent light. In the single-extraction procedure the fluorescence of the initial ethylene dichloride extract is measured; this method will estimate 0·5μg of mepacrine, but is not recommended for use with plasma because of the low concn. of mepacrine therein. F. A. R.

Iodimetric Determination of Methionine. T. F. Lavine (*J. Biol. Chem.*, 1943, **151**, 281–297)—Methionine, like other thio-ethers, gives on treatment with iodine a periodide which is more or less readily hydrolysed to a sulphoxide. The periodide is colourless and water-soluble, and its rate of formation increases and rate of hydrolysis decreases with decreasing acidity. At *p*H 9, however, methionine can be titrated directly with iodine. In presence of iodide, the rate of periodide formation and, still more, the rate of hydrolysis, decreases. The periodide is stable to certain reducing agents such as thiosulphate, so that excess of iodine can be removed by means of thiosulphate, and the resulting colourless soln. of methionine periodide can then be acidified, with liberation of iodine, which can be titrated with thiosulphate soln. This reaction has been made the basis of a method of estimating methionine, but, as other substances, *e.g.*, amines and amino acids, also form periodides, it is important to choose conditions that prevent interference by these substances. It was found that formation of periodides by all classes of amines was greatly restricted in *M* potassium iodide soln. at *p*H 7, and when such periodides are formed they can be readily destroyed by means of thiosulphate. At *p*H 7 amino acids also have only a slight tendency to form periodides, but when such acids are present in quantity, a correction for interference must be made by a blank determination after conversion of methionine into its sulphoxide, which does not form a periodide. Tryptophan forms a coloured ppt. with iodine and this must be removed by centrifuging. Interference by homocystine must be avoided by conversion into the thiolactone of homocysteine. *Method*—Since the amount of reversible periodide formation by various amino acids other than methionine, which constitutes the blank, is a function of the iodine concn., this must be the same in the blank as in the determination. Accordingly a blank test is carried out in such a way that after iodate oxidation the iodine concn. may be adjusted to that in the test sample. Add sufficient 6 *N* hydrochloric acid to 5 ml of the soln. to be tested, so that the final concn. of acid is 0·5–1 *N*. Add sufficient 0·1 *N* potassium iodate, which oxidises methionine to its sulphoxide, to impart a slight yellow colour to the soln. After 10–20 min. sufficient 0·1 *N* potassium iodate to impart a slight yellow colour to the soln. After 10–20 min. convert the excess of iodate into iodine by adding 1 ml of 5 *M* potassium iodide soln. Add half the amount of sodium hydroxide necessary for neutralisation, and then a mixture of buffer soln. of *p*H 7 (7 vols. of *M* K_2HPO_4 and 3 vols. of *M* KH_2PO_4) with the remainder of the sodium hydroxide and sufficient 5 *M* potassium iodide soln. to make the final soln. 1 *M*. The vols. of buffer and iodide solns. are such that each 5 ml of final soln. contains 1 ml of each. Finally, add sufficient water to make the vol. the same as in the determination. Within 10 min. of beginning the blank test, neutralise the test soln. if necessary (methyl red) and then add to a suitable aliquot portion sufficient buffer soln. and 5 *M* potassium iodide soln. to allow 1 ml of each for every 5 ml of final soln. Next, add a 25–50% excess of 0·1 *N* iodine, and, after 10–20 min., destroy the excess with thiosulphate, using starch as indicator. Add 1–1·5 ml of 2 *N* hydrochloric acid for every ml of buffer used, and titrate the liberated iodine with thiosulphate. The iodide concn. should be at least 0·5 *M* at the end of the titration to prevent oxidation of methionine by iodine in the acidified soln. Deduct the blank and calculate the methionine content in the usual way. Methionine added to a mixture of 17 other amino acids gave a value equal to 100·5% of the theoretical. Satisfactory results were obtained with hydrolysed and unhydrolysed casein and zein. When homocystine is present, the mixture must first be treated with zinc to reduce the homocystine to homocysteine (*cf.* Lavine, *J. Biol. Chem.*, 1935, **109**, 141), which is then evaporated to the thiolactone (Riegel and du Vigneaud, *id.*, **112**, 149); cysteine alone is then determined by iodine oxidation in *M* hydriodic acid, and the methionine determination and blank are carried out as previously described to measure the methionine present. F. A. R.

Microbiological Method for the Estimation of Choline by use of a Mutant of Neurospora. N. H. Horowitz and G. W. Beadle (*J. Biol. Chem.*, 1943, **150**, 325–333)—A strain of *Neurospora crassa* for which choline proved to be essential was obtained by irradiation of a wild strain with ultra-violet light. The following method of estimating choline depends on the use of this strain, which is known as No. 34486 or "cholineless." The basal medium used has the following composition (g per litre): ammonium tartrate, 5; ammonium nitrate, 1; KH_2PO_4, 1; $MgSO_4 7H_2O$, 0·5; NaCl, 0·1; $CaCl_2$, 0·1; sucrose, 20; biotin 5×10^{-6}. In addition, the following elements (mg per litre) are added as salts: B, 0·01; Mo, 0·02; Fe, 0·2; Cu, 0·1; Mn, 0·02; Zn, 2·0. The sucrose and biotin are added after autoclaving. Stock solns. of the mutant are maintained on agar slopes composed of the basal medium plus agar, 1·5%, Difco yeast extract 0·2%, malt extract

0·2% and choline 1μg/ml. Assays are carried out in 250-ml conical flasks containing 25 ml of medium; these are inoculated with 1 drop of a spore suspension made up in a few ml of sterile distilled water. The flasks are incubated at 25° C. for 3 days, and the mycelia are pressed out on a filter-paper, dried at 90° C. and weighed to the nearest 0·5 mg. Weigh 100 mg of the test material into a 50-ml flask, add 10 ml of 3% sulphuric acid and autoclave at 15 lb. for 2 hr. Cool, transfer to a centrifuge tube, and neutralise to Congo red with saturated barium hydroxide soln. Centrifuge, wash the ppt. with 3 ml of hot water and again centrifuge. Neutralise the clear filtrate and washings to litmus with sodium hydroxide soln. and then dilute to 30 ml. Run 5 ml of the soln. through a column of Permutit (110 × 0·6 mm) to separate choline from methionine. Wash the column with 5 ml of 0·3% sodium chloride soln. and discard the filtrate and washings. Elute the choline with 10 ml of 5% sodium chloride soln. and dilute the filtrate to 10 ml with water. Add the eluate to the flasks containing the basal media so that the final concentration of choline lies between 0·5 and 20μg per 25 ml. Prepare a series of standards containing 0 to 20μg of pure choline per flask and autoclave the flasks at 15 lbs. for 5–10 min. The weights of mycelium agreed within 5% in duplicate flasks, and estimations of choline on different amounts of the same soln. generally agreed within 10%. Recoveries of added choline were usually within 90–110% of the theoretical. The method proved to be specific, and of 11 water-soluble factors and 22 amino acids tested, only choline and methionine were active. Methionine had only 1/500 of the activity of choline. Lecithin was the only other substance which had any activity. F. A. R.

Determination of Cysteine and Cystine by Vassel's Method. D. K. Mechan (*J. Biol. Chem.*, 1943, **151**, 643–645)—By modifying the method of Vassel (*J. Biol. Chem.*, 1941, **140**, 323; ANALYST, 1941, **66**, 470) a more accurate method of estimating cysteine and cystine, especially in presence of detergents, has been devised. To 1-ml samples of the unreduced or reduced hydrolysate add 5-ml portions of the appropriate reagent, mix and carry out the procedure described by Vassel beginning with the addition of 3 ml of ferric ammonium sulphate soln. To reduce the hydrolysate add 150 mg of zinc dust to 5 ml, and leave until the zinc has dissolved. To prepare the cysteine reagent, add 1·00 g of zinc dust to 20 ml of ferric ammonium sulphate soln. in a flask fitted with a condenser and leave for 10 min., with occasional shaking; then add 2·30 g of granulated zinc and 30 ml of *p*-aminodimethylaniline soln. After 10 min. heat the mixture in a boiling water-bath for 25 min., cool and filter. To prepare the cysteine+cystine reagent, follow the procedure described for the cysteine reagent, but use only 2·00 g of granulated zinc. Thus the final concn. of zinc in the sample plus reagent is the same in the cysteine determination as in the cysteine+cystine determination, allowing the same calibration curve to be used for both. The average error in 20 estimations of cysteine was +1·4% as compared with +2·6% by Vassel's procedure. F. A. R.

