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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Deaths

WE regret to have to record the deaths of the following members:

Frank William Harbord
John Bristowe Pease Harrison
George Alfred Stokes

Joint Meeting with the Food Group of the Society of Chemical Industry

THE Joint Meeting held on December 2, 1942, at the London School of Hygiene and Tropical Medicine, London, W.C.1, was devoted to a symposium on the subject of:

FLAVOURS IN FOOD

The meeting was presided over by Dr. E. B. Hughes, President of the Society and Vice-Chairman of the Food Group, and the papers abstracted below were read. Both papers are to be published in full in *Chemistry and Industry*.

The Naturally Occurring Flavours of Foodstuffs

BY W. A. WAYGOOD, B.Sc., A.R.C.S., F.I.C.

THE taste sensation, located mainly on the tongue, responds to only four types of stimulus—sourness, saltiness, bitterness and sweetness—or combinations of these; certain cells in the mouth also respond to the stimulus of warmth produced by pungent substances. Flavour sensation, experienced in an area in the nasal cavity behind the eyes, is almost identical with odour sensation, being stimulated by very many volatile aromatic substances. Of the numerous classifications of flavours suggested, that of Hennig is sufficient for most purposes. He classified odours as spicy, flowery, fruity, resinous, foul and burnt. It has not yet been found possible to reconstruct from synthetic compounds the natural flavours in primary foodstuffs due to essential oils, largely owing to the difficulty of extracting in unchanged form natural flavouring substances present in only minute proportions. In tasting tests some degree of consistency has been attained, and personal variations have been reduced by the formation of panels and the use of statistical surveys. Tasting tests on normal food should be made at the temperature at which the food is usually eaten. From the point of view of flavour, food may be conveniently considered in 7 groups.

(1) *Fatty Foods.*—The characteristic flavours of different oils are largely due to hydrocarbon constituents present in traces. Diacetyl, mainly responsible for the flavour of butter, can be recognised in a dilution of 1 : 40 million. Four main classes of flavours contribute to the flavour of cheese: (*a*) aromatic sharpness (acetic and propionic compounds), (*b*) cheesy (butyric and valeric acid compounds), (*c*) pungent (caproic, caprylic and capric acid compounds), (*d*) butteriness (diacetyl, etc.). Protein degradation products also contribute to the flavour.

(2) *Flesh Foods.*—Little is known of the flavour constituents. Sodium glutamate has a meaty saline taste, but with no resemblance to the flavours of different meats.

(3) *Eggs.*—The flavour constituents are unknown. Egg fat has a flavour resembling that of chicken, but the distinctive egg flavour is associated with the protein and other constituents of the yolk.

(4) *Fruits.*—Numerous constituents have been isolated, *e.g.*, acetaldehyde, amyl formate, acetate and caproate from apples (Power and Chestnut), methyl anthranilate from grape juice, amyl acetate from bananas, and acetaldehyde from peaches, but the substances giving the flavour to most of the common fruits are unknown.

(5) *Wines and Spirits.*—Acetyl methyl carbinol and diacetyl, furfural, and esters of lower fatty acids and alcohols have been isolated from wines and other fermented products. Whiskey owes its flavour largely to fusel oil, rum to decomposition products of molasses, and brandy to grape constituents. Phenylmethyl carbinol is a flavouring constituent of Kirschwasser and the bouquet of cherry brandy is due to a large number of esters.

(6) *Beverage Materials.*—The natural odorous constituents of tea (green and black) have been studied (references given at the end of paper). They include $\alpha\beta$-hexenal, and a large number of aldehydes, alcohols and acids. It is not easy to separate these with reasonable accuracy. The aldehydes survive fermentation and withering of the tea, although doubtless in reduced quantity. Coffee aroma has been attributed to a series of compounds, and an artificial coffee oil based on the separation of these at low temps. has been patented by Staudinger and Reichstein. The essential oil isolated from cocoa by Bainbridge and Davies contains *d*-linalool and its esters and several lower aliphatic acids.

(7) *Added Flavours.*—These include herbs and spices or their essential oils, vanilla and tonquin beans. Most of the flavour constituents are fairly stable, and some have been quantitatively analysed. The flavours of various liqueurs are built up with blends of flavours in which one is predominant. Other examples are the addition of mixed spices to meat products and of vanilla to chocolate.

Some Aspects of the Chemistry of Flavour

By T. F. WEST, M.Sc., Ph.D.

As sweet taste is an essential background to the appreciation of so many flavours, consideration was given first to the changes in sweetening properties brought about by the exchange of various atoms and groups in the mols. of saccharin and dulcin. The essential oils provided excellent examples of the chemical approach to the problem, being complicated by the effect of secondary constituents on the value of a particular flavouring material. Suitable groups of essential oils containing the same principal constituent were cited as examples of this difficulty. In the course of the argument attention was drawn to various points of chemical interest, *e.g.*, to the fact that the acetate of perillyl alcohol appears to be entirely responsible for spearmint flavour, and that when the free alcohol is oxidised it yields perillaldehyde, the *anti*-oxime of which is reputed to taste 3000 times as sweet as sucrose. In the closely related art of perfumery, the present trend appeared to be intensive investigation followed by the synthesis of chemically identical or similar compounds, but in the flavouring field—when the composition of the natural flavour was known—the synthetic equivalent rarely appeared to bear any relationship to the natural product.

Discussion

Dr. Hughes, opening the discussion, stressed the importance of the science of tasting and deplored the lack of appreciation of flavour by the public, who were influenced far more by appearance. He asked Mr. Waygood how it was that the flavour of olive oil was so modified by refining if, as had been stated, it was due to the presence of hydrocarbons.

Mr. H. S. Redgrove thought that the list of tastes suggested was incomplete; alkaline and metallic tastes should be added and, for practical purposes, pungency could be regarded as a taste. Hennig's classification of odours was too rough and ready; some, *e.g.*, those of butter, cheese and game would not fit in. So far as the pleasant odours of perfumery were concerned, Cerbelaud's more detailed classification, with links between the groups, was, he thought, of far more practical value. Whereas Mr. Waygood was correct in stating that, in general, analytical data on essential oils were incomplete, there were exceptions. For example, perfect synthetic jasmine and violet oils had been produced, shortly before the war, on the basis of Ruzicka's work. Some years ago he (the speaker) had tested the formula of Power and Chestnut for apple flavour containing geraniol and geranyl esters, and thought it good if savoured at correct dilution, although he admitted that it might be improved by "touching up" with traces of fennel oil or ambrette seed oil according to the type of apple flavour desired. One great difficulty in connection with odours and flavours was that we had no exact scientific language in which to describe them, comparable with that available, *e.g.*, for colours. At the best, all that we could say was that one odour was like another. It had, indeed, been maintained that odours belonged to our emotional lives and were not amenable to intellectual treatment.

Dr. J. R. Nicholls said that, whilst it was certainly of importance to know the chemical nature of individual constituents producing flavour and taste, there might be other factors of importance. No perfumer relied solely on solutions of essential oils for his products, but included certain fixatives, without which the "velvety" character of the perfume was missing. Possibly there were substances which modified or enhanced the effects of individual flavouring materials and so produced the characteristics associated with natural flavours. True fruit essences, produced from natural materials such as raspberries and strawberries, were often not very attractive on mere dilution, but gave a very satisfactory effect when mixed with fruit juices and with confectionery. Reference had been made to sugar substitutes and to the fact that mixtures of these might be sweeter than the same weight of either component. This apparent anomaly had been explained by Paul in work referred to by Dr. West. As a result of a large number of tasting tests Paul plotted the relative sweetness of saccharin and sugar and of dulcin and sugar. With neither was the relationship a straight line, and it could be calculated that, whilst saccharin was about 250 times as sweet as sugar at a strength corresponding to about 3% of sugar, it was relatively sweeter at lower strengths; but at 10% of sugar it was less than 200 times as sweet. Similar variations occurred with dulcin. With mixtures of saccharin and dulcin the total sweetness was the sum of the sweetnesses at the respective dilutions. Thus, a solution containing a parts of saccharin and b parts of dulcin would have a sweetness corresponding to the sum of the equivalents of a parts of saccharin + b parts of dulcin;

but to give the same sweetness, more than $a + b$ parts of either saccharin or dulcin would be required. Comparative sweetnesses must always be expressed in conjunction with the strength tested, and this might apply to other flavours.

Mr. E. C. WOOD said that, in his experience, the taste of one ingredient of a mixture must be considered relatively to the other substances present, and he cited as an instance the effect of acidity on apparent sweetness. Pointing out that the sweetness sensation produced by saccharin persisted in the mouth for at least 1 hr., he asked whether the perception of "saccharin sweetness" necessarily proceeded through the same physiological channels as that of "sucrose sweetness."

Mr. L. EYNON drew attention to the effect of the immediately past experience on the nerves of taste.

Mr. W. B. ADAM pointed out that the acid flavour of a fruit was not related to its *p*H, and thought that bitter principles increased the acid flavour. He also distinguished between the functions of an expert tasting panel and a consumer preference panel.

Mr. K. A. WILLIAMS said that those who investigated the flavours of fatty oils were largely precluded from the use of chemical methods, owing to the extremely minute amounts of flavour present, and the tasting panel was the only practical method of approach to the problem. It should not be overlooked that in tasting tests the colour of the material was of primary importance; if it was off-shade, an involuntary prejudice was created.

Mr. A. L. BACHARACH said that the only hope of solving the fundamental scientific problems raised by the papers was by carefully planned and exhaustively analysed investigations involving the collaboration of laboratory chemist, chemical technologist, physiologist and statistician.

Mr. H. E. MONK said that the reaction of Public Analysts to the question of flavour would depend on the advances in knowledge in that field and on the use that was made of them. For instance, it might soon become necessary to require the words "artificially flavoured" to be added to labels when appropriate. Even at the present time, questions of taste were involved in some cases. Thus, in a recent prosecution concerning sage and onion stuffing it had been necessary to set up a standard for the minimum content of onion. One principle adopted in deriving the standard was that the article must contain at least enough onion to taste of it. The occurrence of fatigue in tasting sweet substances also applied probably to most tastes and flavours. It was well known, for instance, that the palate rapidly became accustomed to a saline taste, 50 parts of common salt per 100,000 would be detected by most people in drinking water, but would not be noticed if regularly present in the water supply. The association of sight and the sense of taste could be tested very simply by attempting to name the fruit supposed to be present in a sweet taken blindfold from a bag of "mixed fruit drops."

Mr. WAYGOOD, in reply to Dr. Hughes, said that the hydrocarbons to which the flavour of olive oil was due could, in fact, be recovered from a superheated steam distillate from olive oil. Replying to Mr. Redgrove and other speakers, he suggested that the influence of concentration on flavour would probably explain certain anomalies. To allow for these anomalies, he suggested that tasting should be carried out at or near a minimum concn.

Miss H. M. PERRY, replying for Dr. West, said, with reference to the perfume fixatives that had been mentioned, that limettin might act as a natural fixative in, *e.g.*, lemon oil. She agreed that the exact evaluation of the relative sweetness of saccharin and dulcin was difficult. The question of sourness was equally complex. For example, the sourness of hydrochloric acid was just perceptible in $N/800$ soln., whereas that of acetic acid was perceptible in $N/200$ soln., although the hydrogen ion concn. was considerably less in the acetic than in the hydrochloric acid soln. It was remarkable that the allyl grouping occurred with such frequency in the phenols and phenolic ethers in essential oils; *e.g.*, apiol, eugenol, myristicin, safrole, estragol and others, whereas the presence of the propenyl grouping in such compounds was rare, anethole being the only example which occurred to any extent in nature.

The Micro-Determination of Calcium in Serum

BY P. F. HOLT, PH.D., D.I.C., F.I.C., AND H. J. CALLOW, B.Sc., A.I.C.

(*Read at the Meeting, October* 7, 1942)

MOST of the methods suggested for the determination of the small quantity of calcium in serum depend primarily upon pptn. of the calcium as oxalate, usually in the cold, the ppt. being separated centrifugally and washed with dil. ammonia soln. The oxalate has been determined by gravimetric, volumetric, colorimetric and gasometric means (for a summary, see *Mikrochem.*, 1939, **27**, 145), but the method in general use is to dissolve the ppt. in sulphuric acid and titrate with 0·01 N permanganate.

Clark and Collip, whose procedure[1] has been adopted in most clinical laboratories, separate the oxalate ppt. centrifugally and wash it once only with 3 ml. of dil. ammonia soln. They show that, whereas some of the ppt. dissolves during this washing process, the precipitant (ammonium oxalate) is not entirely removed, and they adjust their technique so that the ammonium oxalate still contaminating the ppt. tends to counteract the loss due to solubility. Their conclusion, based upon experimental evidence and purely chemical reasoning, is that, to obtain correct results, the arbitrary details of their procedure must be closely adhered to. Van Slyke and Sendroy[2] (using the same pptn. method) studied the effect of variation of the washing procedure on the values obtained and showed that, *e.g.*, washing the calcium oxalate ppt. 6 times by the usual procedure might introduce an

error of 12% or, with 9 washings, 24%. The error was greatly reduced if the wash liquor was added carefully so as not to disturb the pad of ppt. at the bottom of the centrifuge tube, *i.e.*, the tube rather than the ppt. was washed. This technique, used with two washings of 3 ml each, gave a better cancellation of errors than was obtained in the procedure of Clark and Collip. At the same time Van Slyke and Sendroy admit the probability of error from the mechanical loss of crystals during each decantation.

The adjustment of procedure to cancel rather than remove errors has been recognised by most authors as undesirable but unavoidable if the simplicity of the method is to be retained. Others have adopted more complicated procedures, converting the oxalate into carbonate (Trevan and Bainbridge[3]) or oxide (Hamilton[4]) and titrating acidimetrically, so that the ppt. can be washed with ammonium oxalate soln. (in which its solubility is negligible) and the excess oxalate removed by ignition. These latter methods, being less simple, have not met with general approval in clinical laboratories.

Van Slyke and Sendroy have shown that the main source of error in the determination of serum calcium by oxalate methods lies in the preparation of the calcium oxalate ppt., not in its determination. Cameron and Moorhouse[5] point out, however, that an additional error (up to 4%) may be introduced through the size of drop delivered by a micro-burette of the usual design. Sobel and Sobel[6] avoid this error in their acidimetric method by the use of a burette of the Pincussen type,[7] from which the reagent is delivered below the surface of the liquid to be titrated; a fine capillary jet prevents leakage of the reagent by diffusion.

The present paper describes an experimental examination of the pptn. and washing stages of the generally adopted micro methods for serum calcium determination. Micro technique reduces the vol. of serum necessary to less than half that required for the centrifuge method and is admirably suitable for routine work. The values obtained by the two methods have been compared. For our purpose the method of Clark and Collip was taken as typical of those in general use.

MICRO METHODS.—The errors shown by various authors to occur in these oxalate methods for the determination of serum calcium are:—(1) solution of the ppt. in wash liquor, (2) incomplete removal of reducing substances in the washing process, (3) loss of ppt. in decanting the liquid after centrifuging, (4) errors due to the size of drop delivered by the burette. The use of micro apparatus minimises errors arising in the washing process by considerably reducing the vol. of wash liquor and yet increasing the number of washings, so that readily sol. substances are completely removed. Centrifuging as a means of separating the ppt. is replaced by micro-filtration with porcelain filter-sticks and the standard suction apparatus described by Briscoe and Matthews.[8] The drop error in titration is avoided by the use of a special micro-burette, precautions as to lighting[9] being taken.

The usual method for the micro-determination of inorganic calcium, with pptn. at 100° C., gives fairly accurate results, provided that at least 3 hrs. are allowed for pptn. (In 7 determinations, each on 1 ml of a soln. containing 0·2 mg of calcium per ml, the errors were between +0·5 and −1·0% when 3 hrs. were allowed for pptn., but the limits of error increased to +2·0 and −5·5% in 9 determinations when the pptn. time was 2 hrs. or less.) As a hot pptn. method cannot be applied to serum, which contains heat-coagulable proteins, the applicability of the micro-technique in the cold was investigated by means of determinations on "known" calcium solns. at room temp., using a slight excess of ammonium oxalate. When 3 or more hrs. were allowed for pptn. the results were as accurate as those obtained by the hot pptn. method.

WASHING TECHNIQUE.—The advantages of micro-filtration over centrifuging for separating and washing the ppt. are threefold: (*a*) a single washing can be completed in about 15 secs., so that the calcium oxalate ppt. has little time to dissolve, (*b*) the ppt. can easily be stirred with the wash liquor by rotating the filter-stick to effect efficient washing, (*c*) only very small quantities of wash liquor need be used for washing, and these are almost completely removed by suction; 0·2 ml (6 drops) of dil. ammonia soln. for each washing is sufficient. In a series of 12 determinations to ascertain the number of washings necessary to effect complete removal of the sol. salts, 0·916 ml of a soln. of calcium chloride containing 10·9 mg of calcium per 100 ml was used in each expt. and the ppt. was washed a different number of times. By plotting the titration values (ml of 0·01 *N* $KMnO_4$) against the number of washings, a curve was obtained (Fig. 1) which showed an initial rapid decrease in titration value corresponding to insufficient washing. A sharp break

after 3 washings, indicating complete removal of the precipitant, was followed by a slight decrease in titration value with increase in number of washings, representing a small solubility of calcium oxalate in the wash liquor. The error that would be introduced by excessive washing, however, is incomparably smaller than is indicated for the centrifuge

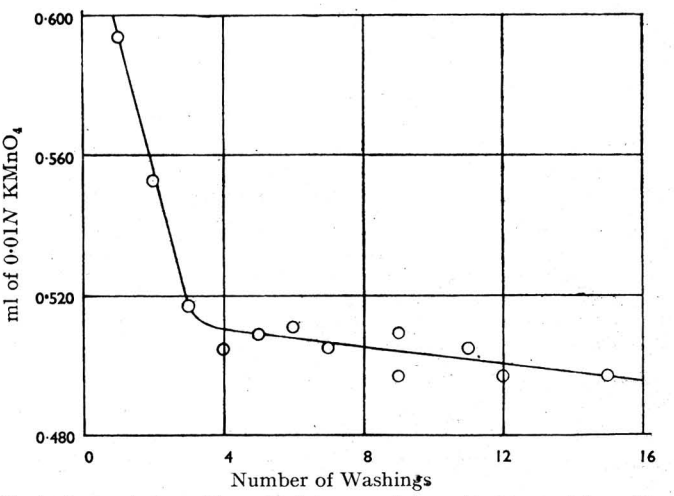

Fig. 1. Effect of repeated washing of calcium oxalate pptd. from calcium chloride soln.

method by the figures of Van Slyke and Sendroy (*supra*). From consideration of this curve, the ppt. was washed 5 times in all subsequent determinations by the micro-filtration method.

MICRO TECHNIQUE APPLIED TO SERUM.—A difficulty in the direct application of this method to serum became apparent during the titration. There was a fading end-point with an attendant error of 10 to 15%. It was assumed that this was caused by the presence of a reducing substance that would not pass through the filter-stick during the washing

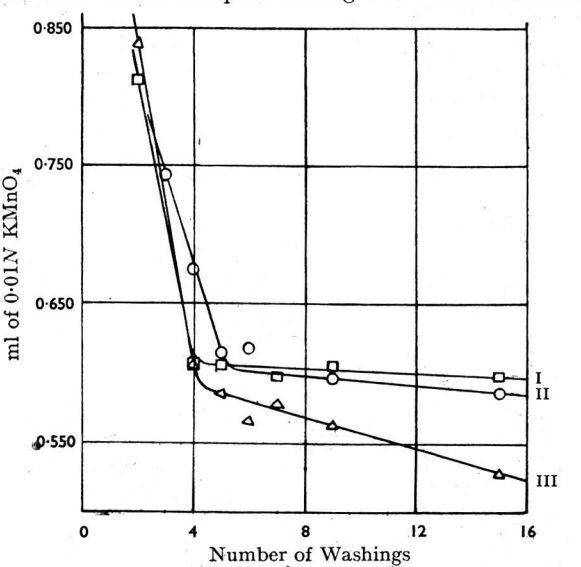

Fig. 2. Effect of repeated washing of calcium oxalate pptd. from serum

process but dissolved in sulphuric acid together with the calcium oxalate. Filtration of the serum before pptn. was therefore indicated, and after this the end-point was sharp.

