FEBRUARY, 1930. Vol. LV., No. 647.

THE ANALYST

Death.

WITH deep regret we record the death of Mr. Arthur Angell, an original member of the Society.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

The Estimation of Fully-Saturated Glycerides as an Aid in the Analysis of Fats.

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IT has been shown that, when neutral fats are carefully oxidised in acetone solution with potassium permanganate, the unsaturated fatty acid groups combined with glycerol are converted quantitatively into acidic products, and any fully-saturated glycerides originally present are left unaltered (*cf.* Hilditch and Lea, *J. Chem. Soc.*, 1927, 3106; Collin and Hilditch, *J. Soc. Chem. Ind.*, 1928, **47**, 261T; Hilditch and (Miss) Jones, ANALYST, 1929, **54**, 75; etc.). The proportion of fully-saturated glycerides present in a fat can thus be determined with fair accuracy, and the data which have been collected in this laboratory show that this proportion is, in most cases, characteristic for a given fat, whilst the saponification equivalent of the fully-saturated glycerides is also, as a rule, fairly well-defined and characteristic. It was, therefore, of some interest to examine how far determination of the amount and equivalent of the fully-saturated glycerides might be useful as an additional means of analysis of mixtures of fats, such as those utilised for various edible purposes.

The method has hitherto been employed with success in studies of the component glycerides of a number of natural fats: in such cases sufficient fat (up to 800 grms.) has been oxidised to ensure sufficient fully-saturated glycerides (usually 50–150 grms.) being available for detailed analysis of their component fatty acids

by the ester-fractionation process. When dealing with relatively large quantities of material in this way, it is possible (bys uitable treatment of the neutral material from the oxidations, in order to eliminate the difficultly removable acidic glyceride complexes produced during the reaction) to obtain an accurate measure of the amount of fully-saturated glycerides, and also to isolate a considerable proportion of them almost entirely free from the acidic by-products, so that an accurate determination of the mean saponification equivalent of the fully-saturated components is obtained.

This process is, however, lengthy, and necessitates the handling of much larger quantities of material than are convenient in analytical practice; and we have, therefore, restricted ourselves, in the present work, to the use of not more than about 50 grms. of fat, and to conditions under which the analytical results may be arrived at in not more than two days. It is readily possible to complete the analysis within this limit of time with this quantity of fat, but the weight of crude fully-saturated glycerides produced is then generally too small, the methods, referred to above, of final purification from acidic by-products, to be utilised. Consequently, the order of accuracy of the percentage of fully-saturated glycerides, and still more that of their apparent saponification equivalents, suffer somewhat by comparison with the more elaborate process. As will be shown, this has been compensated for, to some extent, by the use of partially empirical correction factors, whereby allowance is made for acidic products still included with the fully-saturated glycerides, and the procedure then appears suited, for example, to the examination of butter-fats for the presence of admixed nut fats or carcase (tallow) fats, and may also prove capable of throwing some light upon the general composition of other mixtures, for example, margarine fats.

DESCRIPTION OF THE METHOD.—Prior to the more detailed study of oxidation of the fats in acetone, some attention was given to the possibility of using acetic acid in place of acetone as solvent. After a number of trials it was decided that this change could not be made with advantage: the acetic acid solutions gave much trouble as regards priming, they became distinctly viscous towards the close of the action, and, on the whole, it appeared that the oxidation of the fat was less easily completed than in acetone, unless the acetic acid solution was ultimately boiled for some time. Furthermore, although it is easy to decolorise the final solution by addition of sodium bisulphite alone without use of free mineral acid, recovery of the acetic acid from the final liquor is impracticable, whereas acetone, although perhaps more expensive than acetic acid, can be largely recovered by distillation and used anew in another oxidation. It was, therefore, decided to recommend acetone as the solvent medium for the oxidation, and, after experiments with various modified forms of procedure, the following method was found to give the most generally satisfactory results:

The fat to be examined (about 50 grms., weighed out to 0·01 grm.) is dissolved in acetone (10 ml. per grm. of fat) in a 2000 ml. round-bottomed flask fitted with a glass tube, one inch in diameter and about four feet in length, as an air-condenser.