Rapid Method of Estimating N¹-Methylnicotinamide in Urine. J. W. Huff and W. A. Perlzweig (*J. Biol. Chem.*, 1943, **150**, 395–400; 483–484)—Dilute the urine 10-fold with 25% potassium chloride soln., and to a 10-ml portion of the diluted urine, made alkaline with 2 or 3 drops of 10 *N* sodium hydroxide soln., add 0·5 g of Lloyd's reagent. Centrifuge for 2–3 min. and decant the supernatant liquid; this destroys the fluorescence of N¹-methylnicotinamide without affecting the other pigments of urine. Transfer 1–5 ml aliquot portions of the blank prepared in this way and an equal vol. of diluted urine containing 3–15μg of N¹-methyl nicotinamide to 125-ml separating funnels and add 12·0 ml of *n*-butyl alcohol to each. Stir each mixture with a current of air and add 1·0 ml of 10 *N* sodium hydroxide. Continue stirring for 1 min., run off the aqueous layer and shake the butyl alcohol soln. with 2 g of sodium sulphate. Measure the fluorescence after 20 min. in a fluorophotometer standardised against a quinine sulphate soln. F. A. R.

Estimation of Total Oestrone and Oestradiol from Tissue Sources. C. M. Szego and L. T. Samuels (*J. Biol. Chem.*, 1943, **151**, 587–598)—Oestrone can be estimated in presence of oestriol by means of the guaiacol sulphonic acid method previously described (*Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 263) and small amounts of oestradiol do not interfere. Amounts of the order of 10 to 50μg, however, produced a yellow to pink colour, the variability of which, on the one hand, made it useless for the estimation of oestradiol, and on the other hand made it impossible to apply a correction for the increase in colour produced in the oestrone assay. A modified method is now proposed which gives similar colours with both oestrone and oestradiol, but no colour with oestriol. In this way the total oestrone+oestradiol can be estimated and, since the oestrone can be isolated by means of the Girard reagent, it can then be estimated separately. The use of conc. sulphuric acid in the reaction makes it essential to remove all substances which would otherwise char. Hydrolyse the tissue sample by adding 20% of its vol. of conc. hydrochloric acid and heating under reflux for 1 hr. Extract the soln. 4 times with 15-ml portions of chloroform, filter the combined chloroform extracts and evaporate to dryness on a steam-bath. Dissolve the residue in 20 ml of 70% ethyl alcohol and shake with three 10-ml portions of pentane. Evaporate the alcohol phase to dryness, and dissolve the residue in a 15% soln. of acetone in pentane (both redistilled). Dissolve any insol. residue in water and extract the soln. twice with 15% acetone-pentane. Run the combined solns. through a column of activated aluminium oxide, saturated with the acetone-pentane mixture, and wash the column with 90 ml of the same solvent mixture. Evaporate the filtrate to dryness, dissolve the crystalline solid in absolute alcohol and divide the soln. into two halves. Immerse one half in an ice-bath and add 2 ml of conc. sulphuric acid, mix and immerse in a boiling water-bath for 1 min., stirring at the end of 30 sec., and then replace in the ice-bath for 5 min. Next add 4 ml of a 7·5% aqueous soln. of potassium guaiacol sulphonate and immerse for 2 min. in a boiling water-bath. Cool and leave in a water-bath at 25° C. in the dark for 1 hr. Add 3·6 ml of 50% sulphuric acid and, 15 min. later, measure the colour in an Evelyn photoelectric colorimeter, using a very selective filter transmitting at 520mμ. The colour is proportional to the oestrone and oestradiol present in the soln. Evaporate the other half of the alcoholic soln. to dryness, and to the residue add 0·5 ml of a freshly prepared soln. of Girard's reagent T (100 mg

per ml in glacial acetic acid re-distilled from potassium permanganate). Stopper the tube with a glass marble and heat in a boiling water-bath for 20 min. Cool in ice and add 9·5 ml of ice-cold water and 7·5 ml of cold *N* sodium hydroxide. Transfer the soln. to a separating funnel and extract with three 10-ml portions of chloroform. Wash the chloroform extract twice with 10 ml of cold water, filter, evaporate to dryness and dissolve the residue in hot abs. alcohol. Repeat the colorimetric procedure as described above. This gives the oestrone content, and the oestradiol content can then be calculated by subtracting this value from that previously obtained. The recovery of oestrone and oestradiol from pure solns. of either was 96–106% for oestrone (5μg or over) and 95–105% for oestradiol (10μg or over). The recovery of oestrone from mixtures of pure oestrone and oestradiol was 97–108% of the theoretical, and the results showed that the chromogenic effect of the two hormones was additive and that either compound could be used for constructing a calibration curve. The presence of oestriol in amounts up to 50μg did not interfere. When oestrone was added to biological preparations, the average recovery in 15 estimations was only 55% of the theoretical, whilst with oestradiol the average recovery in 3 estimations was 68%; each step of the procedure appeared to involve a definite loss of hormone.

F. A. R.

New Method for the Bioassay of Antiscorbutic Substances. B. S. Gould and H. Schwachman (*J. Biol. Chem.*, 1943, 151, 439–453)—Guinea pigs on a scorbutogenic diet develop a low serum phosphatase, and this can be prevented or cured by administration of ascorbic acid. The following curative method of assay developed on the basis of this observation was found to be better than a preventive method. Select a group of 16 guinea pigs (6–7 weeks old, weighing 250–275 g) and maintain them for 18–25 days on a scorbutogenic diet composed of equal parts of skim milk (heated at 100° C. for 4–5 hr.) rolled oats and bran, supplemented by 1 ml of cod liver oil every 4–5 days, and then estimate the serum phosphatase activity as follows. Dilute 0·05 ml of serum to 0·50 ml with water, withdraw two 0·02-ml samples and transfer them to Kahn serological tubes. Immerse in a water-bath at 37° C. for a few min. and add to one (the control) 0·4 ml of water and to the other 0·4 ml of substrate (1·06 g of veronal, 1·25 g of sodium β-glycerophosphate and 0·5084 g of magnesium chloride in 250 ml of water). Incubate the two tubes for 1 hr., add 0·4 ml of 10% trichloroacetic acid and centrifuge at 2,000 r.p.m. for 10–15 min. Decant the supernatant liquids and estimate the inorganic phosphate content of each by one of the standard methods. The difference between them is a measure of the phosphatase activity, which is expressed in units each equal to 1 mg of phosphorus liberated by 100 mg of serum in 1 hr. at 37° C. If the phosphatase levels of the animals on the scorbutogenic dict are 3–5 units or less, divide them into 4 groups of 4 animals each, and feed each group daily on a quantity of the sample sufficient to provide 0·2, 0·225, 0·25 and 0·275 mg of ascorbic acid, as determined by a preliminary titration with 2 : 6-dichlorophenolindophenol. On the fifth day after the first dose again estimate the serum phosphatase. Any groups in which at least half the animals show a decrease in serum phosphatase and in which the other animals show no significant increase are regarded as having received less than 0·225 mg of ascorbic acid daily. If most of the animals show an increase and some show no change, this is interpreted as indicating that the group has received the critical dose of 0·225 mg, whilst if all show marked increases it may be assumed that the critical level has been exceeded. A control assay with pure ascorbic acid should be carried out for any new groups of animals. With the aid of this test, dehydro-ascorbic acid and iso-ascorbic acid were shown to have about 80 and 5% respectively of the activity of pure vitamin C, whilst gluco-ascorbic acid and ketogluconic acid were inactive. A complex of iron and ascorbic acid had an activity equivalent to the amount of ascorbic acid present.