With the usual type of micro-burette, from which permanganate is delivered in drops of 0·04 ml, the fading end-point is not so easily detectable, but this must represent an

additional source of error in the usual method of serum calcium determination. Moreover, a sol. reducing substance is also present in the serum filtrate. Insufficient washing of the calcium oxalate ppt. is readily detected, as this reducing substance is transferred with the oxalate after solution in sulphuric acid, and a fading end-point during the titration results.

The washing procedure as applied to serum was checked as previously for the determination of calcium in aqueous solns. The graph obtained (Curve III, Fig. 2) shows a break in the curve after 4 washings, indicating the suitability of the procedure adopted. The large amount of reducing substances in serum accounts for the fact that this break occurs at a later point than in the curve for inorganic calcium.

METHOD.—Filter the sample of serum through a porcelain filter-stick (Berlin porcelain B2, or Royal Worcester porcelain) into a micro-beaker. Transfer an accurately measured vol. of the filtrate (approx. 1 ml) by means of a capillary pipette to a micro-beaker, add 6 drops (0·18 ml) of 0·5% ammonium oxalate soln, rotate the beaker to effect thorough mixing and, after 3 to 4 hrs., filter through a porcelain filter-stick. Wash with 30 drops of 2% ammonia soln., adding 6 drops at a time and sucking the beaker dry after each addition. Dissolve the ppt. in 25 drops (0·4 ml) of 5 *N* sulphuric acid, transfer the soln. through the filter-stick to a second micro-beaker, heat on the water-bath for 3 min. and titrate with 0·01 *N* permanganate until a permanent pink colour is just detectable.

The results in Table I are typical of those thus obtained. In each expt. the vol. of serum was 0·9–1·0 ml.

TABLE I

CALCIUM DETERMINATIONS BY MICRO-FILTRATION METHOD

Serum	Calcium found (mg per 100 ml of serum)			
Normal horse (batch 20.10.41)	12·0	11·8	11·9	12·0
,, ,, (,, 3.11.41)	11·1	11·2	11·0	11·0
Human, pathological	10·3	10·3	10·1	10·3
,, ,, ...	10·4	10·3	10·7	
,, ,, ...	10·6	10·6	10·9	
,, ,, ...	9·65	9·70	9·65	9·75
,, ,, ...	10·4	10·5	10·5	10·2

RECOVERY OF ADDED CALCIUM FROM SERUM.—Added calcium was completely recovered from horse serum. Table II, which gives some of the results obtained by this method, shows that the average figure for calcium in horse serum was 11·1 mg per 100 ml, for a "known" solution of calcium chloride, 10·0 mg per 100 ml. A mixture of equal vols. of the horse serum and the calcium chloride soln. gave the values shown in Table II.

TABLE II

RECOVERY OF ADDED CALCIUM: CALCIUM FOUND

(A) Standard $CaCl_2$ soln., 0·958 ml ..	9·9, 10·0, 10·0, 10·1 (aver. 10·0) mg per 100 ml.
(B) Normal horse serum, 0·958 ml ..	11·1, 11·2, 11·0, 10·9 (aver. 11·1) mg per 100 ml.
Mixtures of (A) and (B)	21·1, 21·3, 21·0, 21·2 (aver. 21·1) mg per 200 ml.

VARIATION OF SERUM VOLUME.—The agreement between the figures for calcium in horse serum obtained in determinations on both 1- and 2-ml amounts (Expts. 7–10, Table III) demonstrates that the accuracy of the method is independent of the vol. of serum taken.

QUANTITY OF REAGENT.—The centrifuge methods for the determination of serum calcium use 1 ml (large excess) of sat. ammonium oxalate to ppt. the calcium rapidly. This is contrary to usual analytical practice and indicates a possible source of error. To investigate this point, a number of determinations were made on horse serum by the micro-filtration method, a small excess of oxalate being used in some expts., and in others an excess comparable with that used in the method of Clark and Collip. The results (Table III) indicate that too large an excess of the precipitant gives high results, possibly owing to partial formation of calcium ammonium oxalate. A proof that the high values are not due to incomplete washing of the ppt. is afforded by curve I, Fig. 2, showing titration values when the calcium was pptd. with the quantities of reagents used in the Clark-Collip method, a different number of washings being applied to the ppt. in each expt. The break in the curve at 4 washings indicates that all readily sol. substances are removed by the method adopted. Curve II shows the effect of reducing the vol. of wash liquor from 0·02 ml to 0·012 ml for each washing. It is probable that the use of a large

excess of ammonium oxalate in the Clark–Collip method introduces an additional error which is counter-balanced by the solubility of calcium oxalate and the losses during decantation after centrifuging.

TABLE III

VARIATION IN QUANTITY OF PRECIPITANT AND VARIATION IN SERUM VOLUME

Expt. No.	Vol. of serum (ml)	Vol. of ammonium oxalate (ml)	Concn. of oxalate soln. (%)	Ca found (mg per 100 ml
Batch 20.10.41				
1	0·958	0·2	0·5	12·0
2	0·958	0·2	0·5	12·0
3	1·916	0·4	0·5	12·1
4	0·958	0·8	4·0	14·0
5	0·958	0·8	4·0	13·5
6	1·916	0·8	4·0	13·1
Batch 27.10.41				
7	0·958	0·2	0·5	11·3
8	0·958	0·2	0·5	11·5
9	1·916	0·4	0·5	11·4
10	1·916	0·4	0·5	11·4
11	0·958	0·8	4·0	13·5
12	0·958	0·8	4·0	13·0
13	1·916	0·8	4·0	12·8
14	1·916	0·8	4·0	13·5
Batch 11.12.41				
15	0·941	0·2	0·5	11·8
16	0·941	0·2	0·5	12·1
17	0·941	0·8	0·5	12·9
18	0·941	0·8	0·5	12·9

COMPARISON BETWEEN THE CLARK–COLLIP AND MICRO-FILTRATION METHODS.—Determinations of calcium in sera were made by both methods. Typical results (Table IV) show that, if the directions of Clark and Collip are strictly followed, using 2 ml of serum and a large excess of ammonium oxalate and allowing exactly 30 min. for pptn., there is little difference between the figures obtained by the two methods; this confirms the statement of these authors that cancellation of errors is effected by the procedure they adopt.

TABLE IV

COMPARISON BETWEEN MICRO-FILTRATION AND CLARK–COLLIP METHODS

Vol. of serum used: Micro-filtration method, 0·916 ml; Clark–Collip method, 2·0 ml.

Serum	Ca content (mg per 100 ml)							
	Micro-filtration method				Clark–Collip method			
Human, pathological ..	12·3	12·3			12·0	12·4		
,, ,, ..	10·1	10·1			10·3	10·2		
,, ,, ..	10·1	10·2	10·2		10·2	10·2	10·0	
Normal horse	12·0	11·5	11·5	12·0	12·3	12·8	12·8	

DISCUSSION AND SUMMARY.—The rapid determination of calcium in serum is clinically important. Because of the known errors in all direct methods in which centrifuging is used for the separation of ppts., numerous variations of the original micro method of Kramer and Tisdall[10] have been introduced. Although the method of Clark and Collip is most widely used, many clinical laboratories have adopted other procedures. The method now devised for use with micro-apparatus avoids the errors which the centrifuge methods attempt to control by a rigid empirical procedure. For this reason the micro-filtration method does not require such rigid adherence to detail; *e.g.*, wide variation in the vol. of serum taken is permissible. On the other hand, the agreement between values obtained by the Clark–Collip and micro-filtration methods is very close, indicating that the procedure adopted in that centrifuge method does, in fact, effect a cancellation of errors, as suggested by the authors.

The micro-filtration method here described has been adopted in these laboratories because accurate results can be obtained on half the vol. of serum required for other methods (thus enabling results to be duplicated even when samples are small) and also because the

time of manipulation is less and there is no risk of mechanical loss during the removal of supernatant fluids. It has the further advantage of ease of manipulation.

We are indebted to Mr. G. F. Stebbing, Surgeon Specialist of Lambeth L.C.C. Hospital, who supplied specimens of blood, and to Mrs. M. Holt and Miss C. A. Westley, who carried out many of the analyses. The work described in this note is part of the research programme of these Laboratories and is published by permission of Mr. J. H. Thompson, Director of Research.

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HOSA RESEARCH LABORATORIES, SUNBURY-ON-THAMES *August*, 1942

Discussion

Mr. N. L. ALLPORT asked Dr. Holt if he had considered the possibility of facilitating the pptn. of calcium as oxalate from a protein-free serum filtrate by adding a water-miscible organic solvent, *e.g.*, dioxan. He had found this device useful for effecting complete and rapid separation. In the present instance it would be necessary to take into consideration possible interaction between the solvent and the permanganate soln. used for the final titration, but presumably the ppt. could be washed free from solvent.

Mr. F. C. HYMAS expressed some surprise that the pptn. of calcium in the cold could be completed in so short a time as 3 hrs. In his experience even with hot pptn. a longer period of standing was desirable. He assumed also that under the controlled conditions with sera there was no necessity to adjust the *p*H.

Mr. A. L. BACHARACH, commenting that in chemical pathology the values required for serum or plasma calcium were generally comparative, but that knowledge of the absolute value might be of theoretical interest, asked whether this could not possibly be more closely evaluated by using extrapolation to the ordinate of the flattened portion of the curves shown by Dr. Holt.

Dr. E. J. KING pointed out that the Clark–Collip procedure included a very convenient technique, *viz.*, the separation and washing of a small ppt. by centrifuging and decantation. This some might be unwilling to discard. The filter-stick technique used by Dr. Holt was undoubtedly more difficult for the routine laboratory. The loss of calcium oxalate in washing the ppt. had been partly prevented by two former collaborators, who retained the centrifuge technique. Pugsley (*Canad. Chem. and Metall.*, 1931), working in Collip's laboratory, had pptd. the calcium with a soln. of potassium oxalate in 50% alcohol and washed the ppt. with alcohol, in which potassium oxalate was soluble. This procedure was successfully applied to both ashed and deproteinised material; there was also some evidence that the calcium could not be completely pptd. from whole serum by ammonium oxalate, as had been done by both Clark and Collip and by Dr. Holt in the present work. Stanford and Wheately (*Biochem. J.*, 1923, **19**, 710) had used a filtered saturated soln. of calcium oxalate for washing, instead of dil. ammonia; there was no doubt that this method prevented loss of calcium oxalate by solution in the wash liquid. He was inclined to think that the solution of the problem of analysing very small quantities of serum (less than 1 ml) for calcium, lay in other directions than the permanganate titration. Ceric sulphate titration of the oxide produced by ignition of the oxalate had been found highly exact for very small amounts of calcium oxalate, and it might prove more satisfactory than the present procedure.

Analytical Studies on some 12-Heteropoly-acids. II. Potentiometric Titration of Molybdate in Presence of Silicic or Phosphoric Acid*

BY A. R. TOURKY, PH.D., AND H. K. EL SHAMY, M.SC.

ELECTROMETRIC METHOD.—The electrometric determination of molybdate has been the subject of several investigations. Brintzinger and Jahn[1] pptd. molybdate as well as tungstate with barium chloride, using electrodes of the respective metals. They inferred that such electrodes responded to the corresponding anions, from the inflections in the titration curves at the equivalence points. Müller and Mehlhorn[2] found, however, that the electrodes respond only to variations in the hydrogen ion activity which take place

* For Part I, see this vol., p. 2.

as the reagent is progressively added. Spacu[3] titrated molybdate with silver nitrate, using a silver wire as electrode against a saturated calomel half-cell. When a sufficient amount of ethyl alcohol was added to reduce the solubility of the silver molybdate formed, all the results were within 1% of the true value. Dickens and Brennecke[4] used lead or mercurous perchlorate for the molybdate titration and a molybdenum plate as an electrode. Klinger, Stengel and Koch,[5] however, did not obtain satisfactory results by this method.

OXIDATION-REDUCTION METHODS.—More interest has centred in the determination of molybdenum by means of oxidation-reduction processes. Stehlik[6] reduced molybdate with several reagents, *e.g.*, zinc, titanous chloride or stannous chloride, and oxidised the reduced product with potassium permanganate or ceric sulphate. He found it necessary to add a small amount of manganese sulphate to accelerate catalytically the decomposition of hydrolytic complexes to ter- and quinque-valent molybdenum, otherwise the results were too high. The titration curves showed sharp inflections corresponding to the stages: Mo^{III}–Mo^{IV}–Mo^{V}–Mo^{VI}, although, according to Müller[2] and Brintzinger and Oschatz,[7] there was no inflection during the transformation of Mo^{IV} to Mo^{V}.

Direct determination of molybdate with a reducing agent involves less error than any of the previous methods. Knecht and Hibbert[8] observed that Mo^{VI} could be reduced to Mo^{V} by means of titanous chloride, but they could not make this the basis of a volumetric process for lack of a suitable indicator for the end-point. Willard and Fenwick[9] applied the method potentiometrically, using the polarised bi-metallic system. They obtained a definite end-point by titrating slowly towards the end, so as to maintain the equilibrium; otherwise there might be an irregular and transient decrease in E.M.F. before the reaction was quite complete. They suggested that possibly the rate of reduction might be accelerated and a better end-point obtained with the usual mono-metallic system, but they did not investigate this point. Willard and Fenwick applied the same method to the determination of molybdenum in ammonium phosphomolybdate. The ammoniacal soln. was acidified and titrated with titanous sulphate. When the ppt. was large, it tended to be re-pptd. on addition of acid. This could be prevented, however, by adding a few drops of phosphoric acid to the ammoniacal soln. This altered the character of the end-point for titration in the cold, but with hot titration the end-point was normal and as distinct as in absence of phosphoric acid. Kolthoff and Tomiček,[10] investigating molybdate titration by means of the mono-metallic system, confirmed several of the observations cited above, but found that phosphoric acid had a disturbing action; too little reagent was required and the colour of the liquid differed from that observed in absence of phosphoric acid; it rapidly became blue and finally turbid.

Using other reductants, *e.g.*, hydrazine, Jakob and Kozlowski[11] found that sexavalent molybdenum was reduced to complex compounds containing Mo^{VI} and Mo^{V} when the medium was weakly acid, and directly to Mo^{V}, without intermediate compound formation, in strongly acid soln.; hydrazine itself was oxidised almost completely to nitrogen. Brintzinger and Oschatz[7] found that molybdenum could be reduced from Mo^{VI} to Mo^{IV} by means of chromous chloride. The potentiometric titration of the strongly acid soln., previously saturated with potassium chloride, showed a noticeable fall in the E.M.F. when the reduction to Mo^{V} was complete. Another, and more distinct, fall could be observed when all the molybdenum was reduced to Mo^{III}, but there was no noticeable change in the E.M.F. curve at the point when all Mo^{V} was transformed into Mo^{IV}. The effect of either silicic or phosphoric acid on the titration was not studied.

The disturbing effect of phosphoric acid on the titration, observed by Kolthoff and also in this laboratory, is to be expected in the light of more recent investigations, *e.g.*, those of Hein, Burawoy and Schwedler[12] and of Krumholz.[13] The former authors showed that the reaction involving the reduction of molybdenum in heteropoly-acids by means of a compound such as stannous chloride proceeds to an equilibrium in which molybdenum blue is one of the products. Molybdenum blue, according to these authors and in agreement with the findings of some other investigators, has the composition Mo_4O_{11}, while the previously assigned formulae were Mo_3O_8 (Muthmann) or Mo_5O_{14} (Guichard). In every instance the formula corresponds with an intermediate stage of reduction between Mo^{VI} and Mo^{V}.

Krumholz proved that the reduction of molybdic acid by iodide proceeds in weakly acid solutions primarily to molybdenum blue. The reduction velocity is catalytically increased in presence of phosphoric, silicic or germanic acid. The formation of the

respective heteropoly-acids as intermediate products in these reactions is independent of the amount of the constituents.

Assuming that molybdenum blue is produced by partial reduction of sexavalent molybdenum to the quinquevalent state, and reaction of part or all of that reduced form with any Mo^{VI} present to form an intermediate product, then it should be possible by adopting an appropriate procedure, which involves the masking and stabilisation of Mo^{V} in a complex compound, to prevent the formation of molybdenum blue. In that event the reduction of molybdenum should proceed very smoothly to the quinquevalent stage and the accuracy of a volumetric method would not suffer in presence of any silicate or phosphate impurity. Such a method would be extremely useful for determining molybdenum in presence of phosphorus or silicon, *e.g.*, in steel, without the necessity of first removing these constituents.

Complex Formation.—Fortunately quinquevalent molybdenum has a great tendency to form complexes. Apart from the great variety of addition compounds formed by interaction of the halides, oxy-halides, and other halogen compounds of Mo^{V}, it enters into combination with phenols, pyrocatechols and salicylates and with formic and oxalic acids. The complex-forming properties of oxalic acid with Mo^{V} have been discussed by Bailhache,[14] who prepared a deep red compound, $R_2[Mo_2O(OH)(C_2O_4)_2]$. According to Barbieri,[15] however, the composition corresponds more closely with $R_2[Mo_2O_2(OH)_4(C_2O_4)_3]$, whilst Spittle and Wardlaw[16] confirm in the main the formula adopted by Bailhache. Rosenheim and Nernst[17] conclude that the compound prepared by them agrees in formula with that of Barbieri save for the water of crystallisation. They represent the ammonium salt as $(NH_4)_4[(MoO_2)_2(C_2O_4)_3]4H_2O$, and the potassium salt as of the same composition but with $9H_2O$ mols. The complex oxalate, as also the formate, formed in water may be regarded as polynuclear complex compounds showing certain analogies with the polynuclear complex anions of tervalent metals, *e.g.*, the complex iron acetates. It is also probable that the complex tartrates are of similar composition.

Titration of Molybdate.—After several preliminary titrations of molybdate with either titanous or chromous chloride in presence or absence of these complex-forming acids and of silicic or phosphoric acid, the following observations were made:

A. *Titrations with Titanous Chloride.*—(1) In presence of even the smallest quantities of either phosphoric or silicic acid, the reduction of molybdate did not proceed smoothly. The solns. became blue on addition of few drops of titanous chloride soln. and the equilibria were not readily attained. The titration curves were irregular and did not show sharp inflections at the equivalence points.

(2) In presence of *ca.* 8% v/v of conc. hydrochloric acid and of oxalic acid in amounts ranging from 5 ml of *N* acid per 0·2 g of MoO_3 up to saturation point, the titration could be carried out successfully. Silicic or phosphoric acid did not interfere under these conditions, nor did pptg. agents, *e.g.*, caesium or ammonium. It was found necessary, however, in presence of any of these ingredients to begin with alkaline solns. and to add the requisite amounts of oxalic acid, so as to prevent heteropoly-acid formation and hence pptn. The titrations could be carried out in the cold but the equilibria were attained much more slowly than in titrations at *ca.* 80° C.

(3) In absence of oxalic acid the titration of pure commercial sodium molybdate did not proceed as smoothly as in presence of that acid. The solns. also became blue after addition of the first drops of titanous chloride soln. This could be attributed to the presence of traces of silica in the molybdate. The equilibria were attained only about 15 min. after each addition of reagent. The curves were not very smooth and the inflections were not sharp. Better, but not quite satisfactory, results were obtained by using sodium molybdate prepared by dissolving re-sublimed molybdic acid in silica-free sodium hydroxide soln.

(4) Although Mo^{V} yields a complex compound with formic acid, it was found that this acid did not influence the titration as favourably as oxalic acid. On the other hand, tartaric acid could serve for the same purpose, provided that the strongly (hydrochloric) acid solns. were saturated with that acid. The titration curves of phosphomolybdates were, however, not very smooth in presence of tartaric acid.

B. *Titrations with Chromous Chloride.*—(1) The reduction of molybdate proceeded smoothly in strong hydrochloric solutions and after saturation with potassium chloride even in presence of small amounts of silicic or phosphoric acid. The inflection points

were not thereby altered, but the equilibria were not attained rapidly. The colour of the solns. changed to green after addition of a few drops of the reagent, then became dark green, and finally turbid and brownish-green.

(2) When, in addition to the above reagents, oxalic acid was added in the same proportions as in the titrations with titanous chloride, the equilibria were attained more rapidly and the solns. remained clear throughout the titration.

(3) In presence of pptg. agents, *e.g.*, ammonium or caesium, it was not possible to titrate the solns. until after acidification with saturated oxalic acid soln. Tartaric, but not formic acid, could also be used. A white ppt. usually formed on addition of tartaric acid and dissolved only when hydrochloric acid was added.