The saponification equivalent and iodine value of the fat are previously determined, and the quantity of permanganate used is based on the latter value: for fats with an iodine value of 50 or less, the amount of permanganate employed is four times the weight of fat taken, but, if the iodine value be higher than 50, the proportion is raised to six times the weight of fat.

The acetone solution of the fat is warmed almost to boiling on a steam-bath, removed, and the first addition of potassium permanganate made (it is convenient to add this in teaspoonfuls, and to use it in powder form ground to pass a 50-mesh sieve); the contents of the flask must be well agitated after each addition of permanganate to prevent caking, and the frequency of addition regulated so that an even, gentle ebullition of the acetone is maintained. When the iodine value of the fat is below 20, it is usually necessary to heat the flask on the steam-bath after each addition until boiling sets in; on the other hand, in the case of fats with iodine value above 30, it is frequently necessary to check the vigour of the reaction by cooling the flask. In general, one-and-a-half to one-and-three quarter hours are required for the addition of the whole of the potassium permanganate. When all the permanganate has been added, ebullition usually continues for some time, during which the flask should be frequently shaken.

When ebullition has finally ceased, the air condenser is disconnected, and as much acetone as possible is rapidly distilled off and collected through a condenser in the ordinary way; as soon as the rate of distillation of the acetone slackens to a few drops per minute, the last portions of solvent are removed by connecting the flask with a water-pump for 5 or 10 minutes while it is still heated on the steam-bath.

If the removal of solvent has been carried out as thus described, the bulk of the residual contents of the flask, when cold, will be removable by careful shaking and tapping; they are shaken out as completely as possible into a large evaporating basin set in a trough of cold water and stirred up well with powdered sodium bisulphite (200 grms.). Some material adheres to the sides of the flask, and a small amount of sodium bisulphite is added and shaken in the flask, after which some water is added and the flask set on the steam-bath until the adherent material is detached. Meanwhile, to the main portion of the product, which has been mixed with bisulphite in the large basin as described, cold water (about 800 ml.) is added cautiously in small amounts and with stirring; the mixture develops considerable heat, and may display a tendency to frothing, so that it is well to have at hand a second basin to receive any overflow. When the first vigorous action has subsided and all the water has been added, the residual contents from the original reaction flask are washed into the main liquor in the basin, and the latter is then heated to boiling. Moderately dilute sulphuric acid is slowly added, with stirring, until the solution is definitely acid to Congo red paper, after which the boiling is continued for a short time, when reduction of the manganese oxides present is usually complete. The cooled contents of the basin are next transferred to a 3-litre separating funnel and extracted with ether (about 1000 ml.), the settled aqueous liquor being re-extracted with a further 400 ml. of ether in another

funnel. Both ethereal extracts are combined in the first funnel and washed with 250 ml. of distilled water four or five times in succession, in order to remove traces of mineral acids and, especially, all traces of manganese salts. Small quantities of manganese salts interfere seriously with the removal by ammonia of the acidic products of oxidation, and, if the washed ethereal solution is more than faintly yellow at this stage, it is well to agitate it with a dilute solution of acidified sodium bisulphite and then repeat the washings with distilled water.

The next part of the procedure is the most critical, since upon the efficiency of removal of the acidic oxidation products from the fully-saturated glycerides depends the accuracy with which the latter can be estimated. The acidic products which render this separation difficult are the semi-azelaic esters of glycerol derived from mixed saturated-unsaturated glycerides, and consequently containing, in addition to one (or two) semi-azelaic ester groups, two (or one) radicles of saturated fatty acid united with the remaining glyceryl hydroxyl groups. The alkaline salts of these bodies are but sparingly soluble in water, and when a slight excess of alkali is present in the aqueous phase, pass fairly readily into solution in ether; also, they are exceedingly active emulsifying agents. (The use of petroleum spirit instead of ether reduces the tendency to solution in the organic solvent, but, in general, increases the difficulty with emulsification.) We find that for the present method the use of ammonia, under the conditions described below, is the most convenient, the ammonium salts being somewhat more soluble than those of sodium or potassium, whilst the employment of a weak base tends to restrict the passage of the complex salts into the ether phase.