F. A. R.

Analysis of Barley from King Tutankhamen's Tomb. E. C. Barton-Wright, R. G. Booth and W. J. S. Pringle (*Nature*, 1944, 153, 288)—The barley (*ca.* 1350 B.C.) was found to be extensively carbonised, but its structure was little damaged, the germ with its scutellum and embryo constituents being intact. It had, however, apparently lost considerably in weight, and its density was only *ca.* 67% of that of fresh English barley. Vitamin B_1 was absent, even from the dissected scutellum, but 0·85μg of riboflavin and 28 μg of nicotinic acid per g were found by micro-biological assay. The total phosphorus was 414 mg and phytase phosphorus 0·4 mg per 100 g (normal vals., 370 and 249 mg per 100 g, respectively), indicating almost complete hydrolysis of the phytic acid present; active phytase or phosphatase, however, was absent. The surprising survival of the riboflavin may be explained by the low relative humidity of the tomb and/or the presence of an oxygen-free atmosphere created by the respiration of the barley and oxygen absorption by other products stored with it; the development of acidity noted (*p*H of a 1% suspension, 4·1; fresh barley 6·25) may also be an influencing factor. Well-defined crystals (at present under examination) were also found in the germ.

J. G.

Determination of Zinc in Biological Material. J. Cholak, D. M. Hubbard and R. E. Burkey (*Ind. Eng. Chem., Anal. Ed.*, 1943, 15, 754–759)—Substitution of di-β-naphthylthiocarbazone for dithizone in the extraction of zinc from solns. of ashed material and the incorporation of other improvements have resulted in the development of sensitive and specific colorimetric and polarographic methods. The substitution has (1) eliminated partition losses occurring in the *p*H range 8·3 to 10·5; (2) also prevented the loss of zinc which otherwise occurs when carbamate is used to form a complex to remove interfering ions in the extraction soln.; (3) made possible standardisation curves which follow Beer's law over their entire range; (4) made possible the addition of carbamate in the initial extraction step—thus enabling the range to be fixed and zinc to be extracted in sufficient purity for either colorimetric or polarographic determination. Substitution of 0·2 *N* for 0·02 *N* hydrochloric acid enables at least 60μg of zinc to be removed from di-β-naphthylcarbazone-carbamate-chloroform soln. by means of a single 50-ml portion of acid, while preventing interference by copper, cobalt, nickel, mercury and silver. Colorimetric determination is recommended for very small quantities of zinc in absence of cadmium. In presence of cadmium, or when the quantities of zinc exceed 0·05 mg per 10 ml of soln., the polarographic method is preferable.

Agricultural

Rotenone in the Yam Bean (*Pachyrrhizus erosus*). L. B. Norton (*J. Amer. Chem. Soc.*, 1943, 65, 2259–2260)—Rotenone was extracted from the insecticidally-active resin of yam seeds, but does not account fully for either the toxicity or the red colour of the bean. A number of other compounds of yet unknown composition have been isolated, some giving the colour test and some showing toxicity to insects. E. M. P.

Organic

Micro-determination of Glycollic and Oxalic Acids. V. P. Calkins (*Ind. Eng. Chem., Anal. Ed.*, 1943, 15, 762–763)—On controlled reduction with powdered magnesium, oxalic acid yields glycollic acid, and the application of a colour test for glycollic acid may be applied to the micro-estimation of oxalic acid. By a suitable modification glyoxylic acid may also be determined, since it is likewise reduced to glycollic acid by powdered magnesium. When glyoxylic and oxalic acids occur in the same soln. the latter can be removed by pptn. from neutral soln. with calcium sulphate soln. When a soln. of glycollic acid reacts with a soln. of 2,7-dihydroxynaphthalene in conc. sulphuric acid, a violet red colour is formed on heating. The colour is probably due to condensation of formaldehyde (split off from the glycollic acid) with dihydroxynaphthalene to form colourless tetrahydroxydinaphthylmethane which is gradually oxidised to a deep red dye. Formic, acetic, oxalic, succinic, tartaric, citric, benzoic and salicylic acids do not interfere with the test. Colour measurement is made on a calibration curve prepared from known solns. of glycollic acid and read in a Coleman spectrophotometer at 530$m\mu$ or a Klett-Summerson photoelectric colorimeter with No. 52 filter. For determination of glycollic acid the soln. should be in 2 *N* sulphuric acid and should contain not more than 100μg of glycollic acid per ml. Measure 0·2 ml of the soln. into a 15-ml conical glass-stoppered centrifuge tube with a serological pipette and add from a microburette 2 ml of 0·01% soln. of 2,7-dihydroxynaphthalene in conc. sulphuric acid, keeping the tube cold in water. Mix by rotating the tube between the hands, and place it in a boiling water-bath for 20 min. and then allow it to cool to room temp. Dilute the soln. with 4 ml of 2 *N* sulphuric acid, shake carefully until the reaction has subsided, then shake vigorously and read the colour. After the colour has formed, practically any dilution vol. can be used if it is desired to reduce the sensitivity of the method so that higher concns. of glycollic acid can be determined. Even the vol. of test soln. can be changed within narrow limits. To determine oxalic acid, add 5 mg of powdered magnesium to 0·2 ml of 2 *N* sulphuric acid containing not more than 200 μg of oxalic acid. Rotate the tube between the hands and leave for 1 hr. Add 2 ml of the dihydroxynaphthalene reagent to the cooled liquid and proceed as already described. The calibration curve shows that Beer's law is obeyed from 0 to 200μg of oxalic acid, and, even with this range, the method is sensitive at low concns. (0 to 20μg). A. O. J.

Method for Studying the Effect of Antioxidants on the Oxidation of Aqueous Suspensions of Unsaturated Fatty Acids. A. Banks (*J. Soc. Chem. Ind.*, 1944, 63, 8–13)—Rapid oxidation caused by addition of small quantities of haematin to linolic acid suspensions in buffered 2% starch solns. can be measured by the Warburg or Barcroft technique at 30° C. Addition of an antioxidant to the system increases the length of the induction period, *i.e.*, the time taken to absorb the first 10 cb. mm of oxygen, and antioxidants can be graded according to their effect on this. *Method*—Heat 0·7 g of linolic acid, 2·5 ml of *N* potassium hydroxide and a little water on a steam-bath under an atmosphere of nitrogen. When the acid is dissolved, add 25 ml of a hot aqueous solution of 1 g of soluble starch, cool under nitrogen, and adjust to *p*H 6·6 and to a vol. of 50 ml by addition of phosphoric acid and water; determine the phosphoric acid required by electrometric titration of potassium linolate with phosphoric acid. For testing antioxidants, pipette 2·9 ml of the suspension into the Warburg differential manometer flask (*cf.* Dixon, "*Manometric Methods*"), together with 0·2 ml of an aqueous soln. of the antioxidant. Pipette 0·1 ml of a soln. of 7 mg of haematin in 100 ml of dil. borate soln. into the side tube and keep the manometer in a thermostat at 30° C. At zero time mix the haematin with the suspension and take readings at convenient intervals. With linolic acid used in test analyses the induction period was usually *ca.* 5 min.; 200 cu.mm. of oxygen were absorbed in *ca.* 30 min. Some antioxidants also affect the rate of oxidation after the induction period has passed. The initial stage of the oxidation is not catalysed by haematin. Progress curves are sinuous, even in absence of antioxidant, the second (rapid) oxidation stage being followed by a third in which oxidation becomes progressively slower. Results of tests, therefore, indicate the action of antioxidants on the spontaneous oxidation of suspensions of linolic acid at 30° C. Of numerous substances tested, certain dried flower petals, logwood extract, haematoxylin, haematein, brazilin and the usual antioxidants were found to be active for the system. According to preliminary tests, compounds containing a carboxyl, keto- or R.CH(OH) group are not good antioxidants. Pyrocatechol and its derivatives are much more active than quinol, and α-naphthol and 1 : 5-dihydroxynaphthalene are good antioxidants, but β-naphthol appears to be slightly pro-oxygenic. A "protection factor" consisting of the ratio of increase in length of induction period caused by antioxidant to the original induction period is used to grade compounds; Tables III–VI give these for the products discussed and others. Table II, giving the oxygen uptake at 30° C. of fish liver oil after oxidation at 100° C. and at room temp., indicates that temp. influences the nature of the oxidation. In the haematin—linolic acid system, the fatty acid is in suspension. No measurable oxidation occurs if haematin and linolic acid are in solution, *e.g.*, in alcohol. In foods, the fats occur in various physical systems and the behaviour of antioxidants in these may differ according to the system. Until the reason for this erratic behaviour of antioxidants is understood there will be difficulty in applying them to increase the keeping qualities of foods containing fats. E. B. D.