METHOD.—To prevent contact of titanous or chromous chloride soln. with atmospheric oxygen, the titration equipment of Zintl and Reinäcker[18] was found the most suitable. Carbon dioxide was used as the inert atmosphere and was freed from traces of oxygen by passing it through a set of wash bottles provided with fritted glass diaphragms and containing titanous or chromous chloride soln.

Titanous Chloride Titration.—Titanous chloride was prepared, as recommended by Kolthoff, by boiling 50 ml of the commercial 20% soln. for 1 min. with 100 ml of conc. hydrochloric acid in a small flask, cooling, diluting, and thoroughly mixing by bubbling carbon dioxide into the storage bottle, in which it was made to occupy the entire capacity (about 2¼ litres). The soln. was then standardised against a ferric chloride soln., the iron content of which was determined gravimetrically each time in three parallel analyses. The titanous chloride soln. was slowly added to the ferric soln. while carbon dioxide was constantly passing. When the soln. was nearly decolorised, potassium thiocyanate was added and the titration was continued until the red colour (ferric thiocyanate) entirely disappeared.

The titanous soln. was also occasionally standardised against a molybdate solution prepared by dissolving weighed quantities of re-sublimed molybdic oxide in pure sodium hydroxide soln. The molybdenum content of that soln. was checked by determining it as sulphide.

Kolthoff and Tomiček[10] noticed that solns. of titanous chloride decreased in strength by 0·2% after 14 days. To obtain trustworthy results, therefore, we determined the titre of the soln. before and after each set of titrations. These were carried out in a well-steamed Pyrex glass beaker, closed with a well-fitting rubber stopper that had been boiled in dil. hydrochloric acid. This stopper was provided with holes for the end of the burette, the stirrer, the electrode and the salt bridge, and for the entrance and exit of carbon dioxide. The titration vessel was kept on an electrically heated plate, the temperature of which could be adjusted at will.

The indicator electrode found to be the most suitable was a perforated platinum plate, 1·5 × 1·5 cm, with holes, about 1 mm in diam., arranged so as to form a gauze-like surface. As a reference electrode, a saturated calomel electrode was used with saturated potassium chloride soln. as the intermediate liquid.

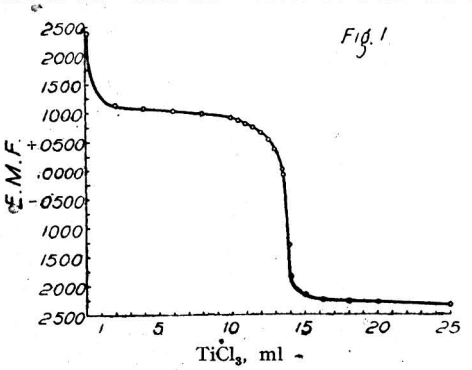
Chromous Chloride Titration.—Several methods have been proposed for the preparation of chromous solns.; they are discussed in Böttger's "*Newer Methods of Volumetric Chemical Analysis*," p. 133. The procedure followed in this laboratory consisted in reducing pure re-crystallised chromate first with conc. hydrochloric acid and then with pure zinc to blue chromous chloride. This was filtered from the excess of zinc through glass wool and converted into the red acetate, which was repeatedly washed by decantation before being dissolved in 2% hydrochloric acid and transferred to a storage bottle. All operations involving filtration, transfer, and washing were carried out in an atmosphere of carbon dioxide. The distilled water and dil. hydrochloric acid were boiled and cooled while carbon dioxide was bubbling through to remove dissolved oxygen. Rubber connections were avoided as far as possible

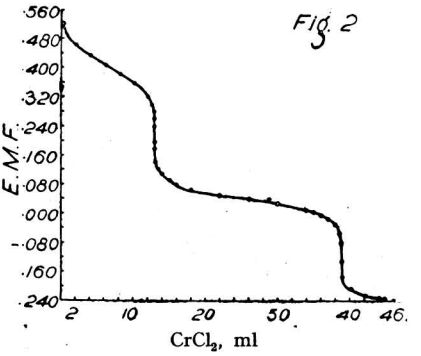
The chromous chloride solutions were standardised before and after each set of expts. against standardised copper sulphate solns. When carefully prepared and stored, a chromous chloride soln. retains a constant titre for several days. The method of titrating was the same as with titanous chloride.

TITRATION CURVES.—Figs. 1 and 2 represent typical titration curves, with the two reagents, of molybdate to which oxalic acid had been added in presence of either phosphoric or silicic acid.

In Fig. 1 the respective solns. contained 7·3467 g of MoO_3 and 1·4484 g of titanous chloride per litre; 25 ml were titrated. Inflection representing the transformation of Mo^{VI} into Mo^{V} occurred when 13·60 ml of the reagent had been added; this corresponds with 0·18363 g of MoO_3 (≡ 7·3452 g per litre), with an error of 0·02%.

In Fig. 2 the respective solns. contained 7·3467 g of MoO_3 and 0·96973 g of chromous chloride per litre; 20 ml were titrated. The first inflection (≡ $Mo^{VI} \rightarrow Mo^{V}$) occurred when 12·925 ml of the reagent had been added; this corresponds with 0·1468 g of MoO_3 (≡ 7·340 g per litre), with an error of 0·09%. No inflection could be observed at the point of transformation of Mo^{V} into Mo^{VI}. The second inflection corresponding to the transformation of Mo^{IV} into Mo^{III} occurred after the addition of 36·775 ml.





In presence of pptng. agents the titration curves do not differ in shape from those here shown, provided that the precautions described above are taken. If the appropriate quantities of either oxalic or tartaric acid together with hydrochloric acid are added, the error in the amount of molybdenum will not exceed ±0·1%.

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CHEMISTRY DEPT., FACULTY OF SCIENCE
FOUAD I UNIVERSITY, ABBASSIA, CAIRO

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Analysis of Beverages containing Citrus Juices

BY I. STERN, B.Sc., F.I.C.

THE determination of the proportion of citrus fruit juice in a beverage is very difficult because the juice contains no known characteristic ingredient that could be used as a quantitative index. For lemon juice, it has been suggested[1] that the percentage and alkalinity of the ash afford useful information. Luhrig[2] has, in addition, determined the phosphoric anhydride in the ash.

In numerous determinations on citrus fruit juices, believed to be genuine, the samples were ashed in an electric muffle furnace at 525° C. and the alkalinity of the ash was

TABLE I.—LEMON JUICE

Sample No.	Origin	Ash % w/v	Alkalinity of ash (ml of *N* acid to neutralise ash of 100 ml)	Phosphoric anhydride % w/v
1	Messina	0·42	4·1	0·022
2	,,	0·42	5·6	0·023
3	,,	0·40	4·2	0·027
4	,,	0·40	4·1	0·027
5	,,	0·44	5·3	0·026
6	,,	0·42	4·8	0·021
7	,,	0·43	4·9	0·035
8	South Africa	0·38	4·9	0·033
9	,, ,,	0·48	5·4	0·024
10	,, ,,	0·39	6·0	0·042
11	,, ,,	0·46	5·3	0·022
12	,, ,,	0·31	3·7	0·023
13	Spain	0·38	4·8	0·022
14	,,	0·42	5·2	0·022
15	Cyprus	0·36	3·8	0·020
16	,,	0·36	5·2	0·037
17	West Indies	0·36	4·3	0·023
18	,, ,,	0·43	5·8	0·029
19	Palestine	0·42	5·0	0·027
	Maximum ..	0·48	6·0	0·042
	Minimum ..	0·31	3·7	0·020
	Average	0·40	4·9	0·027

TABLE II.—ORANGE JUICE

Sample No.	Origin	Ash % w/v	Alkalinity of ash (ml of *N* acid to neutralise ash of 100 ml)	Phosphoric anhydride % w/v
1	Messina	0·46	5·2	0·038
2	,,	0·50	6·1	0·034
3	,,	0·51	4·9	0·032
4	,,	0·52	6·4	0·041
5	South Africa	0·54	6·1	0·052
6	,, ,,	0·53	6·3	0·028
7	,, ,,	0·53	6·7	0·036
8	,, ,,	0·63	7·7	0·041
9	Spain	0·52	6·0	0·035
10	West Indies	0·52	6·6	0·045
11	,, ,,	0·54	5·9	0·033
12	Palestine	0·46	5·3	0·048
13	California	0·39	4·9	0·038
14	,,	0·49	5·1	0·038
15	,,	0·40	4·6	0·034
	Maximum ..	0·63	7·7	0·052
	Minimum ..	0·39	4·6	0·028
	Average	0·50	5·85	0·038

TABLE III.—GRAPEFRUIT JUICE

Sample No.	Origin	Ash % w/v	Alkalinity of ash (ml of *N* acid to neutralise ash of 100 ml)	Phosphoric anhydride % w/v
1	South Africa	0·40	4·4	0·032
2	,, ,,	0·42	4·3	0·030
3	,, ,,	0·56	3·9	0·032
4	,, ,,	0·50	3·9	0·043
5	,, ,,	0·34	3·8	0·030
6	West Indies	0·50	4·2	0·034
7	,, ,,	0·50	4·8	0·030
8	Palestine	0·35	3·7	0·030
9	California	0·48	5·7	0·034
10		0·53	3·9	0·028
11		0·53	3·9	0·037
	Maximum ..	0·56	5·7	0·043
	Minimum ..	0·34	3·7	0·028
	Average	0·46	4·2	0·033

determined by the procedure of the A.O.A.C. (*"Methods of Analysis,"* 5th Ed., 1940). The soln. from the alkalinity determination was then used for the volumetric determination of phosphoric anhydride by the method of Monier-Williams,[3] *viz.*, pptn. with ammonium molybdate in presence of ammonium nitrate, boiling the washed ppt. with excess of $N/2$ sodium hydroxide to expel all ammonia, cooling, and back-titrating with $N/2$ sulphuric acid, with phenolphthalein as indicator.

The figures thus obtained are given in Tables I to IV. They show that, in absence of interfering factors, they enable a close approximation to the proportion of citrus fruit in a beverage to be made.

TABLE IV.—LIME JUICE

Sample No.	Origin	Ash % w/v	Alkalinity of ash (ml of N acid to neutralise ash of 100 ml)	Phosphoric anhydride % w/v
1	West Indies	0·33	3·9	0·025
2	,, ,,	0·36	3·9	0·030
3	,, ,,	0·44	5·4	0·028
4	,, ,,	0·36	4·7	0·023
5	,, ,,	0·37	4·7	0·019
6	,, ,,	0·41	5·1	0·025
	Maximum ..	0·44	5·4	0·030
	Minimum	0·33	3·9	0·019
	Average ..	0·38	4·6	0·025

CONDITIONS AFFECTING THE PROPORTION OF ASH AND ITS ALKALINITY.—(1) TYPE OF PRESERVATIVE PRESENT.—(*a*) *Benzoic Acid.*—A juice preserved with sodium benzoate (the form in which the acid is nearly always added) will give higher figures for ash and alkalinity. However, a correction can be made by determining the proportion of benzoic acid and deducting from the ash an equiv. quantity of sodium carbonate.

(*b*) *Sulphur Dioxide.*—In practice, liquid sulphur dioxide or an aqueous soln. of the gas is not used so often as potassium or sodium metabisulphite or sodium bisulphite. It is not unusual for an imported orange juice to contain as much as 1000 p.p.m. of SO_2. If derived from potassium metabisulphite, this preservative will increase the ash content by 0·108% and the alkalinity of the ash by 1·45 ml ($N/2$) as compared with the figures for juice unpreserved or preserved with sulphur dioxide as such.

TABLE V.—CONCENTRATED JUICES

LEMON	ORANGE	GRAPEFRUIT	The ratios of concentration which *must* be adopted are
When the respective specific gravities at 15·5° C. are not in excess of			
1·080	1·100	1·090	2
1·100	1·125	1·110	2½
1·120	1·150	1·130	3
1·135	1·175	1·155	3½
1·150	1·200	1·180	4
1·170	1·225	1·200	4½
1·190	1·250	1·220	5
1·210	1·275	1·240	5½
1·230	1·300	1·260	6
1·245	1·325	1·280	6½
1·260	1·350	1·300	7
1·280	1·370	1·325	7½
1·300	1·390	1·350	8
1·320	1·415	1·370	8½
1·340	1·440	1·390	9
1·360	1·465	1·410	9½
1·380	1·490	1·430	10

(2) PRESENCE OF SACCHARIN.—In pre-war days saccharin was comparatively little used in beverages containing citrus fruit juices, but the position is very different to-day. The soluble form of saccharin, *i.e.*, the sodium salt, will contribute to the ash. More commonly, however, the ordinary "550" grade of saccharin is used, and, for convenience in handling, this is usually made up into a stock soln. in water with addition of either ammonia or sodium bicarbonate. Ammonia would not affect the ash, but the use of sodium bicarbonate would have some effect. For example, a suggested recipe for

preparing a suitable stock soln. of saccharin involves the use of 10 oz. of sodium bicarbonate for 18½ oz. of saccharin. It should not be difficult to ascertain if ammonia has been used to prepare the saccharin soln., and in absence of ammonia a correction could be made for the sodium bicarbonate used. The importance of the phosphoric anhydride figure lies in the fact that it is not affected by the above factors.

TABLE VI.—CONCENTRATED LEMON JUICE

Sample No.	Origin	Sp.gr. at 15·5° C.	Acidity (as citric) % w/v	Ash % w/v	Alkalinity of ash	P_2O_5 % w/v	Ratio of concentration	Calculated to natural strength juice: Ash % w/v	Calculated to natural strength juice: Alkalinity of ash	Calculated to natural strength juice: P_2O_5 % w/v
1	California	1·245	49·0	1·865	22·6	0·16	6⅓	0·29	3·5	0·025
2	,,	1·235	43·7	1·98	22·65	0·15	6½	0·30	3·5	0·023
3	,,	1·213	38·6	2·20	22·6	0·16	6	0·37	3·8	0·027
4	,,	1·212	38·6	2·07	23·0	0·145	6	0·34	3·8	0·024
5	,,	1·197	34·6	1·80	21·2	0·132	5½	0·33	3·9	0·024
6	,,	1·209	36·15	1·775	22·4	0·122	5½	0·32	4·1	0·022
7	,,	1·223	38·9	2·072	26·2	0·14	6	0·35	4·4	0·023
8	,,	1·214	41·8	2·136	22·8	0·114	6	0·36	3·8	0·019
9	,,	1·197	36·15	1·87	21·7	0·142	5½	0·34	3·9	0·026
10	Palestine	1·136	24·85	1·46	17·7	0·095	4	0·37	4·4	0·024
11	,,	1·137	25·0	1·357	21·0	0·15	4	0·34	5·3	0·038
12	South Africa	1·075	13·25	0·73	8·4	0·065	2	0·37	4·2	0·033
13	Australia	1·087	13·8	0·85	11·6	0·065	2½	0·34	4·6	0·026
	Maximum ..	..	..	..	..	..	..	0·37	5·3	0·038
	Minimum ..	..	..	..	..	..	..	0·29	3·5	0·019
	Average ..	..	..	..	..	..	..	0·34	4·1	0·026

TABLE VII.—CONCENTRATED ORANGE JUICE

Sample No.	Origin	Sp.gr. at 15·5° C.	Acidity (as citric) % w/v	Ash % w/v	Alkalinity of ash	P_2O_5 % w/v	Ratio of concentration	Calculated to natural strength juice: Ash % w/v	Calculated to natural strength juice: Alkalinity of ash	Calculated to natural strength juice: P_2O_5 % w/v
1	California	1·217	9·5	1·67	18·5	0·180	4½	0·37	4·1	0·040
2	,,	1·303	9·1	3·34	30·3	0·260	6½	0·51	4·7	0·040
3	,,	1·330	9·7	3·06	29·1	0·351	7	0·44	4·2	0·050
4	Palestine	1·301	6·65	2·80	27·9	0·369	6½	0·43	4·3	0·057
5	,,	1·146	4·3	1·46	13·8	0·100	3	0·49	4·5	0·033
6	,,	1·287	6·55	2·45	26·1	0·286	6	0·41	4·4	0·048
7	,,	1·343	9·9	3·33	41·0	0·316	7	0·48	5·9	0·045
8	,,	1·146	5·85	1·23	12·5	0·127	3	0·41	4·2	0·042
9	,,	1·319	6·1	2·92	25·2	0·257	6½	0·45	3·9	0·040
10	,,	1·149	6·2	1·32	13·3	0·123	3	0·44	4·4	0·041
11	,,	1·153	4·6	1·33	16·0	0·146	3½	0·38	4·6	0·042
12	,,	1·154	6·3	1·23	13·2	0·123	3½	0·35	3·8	0·035
13	South Africa	1·067	4·05	0·81	8·6	0·057	1½	0·54	5·7	0·038
14	,, ,,	1·157	4·2	1·37	15·9	0·134	3½	0·39	4·5	0·038
15	,, ,,	1·158	4·2	1·35	14·4	0·135	3½	0·39	4·1	0·039
16	,, ,,	1·163	5·3	1·53	15·4	0·170	3½	0·44	4·4	0·049
17	,, ,,	1·254	5·5	2·63	28·8	0·251	5½	0·48	5·2	0·046
18	,, ,,	1·263	5·9	2·98	28·1	0·211	5½	0·54	5·1	0·038
19	,, ,,	1·354	13·3	3·25	36·2	0·406	7½	0·43	4·8	0·054
20	West Indies	1·255	5·5	3·22	33·3	0·274	5½	0·59	6·1	0·050
	Maximum ..	..	..	..	..	..	..	0·59	6·1	0·057
	Minimum ..	..	..	..	..	..	..	0·35	3·8	0·033
	Average ..	..	..	..	..	..	..	0·45	4·65	0·043

CONCENTRATED IMPORTED CITRUS JUICES.—Until fairly recently most of the citrus fruit juice imported into this country consisted of juice of the natural strength. A large proportion of the juice now being received has been concentrated *in vacuo*, and it has been announced that, in order to save shipping space, all the juice to be imported will be concentrated. All imports and allocations of juice are now controlled by the Ministry of Food. The degree of concentration is judged by the sp.gr., according to the table issued by the Citrus Fruit Juice Allocations Control, dated 1/12/41 (Table V). The ratio of concentration is by volume.

Concentrated lime juice is not commercially available at present, but analyses of concentrated lemon, orange and grapefruit juices have been made, and the results are shown in Tables VI, VII and VIII. Comparison of these figures with those in Tables I to IV shows that the ash and alkalinity of the ash of concentrated juices are proportionately somewhat lower than for the corresponding natural strength juices. This difference is probably due to the effect of preservative, as indicated above. The concentrated juices, in general, contain much less preservative (when divided by the ratio of concn. of the juice) than do the normal strength juices. It should be remembered, however, that when making up a fruit squash from concentrated juice it will nearly always be necessary to add further preservative to prevent fermentation, and, as the preservative so added will generally be an alkali sulphite, there will in practice be very little difference whether the juice used is of natural strength or concentrated.

TABLE VIII.—CONCENTRATED GRAPEFRUIT JUICE

Sample No.	Origin	Sp.gr. at 15·5° C.	Acidity (as citric) % w/v	Ash % w/v	Alkalinity of ash	P_2O_5 % w/v	Ratio of concentration	Calculated to natural strength juice: Ash % w/v	Alkalinity of ash	P_2O_5 % w/v
1	Palestine	1·286	9·0	2·12	23·5	0·210	7	0·30	3·4	0·030
2	,,	1·152	6·75	1·16	14·0	0·115	3½	0·33	4·0	0·033
3	,,	1·164	7·0	1·37	14·1	0·122	4	0·34	3·5	0·031
4	,,	1·162	6·9	1·18	12·2	0·105	4	0·30	3·1	0·026
5	California	1·303	16·1	2·67	30·4	0·277	7½	0·36	4·1	0·037
	Maximum ..	..	..	..	..	..	..	0·36	4·1	0·037
	Minimum ..	..	..	..	..	..	..	0·30	3·1	0·026
	Average ..	..	..	..	..	..	..	0·33	3·6	0·031

These Tables show the value of the phosphoric anhydride figure, which is unaffected by the presence of alkali salts, whether derived from preservative or used for preparing the stock soln. of saccharin.

REFERENCES

1. Borntraeger, A., ANALYST, 1898, **23**, 176.
2. Luhrig, H., *id.*, 1906, **31**, 233.
3. Monier-Williams, G. W., *id.*, 1911, **36**, 582.