The washed ethereal solution is gently shaken with 250 ml. of ammonia solution (8–10 per cent. NH_3), allowed to settle for about 15 minutes, and the aqueous layer transferred to another vessel; this is followed by washing twice or thrice with distilled water (250 ml.), the settled aqueous washings being added to the first ammonia washing. Usually there is little emulsification during the first (ammonia) washing, but, on agitation with successive lots of water, a tendency to emulsification sets in as the ammonium compounds left in the ether layer pass into the aqueous phase; if emulsification is very troublesome, it is useful to add a little alcohol or a few drops of the 8–10 per cent. ammonia solution. After the third washing (water), a more dilute solution (250 ml.) of ammonia (5–6 per cent. NH_3) should be added, and then successive water washings should be given until emulsification is practically absent. Frequently it is well to give still a third treatment with dilute (5–6 per cent.) ammonia, and then to repeat the washings with water. All the ammoniacal and aqueous washings are retained and eventually mixed together and extracted with ether (about 500 ml.), the ethereal extract being then washed twice with 250 ml. of distilled water.

It is unnecessary to remove the last traces of ammonia from the ether during the water washings; indeed, if this be done, there is developed a tendency to hydrolysis of the ammonium salts of the acidic glyceride complexes, with the result that the free acidity of the fully-saturated glycerides finally obtained is higher than is desirable.

There are thus obtained:

(i) The main ethereal extract of neutral products of oxidation (*"primary neutral"*);

(ii) The ethereal extract from the primary washings with ammonia and water (*"secondary neutral"*);

(iii) The ether-extracted aqueous solution of ammonium salts of the acidic oxidation products, from which the latter may be isolated if desired by addition of mineral acid, but with which we are not concerned in the present communication.

The *"primary neutral"* and *"secondary neutral"* present in ether extracts (i) and (ii) are recovered by evaporation of the ether, followed by heating in a vacuum at 100°, and are weighed and determinations made of their (*a*) iodine value, (*b*) saponification equivalent, (*c*) acid value, and (*d*) melting point.

CORRECTION OF THE OBSERVED WEIGHTS OF "NEUTRAL" PRODUCTS FOR RESIDUAL ACIDITY DUE TO INCOMPLETE REMOVAL OF COMPLEX ACIDIC GLYCERIDES.—Owing to the peculiar nature, previously discussed, of the complex acidic glycerides, both groups of "neutral products" still retain sufficient acidic material to demand a correction of the observed weights; the acid value of the "primary neutral" may range from 3 to about 20, whilst that of the "secondary neutral" is naturally still higher, for example, from about 30–80.

When large amounts of the crude "neutral products" are available, as in the detailed investigations of component glyceride structure carried out in these laboratories by this method, the crude "neutral product" is conveniently boiled with dilute aqueous potassium or sodium carbonate solution, and subsequently with successive quantities of water, leaving an almost neutral product (acid value usually below 1), which comprises the greater part of the fully-saturated glycerides. Ether extraction of the alkaline and aqueous liquors yields a further quantity of more contaminated saturated glycerides, whilst from the ether-extracted alkaline solution a sample of the contaminating acidic products is recovered by treatment with mineral acid, and the mean equivalent weight of this is employed to correct for acidity in the refined "neutral products," on the assumption that acidic matter still left in them is of approximately the same composition as that extracted during refining.

In the present work, however, it is not possible to determine in any given case the mean equivalent weight of the acidic impurities, since it is not practicable to carry out any further refining process on the small amounts of "primary" and "secondary" neutral products usually obtained. (It is hardly necessary to point out that the acidic products of oxidation (iii) recoverable from the aqueous-ammonia washings in the present method are not comparable with those obtained on further refining of the crude "neutral products," because the former include, in addition to the complex acidic glycerides in question, nonoic, and possibly other easily removable lower fatty acids simultaneously produced in the disruptive oxidation. They are, therefore, of no use as an indication of the complex acidic glycerides left in small quantities in the fully-saturated glycerides.)