Analysis of Rosin Size. Anon. (*Paper Trade J.*, 1944, 118, Jan. 6th, *T.A.P.P.I. Sect.*, 7–10)—The T.A.P.P.I. Tentative Standard described is used for saponified paste, dry and emulsified rosin sizes only. *Free Rosin*—(1) Dissolve approx. 5 g (±5 mg) of the sample in 10 ml of neutralised ethyl alcohol, rinse the soln. into a separating funnel with 40 ml

of acid-free ether and three 10-ml portions of water. Shake, remove the aqueous layer, extract it with 25 ml of the ether, again remove the aqueous layer and re-extract with ether; then extract the combined ethereal extracts with water, reject the aqueous layer, evaporate the ether and dry the residue at 105° C. until of const. or min. wt. Addition of 10 ml of alcohol to the rosin facilitates drying; over-drying may result in oxidation and an increase in wt. This method includes unsaponified material, and there is a danger of high results through hydrolysis of some of the combined rosin. (2) The alternative titration method proposed is more rapid, but the acid val. of the rosin present must be known or assumed. Dissolve 4·0–4·5 g (±5 mg) in 100 ml of anhyd. *iso*propyl alcohol added a few drops at a time, stir to a smooth paste, and dilute with the rest of the alcohol so as to avoid lumps. Then add exactly 0·5 ml of a 1% soln. of thymol blue, and titrate with a filtered 0·5 *N* soln. of potassium hydroxide in *iso*propyl alcohol (standardised against 0·5 *N* hydrochloric acid to thymol blue). If the titration is carried out in the light of a Wratten filter the transmission band which is almost the same as the absorption band of the indicator in alkaline soln., the colour of the soln. changes from yellow-green to dark green, and at the end-point there is a sharp decrease in the amount of light transmitted by the soln. This procedure minimises difficulties due to the colour of the rosin soln. For most purposes an acid val. of 162 may be assumed. *Free Alkali*—The method is based on the insolubility of sodium carbonate and bicarbonate in anhyd. *iso*propyl alcohol, which dissolves the rosin. Dissolve 5–7 g (±5 mg) in 35 ml of the neutral alcohol in a 100-ml beaker as described above, put 15 ml of the alcohol over the surface of the soln. to prevent creeping, and place the beaker in an air-tight container overnight (to protect it against acid fumes and moisture). Filter through a dry No. 40 Whatman filter-paper, and wash the residue with small portions of the hot alcohol until free from rosin. Then wash it with ten 10-ml portions of cold distilled water (previously boiled to remove carbon dioxide), add to the new filtrate so obtained 5–8 drops of a neutral 10% soln. of phenolphthalein in 50% alcohol, and titrate with 0·1 *N* hydrochloric acid until the pink colour disappears; then titrate with 2 drops of 0·1% methyl orange soln. as indicator in the usual way. To determine moisture and volatile matter heat at 105° C. until there is no further loss; determine total rosin by extraction with ether and washing the extracts with water, and unsap. matter, ash and alkalinity of the ash by the usual methods. J. G.

Polymer Distribution of Varnish Resins. H. E. Adams and P. O. Powers (*Ind. Eng. Chem., Anal. Ed.*, 1943, 15, 711–714)—Schulz has shown (*Z. phys. Chem.*, 1937, A179, 321) that the amount of non-solvent required to ppt. a polymer from solution is a measure of its degree of polymerisation. Thus the distribution of polymers in a resin may be estimated if the optical transmission during pptn. and the concn. of non-solvent required to ppt. a polymer are known. Even where the relation between the polymer and the concn. of non-solvent is not known, the results are valuable in comparing resins of the same class. The titration was conducted in a square pint jar painted black except for two windows on opposite sides (2·5 × 7·5 cm). Light entered through one window from an automobile head lamp with a parabolic reflector, which was covered except for a small window. The photronic cell (Weston No. 594) was placed in front of the other window. The potentiometer included 5 high resistances (carbon resistors ranging from 957 to 97,000 ohms) through any one of which the circuit could be closed, and these made it possible to titrate solns. of dark coloured resins, since changes in transmission at low intensities can be measured if a high resistance is used. A 1·5 volt cell was the source of current. The soln. of resin and the non-solvent were brought to 25 °C. For titration 80 ml of resin soln. (usually in toluene at 0·05% solids) were placed in the jar and the non-solvent (usually methanol) was added, the mixture being stirred by an air-driven stirrer at a rate that ensured complete mixing without introducing air bubbles. When pptn. was about to occur, the microammeter was balanced, the resistance giving the highest reading on the potentiometer scale being used. Small amounts of methanol were added and the circuit was again balanced. The reading just before precipitation was taken as a measure of the initial transmission I_0, and the transmission I was determined after each small addition of precipitant. Decrease in transmission occurs rapidly at first, and titration is complete when further addition does not decrease the transmission. Contrary to expectation, transmission did not increase with dilution. To establish the range of pptn. and conditions for titration, 0·5% solns. in toluene of various varnish resins (*e.g.*, coumarin-indene resins of successively decreasing mol. wt., malein-rosin resin and a rosin-modified phenolic resin) were pptd. by addition of methanol. The curves obtained with concn. of non-solvent as abscissa and extinction ($-\log I/I_0$) as ordinate show the wide range of composition of commercial resins. The titration curves appear to be a sufficient basis for comparison of commercial varnish resins, but to correlate the titration with polymer distribution, a coumarin-indene resin was fractionated by partial soln. and the mol. wts. of the 7 fractions were determined by depression of m.p. of benzene. The first 2 more sol. fractions did not ppt., the other fractions gave varying amounts of ppt., only the last being entirely pptd., showing presence of lower polymers in these fractions. Since the decamer was pptd. at 37% and the tetramer at 73% of non-solvent, the Schulz equation (*loc. cit.*) $\gamma = A + B/X$, where γ is concn. of non-solvent, X the degree of polymerisation and A and B constants, could be used to estimate the range in which each polymer is thrown down. From the above-mentioned values $\gamma = 0{\cdot}13 + 2{\cdot}42/X$. The amount in each fraction can be estimated by the differential of $-\log I/I_0$. A similar estimate of the distribution made from the titration of the original resin shows a somewhat narrower range, and apparently the lower polymers are not estimated by this method. The high polymers often determine the character of a resin and an estimate of their range and amount affords significant information. With rosin-modified phenolic resins, pptn., as measured by the extinction at low non-solvent concn., indicated the more viscous, higher melting and less soluble resins. A. O. J.