KIA-ORA, LTD., RUSHWORTH STREET, LONDON, S.E.1. *December*, 1941

Methods of Analysis for the Purposes of the Cake and Flour Confectionery (Control and Maximum Prices) Order, 1942 (S.R. & O. No. 2103 of 1942)

(*Read at the Meeting, February* 3, 1943)

THE Cake and Flour Confectionery Order, 1942, restricts the amounts of sugar and of fats and oils which may be present in any cake, including fruit loaves, bun loaves and flour confectionery of any description with certain specified exceptions. The Order prescribes that the percentage of sugar and of oils and fats shall be determined in accordance with the following provisions:—(*a*) the percentage shall be determined by reference to the weight of the cake taken at any time; (*b*) the percentage shall be ascertained by analysis of a sample representing a fair average of the whole article; (*c*) all oils and fats and sugar contained in or added to the cake shall be taken into account in whatsoever form they may have been introduced; (*d*) the percentage of sugar in the sample shall be determined by adding the percentage of sucrose to the percentage of the total reducing sugars expressed in terms of dextrose; (*e*) the percentage of oils and fats shall be determined by ascertaining the percentage of the sample which is extractable with ether after the sample has been suitably digested with diluted hydrochloric acid.

For the purposes of The Cake and Pastry Order, 1917, methods of analysis for sugar were drawn up and agreed between The Government Laboratory, this Society and representatives of Cake Manufacturers. The Analytical Methods Committee of this Society

considered that a useful purpose would be served if a similar procedure were adopted for the current Order, and requested The Government Laboratory and The Chemists' Panel of The Cake and Biscuit Manufacturers War Time Alliance, Ltd., to draw up agreed methods.

We considered that any methods put forward should be suitable for manufacturing control as well as for enforcement, that they should not be elaborate or time-consuming, and that latitude should be allowed for variations in detail which would not affect accuracy. The Appendix gives the methods which we recommend.

As regards the method for fat we have adopted a mixed solvent for extraction because its use is more widely applicable than ether alone and avoids certain practical difficulties. We are of opinion that the results obtained by the proposed method are the same as those obtained by more elaborate and longer methods using ether alone.

It will be observed that the Order does not include a tolerance. We consider that manufacturers should work to some reasonable margin below the prescribed limits, and that public analysts or other chemists concerned with enforcement should use their discretion as to the tolerance to allow for variation in the materials used in manufacture, in sampling and in analysis.

J. R. NICHOLLS, representing The Government Laboratory.

R. T. COLGATE, L. S. FRASER, E. B. HUGHES } representing The Cake and Biscuit Manufacturers War Time Alliance, Ltd.

APPENDIX

METHODS OF ANALYSIS FOR THE PURPOSES OF THE CAKE AND FLOUR CONFECTIONERY (CONTROL AND MAXIMUM PRICES) ORDER, 1942

1. PREPARATION OF THE SAMPLE FOR ANALYSIS.—The whole sample shall be passed through a mincing machine and shall then be mixed by hand to ensure an even distribution of any fruit, peel, or pieces of nuts, after which the whole sample shall again be passed through the mincing machine. These operations must be conducted quickly and adequate precautions must be taken to avoid loss or gain of moisture.

2. DETERMINATION OF SUGAR.—About 10 g, or 5 g if the proportion of sugar is high, of the prepared sample, accurately weighed, shall be ground with water in a mortar and transferred to a 250 ml flask, using in all about 200 ml of warm water (not above 50° C.). The flask shall be kept at a temperature of 45° to 50° C. for one hour, being shaken at intervals. The contents of the flask shall then be cooled, and if it is necessary to use a clearing agent, a small quantity of alumina cream or dialysed iron shall be added. The liquid shall be made up to 250 ml excluding the layer of fat (if any), well mixed and filtered.

(*a*) *Reducing Sugars.*—The reducing sugars in the filtrate shall be determined by a copper reduction method, the reducing power being expressed in terms of dextrose. The figures so obtained shall be multiplied by 0·97 if 10 g, or by 0·99 if 5 g, of the sample have been taken for the analysis, and the result shall be calculated as a percentage of dextrose on the original sample.

(*b*) *Sucrose.*—Fifty ml of the filtrate shall be measured into a 100 ml flask and 25 ml of dilute hydrochloric acid (containing 5 ml of hydrochloric acid of sp.gr. 1·18) shall be added and a thermometer inserted in the flask. The flask shall be placed in a water-bath at or a few degrees above 70° C. and shall be kept in continuous motion so that the contents attain a temperature between 67° and 70° C. in from 2½ to 3 minutes. The temperature shall then be maintained between 67° and 70° for a further period of 5 minutes, after which the flask shall at once be withdrawn and cooled. The contents shall be neutralised with sodium hydroxide solution, diluted to 100 ml and filtered. The total reducing sugars in the filtrate shall be determined by a copper reduction method, the reducing power being expressed in terms of dextrose. The figure so obtained shall be multiplied by 0·97 if 10 g, or by 0·99 if 5 g, of the sample have been taken for the analysis and the result shall be calculated as a percentage of dextrose on the original sample. This percentage of dextrose shall be reduced by the percentage of dextrose determined under 2(*a*) and the result shall be multiplied by 0·95 to obtain the percentage of sucrose.

(*c*) *Sugar.*—The percentage of sugar in the sample shall be determined by adding the percentage of sucrose determined under 2(*b*) to the percentage of total reducing sugars expressed in terms of dextrose determined under 2(*a*).

3. DETERMINATION OF OILS AND FATS.—About 2 g of the prepared sample, accurately weighed, shall be transferred to a stoppered cylinder and 2 ml of alcohol (or industrial methylated spirit) added so as to moisten all the particles. Ten ml of hydrochloric acid (sp.gr. 1·18) and 5 ml of water shall be added and the cylinder shall be placed in a water-bath maintained at 80° C., the contents being stirred occasionally. After half-an-hour the cylinder shall be removed, and the contents cooled and mixed with 15 ml of alcohol (or industrial methylated spirit), followed by 25 ml of ether. The cylinder shall be stoppered and shaken vigorously. The stopper shall then be removed, 25 ml of light petroleum (boiling range 40° to 60° C.) shall be added, the cylinder re-stoppered and again shaken vigorously. The cylinder shall be allowed to stand for not less than half-an-hour, or shall be centrifuged at a low speed. The ethereal layer shall be transferred to a flask by means of a wash-bottle fitting with the delivery tube turned up at its lower end to avoid disturbing the lower layer. If the ethereal layer is not clear it shall be filtered through a small plug of cotton wool. The extraction shall be repeated twice more using on each occasion 20 ml of ether and 20 ml of light petroleum. The mixed ethers shall then be distilled off and the residual fat shall be dried at 100° C., cooled and weighed. The residue in the flask shall be dissolved in light petroleum, and the solution carefully poured off and washed out with light petroleum till all the fat is removed. The flask shall be dried at 100° C., cooled and weighed, and the difference in weight before and after extraction shall be calculated as a percentage of oils and fats on the original sample.

Notes

ESTIMATION OF HYDROCYANIC ACID PRODUCED BY ALMONDS

FOR an investigation of the toxicity of English-grown almonds, a convenient method for estimating small amounts of hydrocyanic acid was developed. Based on the D.S.I.R. method for detection of hydrocyanic acid vapour (Abst., ANALYST, 1938, **63**, 658), the method is suitable for proportions ranging from a few p.p.m. upwards, and can be applied to one almond or less.

AUTOLYSIS.—Transfer 0·5 g of the ground-up sample to a stout test-tube (6 × ¾ in.) with 4 ml of water, mix, close the tube with a rubber stopper and leave for 1 to 1½ hr. at room temp., with occasional shaking. Place the tube, tightly corked, in a beaker of cold water, heat just to boiling, then remove the tube and cool to 15·5° C. or other selected temp.

ANALYSIS.—Fit up a suitable apparatus so that a known vol. of air can be bubbled through the suspension at a controlled rate, and passed thence through a glass tube of slightly over ¼" bore containing a strip of Congo red silver nitrate test-paper. A 250 ml measuring cylinder connected to a water supply is probably the simplest means for controlling the air flow.

When autolysis is complete, introduce 1 ml of 5% tartaric acid soln. into the test-tube, the rubber cork being raised sufficiently to admit it; then replace the cork by one fitted with glass tubes for bubbling, taking care to minimise loss of hydrocyanic acid. Mix the suspension and connect the outlet tube to the test-paper tube; then pass 200 ml of air through the suspension and over the strip at a steady rate of 20 ml per min., keeping the temp. constant and shaking the suspension if there is any tendency to form a sediment.

Next remove the test-paper strip, mark the end of the blue stain with pencil on each side, and read the average length of the stain as hydrocyanic acid on a graph prepared from standard stains produced under similar conditions from dil. potassium cyanide soln., *i.e.*, with 1 ml of tartaric acid added and water up to 5 ml. If desired, 0·5 g of sweet almonds, free or freed from amygdalin, may be added to the standard test mixtures, but this did not appear to be necessary.

To prepare extra-sensitive test-paper strips, soak Whatman's No. 1 filter-paper, cut into 4-in. strips, for 1 min. in a freshly diluted 0·05% aqueous soln. of Congo red, pressing the strips between blotting paper and drying at about 60° C. Immerse these strips evenly in a 0·5% soln. of silver nitrate (A.R.) for 30 sec., press them again and dry at *ca.* 60° C. in a clean atmosphere (free from hydrogen sulphide and acid or alkaline fumes). Cut off a margin of ½ in., reject any discoloured parts, and cut out strips of 3 × ½ in. Keep them in an air-tight bottle in the dark.

These strips give a sharp stain of about 14 mm with 0·1 mg of hydrocyanic acid, but the length and consistency of the stains naturally depend upon the quality of the strips. The stains appear to be directly proportional in length to the amount of hydrocyanic acid, and two or more strips, placed end to end, may be used if high results are expected.

The method is not, of course, specific for hydrocyanic acid, but perfect "blanks" have been obtained on almonds free from hydrocyanic acid, and no interfering substances are likely to be encountered. The results agreed well with those obtained by the A.O.A.C. method of steam-distillation followed by thiocyanate titration.

RESULTS.—Considerable variations in potential hydrocyanic acid were found between different almonds from a single tree (10 nuts from one tree gave results ranging from 0·004 to 0·011% of hydrocyanic acid); further variations were found in different parts of a single almond, the outer layer of the cotyledons next to the skin yielding twice as much hydrocyanic acid as the inner part. Hence, to obtain a representative sample, it is necessary to grind up several almonds together. By this method the following results were

obtained on samples of English-grown almonds from known varieties of trees; these varieties are believed to include all that bear fruit in this country.

Variety	Hydrocyanic acid, yield %
Prunus amygdalus amara (imported nuts) ..	Recent, 0·21, 0·22, 0·23 Old, 0·18, 0·14, 0·10 (1851)
P. a. Pollardii	1942, 0·11, 0·13
P. a. nana (*P. tenella*)	1942, 0·12
P. amygdalo-persica	1942, 0·07
P. amygdalus (*communis*)	1942, 0·012, 0·024, 0·010, 0·013
P. a. praecox	1942, 0·011
P. a. macrocarpa	1942, 0·0008, 0·0005
P. a. dulcis (imported nuts)	Recent, 0·001, 0·0005, 0·0007, 0 Old, 0, 0

It was noticed that, whilst the nuts from the first four varieties mentioned above tasted unmistakably and unpleasantly bitter, the last four had a relatively sweet and agreeable taste; this is a useful indication of the toxicity.

Almonds from Decorative English Trees.—To ascertain the potential toxicity of the almonds borne by decorative English trees, samples were collected from trees, of unidentified varieties, growing chiefly in or near the London area. The general characters of these nuts, *e.g.*, size, shape, texture of shell, were in every instance similar to those of *P. amygdalus* (*communis*); the taste of the kernels was also similar, being relatively sweet and having the characteristic "almond" flavour indicative of amygdalin. The amounts of hydrocyanic acid produced by these samples were as follows:

No. of samples	7	22	24	13	3	1	Total 70
HCN yield, %	0·008–0·009	0·010–0·011	0·012–0·013	0·014–0·015	0·016–0·017	0·020	Aver. 0·012

These results show remarkably little variation between the samples, and confirm the general indications that they were all derived from the common almond. The variety *P. a. praecox* cannot be entirely excluded, but may be considered unlikely, since its early flowering season usually precludes the formation of fruit.

According to information received from the leading growers, the common almond, which is probably a hybrid between the bitter and the sweet almond, does not grow well from seed in this country, and is propagated by budding or grafting on to plum stock; the great majority of trees grown here may well have been produced from the same original source, without admixture of other varieties in the strain. This would explain the agreement noted above, and would support a wider inference. All the samples referred to were from the 1942 crop; some, freshly picked, had very light skins and were barely ripe. Comparison with old, dark-skinned, over-ripe nuts from the same tree showed only slight differences in hydrocyanic acid yield, the older nuts giving results 10% lower.

A general analysis of a mixture of 12 samples of the fresh, unblanched, common almonds, gave the following results:—moisture, 8·5; oil, 48·8; protein, 28·3; mineral matter, 3·0; carbohydrates, fibre, etc., 13·4%.

H. Amphlett Williams

Analytical Laboratory, 4, Southwark Street, London, S.E.1 *December*, 1942

O-DIANISIDINE AS INTERNAL INDICATOR FOR ZINC-FERROCYANIDE TITRATIONS

Diphenylamine and diphenylbenzidine have both been suggested for the zinc-ferrocyanide titration, but the end-point often proves uncertain (*cf.* Garratt, "*Drugs and Galenicals*," p. 310) *o*-Dianisidine provides an end-point colour change from red-brown to pale blue-green, which is not only easily detected but also very sharp in *N*/20 solns.

Preparation.—Mix 0·1 g of *o*-dianisidine with 0·5 ml of conc. sulphuric acid and dilute to 100 ml with water.

Titration.—Add 10 g of ammonium chloride and 5 ml of hydrochloric acid to the zinc soln., heat to boiling and remove from the source of heat. Begin the titration and add sufficient indicator to produce a suitable depth of colour. If the ferrocyanide soln. contains insufficient ferricyanide to produce the colour, add 0·1% ferricyanide soln., drop by drop, until the red-brown tint is obtained. Continue the titration with *M*/20 ferrocyanide until the soln. is colourless or, more usually, pale blue-green. H. F. Frost

Harris Technical College, Corporation Street, Preston *November*, 1942

ACRIFLAVINE AS INTERNAL INDICATOR FOR SULPHANILAMIDE-NITRITE TITRATIONS

The determination of members of the sulphanilamide group by diazotisation is rapid and accurate (Calamari, Hubata and Roth, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 534), but the use of starch iodide paper as external indicator takes time when individual tablets of varying sulphanilamide contents are being assayed. I have found that a very dil. soln. of acriflavine can be used as internal indicator to indicate the approach of the end-point (and with practice the end-point itself). The colour change is from yellow to violet. Just before the end-point the colour is somewhat purple, owing to the presence of unchanged acriflavine, but the true end-point colour, once seen, is unmistakable. The change is irreversible, the utility of the indicator depending upon the relative reaction rates of acriflavine and the sulphanilamides. If the acriflavine is added too soon, more must be added during the titration, but the intensity of the colour makes the correction negligible. At the end-point the indicator becomes violet as it enters the soln.

The use of starch iodide paper requires some experience, for near the end-point the oxidation of the iodide is very rapid. A true excess of nitrous acid is indicated by the production of a deep blue-black edge to the test drop, whereas in the earlier stages the whole of the moistened area becomes purple-blue. For routine analyses, the use of ice is not essential, and the nitrite may be standardised against permanganate, it being borne in mind that the *N*/10 nitrite used is *N*/20 with respect to sulphanilamides. H. F. Frost

Harris Technical College, Corporation Street, Preston *November*, 1942

Official Appointments

THE following Amendments to the list of Public Analysts appointed by Local Authorities with the approval of the Minister of Health were notified by the Ministry on January 4th, 1943:

Authority	Public Analyst
Southport County Borough	Miss MURIEL ROBERTS (Deputy)
Widnes Borough	WILLIAM HENRY ROBERTS
Cheltenham Borough	ROWLAND HOLLIDAY ELLIS
Colne Borough	MALCOLM MCFARLANE LOVE
Stepney Metropolitan Borough	WILLIAM MINTERNE PAULLEY (Deputy)
Malden and Coombe Borough	DANIEL DONALD MOIR (Deputy).

FERTILISERS AND FEEDING STUFFS ACT, 1926

THE Ministry of Agriculture and Fisheries has notified the Society of the following changes in the appointments of Agricultural Analysts which have taken place since July 2nd, 1942 (ANALYST, 1942, **67**, 261).

H. J. EVANS, B.Sc., F.I.C., Agricultural Analyst for the County of Carmarthen and the County of Pembrokeshire, *vice* C. A. Seyler, resigned.

F. W. F. ARNAUD, F.I.C., Agricultural Analyst for the County of Kent—re-appointed as from April 1st, 1943.

F. W. M. JAFFÉ, B.Sc., F.I.C., Deputy Agricultural Analyst for the County Borough of Dewsbury

January 7th, 1943

Ministry of Food

STATUTORY RULES AND ORDERS*

1942 No. 2348. Order dated November 14, 1942, amending the Soft Drinks (Licensing and Control) Order, 1942, and the Soft Drinks (Current Prices) Order, 1942. 1d. net.

An amendment of Article 2 of S.R. & O., 1942, No. 1337, *prohibits the manufacture or packing of any soft drink by way of trade or business except by licence.*

In S.R. & O., 1942, No. 1336, *paragraph (b) of the definition of "concentrated drink" is amended by substituting the following paragraph, "(b)* any egg product or any cereal product other than flavoured barley water and other than any product for the preparation of flavoured barley water."

— **No. 2350. The Mustard Seed (Control and Maximum Prices) Order, 1942. Dated November 14, 1942.** 1d. net.

In this Order "Mustard seed" means threshed white (or yellow) mustard seed and threshed brown (or black) mustard seed.

The Order prohibits the grinding of any mustard seed by way of trade or business except by licence. Mustard seed may be supplied, obtained, bought or sold only by (a) the grower to an approved buyer or licensed manufacturer; (b) an approved buyer to another approved buyer; (c) one licensed mustard seed merchant to another or to a licensed manufacturer; (d) one licensed manufacturer to another; (e) any person to a retailer or a person buying for the purpose of sowing. "Retailer" here means a person who sells the mustard seed for the purpose of sowing.

Schedule II, fixing max. prices for sales to a licensed manufacturer, classifies mustard seed into two kinds: (i) *white (or yellow) or any mixture of white (or yellow) with brown (or black);* (ii) *brown (or black). The max. prices for the two kinds are the same if the brown (or black) seed was harvested before Jan.* 1, 1943; *if harvested on or after that date,* 20s. *per cwt. net more may be charged for the brown (or black) seed.*

— **No. 2451. Order dated November 30, 1942, amending the Sampling of Food Order, 1942.** 1d. net.

The original Order (S.R. & O., 1942, Nos. 531 and 1199) *is further amended by inserting after the words "the Fertilisers and Feeding Stuffs Act, 1926," the words "or the Sale of Food (Weights and Measures) Act, 1926."* (*Cf.* ANALYST, 1943, **68**, 16.)

— **No. 2476. Order dated December 2, 1942, fixing Appointed Days under the Manufactured and Pre-Packed Foods (Control) Order, 1942.** 1d. net.

Under the Principal Order (S.R. & O., 1942, No. 1863; cf. ANALYST, 1942, **67**, 357) *appointed days are now fixed in respect of suet, gelatine, isinglass, agar-agar and gelatine products.*

In this Order "gelatine product" means any food containing either gelatine, isinglass or agar-agar and one or more of the following products, that is to say, sugar, glucose, saccharin or any other sweetening agent, starch, citric acid, tartaric acid, flavouring, gum, arrowroot, or flour or any other cereal product.

— **No. 2482. The Jam and Marmalade (Maximum Prices) Order, 1942. Dated December 4, 1942.** 3d. net.

This Order revokes S.R. & O., 1941, Nos. 1735 and 1736, and amending Orders, 1942, Nos. 545 and 714. The 15 Articles are essentially the same as in S.R. & O., 1941, No. 1736 (cf. ANALYST, 1942, **67**, 19) *except that "Jam" now includes marmalade, vegetable jam and jam in the form of jelly. "Marmalade" is defined as in S.R. & O., 1941, No. 1735* (ANALYST, 1942, **67**, 17).