Application of Alkaline Nitrobenzene Oxidation of Plant Materials to Taxonomic Classification. R. H. J. Creighton, R. D. Gibbs and H. Hibbert (*J. Amer. Chem. Soc.*, 1944, 66, 32–37)—In the expts. described the air-dried wood chips were ground to pass a 40-mesh sieve, and the resulting wood meal was extracted successively with ethanol-benzene (50 : 50) for 48 hr., ethanol

(24 hr.) and hot water (12 hr.) and then air-dried. The moisture was determined by drying 2 g overnight at 105° C., and the Klason lignin was determined on the oven-dried sample by the method of Ritter, Seborg and Mitchell (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 202) involving the use of 75% sulphuric acid. Alkaline nitrobenzene oxidation was carried out on the extracted wood residue by the method previously described (*Ind. Eng. Chem., Anal. Ed.*, 1941, **63**, 3049; *cf.* next abstract).

The commonly accepted pteridophytes and gymnosperms (*e.g.*, bracken, white spruce, hemlock, white pine, redwood) gave only vanillin in yields of 15–24% calculated on the Klason lignin content of the wood, whilst the dicotyledons (*e.g.*, red maple, aspen, yellow birch, slippery elm, black walnut, jute fibre) yielded 35–51% of a mixture of vanillin and syring-aldehyde in approx. ratio of 1:3. The monocotyledons (*e.g.*, *Aloe abyssinica*, bamboo, rye straw, corn stalks, sugar cane stem) gave lower yields of mixed aldehydes (16·3–37·1%). In the grasses (except sugar cane stem) the presence of a third (non-methoxy) aldehyde, admixed with the vanillin, was indicated by the lower methoxyl values (*cf.* next abstract). On the assumption that native maple lignin is derived essentially from a mixture of anhydro polymeric forms of coniferyl and syringyl alcohols, the highest combined aldehyde yield (silver maple 51%) would amount to 61% of the Klason lignin. Exceptions to the above ratio (1 : 3) were observed with certain primitive angiosperms (*e.g.*, *Belliolum haplopus*, *Zugogynum vieillardi*) characterised by absence of vessels; with these the ratio was 1 : 1. All genera of the *Gnetales* (*e.g.*, *Sorbus americana*, *Robinia pseudacacia*) yielded both vanillin and syringaldehyde, a result in harmony with their high lignin and methoxyl values and the pronounced red colour obtained with Maule's lignin reagent. Their relationship to the angiosperms rather than the gymnosperms is thus indicated. Only a very few members of the *Coniferales* (*Podocarpus amarus*, *P. pedunculatus*, *Tetraclinis articulata*) yielded, on oxidation, a mixture containing both vanillin and syringaldehyde and gave a definite Mäule reaction. The results of the colour reactions in the Mäule test given by the 55 plants investigated agreed completely with those obtained on alkaline nitrobenzene oxidation.

Alkaline Nitrobenzene Oxidation of Corn [Maize] Stalks. R. H. J. Creighton and H. Hibbert (*J. Amer. Chem. Soc.*, 1944, **66**, 37–38)—In the alkaline nitrobenzene oxidation of woods (*cf.* preceding abstract) certain monocotyledons, notably corn, bamboo and rye straw, yielded, in addition to vanillin and syringaldehyde, a non-methoxylated carbonyl derivative which could not be separated from vanillin by sublimation. A separation has been based on solubility differences in cold benzene, and the constituent has been identified as *p*-hydroxybenzaldehyde. In the expts. described 50 g of corn-stalk meal (previously extracted successively with ethanol-benzene, ethanol and hot water) were heated at 160° C. with 30 ml of nitrobenzene and 600 ml of 2 *N* aqueous sodium hydroxide for 3 hr. with vigorous agitation. The resulting crude bisulphite-soluble oil (1·503 g) yielded on distillation two crystalline fractions: (i) b.p. 130°–160° C. (0·2 mm), 0·825 g; (ii) b.p. 210°–230° C. (0·25 mm). The second fraction was recrystallised from water and gave syringaldehyde, m.p. 110°–112° C. Fraction (i) was mixed with 10 ml of dry benzene and stirred for 1 hr. at 20° C., the mixture was then centrifuged, and the benzene-soluble portion was evaporated and re-treated in the same way with a further 5 ml of benzene. Recrystallisation of the sol. fraction yielded 0·451 g of pure vanillin, m.p. 81–°82° C. The benzene-insol. fractions, recrystallised from water, yielded 0·126 g of *p*-hydroxybenzaldehyde, m.p. 116°–117°C. The respective yields of the three aldehydes were 4·5, 2·6 and 1·4% based on the Klason lignin content. The presence of free *p*-hydroxybenzaldehyde may serve as a distinguishing characteristic between the mono- and dicotyledons.

Inorganic

Analysis of Brass by Vacuum Distillation. W. D. Treadwell and G. Frey (*Helv. chim. Acta*, 1944, **27**, 42–50)—The sample (0·5–1 g) of the brass in the form of fine filings is heated at 800–850° C. in a vacuum of 10^{-4} mm of mercury. The sample is contained in a porcelain boat, which is introduced about 25 cm into a steel tube about 16 mm diam. × 50 cm long. The middle part of the tube around the boat is surrounded by an electrical resistance wiring as source of heat, and around the tube on either side of the heater is a welded-on water jacket to provide cooling surfaces for condensing the zinc. Connections to the steel tube for exhaustion and for insertion of a thermocouple are made by flanged joints. The method was tested on samples covering a range of copper contents. Brasses with up to *ca.* 70% of copper lose zinc quantitatively in 2½–3½ hr., leaving a porous residue of bright red copper. Lead is also quantitatively volatilised. Brasses with higher copper, *e.g.*, 85%, lose zinc much more slowly, and require heating for *ca.* 10 hr. The initial addition of zinc to the sample of high-copper brass filings is advantageous; zinc vapour is absorbed during the heating-up, causing a broadening of the lattice, which allows zinc to be lost more readily at the higher temperature by reason of the increased internal surface. The effect is said to be analogous to the behaviour of silver-gold alloys in acid attack. Heating the brass sample to the m.p. slows down the rate of loss of zinc, while overheating may result in mechanical loss through boiling-out of zinc. A factor controlling the time required in the method is the rate of diffusion of zinc from the centre of the particles of brass. The filings should therefore be as fine as possible (about 0·1 mm).

S. G. C.

Determination of Tetraethyllead in Gasoline [Petrol]. H. Gonick and J. J. Milano (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 4–6)—To 100 ml of gasoline in a conical 500-ml flask add 50 ml of a saturated soln. of iodine in carbon tetrachloride and set aside for at least 5 min. Evaporate to dryness with the aid of a hot air stream. Add 25–50 ml of strong nitric acid and manipulate the flask over a free flame for the expulsion of iodine and nitrogen peroxide, dislodging organic matter from the walls of the flask. Add solid potassium chlorate to the briskly boiling soln., avoiding evaporation to dryness. Usually 2–3 g will destroy the whole of the organic matter, otherwise add another 2–3 g to the boiling soln. Evaporate the clear liquid to dryness, adding more chlorate if darkening occurs, and fuse the dry residue, which should be white. Cool, boil with hydrochloric acid (1 : 1), and determine the lead in the soln. (*cf.* ANALYST, 1943, **68**, 345). The authors describe an acidimetric method based on formation of a compound of lead with 8-hydroxyquinoline, with liberation of an equivalent quantity

of acid. The operator is advised to wear a mask while working with chlorate, although the authors did not experience serious explosions. W. R. S.

pH Determinations of Water in Contact with Stones. H. G. Williams (*J. Soc. Chem. Ind.*, 1943, **62**, 209–212)—Determinations, by means of the glass electrode, of the *p*H of water kept in contact with stones in Pyrex glass bottles, with slight hand-shaking, shows that the classification into "acidic" stones and "alkaline" stones is erroneous. Of the samples of English and French stones tested, all were found to be more or less alkaline to water, except quartz and some gravels; the order of decreasing alkalinity was: limestones, porphyre, granites, quartzites, and some gravels. Typical values for English stones are shown in the table.

Stone	Source		*p*H value after (days) 1	2	4	7	14	26
Limestone	Derby	(*a*)	9·09	8·95	8·60	8·38	8·28	—
,,	,,	(*b*)	8·97	8·79	8·45	8·29	8·23	—
,,	Weston-super-Mare		—	—	8·18	8·04	—	—
,,	Wells		8·87	8·67	8·42	8·30	8·24	—
,, (2-in size)			8·50	8·42	—	8·14	8·07	—
Granite	Guernsey	(*a*)	8·47	8·37	—	8·20	—	—
,,	,,	(*b*)	8·47	8·39	—	8·23	—	—
,,	Mt. Sorrel	(*a*)	8·29	8·28	—	—	—	—
,,	,,	(*b*)	8·37	8·28	—	—	—	—
Quartzite	Glos.	(*a*)	7·14	7·46	7·50	7·27	6·84	—
,,	,,	(*b*)	7·18	7·47	7·51	7·29	6·85	—
Gravel	Ham River	(*a*)	6·37	6·75	6·50	6·45	5·67	—
,,	,,	(*b*)	5·76	6·26	6·04	6·03	5·28	—
,,	,,	(*c*)	6·80	—	6·67	—	—	—
Gravel (crushed)	Thames Valley	(*a*)	7·44	7·67	7·91	7·93	—	7·93
,,	,,	(*b*)	7·27	7·41	7·60	7·39	—	7·28

Distilled water used	initial *p*H	1	2	3 (days)
	5·84	6·25	6·24	6·25

The change in *p*H with time indicates that the isolated *p*H values after a given time, which are usually quoted, are not representative of the behaviour of the stone in contact with water. It was further found that the smaller the grading size of the stone, the higher is the max. *p*H reached and the more rapid is the rise in *p*H, where alkaline; any exceptions to this appear to be due to slightly more soluble salts, present only on the surface of the larger stones. Washing the stones, or soaking, followed by contact with fresh water, lowers the alkaline *p*H values, owing to the removal of the more soluble alkalis and to the small amount of dust left after air-blowing. The amount of the dust is insufficient to explain the *p*H obtained, and if it is washed off the salts are also removed, altering the "characteristic" *p*H of the stone; the presence of dust, however, does increase the *p*H slightly over the "true" value for a given grading and the increase would be relatively greater for the larger sizes. Qualitative analysis of the contact waters gave the following results.

Stone	Source	Found	Traces found	*p*H
Limestone	Derby	Ca, Cl, silicate, CO_3	Na, Fe, SO_4	9·82
	Weston-super-Mare	Ca, Cl, CO_3	Na, SO_4, silicate	9·51
Granite	Guernsey	Na, Ca, Mg, CO_3	Fe, K, silicate, Cl	9·13
Porphyre	St. Raphael, France	Na, Cl, CO_3	SO_4	—
Gravel	Thames Valley	Mg, CO_3	Na, K, Cl, SO_4	—

E. M. P.

Polarographic Analysis of Dilute Solutions of Bismuth. D. F. Swinehart, A. B. Garrett and W. M. McNevin (*Ind. Eng. Chem., Anal. Ed.*, 1943, **12**, 729–731)—Polarograms for the reduction of bismuth in hydrochloric acid exhibit erratic maxima and are generally unsuitable for the determination of bismuth. More promising results are obtained from the polarographic analysis of solns. of bismuth in the form of the complex tartrate in acetate-buffered solns. Over the range 10^{-5} to 3×10^{-3} *M* these solns. can be analysed with an average error of 1 mg of bismuth per litre. B. S. C.

Spectrographic Determination of Nickel and Chromium in Stainless Steels. J. H. Coulliette (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 732–734)—With an A.R.L.–Dietert commercial type high-voltage spark circuit with synchronous interrupter spectra of stainless steels can be excited with sufficient reproducibility to determine nickel and chromium to within 5% of the value obtained by routine chemical determination. The lines Ni 3087·1A, Cr 3169·2A and Fe 3259·0A are suitable for the construction of working curves. The range of compositions investigated was 27·7% to 32·2% of chromium and 14·3% to 24·5% of nickel. The primary advantage of the method is speed, the precision being sufficient for the control of melting operations of stainless steel. Analyses have been made in 9 min. from the time of taking the samples to reporting the results. B. S. C.

Volumetric Determination of Iron with the Silver Reductor. J. L. Henry and R. W. Gelbach (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 49)—In the method of Walden, Hammett and Edmonds (*J. Amer. Chem. Soc.*, 1934, **56**, 350) a silver reductor is used and the ferrous iron is titrated in sulphate soln. with ceric sulphate. The authors

reduce the ferric chloride soln. in *M* hydrochloric acid with the silver reductor and titrate with dichromate after addition of diphenylamine indicator and 5 ml of 85% phosphoric acid. Manganese, chromium, titanium, and vanadium at concns. of less than 0·1 g in 200 ml, do not interfere.
W. R. S.

New Gravimetric Reagent for Tungsten. J. H. Yoe and A. L. Jones (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 45–48)—Anti-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene is proposed as a substitute for cinchonine, unobtainable in wartime. The compound is supplied by La Motte Chemical Products Co., Baltimore, Md. It forms yellow needles soluble in alcohol (0·766 g in 100 ml), insoluble in water. The alcoholic soln. is added to the cold acidified tungstate soln. (hydrochloric acid to 0·2 *N*). The yellow ppt. is set aside for 3 hr., washed with cold acidulated water containing a little reagent, and ignited to WO_3. The pptn. of molybdenum is of the same order as with cinchonine, and the weighed WO_3 must be tested for molybdenum and iron by the usual methods. Detailed descriptions of assay methods for ores and alloys are given. W. R. S.

Colour of Dichromate Solutions. R. E. Kitson and M. G. Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 42–44)—Solns. of potassium dichromate and chromate are used as colorimetric standards in the determination of silicomolybdic acid, carotene, etc. The effect of various factors on the colour was studied spectrophotometrically. A low *p*H gives orange tints, a high *p*H yellow ones. There is an intermediate range (*p*H 5–8) in which the tint is very sensitive to small variations. Sulphuric acid causes greater variations in colour than other acids. Solutions containing hydrochloric acid fade (about 2% in 48 hr. with 0·8 *M* acid) and should not be used as permanent standards. Excess of caustic alkali has little effect in chromate solns., but strongly alkaline solns. attack the glass and cause turbidity. When acidified dichromate solns. are to be used, the nature and amount of acid or acids added should be accurately specified.
W. R. S.

Stability of Solutions of Copper Perchlorate and Potassium Iodate. J. J. Kolb (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 38–39)—The solns. can be used as primary standards for thiosulphate titrations. Solns. of copper perchlorate are more stable than those of potassium iodate. Rubber-stoppered bottles may be used for the former, glass-stoppered ones must be used for the latter. Thymol should not be used as a preservative.
W. R. S.

Microchemical

Micro-determination of the Formyl Group. J. F. Alicino (*Ind. Eng. Chem., Anal. Ed.*, 1943, 15, 764)—Micro-determination of the acetyl group is now widely used, but little attention has been given to analysis of the formyl radical. It was found that the formyl group can be determined under the same conditions as the acetyl group and that since formic acid and bromine react quantitatively in 0·01 *N* soln., the determination can be made specific. Formic acid (0·01 *N*) was standardised by means of excess of potassium iodate and potassium iodide, and 0·01 *N* bromine soln. was standardised by titration of iodine liberated from potassium iodide. A measured amount of formic acid was shaken thoroughly in a stoppered flask with excess of the bromine soln., and after 5 min. the residual bromine was determined. The results agreed satisfactorily with the calculated results. The Elek-Harte method for determination of the acetyl group (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 267) was applied to the following formyl derivatives —triformyl cholic acid, diformyl desoxycholic acid, cholesterol formate, S-benzyl-N-formyl-*dl*-cysteine, S-benzyl-N-formyl-*d*-cysteine and methyl formanaline, and where material was available (*i.e.*, with the first 3 compounds) the modified method, using the reducing action of the liberated formic acid, was followed. Correction for the amount of sulphur dioxide liberated from the saponifying agent is seldom necessary if the procedure is carefully controlled, and water can be used in the receiver where there is a question of the nature of the acid group. The distillate is treated quickly in a stoppered flask with excess of the bromine soln. and the residual bromine is titrated as described. If both formyl and acetyl may be present, a total acid titration should be made with an aliquot portion of the distillate. The method gave satisfactorily accurate results both for formyl found as formic acid and for formyl found as reducing agent. The bromine soln. must be stored in a refrigerator and its normality checked frequently. A. O. J.