* A summary of some recent Orders. Italics signify changed wording. H.M. Stationery Office, 1942.

In Schedule I (Quality Standards) "Fruit Content," "Percentage of Soluble Solids," "Fresh Fruit Standard Jam" and "Full Fruit Standard Jam" are defined as in S.R. & O., 1941, No. 1736 (ANALYST, 1942, **67**, 19), *the words "or vegetable(s)" being added after "fruit" where required.*

"Special Standard Marmalade" means marmalade : (i) *with a min. content of 68·5% of soluble solids ;* (ii) *which is jelly marmalade or coarse cut marmalade ;* (iii) *which has a min. content of 30% of fruit.*

The min. fruit contents tabulated agree with those in Column II of the Table in S.R. & O., 1941, No. 1736 (loc. cit.) with the following differences :—"Apple and Raspberry" becomes "Apple and Raspberry and/or Loganberry," 40 (30/10) ; Damson and Damson Jelly, 38 (instead of 20) ; Greengage, 38 (instead of 20) ; Loganberry, 20 ; Peach and Mixtures of Peach with Citrus Fruit, 40 ; Plum and Plum Jelly, 40 (instead of 35) ; Plum and Strawberry (instead of Strawberry and Plum), 40 (30/10) ; Quince Jelly, 40 ; Raspberry and Raspberry Seedless or Jelly, 20. Marmalade (other than Special Standard Marmalade), 20.

In Schedule II (Labelling) the provisions are as in the previous Order, but there is no reference to Fresh Fruit Standard Jam or to jam manufactured before Sept. 1, 1940.

Schedules III and IV give max. prices for Home-produced and Imported Jams, respectively.

Notes from the Reports of Public Analysts

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

CITY AND COUNTY OF KINGSTON UPON HULL: REPORTS FOR THE 2ND AND 3RD QUARTERS, 1942

POTTED MEAT.—A sample contained 31·5% of excess water and 16% of undeclared wheat flour.

DRESSED CRAB.—A sample contained *ca.* 20% of undeclared farinaceous matter (calc. as wheat flour).

FISH CAKES.—One sample contained only *ca.* 12% and 3 others *ca.* 20% of fish. Even having regard to war-time conditions, I am of opinion that fish cakes should contain not less than 25% of fish.

FRUIT CORDIAL.—A sample was labelled "Orange Flavoured Cordial . . . does not contain fruit juice or sugar," the word "cordial" being in large type and the declaration in relatively small type. It was sweetened with undeclared saccharin. In my opinion the word "Cordial" should not be applied to a beverage containing neither fruit juice nor sugar, even though their absence is declared.

LEMON, GLYCERIN AND HONEY.—Of 5 samples, (*a*) contained *ca.* 10% of glucose syrup and *ca.* 8% of sucrose; (*b*) *ca.* 17% of sucrose; (*c*) *ca.* 15% of sucrose; (*d*) no glycerin; (*e*) *ca.* 12% glucose syrup.

COD-LIVER OIL EMULSION.—Two samples, labelled ". . . with Hypophosphites," contained 31·5 and 36·9% of cod-liver oil, whereas the B.P. Codex 1934 requires 50% v/v. Moreover, by the Pharmacy and Medicines Act, 1941, the strengths of therapeutically active ingredients of medicines must be notified on the label. Cautions were issued in this and all the other cases mentioned above. D. J. T. BAGNALL

CITY OF LEICESTER: ANNUAL REPORT FOR 1941

MILK SUBSTITUTES.—One sample was essentially a mixture of flour and salt and another contained a large proportion of dried mashed potatoes. Since the issue of the Food Substitutes (Control) Order, 1941, milk substitutes have disappeared from the retail trade.

GROUND ALMOND SUBSTITUTES.—One sample (price 4s. per lb.) consisted of 37% of flour (2½d. per lb.) and 63% of soya bean flour (5d. per lb.) flavoured with essence of almond. Another preparation (5s. per lb.) contained 75% of ground peanuts and 25% of flour.

SALAD SPREAD.—This was essentially a very wet (79% water) soft cheese made from partly skimmed milk and was sold at a min. price of 1s. 4d. per lb. It was variously described as "Salad Spread," "Tea-time Spread" and "Tea-time Salad Spread." Had it been sold as cheese it would have been controlled by S.R. & O., 1941, No. 945 (max. water content 50%; price 10d. per lb.).

SARDINE SANDWICH SPREAD.—This consisted of: Bread, 42·6; moist lean fish, 33·9; fat, 0·7; water, 22·8%. In view of its low fat-content, it was certified to be deficient of at least 78% of sardine. It would not spread by any of the ordinary methods. The label bore the surprising information: "This product complies with the Food and Drugs Act, 1941." The vendor was fined £10.

COFFEETTE.—This was a thin brown liquid which the purchaser was warned to "use sparingly. For making a beverage this advice was misleading, for the manufacturer had already applied it to the coffee he had used. The composition was: water, 98·12; coffee extractives and added flavouring, 1·88%; this hardly justified the description on the label as a "Concentrated Coffee Essence."

BACTERIA IN SYNTHETIC CREAMS.—Examination showed that wholesome materials were being used for these cream substitutes, but that, owing to slipshod methods of manufacture, some of them were of very poor bacterial quality. Of 38 samples examined, 10 were passed as satisfactory, yielding from 600 to 180,000 counts per ml (48 hr. at 37° C.) and containing no *B. coli* in 0·01 ml. Of the unsatisfactory samples, the counts ranged from 60,000 to 130,000,000 per ml. The samples with the relatively low counts contained *B. coli* in 0·01 ml; some of the other samples contained *B. coli* in 0·0001 ml. F. C. BULLOCK

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT FOR 1941

IN 1939 it was decided that the Fruit and Vegetable Canners' Association should be the representative body for the canning industry, and the Director of the Research Station (Mr. F. Hirst, M.Sc.) was invited to act as Emergency Secretary. This has brought the Station into still more intimate contact with the industry. The work summarised in the Report has been confined to nutritional problems, and bacteriological and mycological research has had to be curtailed. Investigations were continued on the following problems.

HYDROGEN SWELLS.—Expts. were made to ascertain the risk incurred in using sugars containing sulphur dioxide (2, 8 and 20 p.p.m.) in the canning of gooseberries and plums. No appreciable increase in the rate of formation of hydrogen swells was observed. This agreed with earlier results of practical tests which showed that sulphur in elemental or easily reducible form or as sulphide acts as an accelerator of corrosion. In practice, sulphite is much less active, notwithstanding its power of accelerating the corrosion of steel strips in buffer solns. of citric acid.

GUMMING OF VICTORIA PLUMS.—It was found previously that injection of boric acid into the branches or spraying it on to the leaves of Victoria plum trees reduced the percentage of fruits containing gum. In tests during 1941 the soil surrounding the trees was treated with borax, but no significant reduction in gumming of the fruit was observed.

FACTORS AFFECTING THE VITAMIN C CONTENT OF CANNED FRUIT AND VEGETABLES.—In his progress report the Director draws the following conclusions from the expts. described in detail:—(*a*) The preparation of vegetables, including blanching, causes some loss (*vide infra*). (*b*) Headspace, even in cans closed at 80–85° C., has a pronounced effect, *e.g.*, a loss of 20–30% of the vitamin C originally present. (*c*) Plain cans afford more protection than lacquered cans. (*d*) Normal variations in the method, time and temp. of processing do not usually affect the vitamin C content (sprouts form an exception). (*e*) Storage at normal temps. for periods up to 6 months had no significant effect with canned gooseberries, peas, broad beans or stringless beans. Some loss of vitamin C was observed in canned loganberries and blackberries.

EFFECT OF BLANCHING ON THE NUTRITIVE VALUE OF CANNED VEGETABLES.—Expts. by W. B. Adam and G. Horner showed that steam-blanched vegetables retain their nutritive value better than the water-blanched, although it is questionable whether the substitution of 2 to 3 min. steam blanching for water-blanching would justify the alterations in practice that it would involve. The aver. retention of sugar by all vegetables was 94, 89 and 86% for 1, 3 and 6 min. blanch in water and 92% for 3 min. steam blanching. Fresh peas and sliced and diced vegetables showed a lower retention of sugar than larger units (except potatoes). Somewhat similar figures were recorded for the % retention of mineral salts. The protein figures were 86, 81 and 78% for water-blanched and 89% for steam-blanched vegetables. The aver. retention of vitamin C was 72, 64 and 58% for the 3 successive blanching periods in water and 75% for the steam blanching.

PHYSICAL CHANGES DURING THE BLANCHING OF VEGETABLES.—Blanching generally causes a reduction in weight, owing apparently to expulsion of water-sol. cell contents, which is most rapid during the first 1–2 min. In soaked peas and beans the loss is more than counterbalanced by absorption of water. There is also a sharp increase in sp.gr., due to expulsion of gases and collapse of the tissues, especially in the first 1 or 2 min. of blanching. It is not noticeable in potatoes, soaked peas or soaked beans. A sudden shrinkage (about 5% in vol.) takes place; thus, blanching for 3 min. in water enables 6–10% more vegetables to be filled into the cans than when unblanched vegetables are used. Steam is not quite as effective as water in producing this shrinkage.

RIPENING OF PLUMS BY MEANS OF ETHYLENE.—Three varieties of plums were divided into: (*a*) untreated fruit stored in a well-ventilated room at *ca.* 58°–65° F., (*b*) fruit treated for 24 hr. at 60°–65° F. with ethylene (dilution 1 : 1000) and stored as (*a*), and (*c*) fruit treated for 48 hr. with ethylene (1 : 1000) and stored as (*a*). Fruits of each variety and state of ripeness were treated separately, *ca.* 50–80 lbs. being placed in a room into which the requisite vol. of ethylene was injected at floor level and circulated by means of a fan. The general conclusions drawn from the analyses (given in detail) are that the method of ethylene treatment used produced no material increase in the sweetness or decrease in the acidity of plums, but stimulated slightly the ripening changes associated with colour and flavour. The percentage of mouldy fruits occurring after storage of the fruit for 4–5 days was greater in the samples treated with ethylene than in the untreated fruit. In view of these facts, there would be little advantage in treating with ethylene plums for canning.

The British Pharmaceutical Codex, 1934. Fifth Supplement

QUININE PREPARATIONS AND CUMULATIVE INDEX*

THE use of quinine is restricted in so many preparations that new formulae have been included in the Fifth Supplement. In June, 1942, the General Medical Council authorised the omission of quinine sulphate from Easton's Syrup, now termed "Easton's Syrup without Quinine," and ordered that the new syrup should be supplied when Syr. Ferr. Phosph. c. Quinin. et Strych. (synonym, Easton's Syrup) was prescribed. The Codex Revision Committee has also ordered the omission of quinine from certain preparations (*e.g.*, Compound Syrup of Hypophosphites), whilst for other quinine preparations which may still be legally prescribed for non-malarial conditions, they have provided standard alternative formulae.

The new alternative preparations, without quinine, are:—Liquid Extract of Malt with Strychnine (Ext. Malt. Liq. c. Strych.), Solution of Strychnine for Syrup of Ferrous Phosphate with Strychnine (Soln. of Strychnine for Easton's Syrup without Quinine; Liq. Strych. pro Syr. Ferr. Phosph. c. Strych.), Iron Phosphate Pills with Strychnine (Easton's Pills without Quinine; Pil. Ferr. Phosph. c. Strych.), Pills of Iron and Zinc Valerianates (Pil. Ferr. et Zinc Valer.; as substitute for Pilulae Ferri Valerianatis Compositae), Podophyllin and Belladonna Pills (Pilulae Podophyllini et Belladonnae), Syrup of Ferrous Bromide (Syr. Ferr. Brom.; for Syr. Ferr. Brom. c. Quin.), Syrup of Ferrous Bromide with Strychnine (Syr. Ferr. Brom. c. Strych.; for Syr. Ferr. Brom. c. Quinin. et Strych.). In Syrupus Triplex, Syr. Phosph. c. Quinin. et Strych. may be replaced by Syr. Phosph. c. Strych.

Quinine is to be omitted from the following preparations:—Glycerinum Hypophosphitum Compositum, Syrupus Hypophosphitum Compositus, Tabellae Hypophosphitum Compositae, Tabellae Phosphatum

* Pp. 38. The Pharmaceutical Press, 17, Bloomsbury Square, W.C.1. 1942. Price 2s. 6d.; post free 2s. 8d.

et Hypophosphitum Compositae, Tinctura Antiperiodica; also from various preparations compounded with Acidum Hypophosphorosum Dilutum in Part I of the Codex.

Lonchocarpus (Cube Root) may be used as an alternative to Derris in the preparation of Derris Praeparata (Prepared Derris Powder). Microscopical characters of Derris and Lonchocarpus, and the Standard (not less than 2% of rotenone) and Action and Uses of Lonchocarpus are given.

The Cumulative Index to the five Supplements occupies 24 pages.

British Standards Institution

B.S. 1079—1942. HAEMOGLOBINOMETERS (HALDANE TYPE)*

THIS Specification defines a colorimetric basis for haemoglobinometer colour tubes. The colour specification is derived from a standard carboxyhaemoglobin soln. prepared by saturating a 1% v/v soln. of normal blood containing 18·5 ml of oxygen (≡ 13·8 g of haemoglobin) per 100 ml. Hitherto blood samples have been diluted equally for any given reading in graduated haemoglobinometer tubes irrespective of the tube diameter, with the result that only graduated tubes agreeing in diam. could be interchanged; moreover, manufacturers were restricted in their choice of tube diam. The new Standard defines the colour tubes on the trichromatic scheme adopted by the International Commission on Illumination in 1931.† This enables tubes to be permanently defined and any individual tube to be rapidly verified. All tubes that comply with the Specification are interchangeable and can be used in conjunction with any standard colour tube. Evidence is adduced to show that the new standard tubes agree closely with Haldane's original standard. No change in the technique of testing a sample of blood is required, and the use of the new standard tubes will enable different workers to obtain more comparable results than has hitherto been possible. The National Physical Laboratory is prepared to examine haemoglobinometer colour tubes and graduated tubes for conformity with the Specification. The method of deriving the colour specification is described in detail in Appendix A.

HANDBOOK OF BRITISH STANDARDS

THIS edition,‡ revised to July, 1942, with October Supplement, gives the names of the Members of the General Council, the Executive Committee, Divisional Councils and Industry Committees. Then, after a summary of the aims and objects of the Institution, there are lists of New Standards issued and in course of preparation, a subject index and a numerical list of British Standards, B.S. Aircraft material specifications, etc.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Improved Method for Determining the Distribution of Salt and Water in Cured Hams. A. K. Besley and F. Carroll. (*J. Agric. Res.*, 1942, **64**, 293–306.)—A method of sampling cured hams is recommended, in which the salt (sodium chloride) and water contents are determined in the muscle segments, carefully dissected from adhering fat and connective tissue, in the chosen slice. The salt contents of muscles in the same cross-section slice vary widely, as shown by the following figures for muscles from one slice of a 30-days brine-cured ham: adductor, 10·5; biceps femoris, 3·8; gracilis, 21·6; rectus femoris, 13·1; sartorius, 20·7; semimembranosus, 11·7; semi-tendinosus, 2·6; vastus intermedius, 2·3; vastus lateralis, 8·9; vastus medialis, 8·3%. This variation makes it essential to include as many muscles as possible in the sample used for determination of salt distribution. In the proposed method, five cross-section slices of equal width are taken between the aitchbone and the stifle joint, and the second slice from the aitchbone is used for the determinations; this slice represents the thickest and widest part of the ham, contains segments of all the muscles between the aitchbone and the stifle joint, and has salt and water contents approximating to the average for the five slices. Each muscle segment is cut into fine pieces with scissors, and the water content is determined by drying for 16 hrs. at 214° F. The dried material is finely ground in a mortar, and a portion is used for salt determination by the second method suggested by Kerr (*J.A.O.A.C.*, 1933, **16**, 543–546). Greater concns. of silver nitrate and thiocyanate solns. than are recommended in the Kerr wet procedure may be necessary, as dried material is being used. E. M. P.

Estimation of Phenols in Meat and Fat. I. W. Tucker. (*J. Assoc. Off. Agric. Chem.*, 1942, **25**, 779–782.)—The method is based on the reaction of phenols with 2,6-dichloroquinonechloroimide to form blue dyes of the indophenol series (*cf.* Gibbs, *J. Biol. Chem.*, 1927, **72**, 649; Houghton and Pelly. ANALYST, 1937, **62**, 117) and measurement of the colour in a photoelectric colorimeter within the range 500–700*mμ*. Repeated tests of extracts of fresh ham have shown that this tissue normally contains no constituents that react. The logarithm of the transmission at *ca.* 635*mμ* showed a straight line relationship with the concn. of phenol or guaiacol originally taken. The indophenol formed in the reaction may be completely extracted from 50% alcoholic soln. by means of butyl alcohol, whilst brownish decomposition products of the chloroimide remain in soln. *Method.*—Beat a 50-g sample of meat (or fat) with 200 ml of 50% alcohol for 5 min. in a mechanical beater, filter off and chill the extract, and filter again in the cold to remove most of the fat (a small amount of colloidal fat in the filtrate from lard does not interfere with the test). If necessary, decolorise the extract (without heating) with activated carbon prior to

* British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. net, post free 2s. 3d.

† Commission Internationale de l'Eclairage, *Compt. rendu des Séances*, 1931, p. 19.

‡ 1942. Publications Dept., British Standards Institution. Price 1s. 6d. net.

the second filtration. Add to 5 ml of the extract (previously diluted if necessary) 5 ml of a 0·5% soln. of sodium borate ($Na_2B_4O_7.10H_2O$) (which promotes the reaction) and 1 ml of a 0·05% soln. of 2,6-dichloroquinonechloroimide in 7% alcohol, leave for 1 hr. at room temp. and then extract the blue indophenol with 15 ml of *n*-butyl alcohol. Drain off the aqueous soln. (if 5 ml of undiluted extract were originally used, add an additional 5 ml of water to reduce the amount of ethyl alcohol dissolved in the butyl alcohol) and add 2 ml of butyl alcohol saturated with ammonia. Measure the vol. of butyl alcohol containing the dye, make up to definite vol. to correct for any vol. changes due to mutual solubility of water, alcohol and butyl alcohol, filter, and read the colour intensity of the filtrate in a photoelectric colorimeter, using a filter that transmits a band at *ca.* 635$m\mu$. In test expts., in which known quantities of phenol or guaiacol were added to sweet pickled ham, fresh pork and lard, the recoveries and estimation were satisfactory with as little as 0·05 mg per 100 g. With larger amounts (*e.g.*, 48·3 mg per 100 g) agreement was not so close (*e.g.*, 52 mg per 100 g). The distribution of phenol in a commercial ham smoked for 24 hr. and analysed 7 days later was as follows:—fat just below skin, 0·45; exposed fat (not covered by skin), 1·89; lean surface tissue, 1·36; lean tissue ½ in. below lean surface, 0·32; lean tissue beneath fat layer, <0·03; lean tissue in centre of ham, <0·03 mg per 100 g.