Physical Methods, Apparatus, etc.

Fused Salt Technique in Spectrochemical Analysis. N. H. Nachtrieb, D. H. Johnson and K. S. Dress (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 734–736)—The spectrographic determination of antimony, vanadium, chromium and iron in titanium oxide may be made by fusing the powdered sample with potassium bisulphate and coating the tips of graphite electrodes with the molten soln. In addition to providing a simple means of preparing homogeneous standards, this technique eliminates the undesirable sputtering characteristics of the titanium dioxide arc. Procedure—Fuse together 100 g of spectrographically pure potassium bisulphate and 50 g of pure titanium dioxide in a porcelain dish. Add to the melt sufficient antimony trioxide, ferrous ammonium sulphate, vanadous chloride and potassium dichromate to give 1% of antimony, 0·1% of iron, 0·2% of vanadium and 0·1% of chromium, all based on titanium dioxide. After mixing the melt, dip the tips of preheated graphite electrodes into it so as to obtain a thin vitreous coating. After solidification, crush and weigh the remainder of the melt and re-fuse with additional potassium bisulphate and titanium dioxide to obtain a fresh set of standards containing half the impurity content of the former set. Repeat this process to cover the impurity range desired. Prepare similar electrodes with the samples of titanium dioxide of unknown purity. Excite the spectra, using a 2200v A.C. arc with a gap of 1 mm and current of 4·5 amp. On a large quartz spectrograph with wavelength range set at 3400A to 2500A the exposure needed will be 1 min. with a slit width of 30μ. From microphotometer readings on lines of the known standards construct working curves, using the following line pairs: Sb 2598/Ti 2590·3, V 3183·4/Ti 3179·3, Fe 3020·6/Ti 3002·7 and Cr 3021·6/Ti 3002·7.
B. S. C.

Simple Device for Quantitative Ultra-Filtration in Chemical Analysis. W. R. Thompson and J. J. Quigley (*J. Biol. Chem.*, 1943, **151**, 343–347)—For the preparation of filtrates which would not foam excessively in the van Slyke–Neill chamber during estimation of sugar by direct yeast fermentation, ultra-filtration was adopted to remove the proteins responsible for frothing. The membranes were prepared in alundum crucibles as follows. Ignite the crucibles at 555–560° C. and store them at room temp. in a desiccator until required. Prepare a nitrocellulose sol by shaking nitrocellulose with glacial acetic acid intermittently for 2–4 weeks, heat the mixture to 90° C. and then allow it to cool slowly to room temp.; store the sol in the refrigerator until required. Put about 25 ml of the sol into a crucible and transfer this to a crystallising dish in a vacuum desiccator evacuated to a pressure of 80 mm of mercury. After 1 hr. admit air slowly to the desiccator, remove the crucible and decant the contents. Coagulate the nitrocellulose sol in 25% alcohol to form a semi-permeable membrane and, after 30 min., transfer the crucible to a bath of running tap-water for 24 hr. The crucibles may be stored in distilled water containing a small amount of hypochlorite.

F. A. R.

Potentiometric Method to Determine Cations and Anions with Collodion and Protamine-Collodion "Membrane Electrodes." K. Sollner (*J. Amer. Chem. Soc.*, 1943, **65**, 2260–2261)—Collodion membranes of high ionic selectivity (Sollner, Abrams and Carr, *J. Gen. Physiol.*, 1941, **25**, 7; Carr, Thesis, Univ. of Minnesota, 1943) can be used for the potentiometric determination of K^+, Na^+, Li^+, NH_4^+, Mg^{++}, and possibly some other cations. Also, collodion membranes impregnated with protamine are electropositive, and can be used for the determination of Cl^-, Br^-, I^-, F^-, ClO_3^-, ClO_4^-, BrO_3^-, IO_3^-, NO_3^-, acetate$^-$, etc. The determinations may be made by evaluating on the basis of a theoretical semi-standard curve the potential difference between a known soln. on one side of the membrane and a soln. of unknown concn. on the other side. Or the potential values may be compared with an empirical curve, determined in advance for a specific membrane. In a third method, the membrane separates water from a soln. of unknown strength, and an electrolyte soln. of known higher concn. is added to the water until the potential difference is reversed. Some results obtained by the third method are cited.

E. M. P.

Cellulose Acetate Mounts for Rock and Mineral Fragments. A. J. T. Dollar (*Nature*, 1944, **153**, 226)—The embedding of rock or mineral particles in a uniform thin sheet of cellulose acetate facilitates their optical examination, transport and storage, especially during field studies; more than 100-fold the quantity of such particles can be mounted together than in any normal Canada balsam/glass mount, although the main microscopical advantages of the latter are retained. More accurate quantitative mineralogical analyses of certain sands are thus possible, *e.g.*, by accommodating in a single mount the whole heavy-mineral concentrate from a bulk sample of greater vol. than it has hitherto usually been convenient to study. Areas of the film containing grains of special importance can be marked with ink, greasy crayon or gummed paper masks, or cut out for special treatment. The original mount should be colourless, transparent and isotropic when unstrained, with n_D^{20} approx. 1·4, depending upon the exact composition of the dispersion. Its composition is identical with that of the laminar moulds used by the author (ANALYST, 1943, **68**, 347) for studying the finer structures of rocks, mineral and metal surfaces. Allow a cold dispersion of cellulose acetate in a mixture of 1 vol. of tetrachloroethane with 2 vol. of "Cerric Thinner T10" (Cellon, Ltd., Kingston-on-Thames) containing about 20% (on the wt. of cellulose acetate) of a plasticiser (*e.g.*, triphenyl phosphate or dimethyl phthalate) to flow over a level sheet of clean plate glass (23 × 10·5 × 0·6 cm.) to a depth of 0·1–0·15 cm, according to the max. diam. of the particles concerned. Immediately moisten the sample or its sieved fractions with tetrachloroethane, and shake it as evenly as possible over the dispersion layer, into which it sinks. After about 8 hr. the compact film remaining after the volatile portions of the dispersing medium have evaporated, has a thickness equal to about 10% of that of the original fluid layer. Then strip the film from the glass, label it, and trim its edges. A trace of a suitable dye, added to the parent dispersion, serves to distinguish any particular set of mounts. Rectangular films (23 × 1·5 × 0·01 cm) are of a convenient shape and size for microscopical examination and can be stored or posted in standard foolscap paper envelopes without further protection. Such mounts develop no appreciable discoloration, brittleness or shrinkage after 3 years of storage in these envelopes.

J. G.

Measurement of the Permeability of Paper and Paperboard. Anon. (*Paper Trade J.*, 1944, **118**, 3rd Feb., *T.A.P.P.I. Sect.*, 37–39)—The method described is the T.A.P.P.I. Official Standard, T 448-m-41, and is suitable for sheet materials up to 1 in. in thickness. Place sufficient calcium chloride or anhyd. magnesium perchlorate (in small lumps, free from fines passing a No. 30 screen) in an open-mouthed cup or dish (area, at least 30 sq.cm.) to a depth of at least 15 mm. Suitable designs for the dish, with supporting flanges and rings, are illustrated. Cut a circle of the specimen (with the aid of a template) with such diam. that it closes the aperture of the dish and rests on the supporting flange. Seal the specimen to the edge of the dish with a molten mixture of equal parts of rosin and beeswax, so as to obtain an air-tight joint. Weigh the assembly to 1 mg, using as a tare a similar dish covered with the specimen, but containing no desiccant. Place the dish on a rack in a cabinet or room at 50% relative humidity and 73° F. in an inverted position, so that an even layer of desiccant is in contact with the inside face of the test sheet, and that free access of the conditioned air, circulating continuously at not less than 500 ft. per min., is available over the other. Weigh the assembly at suitable intervals until the gain in wt. is const. With relatively pervious papers, complete the test by making frequent weighings before the desiccant cakes or forms drops of liquid agglomerate (indicated by a "drift" from the const. rate of gain). Plot the gain in wt. against the time; the slope of the linear portion of the curve is a measure of the permeability, and results are reported to 3 significant figures as g per sq.m. per 24 hr., for each side of the paper, separately. Duplicate determinations should agree within 10%.

J. G.

Reviews

LUMINESCENCE OF LIQUIDS AND SOLIDS AND ITS PRACTICAL APPLICATIONS. P. PRINGSHEIM and M. VOGEL. Pp. x + 201, 72 Figs. New York: Interscience Publishers, Inc.; London, Imperia Booksellers, Ltd. 1943. Price $4.00.

No one book treats of the whole subject of luminescence; indeed, such an undertaking might well require the services of a team of specialists. The present volume sets out to fill a gap, defined by its title, in the literature of luminescence and adequately fulfils its object.

Where luminescence phenomena can be made useful for analysis or for light production, the physical laws underlying such uses, or the techniques employed, are succinctly discussed in the first half of the book. This portion includes a discussion on the theories of luminescence, a chapter on sources of primary radiation, filters, photometry, spectroscopy, phosphoroscopes and fluorimeters, and fluorescence microscopy. A further chapter deals with luminescent materials and their properties. Much in these chapters is of interest to those engaged in analytical and control work who can adapt the information set forth to their own particular problems.

In the chapter on luminescent materials adequate stress is laid on the necessity for caution in accepting any statement concerning the fluorescence of a solid material, for often the fluorescence probably depends upon the presence of a trace of an impurity. The fluorescence appearances of solid alkaloids have been described in the literature by several workers and the fact that the same alkaloid has a different appearance ascribed to it by each worker is cited as an example of pitfalls attendant on too implicit confidence being placed in the results of the fluorescence examination of solids. Many other examples could be given, by others working in this field, to support the authors' contention. This chapter deals with organic and pure inorganic compounds, with synthetic inorganic phosphors and with minerals and glasses. The section on synthetic inorganic phosphors emphasises the difficulties of manufacturing reproducible products on any appreciably commercial scale.

The second half of the book deals with the applications of luminescence, Chapter V with fluorescence analysis and testing and Chapter VI with luminescent substances as light sources (such as fluorescent and phosphorescent paints), fluorescent television and X-ray or photographic intensifying screens, and fluorescent lamps. A final chapter gives some useful tables of the more important luminescent substances.

Professor Pringsheim considers that the potentialities of fluorescence analysis have been over, rather than under, estimated and devotes some thirty pages to this aspect of the subject. In the Preface he has stated that a certain degree of criticism is desirable to serve as an antidote to the over-enthusiasm of those inclined to exaggerate the possibilities of fluorescence analysis. In this chapter he therefore gives a number of tests, based on spectrographic records of fluorescence, which are of unimpeachable reproducibility. He emphasises that the fluorescence method will only be useful when combined with other tests. His conclusion is that "from any organic compound some fluorescent substance may be derived somehow. The question is whether other compounds might not produce similar fluorescence after having undergone the same procedure." Although this is true, few chemists would be content to apply a single test to a substance or mixture, and let it go at that. Every year brings forward fresh work to swell the number of confirmatory fluorescence tests for various substances. For any compound, each test requires a different procedure, and the number of compounds reacting similarly is disproportionately reduced with each succeeding test applied.

Fluorescence as a test for purity is dealt with by pointing out the many faulty inferences that can be drawn when unknown impurities are present. A useful survey of quantitative fluorescence analysis and of fluorescent indicators follows and is of particular interest to analysts. In a section on the "Technical Identification of Materials" the author appears to share in the over-enthusiasm he has earlier deprecated. "Not only will almost every white or colourless material like paper, linen, porcelain or glass, coming from different sources and hardly distinguishable in daylight have very different fluorescent properties, but the same is true for coloured materials. . . . It is utterly improbable that two samples show the same daylight colour and the same fluorescence if they are not really identical." The first statement is hardly borne out by practice and is of the type that brought fluorescence analysis into disrepute in the early days of its inception. The second is also much too sweeping.

As a well-known, distinguished doctor of philosophy would say, "It all depends what you mean by 'same'."

The author's surmise as to the reason for adding a fluorescing agent to mineral oils is incorrect. It is done to restore the natural fluorescence, which is destroyed in refining, and which makes the high viscosity of the oil apparent to the eye.

Throughout the book there appear a number of self-evident misprints, the most serious being the value for Planck's constant on p. 9. German and English spellings of the same compound sometimes appear on the same page. The book is nicely produced, on good paper, and should give several hours' pleasant and instructive reading to all interested in luminescence.

J. A. RADLEY

SOLVENTS. T. H. DURRANS. 5th Edition. Pp. 216. London: Chapman & Hall, Ltd 1944. Price 17s. 6d. net.

This edition occupies less bulk than its predecessor but the material content is much the same—some obsolescent material has been removed and some new material added here and there—and the work still stands as an excellent up-to-date monograph on the subject of solvents and the uses to which they are put in industry.

To have reached five editions since 1930, with one third of the time given over to war, during which the use of solvents has necessarily been circumscribed by war priorities, is sufficient testimonial of its value. The main change in the structure and content of the book took place in the 4th edition (1938) when the author introduced much new matter touching the scientific principles involved in the use of solvents and re-wrote the chapter on plasticising solvents.

In dealing with toxicity the author has adopted a cautious attitude to the subject as a whole, but has attempted to give guidance upon dangers that may be encountered through inhalation of air charged with solvent vapour under practical working conditions.

L. A. JORDAN

INAUGURAL MEETING OF THE MICROCHEMICAL GROUP

IN view of the present uncertain conditions and of the extreme difficulties of travelling long distances, the Council has decided to postpone the inaugural meeting of the Microchemical Group until the day of the Ordinary Meeting of the Society in October. Members of the Group will receive notification of the meeting in due course.

PROPOSED FORMATION OF A GROUP DEALING WITH PHYSICAL METHODS OF ANALYSIS

IN pursuance of the policy for the formation of Groups for special branches of analytical chemistry proposed by the Council and approved by a meeting of the Society on November 3rd, 1943, the Council has had under consideration a proposal to form a Group dealing with physical methods of analysis. The Group would deal with such methods as for example:

1. Spectrographic methods
   (*a*) Emission spectrograph;
   (*b*) U.V. and visible absorption spectrograph;
   (*c*) Infra-red absorption spectrograph;
   (*d*) Mass spectrograph.
2. Quantitative photometric methods by means other than spectrophotometry.
3. Polarographic methods.
4. X-Ray diffraction.

The Council's decision as to the formation of the Group will depend on the number of members of the Society desirous of joining it. Members of the Society who wish to become members of the Group are asked to notify the Hon. Secretary of the Society, 7/8, Idol Lane, London, E.C.3.

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Printed for the Society of Public Analysts by W. Heffer & Sons Ltd., Hills Road, Cambridge.
Communications to be addressed to the Editor, C. Ainsworth Mitchell, The Close, Weedon, Aylesbury, Bucks.