Determination of Solids in Blackstrap and High Test Molasses. F. W. Zerban, J. E. Mull and J. Martin. (*J. Assoc. Off. Agr. Chem.*, 1942, **25**, 763–769.)—The U.S.A. Sugar Act, 1937, established quotas for "liquid sugar," which is defined as a product containing "soluble non-sugar solids (excluding any foreign substance that may have been added) equal to 6% or less of the total soluble solids." The sol. non-sugars equal the diff. between the total sol. solids and the total sugars. There are 3 official A.O.A.C. methods for the determination of solids in sugar products, *viz.*, drying, and evaluation from sp.gr. or from the ref. index. The commercial procedure generally used in U.S.A. for determining Brix degrees of blackstrap molasses is the so-called double-dilution method ("*Methods of Analysis*," A.O.A.C., 1940, 485–6). Comparative analyses have shown that the sp.gr. (Brix) of blackstrap molasses by the double-dilution method averages about 5 degrees higher, that of the undiluted molasses about 3 degrees higher, and the refractometric result (Brix) more than 2 degrees higher than the dry substance determined *in vacuo* at 70° C. With high-test molasses the sp.gr. (Brix) by double dilution is within 1 degree, up or down, of the dry substance, and averages only 0·25 degrees higher; the Brix value of the undiluted molasses is generally lower (aver. 0·7 degree) than the amount of dry substance, but when it is corrected for the invert sugar the average minus error is reduced to 0·4 degree; the refractometer (Brix) value averages about 1 degree lower, but when corrected for invert sugar it gives results in close approximation to the amount of dry substance (aver. error, 0·18 degree). The method of the U.S. Treasury Dept. for sol. dry substance, *viz.*, filtration and determination of dry substance in the filtrate, invariably gives higher results (*e.g.*, aver. of 15 determinations, 0·38%) than does the difference method, in which the insol. dry substance, determined in a separate portion of the sample, is deducted from the total dry substance. The difference between the results obtained by the two methods is probably due to evaporation of the soln. during the filtration prescribed in the Treasury method. The sp.gr. of the mixture of salts (8–12%) in cane molasses is higher than that of sucrose solns. of the same concn. The effect of the salts on the ref. index is less pronounced than it is on the sp.gr.; as a rule, the ref. index of blackstrap molasses is somewhat higher than that of a sucrose soln. of the same concn. The amount of organic non-sugar in blackstrap molasses is usually equal to or somewhat higher than that of the ash. The nature and quantity of non-sugar constituents vary widely, and the combined effect on the sp.gr. Brix or refractometric Brix values is uncertain.

Necessary Precaution in the Use of Takadiastase for the Estimation of Maltose. A. H. Bunting. (*Biochem. J.*, 1942, **36**, 639–640.)—In the enzymic hydrolysis of carbohydrates it is usual to add toluene to ensure sterility during incubation. The efficiency of toluene for this purpose is questionable, and an alternative method is now proposed. Yemm (*Proc. Roy. Soc.*, B., 1935, **117**, 483) used takadiastase for the hydrolysis of maltose in sugar mixtures derived from barley seedlings, and assumed that on hydrolysis, maltose would show an increase in reducing power of 100%. He actually observed increases of only 90 to 97%, and suggested that the low value was due to an equilibrium between glucose and maltose. A calculation shows, however, that an increase of 109% would be expected for complete hydrolysis, and the discrepancy between the theoretical and Yemm's recorded values is explained by the observation that takadiastase is heavily infected with bacteria. Hydrolysis was therefore carried out with a solution of takadiastase sterilised by Seitz-filtration, and the reducing value was then increased by 110%, in good agreement with the theoretical value. It is therefore recommended that, wherever possible, sterilisation should be employed, but where this is impracticable, a more effective antiseptic, such as thymol, should be used instead of toluene.

F. A. R.

Bitter Principles of Neem Oil. A. L. N. Murti, S. Rangaswami and T. R. Seshadri. (*Indian J. Pharm.*, 1940, **2**, 206–212.)—Freshly prepared neem oil (*Melia azadirachta*), used in India as an anthelmintic and for skin diseases, yields, after about 6 months, a thick solid deposit separable into 3 parts:—(A) Sol. in alcohol and benzene; (B) sol. in alcohol, insol. in benzene; (C) sparingly sol. in alcohol (mainly fatty acids). Alcoholic extraction of the expressed oil and residual oilcake yields substances (A) and (B) identical with those in the deposit. These non-crystalline compounds ($C_5H_7O_2$ and $C_4H_7O_2$) are odourless but very bitter and are responsible for the bitterness of the oil; the taste of (B) is less disagreeable than that of (A). Both are insol. in water, and in dil. sodium carbonate soln., but sparingly sol. in ether. They are non-toxic to small fish and earthworms in a concn. of 1 in 5000. They reduce alkaline potassium permanganate and Fehling's solution, but do not give the reactions of glycosides; with ferric chloride no colour is formed. (A) is an amorphous grey powder, which decomposes slowly at 115° C. and more markedly at 155°–175° C. It is difficultly sol. in dil. aqueous sodium hydroxide soln. (B) is an amorphous scaly dark brown powder, which liquefies at 72° C.

and decomposes at 110°–115° C.; readily sol. in dil. aqueous sodium hydroxide soln. (C), after re-crystallisation from alcohol, is a colourless waxy crystalline solid, m.p. 72–74° C., consisting mainly of arachidic acid with a small amount of stearic acid. The obnoxious odour of the original oil is attributed to volatile decomposition products, devoid of sulphur (*cf.* Watson *et al.*, *J. Soc. Chem. Ind.*, 1923, **42**, 387T). E. B. D.

(ABSTRACTOR'S NOTE.—The Director of Scientific and Industrial Research, India, has recommended a malodorous neem oil extract as denaturant for alcohol; *cf.* Dunnicliff, ANALYST, 1942, **67**, 394.)

Some Reactions of Morpholine. A. R. Ingram and W. F. Luder. (*J. Amer. Chem. Soc.*, 1942, **64**, 2506–2507.)—The addition compound $SnCl_4.2C_4H_9ON$, formed as a white ppt. by the reaction of morpholine with stannic chloride (*cf. J. Franklin Inst.*, 1938, **226**, 293; *Chem. Rev.*, 1940, **27**, 547), is similar to the compounds prepared by Haendler and Smith (*J. Amer. Chem. Soc.*, 1941, **63**, 1164). It is insol. in the common organic solvents, water and dil. acids, sol. (with decomp.) in hot conc. acids; m.p. (decomp.) 215°–235° C. Morpholinium chloride can be prepared from morpholine and carbon tetrachloride in a few hrs. by warming approx. equal vols. at 50–100° C., or within 1 day (room temp.), by mixing in mol. ratio 2 : 1 each component after being dried over calcium chloride or sulphate. The reaction occurs even in presence of calcium oxide. With chloroform, morpholine reacts similarly. The chloride is identified by its m.p. (177° C.), by forming a white ppt. with silver nitrate in aqueous soln., and by its mixed m.p. with morpholinium chloride prepared from hydrogen chloride and morpholine. E. B. D.

Colour Test for Citrinin. H. Tauber, S. Laufer and M. Goll. (*J. Amer. Chem. Soc.*, 1942, **64**, 2228–2229.)—The following is a test for citrinin prepared according to Raistrick and Smith (*Chem. and Ind.*, 1941, **60**, 828.) Dissolve 1 mg of the sample in 0·5 ml of 95% alcohol and add 0·3 ml of 3% hydrogen peroxide. Agitate for 1 min. The intense yellow soln. becomes colourless and then light brown. Add 0·3 ml of 0·2 *N* sodium hydroxide. A deep wine-red colour forms at once. Add 0·3 ml of 0·2 *N* sulphuric acid, which changes the colour to orange-yellow, becoming wine-red again on addition of 0·3 ml of 0·2 *N* sodium hydroxide. Prepare a control tube using water instead of hydrogen peroxide; an orange-yellow colour develops in this tube. When 0·25–0·5 ml of 0·02 *N* sodium hydroxide is added to 1 mg of citrinin in 0·5 ml of ethyl alcohol, a very light pink colour develops, and does not change in intensity on further addition of alkali or on standing. Continued exposure of citrinin to dioxane results in a hydrogen-peroxide-like reaction. On long exposure to air, alcoholic citrinin solutions undergo certain changes apparently not identical with hydrogen peroxide oxidation. The hydrogen peroxide-sodium hydroxide colour reaction is also given by the original cultures, by the acid-pptd. crude citrinin, and by citrinin recrystallised from 95% alcohol. Citrinin solns. after treatment with sodium hydroxide and readjustment to the original *p*H do not give the reaction. Penicillin from *P. notatum* gives a lemon-yellow colour in the test. E. M. P.

Biochemical

Micro-diffusion Methods. Ammonia and Urea using Buffered Absorbents. (Revised Methods for Ranges greater than 10μg of Nitrogen.) E. J. Conway and E. O'Malley. (*Biochem. J.*, 1942, **36**, 655–661.)—The micro-diffusion method for the determination of ammonia and urea have been criticised on the grounds that, for clinical use, the upper limit of 100 mg of urea per 100 ml is too low, since blood ureas occasionally exceed this figure. This difficulty is overcome in the method described, which is applicable at almost any concn. either for occasional blood urea determinations or for a series. A different soln. is used for absorbing the ammonia, and the indicator, which changes colour at an end-point where the buffering is minimal, is more sensitive than that previously employed when titrating with acid. The end-point is very sharp, and the results are as accurate as by the original method. For the estimation of ammoniacal nitrogen in amounts exceeding 10μg, transfer a vol. of soln., not exceeding 2 ml, to the outer chamber, and add to the central chamber 1 ml of boric acid reagent (mix 5 g of purest boric acid with 200 ml of alcohol, add 700 ml of water and then 10 ml of an alcoholic soln. of bromocresol green, 0·033%, and methyl red, 0·066%; adjust the soln. to the desired end-point by adding acid until the colour is faintly reddish, and dilute to 1 litre). Alternatively, use 1 or 2 ml of half-saturated magnesium chloride soln. with the mixed indicator in place of the boric acid. Smear the lid of the apparatus with white vaseline to serve as fixative or, if heat has to be applied, a mixture of 3 parts of vaseline with 1 part of paraffin wax. Add 1 ml of saturated (69%) potassium metaborate soln. to the outer chamber and place the lid over the cell. Mix the contents of the outer chamber by rotation and leave for a time varying according to whether the apparatus is rocked or not, and according to the temp. For example, with 1 ml of ammonia soln., 2 hrs. are required without rocking, 1 hr. with rocking, and 0·6 to 0·7 of these values at 38° C. Next, titrate the soln. in the central chamber, into which the ammonia has diffused, with 0·005 *N* hydrochloric acid from a horizontal burette. The end-point is normally sharp, but when the ammonia content of the soln. is very high, and the titration has taken a comparatively long time, the colour may fade at the end-point, but on standing, a stable end-point is obtained by addition of a little more acid. For determining blood urea, mix 0·2 ml of blood in the outer chamber with 0·5 ml of urease-phosphate soln.* Pipette 2 ml of the boric acid or magnesium chloride reagent into the central chamber and, after 15 mins., add 1 ml of saturated metaborate soln. and mix by rotation. After 1¾ hr. titrate the soln. in the central chamber with 0·02 *N* hydrochloric acid. For a more rapid method, mix 0·5 ml of blood with 0·5 ml of the urease-phosphate mixture (diluted 2-fold) in a test-tube and, after 10 min., transfer

* The urease-phosphate mixture is made by stirring or shaking 10 g of jack bean meal with 20 ml of water for 15 min., adding 100 ml of phosphate buffer of *p*H 7·4 (3 g of anhyd. Na_2HPO_4 and 2 g of anhyd. KH_2PO_4 in 100 ml of water) and 20 ml of glycerin, and decanting the soln. through a folded filter. The filtrate is freed from ammonia by shaking with acetic acid-washed Permutit and diluted 3-fold immediately before use.

0·2 ml to the outer chamber and mix with 1 ml of saturated metaborate. Titrate after only 45 min. with 0·01 *N* hydrochloric acid. For large amounts of urea, add to the absorbents, in addition to the other two indicators, a soln. of phenol red in the same concn. as that of the methyl red. Determine urea in urine by putting 0·2 ml of the suitably diluted sample in the outer chamber, and determine ammonia by using 0·2 ml of undiluted urine. The standard deviation of an individual determination in 51 duplicate tests was 0·34 mg of urea per 100 ml.

F. A. R.

Nitrogen of the Potato. A. Neuberger and F. Sanger. (*Biochem. J.*, 1942, **36**, 662–671.)—The nitrogenous constituents of the potato were examined by various methods, and it was shown that the ninhydrin method was more reliable than the nitrous acid method for the determination of α-amino acid nitrogen. Asparagine, glutamine, arginine, tyrosine, leucine, phenylalanine, trigonelline and choline were isolated, and the amounts of asparagine, glutamine, α-amino-acid and basic nitrogen were determined. They varied widely in different varieties and in different layers of the potato. The total nitrogen in different varieties ranged from 0·236 to 0·359%, and the protein from 0·657 to 1·2% of the fresh weights, whilst the protein nitrogen, expressed as a percentage of the total nitrogen, ranged from 37 to 63·7%. Majestic potatoes contained up to 27% of the total nitrogen as asparagine, up to 15% as glutamine, about 10% as α-amino nitrogen, and 21–23% as basic nitrogen. F. A. R.

Effect of Formaldehyde on the Volatilisation of Ammonia, Mono-, Di- and Trimethylamines. G. J. Benoit, Jr., and E. R. Norris. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 823–825.)—A modification of Delépine's method (*Compt. rend.*, 1896, **122**, 1064) has been evolved for the separation of trimethylamine (especially in biological material) from ammonia and mono- and dimethylamines in presence of formaldehyde. Pipette the test soln. into the distillation flask of a Beatty and Gibbons all-glass micro-Kjeldahl apparatus (*J. Biol. Board Canad.*, 1936, **3**, 77), the condenser-outlet of which fits (by means of a glass joint) into a receiving vessel which is connected to a water aspirator. Then add the formaldehyde (U.S.P. grade), and sufficient water to adjust the concn. of formaldehyde to a suitable val. (*vide infra*). Put sufficient 0·07 *N* sulphuric acid in the receiver to cover the condenser outlet, and then add the necessary amount of alkali through the stop-cock provided. Close the system, evacuate the apparatus by means of the aspirator, and cautiously admit steam at 30° C. When distillation is complete (20–30 min.), close the system to the aspirator and steam, and admit air gradually. Titrate the distillate with 0·018 *N* sodium hydroxide (bromocresol green as indicator), unless the amount of amine recovered is less than 25 micromoles, when chlorophenol red should be used. No significant differences in yield occurred if the formaldehyde and amine solns. were mixed and left overnight. A blank expt. should be made. In tests with various quantities of reactants with and without formaldehyde the effect of the latter was to render the ammonia almost completely non-volatile; it had no effect on the recovery of trimethylamine, and it did not always prevent the volatilisation of mono- or di-methylamine. Anomalies were shown by these two last substances; those of dimethylamine were probably due to the methanol in the formaldehyde or formed from it by the Cannizzaro reaction in alkaline soln.; thus, addition of methanol caused an increase in the recovery of dimethylamine, but only a slight increase in that of monomethylamine. Formaldehyde equiv. to a concn. of more than *ca.* 4% in the mixture distilled (or to 0·8 ml of added formaldehyde) was necessary to obtain max. prevention of volatilisation of ammonia. Min. recoveries of mono- and di-methylamines were obtained with formaldehyde concns. of *ca.* 0·6 and 2·0%, respectively, and the removal of the last traces of formaldehyde from the apparatus was necessary if subsequent expts. were to be made. Curves illustrate the effects of time and of the vols. of added formaldehyde or of methanol on the % recoveries. They show that if the amounts of mono- and di-methylamines are small relative to those of the ammonia and trimethylamine, then the amounts recovered of the first two may be below the limit of detection.

J. G.

The Quantitative Estimation of both Cystine and Cysteine in Mixtures. M. X. Sullivan, W. C. Hess and H. W. Howard. (*J. Biol. Chem.*, 1942, **145**, 621–624.)—Cysteine reacts with sodium 1:2-naphthaquinone-4-sulphonate in alkaline soln., with formation of a red colour, and the test can be used for detecting cystine after reduction, for which purpose 5% sodium cyanide soln. has been most generally used. A method of estimating cystine and cysteine in presence of each other has now been based on this reaction. Use is made of the fact that reduction of cystine with sodium cyanide gives only one-half the colour produced by pure anhydrous cysteine, whilst reduction with sodium amalgam gives a quantitative conversion to cysteine. Add 2 ml of freshly prepared 5% sodium cyanide to 5 ml of a soln. containing 1 mg of cystine, stand for 10 mins. at 20–25° C. and add 1 ml of 1% sodium 1 : 2-naphthaquinone-4-sulphonate and shake for 10 sec. Add 5 ml of 10% anhydrous sodium sulphite in 0·5 *N* sodium hydroxide, followed, after 30 min., by 1 ml of 20% sodium hydrosulphite in 0·5 *N* sodium hydroxide. Match the colour against that of a cystine standard similarly treated. To another 7 ml of the cystine soln., 0·1 *N* with respect to hydrochloric or sulphuric acid and containing 100–200 p.p.m. of cystine, add 1 ml of 0·2% sodium amalgam. After shaking for 1 hr., transfer 5 ml of the reduced solution to a test-tube and compare the cysteine content with the amount of cysteine produced by treatment with sodium cyanide. The difference between the two results gives the cysteine present, and this, subtracted from the value found in the second reaction, gives the amount of cystine present.

F. A. R.

Extraction of Phytin of Foodstuffs with Trichloroacetic Acid. M. L. Sarma. (*J. Indian Chem. Soc.*, 1942, **19**, 308–310.)—The following modification of the McCance and Widdowson method (*Biochem. J.*, 1935, **29**, 2694) is proposed for the extraction of phytin from foodstuffs:—Extract 25 g of powdered cereal with 20 ml of 10% trichloroacetic acid for *ca.* 30 min., shaking occasionally, and filter, keeping in a refrigerator throughout. Repeat the extraction similarly, neutralise the combined extracts with *N*/10 sodium hydroxide (indicator, phenolphthalein), acidify slightly with *N*/10 hydrochloric acid and measure total vol. Dilute 20 ml to 50 ml and determine phytin by the McCance and Widdowson method

(*loc. cit.*). Results by this method and by the hydrochloric acid extraction method are compared for various cereals (Table I). In some instances slightly higher values were obtained by the modified method; it is said that these may be due to the more efficient extraction of the phytin. The optimum concn. of trichloroacetic acid was 5–10%. The phytin P in rice (total P, 0·355%) was 0·277 and 0·006% in the first and second extractions respectively, indicating that for practical purposes one extraction is sufficient. This method is considered best when the distribution of acid-soluble phosphorus compounds (for which all determinations should be on the same extract) is required.

E. B. D.

Determination of Inulin. R. S. Hubbard and T. A. Loomis. (*J. Biol. Chem.*, 1942, **145**, 641–645.)—Inulin can be estimated by hydrolysis to fructose and colorimetric estimation with diphenylamine and hydrochloric acid, or by hydrolysis and treatment with resorcinol in the presence of hydrochloric acid. The diphenylamine method is the more sensitive, but an error is introduced if glucose is present; this does not affect the result by the resorcinol method. Both methods can be simplified by omitting the preliminary hydrolysis, and relying on the hydrolysis taking place during the estimation. Interference is caused in both methods by the presence of non-fermentable substances and in the second by reaction between resorcinol and urine. This second reaction can be allowed for by making simultaneous determinations in which resorcinol is omitted. To 1·0 ml of plasma, add 8·0 ml of acid zinc sulphate (12·5 g of $ZnSO_4.7H_2O$ in 125 ml of 0·25 *N* sulphuric acid diluted to 1 litre) and 1 ml of 0·75 *N* sodium hydroxide. After 30 min. filter and dilute the filtrate to contain 0·5–4·0 mg per 100 g of inulin. To 1 ml of the diluted filtrate add 1·0 ml of 0·1% alcoholic resorcinol soln. and 3·0 ml of 30% hydrochloric acid. Mix, immerse for *exactly* 8 min. in a water-bath at 80° C., and cool. Adjust the vol. to 5 ml with 95% alcohol and compare the colour with that given by standard inulin solns. subjected to the same procedure. A photoelectric colorimeter transmitting maximally at 450$m\mu$ is suitable. Protein-free urine can be analysed directly by the same method after dilution so that it contains 0·5–4·0 mg of inulin per 100 g. To 1·0 ml of the diluted urine, add 0·1 ml of alcoholic resorcinol soln. and 3·0 ml of 30% hydrochloric acid. To 1·0 ml of diluted urine in a second tube add 1 ml of 95% alcohol and 3 ml of 30% hydrochloric acid without resorcinol, and measure the colour of both tubes in the same way. Subtract the value of the blank from that of the test soln., and calculate the inulin content from the colour given by a standard solution.

F. A. R.

Activation of (and Activation Tests for) Dicalcium Phosphate for the Chromatographic Determination of Carotene. L. A. Moore. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 707–708.)—Since some dicalcium phosphates of the brand specified in the author's method for the determination of carotene by the chromatographic removal of other pigments (ANALYST, 1941, **66**, 169) have now been found to have inadequate adsorbing powers, activation by treatment with alkali under various conditions has been studied. The following method was finally adopted:—To 100 g of the inactive dicalcium phosphate (preferably Merck's reagent or another coarsely-granular grade) in a 2-litre flask add a soln. of 5 g of potassium hydroxide in 1 litre of water, boil for 15–30 min., filter on a Buchner funnel, and wash with 0·5–1 litre of water. Dry the residue for 24 hr. at 100° C., and break it up in a mortar. The adsorbing activity of a calcium phosphate may be estimated as follows:—Expose dehydrated alfalfa leaf meal for 2–3 weeks in air at *ca.* 20° C. (to develop the non-carotene pigments), shake 10 g with 50 ml of light petroleum for *ca.* ½ min., and decant 20 ml of the extract on to the column of phosphate to be tested. If activation is satisfactory the carotene will pass through, while the chlorophyll will be held at 3–7 mm from the top of the column, and a lower yellow band with a shade of red above it will form 12–25 mm below; the lower yellow band should not pass into the filtrate after considerable washing with light petroleum. The filtration rate may be measured (for a vacuum of 65 cm of mercury) in terms of the time (in sec.) for 100 ml of light petroleum to pass through a column (diam. 2·2 cm) containing 8 g of adsorbent packed with the aid of vacuum and slight tamping; the mean of several vals. gives reliable results. A column 7·5–10 cm deep should permit the passage of 100 ml of light petroleum in not more than 6 (preferably, 4–5) min. Since in general the filtration rate is decreased by activation, the addition of a filter aid may be desirable to save time and prevent destruction of carotene; a commercial dextrose (Dyno) was found suitable. The height of the column before and after application of the vacuum should also be recorded. Quantitative data for the degree of activation are obtainable as follows:—Add 0·5 g of adsorbent to 1–10 ml (according to the activity anticipated) of a soln. of cryst. chlorophyll (1 mg per ml) in light petroleum in a 30-ml test-tube; dilute to 15 ml with light petroleum, shake well, and centrifuge the mixture. If all the chlorophyll is adsorbed, remove an appropriate vol. of the liquid, replace it by the same vol. of the chlorophyll soln., and shake again. When the final soln. remains green pipette the soln. into the micro-cell of an Evelyn photoelectric colorimeter fitted with a M-440 filter, and determine the vol. to be added to bring the galvanometer reading down to an arbitrarily-chosen val. (*e.g.*, 60·0); from this the chlorophyll (in mg) adsorbed per g of adsorbent is calculated. The fact that freshly-dried activated dicalcium phosphate will retain carotene if more than 5 g of sodium hydroxide per 100 g are used in the activation process has been used for making chromatographic determinations of extracts of yellow maize; this property is however, lost after a few days' exposure at *ca.* 20° C. Poorer degrees of activation were obtained by treatment with sodium hydroxide, trisodium phosphate or disodium phosphate, and in general the greater the alkalinity of the activating agent, the greater its effect. Barium hydroxide and aluminium oxide adsorbents may be activated with alkali by a similar procedure.

J. G.

Modified Micro-Fermentation Method for the Estimation of Thiamine. E. S. Josephson and R. S. Harris. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 755–756.)—The method of Schultz, Atkin and Frey (ANALYST, 1942, **67**, 170) has been improved as follows:—Prepare suspensions of solid samples (clear liquid samples require no preparation), and make a preliminary test by diluting until a 1-ml aliquot portion, diluted to 1000 ml, stimulates the production of carbon dioxide by yeast to an extent equiv. to 10–20 × 10^{-9} g of cryst. thiamine. To one of 2 aliquot portions of sample in 100-ml

glass-stoppered flasks add 5 ml of fresh 4% sodium sulphite ($7H_2O$) soln., to inactivate active substances other than thiamine, and 20 ml of water; to the other add 25 ml of water. Adjust the *p*H of both to 5·2 (to a buffered methyl red indicator) with 15% sodium hydroxide soln. or 8% sulphuric acid. Plug the flasks with cotton wool, heat them in flowing steam at 100° C. for 30 min., cool, and destroy the excess of sulphite (important) by 3% hydrogen peroxide, adding 2 drops in excess; a soln. containing 5% of potassium iodide, 1% of sol. starch and 32% of sulphuric acid is used on a spotting-plate as outside indicator. Adjust both treated and untreated solns. to *p*H 6·2 with 15% sodium hydroxide soln. (neutral litmus as indicator), and dilute to 100 ml. The concn. of carbon-dioxide-stimulating substances in the untreated soln. should be equiv. to 10–20 × 10^{-9} g of thiamine per ml; it may be below this range in the treated soln., but if it is, 10 × 10^{-9} g of thiamine should be added (as a standard soln., *vide infra*) before dilution. This is often necessary with foodstuffs, but not with urines. Fermentation is carried our preferably in a Warburg–Summerson micro-fermentation apparatus, (Summerson, *J. Biol. Chem.*, 1939, **131**, 579). In the main reaction-chamber place 1 ml of water, and in the inset chamber place 1 ml of the test soln. or of the thiamine standard *A* or *B* (*vide infra*). Mix in a 100-ml flask 15 ml of soln. *Y*, 10 ml of soln. *X* (*vide infra*), 7 ml of 15% ammonium sulphate soln. and 25 ml of a fresh 2% baker's yeast suspension, and note the time when the yeast is added; dilute to 100 ml., and add 1 ml of the mixture to the inset chamber of each flask. Fix the flasks to manometers, immerse them in a water-bath at 30° C., flush them through with nitrogen at the end of 10 min. after the yeast addition, and shake the flasks at 70 double-oscillations (amplitude, 3 cm) per min. Stop the nitrogen after 50 min., close the gas valves, and release excess pressure 5 min. later. Make a zero reading at exactly 60 min., close the system, and make a final reading 60 min. later. The calculation is based on the comparison of the vol. of gas evolved from the test soln. (after correcting for the non-thiamine stimulating substances inactivated by sulphite treatment) with that from the standard thiamine solns. *A* and *B* (which must be determined each time); a linear relationship between thiamine concn. and carbon dioxide evolution is assumed for the range 10–20 × 10^{-9} g. Soln. *X* contains 7·0% of citric acid ($1H_2O$) and 9·08% of dipotassium hydrogen phosphate; soln. *Y*, 20 g of anhydrous dextrose, 2·2 g of monopotassium dihydrogen phosphate, 1·7 g of calcium chloride ($2H_2O$), 10 g of magnesium sulphate ($7H_2O$), 0·01 g each of manganous sulphate ($4H_2O$) and ferric chloride ($6H_2O$), with 0·067 g of cryst. nicotinic acid per litre. Prepare a stock thiamine soln. by dissolving 50 mg of thiamine hydrochloride (dried to const. wt. over phosphorus pentoxide) in a mixture of 26·4 ml of non-denatured 95% alcohol and 0·42 ml of conc. hydrochloric acid, and dilute to 100 ml; this is stable for several months at 4° C. in a stoppered flask. Immediately before each expt. wet the walls of a 500-ml flask with 5 ml of 1% gelatin soln. and 100 ml of water, add 1 ml of the stock thiamine soln., and dilute to the mark. Prepare thiamine standards *A* and *B* similarly, so that 100 ml contain 1 ml of the gelatin soln. and 1 and 2 ml, respectively, of the thiamine substock soln. (final concns., 10 × 10^{-9} and 20 × 10^{-9} g per ml, respectively). Sterilise solutions *X* and *Y*, and the ammonium sulphate and the gelatin solns., in flasks plugged with cotton wool and covered with tin foil, in flowing steam at 100° C. for 30 min. on 3 successive days, and store at 4° C. after opening. The method is very accurate, and particularly suitable for plant and animal tissues or fluids. Duplicates agreed to within 2%, and were within 5% of the results obtained by fluorometric assay. The method is preferable to the latter for very small concns. of thiamine (*e.g.*, 10^{-8} g per ml), or to obtain information on what happens to thiamine during the handling of food and in tissue metabolism. J. G.

Colour Reaction between Ergosterol and Methyldichlor-arsine. P. M. Baranger and J. M. Mercier. (*Biochem. J.*, 1942, **36**, 703–705.)—When a 3% chloroform soln. of ergosterol is added to a dil. chloroform soln. of methyldichlor-arsine, a golden-yellow colour is formed, the intensity of which reaches a maximum after a certain time. The value of this maximum is a linear function of the concn. of methyldichlor-arsine, and the method can be used for estimating this compound. Add a measured vol. of the test soln. (0·04–0·12 mg/ml) to 5 ml of a freshly prepared 3% soln. of ergosterol in pure, dry chloroform and dilute to 10 ml with chloroform in the cell of a Pulfrich photometer maintained at 46–47° C. At lower temps., colour development is slow. Use pure chloroform in the blank cell and measure the extinction coefficient at 430$m\mu$ at intervals. Plot the values as obtained and calculate the max. absorption; from this, by reference to a standard curve, calculate the concn. of methyldichlor-arsine. F. A. R.

Bacteriological

Determination of the Bacterial Count on Restaurant Glassware. M. Novak and A. M. Lacy. (*Amer. J. Hyg.*, 1942, **36**, 316–320.)—A review of the literature reveals that a swabbing technique with various modifications has been most widely used in the examination of drinking vessels for bacterial contamination, and that there are many variable factors in the swabbing procedure which may affect the quantitative accuracy of the results. Other methods, such as the use of agar discs pressed on the surface of the vessel, the making of rim impressions on hard and soft agar, and the swabbing of the inner surface of the vessel into sterile water contained in it with subsequent disintegration of the swab in the water, all have disadvantages. The following method overcomes some of the difficulties in existing methods. Cool the vessel (*e.g.*, a tumbler) in a refrigerator for a few min., pour into it 20 ml of double-strength nutrient agar, previously melted and cooled to 45° C. and, holding the vessel almost horizontally and rotating it, cover the entire inner surface smoothly and evenly with agar, which sets immediately. Dip the open end of the vessel into another 20 ml of melted agar in a Petri dish and rotate it so that the rim and *ca.* 2 cm of the outer surface are similarly covered with agar. Incubate the tumbler in a sterile beaker covered by a Petri dish lid and incubate the Petri dish (containing the residue of the second 20 ml of agar) to include any organisms washed off during immersion of the rim of the tumbler. By the use of blood agar, haemolytic species of bacteria may be detected. When the count is low the entire surface of the agar may be counted, but, as a rule, the counting of a fraction of the area is preferable. The method eliminates all the variable factors in the swabbing

technique, dispenses with pour plating and dilution and, when counts are high, the vessel can be used as an ocular demonstration to the restaurant proprietor of the imperfection of the washing methods. The method may also be used to test the efficiency of germicidal agents on vessels artificially inoculated with known spp. of bacteria, and relatively accurate quantitative results are obtained. A. O. J.

Agricultural

Fractionation of the Organic Matter including Nitrogen of certain Soils and its Relation to their Quality. M. R. F. Ashworth. (*J. Agric. Sci.*, 1942, **32**, 349–359.)—The procedure is a slight modification of the scheme of proximate analysis used by Shewan (*J. Agric. Sci.*, 1938, **28**, 324), itself a modification of that of Waksman and Stevens (*Soil Sci.*, 1928, **26**, 113, 239; **27**, 271, 389; 1930, **30**, 97; *Amer. J. Sci.*, 1930, **19**, 32). *Water extraction.*—Boil the soil (12–100 g according to the amount of organic matter present) with 300 ml of water under reflux for 1 hr. Allow to stand for 24 hr., filter through poplin on a Buchner funnel and dilute the filtrate and washings to 1 litre. Concentrate an aliquot portion and determine nitrogen by the Kjeldahl method. Evaporate a similar aliquot portion to dryness, dry the residue at 105° C. for ½ hr., weigh, ignite at *ca.* 500° C. and re-weigh to obtain the total water-soluble organic (ash-free) matter. *Alcohol-benzene extraction.*—Extract the bulk of the air-dried residue from the water extraction (8–80 g) in a Soxhlet extractor with alcohol and benzene mixture (1 : 1) for 30 to 36 hr. Dry the sol. matter at 105° C. for 15 min. and for successive 10-min. periods until the wt. is constant. *Dilute acid hydrolysis.*—Reflux the oven-dried residue from the organic solvent extraction (4–30 g) with 300–350 ml of 2% hydrochloric acid for 5 hr. and, after 24 hr., filter through No. 50 Whatman paper in a Buchner funnel, wash the residue well with water and make the filtrate and washings up to 1 litre. Determine the sol. nitrogen in an aliquot portion (500–700 ml). Determine reducing sugars by the modified Lane and Eynon method as follows:—Make an aliquot portion (25–100 ml) just alkaline to bromocresol purple with 2·5% sodium hydroxide soln. and add 5 ml each of the Lane and Eynon solns. After boiling for 1 min. add 4 drops of 1% methylene blue soln. and titrate with standard glucose soln. (0·2%), 5-ml portions being added at 20-sec. intervals to obtain an approx. titre. In subsequent accurate titrations add as much as possible of the standard glucose soln. before boiling and sufficient water to ensure that the vol. at the end-point shall be the same in every determination. Add the final 0·3–1·0 ml of glucose soln. slowly in drops to the boiling liquid. Standardise the Fehling's soln. against the glucose soln. at the same final vol. Each ml of glucose soln. × 0·9 ≡ mg of hemicellulose. The method differs from that of Shewan chiefly in omitting the removal of sesquioxides by pptn. and thus avoiding errors due to adsorption of glucose by the ppt. *Conc. acid extraction.*—Treat the finely powdered residue with 25 ml of 80% sulphuric acid for 2½ hr. at 12–14° C. with frequent stirring. After dilution to 900 ml, reflux the mixture in a 1500-ml flask for 5 hr., leave for 24 hr., filter through a No. 50 Whatman paper, wash the residue with water and make the filtrate up to 1 litre. Determine reducing sugars as before. *Residue.*—Weigh the oven-dried residue and determine the nitrogen and ash (at *ca.* 900° C.) on portions. Deduct the protein content (N × 6·25) from the organic (ash-free) residue and regard the remainder as lignin and allied compounds. On the original sample determine carbon by Robertson and Shewan's wet combustion method (*J. Soc. Chem. Ind.*, 1935, **54**, 35T), nitrogen, ash and moisture. For the ammonia and nitrate contents use Olsen's method (*C.R. Trav. Lab. Carlsberg*, 1928, **17**, 1), but use thymol blue papers instead of Congo red, and filter the extracts through Postlip Mill paper instead of through a Berzelius filter. Express the proximate fractions as % of the total organic matter (C × 1·724) and the nitrogen as mg of nitrogen per 100 g of total organic matter. The seven soils investigated were (1), hill peat; (2) Scots pine woodland; (3) *Calluna* heath; (4) open *Deschampsia* pine woodland; (5), deciduous woodland; (6) lawn; (7), old pasture, the order being that of improving quality. This gradation of quality was brought out by many of the figures of the proximate analysis. The better-quality soils (5, 6 and 7) were associated with:—(*a*) lower average contents of fats, waxes, hemicelluloses and cellulose; (*b*) a rapid decrease in cellulose with increasing depth; (*c*) higher total nitrogen, ammonia and nitrate and higher nitrate/ammonia ratio; (*d*) a higher proportion of the more sol. nitrogen compounds and a ratio of average dil. acid-soluble nitrogen to average residual nitrogen increasing with quality. In all the soil profiles the total nitrogen and its fractions generally increased with the depth. Water-sol. organic matter and lignin showed no obvious correlation with quality; in general, the water-sol. organic matter decreased and the lignin increased with the depth. A. O. J.

Accurate Wet Combustion Method for the Determination of Carbon in Soils. P. Alper. (*J. Agric. Sci.*, 1942, **32**, 389.)—In Alper's method for the determination of carbon in soils (*J. Agric. Sci.*, 1938, **28**, 187) the soil is digested with sulphuric acid and potassium dichromate, the products of combustion are passed through a heated combustion tube, and the carbon dioxide is absorbed in barium hydroxide soln. Digestion and absorption take place in a closed system, the absorption flask being evacuated and separated from the digestion flask by a glass tap which is manipulated as the pressure in the system (indicated by a mercury gauge) increases. To avoid constant manipulation of the tap, the pressure gauge has been replaced by an elastic bladder (a toy rubber balloon is recommended), which is connected to the system by means of a glass T-piece. At the beginning of the expt. the pressure is below atmospheric pressure, owing to the suction of air through the system, and the balloon is deflated. As digestion proceeds the balloon distends and manipulation of the tap may be necessary, the tap being opened when distension of the balloon becomes too great and closed when the balloon is deflated. When the digestion is complete the washing of the apparatus by air deflates the balloon completely. It is unnecessary to watch the expt. closely, and manipulation of the tap is required only during the first few min. of the digestion, when gases are quickly evolved. A. O. J.

Organic

Estimation of Carboxyl Groups in Commercial Starches Modified by Oxidation. L. H. Elizer. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 635–636.)—Oxidation of starch may result in the

conversion of (*a*) aldehyde groups or (*b*) primary alcohol groups to carboxyl groups; (*c*) secondary alcohol to ketone groups; (*d*) glycol to carboxyl groups, with rupture of the pyranose ring. The number of carboxyl groups is, therefore, a measure of the degree of oxidation, and is preferable to a viscosity measurement in this respect, because hydrolysis may also modify the viscosity. Three methods have been developed. *Silver method* (*cf.* Sookne and Harris, *Amer. Dyestuff Reptr.*, 1941, **30**, 107).—Prepare the sample by stirring 35 g with 150 ml of 2·5% v/v sulphuric acid, and after 2 hr. at *ca.* 20° C., filter on a Buchner funnel and wash with water until the filtrate gives no reaction with silver nitrate soln. Dry at below 60° C., transfer to an air-tight container, and determine the moisture content. This procedure converts any carboxyl groups which may have been neutralised by processing back again to the free acid grouping. The reagent is prepared by adding 14 g of silver oxide to 14 g of molten *o*-nitrophenol, and making a satd. soln. (approx. 0·01 *M*) of the resulting cake. Pipette 50 ml of reagent into a 125–ml flask containing 0·75 g of sample; stopper, shake well, and after *ca.* 18 hr. pipette out 25 ml of the clear supernatant liquid. To this add 5 ml each of dil. (1 : 5) boiled nitric acid, and ferric alum indicator, and titrate with standard 0·01 *M* ammonium thiocyanate; allowance is made for a blank expt. with 25 ml of silver reagent. The results are recorded as mg-equiv. per 100 g of dry starch. It may also be advisable to wash the sample with distilled water until the washings give no ppt. with silver nitrate soln., then to wash with dil. sulphuric acid, and finally to repeat the water wash. *Copper Method.*—Shake 0·75 g of washed (*vide supra*) sample in a stoppered 125–ml flask with 50 ml of a 0·01 *M* soln. of neutral cupric acetate, and after *ca.* 18 hr. remove 25 ml of the clear liquid and add to it 5 ml of 2 *N* acetic acid and sufficient potassium iodide to dissolve the cuprous iodide formed. Titrate with standard 0·01 *M* sodium thiosulphate, with 1 ml of starch soln. as indicator, allow for a blank on 25 ml of copper reagent, and express the result as mg-equiv. per 100 g of starch. The values obtained are slightly high as compared with those by the silver method when oxidation is moderate or high, and this may be due to the higher concn. of copper ions at equilibrium (*e.g.*, twice as many univalent silver ions as bivalent copper ions are removed from soln. for a given number of carboxyl groups). *Colorimetric Method* (recommended for routine work).—Stir well 1 g of sample and 25 ml of a 1% copper acetate soln., allow to stand for 10 min., filter on a Buchner funnel, and wash with water until the filtrate gives no copper reaction with 1% potassium ferrocyanide soln. Add to (presumably) the residue 25 ml of the potassium ferrocyanide soln., stir well, filter after 2 min., wash with water until the washings are colourless, dry and compare the resulting colour with those of standards prepared by heating oxidised starches of known properties in the same way. Data are given for 7 commercial oxidised maize starches, 1 unmodified maize starch, and 1 hydrolysed, thin-boiling maize starch. To prepare oxidised starches for testing purposes, stir 50 g of starch with 100 ml of water and 50 ml of sodium hypochlorite soln. (5·25% available chlorine) for 3 hr. at *ca.* 20° C. filter, wash and dry. J. G.

Preparation of Extractive-free Plant Material for Analysis. Anon. (*Paper Trade J.*, 1942, **115**, *T.A.P.P.I. Sect.*, 226.)—For many analytical purposes it is necessary to subject plant material to a preliminary extraction process (*e.g.*, prior to cellulose determinations, *cf.* Bloom, Jahn and Wise, ANALYST, 1943, **68**, 27. A standardised method applicable to woods, bark, straw and similar fibrous materials has therefore been proposed by *T.A.P.P.I.* Place the sample in a fritted-glass or alundum Soxhlet extraction thimble, or in a well-washed bag of cotton cloth of fine weave, and extract for 4 hr. in a Soxhlet apparatus with a mixture of *ca.* 95% ethyl alcohol and benzene (1 : 2 vol.); extraction rate, not less than 4 siphonings per hr. Transfer the residue to a Buchner funnel, wash the benzene out of it with the alcohol, return the residue to the thimble, and extract it with the alcohol alone for 4 hr., or until the alcohol liquid siphoning over is colourless. Spread the residue in a thin layer and, after evaporation of the alcohol, extract it in a conical flask with 3 successive 1-litre portions of water for 1 hr. in a boiling water-bath. The water should be boiling before immersion of the flask, which it should surround completely. Finally, filter the residue in a Buchner funnel, wash it with 500 ml of boiling water, and allow it to dry in air. J. G.

Inorganic

Pyrex Brand Glass Wool as a Filtering Medium. G. B. Heisig. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 766.)—When this material was used as a filtering medium in semi-micro-analysis involving extraction with fairly strong acids or bases, aluminium was incorrectly reported (*e.g.*, 1 ml of 0·1 *N* hydrochloric acid gave a positive aluminon reaction after extraction of *ca.* 1 g of the wool). After extraction several times with dil. hydrochloric acid and thorough washing, the aluminium reaction obtained from a further acid extraction was positive but very faint. Extracts in hot 5 *N* hydrochloric acid or 2 *N* sodium hydroxide of a sample of high-silica glass wool submitted by the Corning Glass Works (not yet available commercially) gave no aluminon reaction. J. G.

Detection of Lead as Rhodizonate. F. Feigl and H. A. Suter. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 840–842.)—Neutral lead solns. give with sodium rhodizonate a blue-violet amorphous ppt., $PbC_6O_6.Pb(OH)_2.H_2O$; in slightly acid solns. a crystalline scarlet red ppt. is produced. The buffer soln. (*p*H 2·79) contains 15 g of tartaric acid and 19 g of sodium bitartrate in 1 litre. Place a drop of test soln. on filter-paper, and touch the spot with a drop of 0·2% sodium rhodizonate soln. Lead produces a blue-violet colour. Sensitivity: 0·1μg at 1 : 500,000. Thallium, silver, cadmium, barium, and stannous ions also react at *p*H 2·8. *Mixed chloride ppts.*—Dry the ppt. in a crucible and heat to redness; thallous and mercurous chlorides volatilise. Treat the cold residue with 4 drops of strong ammonia, evaporate to dryness, add 3 drops of buffer soln. and 1 of reagent; a red colour or ppt. indicates lead. *Mixed sulphate ppt.* (Ba, Pb).—Wash the ppt. free from sol. sulphate, transfer 0·05 g to a spot plate, mix well with 5 drops of saturated sodium acetate soln. in 6 *N* acetic acid, dry in a current of hot air, and add 1 drop of water and 1 of reagent. The mass becomes violet on stirring if Pb : Ba > 1 : 10,000. *Lead ores.*—Place 1 mg on a spot plate, add 3 drops of buffer soln. and, after 2 min., 1 drop of reagent; lead gives a red ppt. or colour. *Alloys, pigments, glass.*—Treat a few mg of filings (free from grease) or powder with

3 drops of buffer soln. on a spot plate, stir for 2 min., and add a drop of reagent without stirring; lead gives a red ppt. or colour. If the alloy is very low in lead, run a blank; this becomes colourless after a few minutes. W. R. S.

Determination of Oxide Copper in Ores. R. S. Young and D. G. M. Graham. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 787–788.)—The extraction of oxidised copper by the solvent usually recommended (3% sulphur dioxide soln.) was found to be incomplete, but 5% sulphuric acid saturated with sulphur dioxide gave theoretical results with chrysocolla, malachite, and azurite. No satisfactory solvent was found for cuprite (Cu_2O) in presence of sulphides. Iron as sulphide or oxide does not attack sulphide copper when the acid sulphur dioxide solvent is used, but ferric sulphate (if initially present) attacks sulphides; hence the determination of oxide copper becomes unreliable. W. R. S.

Colorimetric Determination of Titanium in Chromium Steels. L. Silverman. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 791–792.)—Chromium trioxide is insol. in cold perchloric acid (69 to 72%). The following method is proposed for 18–8 chromium nickel steel (Bur. St. No. 121). Dissolve 1 g in 15 ml of acid mixture (hydrochloric acid 300, nitric acid 100, water 400 ml), add 30 ml of perchloric acid, and heat for some min. after pptn. of red chromium trioxide. Cool, filter on a Gooch crucible (asbestos), wash with cold perchloric acid previously heated until heavy white fumes appear. Evaporate the filtrate to 15 ml in a 150-ml beaker, dilute with 25 ml of water, and expel chlorine by boiling. Dilute with 5% sulphuric acid and determine titania with hydrogen peroxide. To analyse Standard No. 121 (0·394% Ti), dissolve the red ppt. in water, re-ppt. by heating with perchloric acid, filter, and combine the filtrates. Vanadium, if present, interferes. Decolorise the yellow titanium soln. with hydrofluoric acid; a brown residual soln. proves the presence of vanadium. W. R. S.

Volumetric Determination of Sulphate. S. W. Lee, J. H. Wallace, W. C. Hand and N. B. Hannay. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 839–840.)—A 10% soln. of the sodium salt of tetrahydroxyquinone is used as a spot test on filter-paper. Titrate the neutralised assay soln. with standard barium chloride soln., adding from time to time a drop of assay soln. to a wet spot of indicator on filter-paper. The first change (from yellow to pink) occurring in 2 to 3 sec. indicates the end-point. Other spots (15 sec. or more) should be disregarded. Free acid, aluminium, and much sodium chloride interfere. Aluminium may be removed by ammonia pptn. Dilute solns. containing sodium chloride with an equal bulk of alcohol, keeping the total vol. at 30 to 40 ml. Put a drop of 15% silver nitrate soln. near the indicator spot to make the spreading boundaries meet, and then place a drop of assay soln. inside the silver nitrate spot close to the indicator spot. W. R. S.

Standardisation of Thiosulphate Solution. S. O. Rue. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 802–805.)—The standardisation and the stability of thiosulphate solutions in hot weather are discussed in detail. *Recommended method.*—Dissolve 0·2–0·22 g of potassium dichromate in 125 ml of water in a 500-ml conical flask, add 5 g of pure potassium iodide, rotating until dissolved, and then add 5 ml of 6 *N* hydrochloric acid during swirling. Rinse down the sides so as to form a supernatant layer, stopper and keep in the dark for 6 min., add 165 ml of water, titrate with 0·1 *N* thiosulphate during constant agitation until the liquid is greenish-yellow, and finish the titration after addition of 0·6% starch soln. (2 ml at 20°, 3 ml at 40° C.). Results are tabulated showing stability during 4 months' storage at various temperatures and with a variety of preservatives. Chloroform and mercuric iodide, the most satisfactory stabilisers, were effective for about 2 months at 40° C., whilst sodium hydroxide and carbonate actually accelerated decomposition. W. R. S.

Electrometric Titration of Alkali Carbonates, Sulphides, Hydroxides and Acetates. G. J. Crocker, L. A. Dow, N. F. Ripanti and W. J. Nolan. (*Paper Trade J.*, 1942, **115**, *T.A.P.P.I. Sect.*, 243–250.)—Two new methods are described:—*Modified Wheatstone Bridge Method.*—The 4 arms of the bridge comprise respectively a 100-ohm and 1000-ohm resistance (wound non-inductively), the cell, and a variable resistance to balance it. It is activated by a 2–20 volts A.C. supply (obtained from the 110-volt, 60-cycle mains through a variable transformer), and the end-point is indicated visually by a copper oxide rectifier and galvanometer. The cell comprises 2 platinised platinum electrodes (diam., 0·5 in.), 1·5–2 in. apart, in a 600-ml beaker fitted with a glass stirrer. To 10 ml of soln. to be titrated add 250–300 ml of water, start the stirrer, set the transformer output to 2–5 volts, and adjust the resistance to obtain the min. galvanometer deflection; the use of a low initial voltage gives increased accuracy in the initial stages of titration. Raise the voltage to 18 v., again adjust the resistance to obtain a min. deflection, and note the resistance setting. Add 0·1 or 0·5 *N* sulphuric acid and balance the bridge for each 1-ml increment, noting the resistance setting each time. Titration-time, 45 min.; error, *ca.* 1%. *Measurement of the Cell-Voltage Drop.*—A simpler circuit for rapid routine work requires only 10–15 min. for a titration, and has only a slightly lower accuracy. A 10-ohm, 2-ohm, and a water-cooled 100-ohm resistance are connected in series with the cell (which should be water-cooled), and the 110-volt, 60-cycle, A.C. mains supply. A 2000-ohm voltmeter (range, 0–150 volts) is connected in shunt across the cell, and a similar voltmeter (also in shunt) across the first voltmeter and the 100-ohm resistance. The cell comprises two 3-in. 20-gauge Nichrome (or other alkali-resistant) wire electrodes, wound in close spirals on ⅛-in. centres, in a 600-ml beaker, and they are kept always 2 in. apart by slipping them over the arms of a U-shaped glass rod. To 10 ml of the soln. to be titrated add 500 ml of water, start the stirrer, and adjust the 100-ohm resistance until the voltage drop across the cell is *ca.* 50% of the total line-voltage (shown by the first and second voltmeters, respectively, *cf. supra*). This adjustment must remain constant throughout the titration. Add 0·5 *N* sulphuric acid in 1-ml increments, and record the voltage drop across the cell (first voltmeter, *supra*) continuously, maintaining the const. line-voltage by adjusting the 10- and 2-ohm resistances. Titration curves (vol. of acid added against resistance of cell) are reproduced for sodium hydroxide, carbonate, sulphide and acetate, and for sodium-organic compounds in the form of black liquor from the soda process for cooking wood, both singly and in certain mixtures. Sharp end-points were obtained with all the solns. of pure

substances tested. With mixtures of hydroxide and carbonate the results were satisfactory only when 0·1 *N* acid was used, and the increments were small; otherwise insufficient points are obtainable in the neighbourhood of the bicarbonate end-point, which may be in error to the extent of 6%. With mixtures of hydroxide, carbonate and acetate the slopes of the acetate and bicarbonate-to-sulphate lines are so similar that an accurate end-point is not obtainable; however, the hydroxide and carbonate-to-bicarbonate end-points are so sharp that the acetate may be determined accurately by difference. With mixtures of hydroxide and sulphide the hydroxide and sulphide-to-hydrosulphide curves are so similar that the end-point is difficult to determine, although the hydrosulphide-to-sulphide conversion is sharply defined; the hydroxide may therefore be obtained by difference. Black liquors are titrated directly to obtain the total sodium, and again after addition of barium chloride soln. (which ppts. organic matter) to obtain the hydroxide and sulphide; the second titration is made without removing the ppt. The resulting data and moisture, ash and sp.gr. determinations (methods for which are described) enable the % wood-removal and concn. of chemicals to be calculated. This method was used to follow the wood digestion process.

J. G.

Alkalimetric Standardisation of Iodine Solution. F. L. Hahn. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 773.)—Decolorise a measured vol. of iodine soln. with a soln. of pure alkali sulphite slightly alkaline to thymolphthalein; 1 or 2 drops in excess do not matter.

$$Na_2SO_3 + H_2O + I_2 = Na_2SO_4 + 2HI.$$

Then titrate the acid formed with standard alkali, using phenolphthalein as indicator. W. R. S.

Reviews

SPECTROSCOPY AND COMBUSTION THEORY. By A. G. GAYDON, M.Sc., Ph.D. With a Foreword by Prof. A. C. EGERTON, F.R.S. Pp. x + 191. London: Chapman & Hall, Ltd. 1942. Price 17s. 6d.

Human curiosity has always been excited by the nature of flame, but, although considerable progress has been made during the last few years, much remains to be discovered concerning the mechanism of combustion. Spectroscopy is a powerful weapon in the hands of workers in this field and, as various observed combustion spectrum bands yield themselves to analysis, the problems begin to be solved. Dr. Gaydon, who is well known for his own contributions to the technique of flame spectroscopy and molecular spectrum analysis, has summarised in this book the results obtained and the theories formulated during recent years.

In his preface the author states that he has endeavoured to present the subject so that it may be read easily by the beginner; hence the first two chapters are devoted to an explanation of molecular spectra, with particular reference, of course, to those of diatomic molecules, while the factors governing the band systems of an emitter are considered. This part of the book might perhaps have been expanded to advantage, for subsequently more knowledge is assumed than any "beginner" could hope to possess. In the next four chapters the spectra of individual flames are described and possible combustion mechanisms are discussed on the basis of the results. The flames of hydrogen, hydrocarbons, and carbon monoxide and cool and atomic flames are dealt with individually and a special chapter is devoted to explosion spectra and the contribution of spectral investigation to the problems of knock in the internal combustion engine. The emission spectra of flames of nitrogen compounds, sulphur, hydrogen sulphide and halogen compounds all receive individual description.

With the ninth chapter begins the study of the absorption spectra of flames. The whole spectrum is used for this work, but particularly the infra-red, in spite of the difficulty of working at longer wavelengths. Life times of activated molecules, considered mainly from a theoretical standpoint, and the after-burning of carbon monoxide are discussed and, after a chapter devoted to measurement and calculation of flame temperature and another on dissociation energies, the author indicates "a few personal ideas of the lines along which the application of spectroscopy to the theory of combustion may be developed most profitably." Dr. Gaydon emphasises the need for quantitative measurement both in emission and absorption studies and points out that very little work has been done on the spectra of flames in the photographic infra-red, a region which might well be studied with profit.

An appendix contains details of the principal band systems occurring in organic and inorganic flame spectra, atomic and molecular energy levels and spectra of various flames. The book concludes with 258 references to original literature.

Every page reveals evidence of careful compilation; as Prof. Egerton says in his foreword, "many will find useful a book which collects together what the spectroscope tells about flames."

R. A. FINCH

A TEXT-BOOK OF BACTERIOLOGY. By R. W. FAIRBROTHER, D.Sc., M.D., F.R.C.P., F.I.C. Fourth Edition. Pp. vii + 463. London: Heinemann. 1942. Price 17s. 6d.

Students of bacteriology, especially those studying medicine, are fortunate in having available a text-book which presents in such a clear and concise manner the fundamentals of bacteriology, as related to the prevention, diagnosis and treatment of disease. The fact that four editions have been called for in as many years is proof of the welcome that this book has received. Part I deals with general bacteriology and includes chapters on the morphology, biology, cultivation, multiplication and classification of bacteria, infection, immunity, antigens, hypersensitiveness and bacteriology and medicine. A new chapter on chemotherapy, dealing mainly with the sulphonamides, is also included; in this the author makes it clear that the normal administration of this class of drugs may produce severe toxic effects in susceptible persons, whereas penicillin, when given intravenously, has no toxic effects. The chemical synthesis, if possible, of this latter substance is eagerly awaited, so that it may be produced in quantity. Part II, consisting of 259 pages, contains detailed, up-to-date, information concerning the various disease organisms, under the headings of morphology, cultural characteristics, resistance, biochemical activity, serology and pathogenicity. The particulars given should enable the student to classify an organism, but the bacteriologist must be prepared to encounter variants which do not strictly conform to type. This section of the book also contains revised chapters on cerebrospinal meningitis, tuberculosis, viruses, rickettsia and the bacteriology of water, milk and shellfish. Part III contains instructions for the preparation of media and the common staining solutions and also for the carrying out of the serological technique. In a future edition the modified desoxycholate-citrate selective medium, which has proved valuable for the isolation of organisms of the dysentery and Salmonella groups, might be described.

This well-produced, carefully compiled and fully indexed text-book can be strongly recommended to all who are engaged in the study of medical bacteriology.

D. J. T. BAGNALL

WAR GASES. THEIR IDENTIFICATION AND DECONTAMINATION. By M. B. JACOBS, Ph.D. Pp. 180. New York: Interscience Publishers, Inc.; London: Imperia Book Co., Ltd. 1942. Price $3.00.

It is quite depressing to note the volume of literature which now exists on chemical warfare, how much scientific investigation and how many papers written originally for quite other purposes have now been diverted to this inglorious subject. Even our food has now to be considered from this point of view. With apologies to Henry Hart Milman it can be said truly:

"Death rides on every passing breeze,
He lurks in very flour;
Each packet has its own disease,
Its peril every hour."

Such are the sordid facts.

This volume is written primarily for gas identification officers, chemists, medical officers and wardens, who may have to deal with civilian defence against gas. It is, therefore, a blend of the theoretical with the practical, of science with common sense. The subject-matter has been culled from recent literature and the advice or opinions are those of the author and not of any official department. There are frequent citations from official publications of the Ministry of Home Security and references to other works, with due acknowledgment of the sources. Dr. Jacobs has done a useful service in compiling a volume in which are collected and sifted most of the published methods for the identification and often the quantitative estimation of all the well-known war gases. There are useful tables of data much to the point, such as lethal limits, limits of detectable odour, physiological responses and the action of water and of alkali. The book is ancillary to those we already have, such as the works by Sartori or Prentiss, in that it deals solely with tests, effects and decontamination, not with the manufacture or the pure chemistry of these substances. The effect of gases on foods is considered and some methods of analysis are given, but we

should like to have seen much more experimental detail on the examination of foods, limits of sensitivity and the like. In my opinion the work has been well done, but of necessity it suffers from the defect that it is a compilation and, so far as concerns sampling and decontamination technique, is based on common-sense expectation and probability rather than on actual test or experience, which happily we are all without. Nevertheless there are valuable data based on experiment, such as the quantity of decontaminating agents likely to be required for different surfaces. There is also information on the effect of gases on such materials as clothing, leather, and water, different foods and packing materials. Arsenic is well discussed, including the special difficulty attending its determination in toxic smokes, such as was recently debated in our Society. It is of interest to note that no limit of tolerance is recommended for arsenic pollution, although there is mention of 0·025 grain per lb. for spray residues on fruit. Physiological effects of gases are catalogued, but first-aid is not included

It is useful to have all this information to hand in one volume, and Jacobs's book will be welcome to analysts and to gas identification officers whose unpleasant duty it is to think on these things. H. E. Cox

PHYSICAL AND CHEMICAL METHODS OF SUGAR ANALYSIS. By C. A. BROWNE, Ph.D., and F. W. ZERBAN, Ph.D. Third Edition. Pp. xiv + 1353. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1941. Price $15; in London 90s.

Sugar chemists and many other analysts will be grateful for this revised edition of Browne's "*Handbook of Sugar Analysis.*" Thirty years have elapsed since that standard work appeared, and a new edition has long been eagerly awaited. In the task of revision Dr. Browne has been joined by his successor at the New York Sugar Trade Laboratory, and the outcome is a monumental work, which retains the format and style of its predecessor and incorporates the most important developments of the subject to date.

In the previous edition 523 pages of text were occupied by analytical matter and 259 pages (Part II) by a description of the chemistry, occurrence and properties of the sugars. That purely descriptive part has been omitted from the new edition and the 523 pages of analytical text have been expanded to 1183 pages. An Appendix of tables occupies 108 pages and Author and Subject Indexes occupy 60. Apart from the omission mentioned the arrangement of matter is as before, with substantially the same chapter headings. All the chapters have been much enlarged, particularly those dealing with miscellaneous physical methods, reduction methods and miscellaneous determinations.

The sub-title describes the work as "A Practical and Descriptive Treatise for Use in Research, Technical and Control Laboratories." It is by far the most comprehensive and authoritative book on methods, old and new, for determining the different sugars, and gives valuable guidance as to their applicability and limitations. But it deals also with many other determinations required by modern sugar technology, including the measurement of viscosity, surface tension, electric conductivity, *p*H value, colour and turbidity, as applied to sugar products, the determination of various impurities in sugars, and the determination of starch and diastatic power, cellulose, pentosans, uronic acids, ascorbic acid and other substances related to the sugars.

The book maintains the high standard of its predecessor and is certain to remain the standard work on the subject. J. HY. LANE

Society of Public Analysts and Other Analytical Chemists

ANALYTICAL METHODS COMMITTEE

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