The method adopted has, therefore, been as follows:

Eight individual fats were analysed according to the procedure outlined; six of these were samples which have been studied independently on a larger scale, and in full detail, by other workers in the laboratory, namely, coconut and palm-kernel fats (Collin and Hilditch, *J. Soc. Chem. Ind.*, 1928, **47**, 261T), butter-fats "A" and "B" (Hilditch and Jones, ANALYST, 1929, **54**, 75), mutton tallow (Collin, Hilditch and Lea, *J. Soc. Chem. Ind.*, 1929, **48**, 46T), and palm oil (Belgian Congo plantation oil, detailed results not yet published). From consideration of the already known fully-saturated glyceride contents of these six fats (given in the various papers cited), it was found that approximately concordant figures resulted from the present analyses when the acid value of the residual contaminating acidic glycerides in the "primary" and "secondary" neutral products obtained was assumed to be 127 in the case of coconut and palm-kernel fats, and 100 in the case of the butters, tallow and palm oil. These values have, therefore, been adopted as a first approximation for the purpose of the present paper.

APPLICATION OF THE METHOD TO INDIVIDUAL FATS.—The data obtained for the six fats mentioned, together with those for a sample of beef tallow and of refined ground-nut oil, are collected in Table I in connection with which the following points may be noted:

(i) In general investigations of component glyceride structure we find it more convenient to deal with *saponification equivalents* rather than *saponification values*, but, in the present paper, we express this characteristic in the form of *saponification value* throughout, in order to conform with the more prevalent analytical custom.

(ii) The "melting-points" of the "primary neutral" products appear to be of value in characterising different fats, especially if they are determined by both closed-tube and open-tube methods. The figures given under "closed tube" refer to the range of temperature between the first signs of melting of the fat in a closed capillary tube and the appearance of a clear liquid phase. The "open tube" "melting points" were determined by allowing a layer of fat 1 cm. long to set at room temperature for 24 hours in one end of an open melting-point capillary tube, and observing the temperature at which the fat became sufficiently fluid to be forced up the capillary when the tube was immersed to a total depth of 3 cm. in water which was heated at the rate of about 0·5° C. per minute.

(iii) With the exception of mutton tallow, results are given for duplicate determinations on each fat.

Arachis oil yielded less than 1 per cent. of neutral products from the oxidation, and these appeared to be mainly of a non-fatty nature; a similar result was obtained by one of us and Lea (*J. Chem. Soc.*, 1927, 3106) from cottonseed oil, and there is good reason to believe that other vegetable kernel fats of the "soft oil" type, in which the proportion of saturated fatty acids does not exceed about 30 per cent. of the total mixed fatty acids, will be found to contain negligible amounts of fully-saturated glycerides (*cf.* Collin and Hilditch, *Biochem. J.; in the press*).

TABLE I.

	Coconut fat.		Palm-kernel fat.		Butter-fats. "A."		Butter-fats. "B."		Mutton tallow.	Beef tallow.		Palm oil (Belgian Congo).	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)		(i)	(ii)	(i)	(ii)
Wt. taken (grms.) ..	57·63	54·92	56·07	52·40	60·88	59·49	58·03	59·38	64·78	56·77	58·61	52·36	52·52
"Primary Neutral."													
Grms.	39·18	46·00	36·40	33·45	19·63	22·25	16·84	18·65	20·41	9·15	10·46	5·95	5·58
Sap. value	264·2	—	256·5	257·4	244·1	244·4	242·4	244·4	210·9	204·4	206·5	209·2	210·9
Acid value	2·9	3·3	3·9	3·7	5·3	18·8	7·3	11·9	18·1	15·6	20·9	20·0	19·6
M.pt. (closed)	26–28°	26–27°	30–32°	30–31°	34–36°	35–36°	35–37°	32–36°	47–56°	50–53°		54–57°	
,, (open)	25–26°	26°	30–30·5°	30–31°	37–39°	38–39°	37–38°	38–39°	53–54°	52°		56°	
"Secondary Neutral."													
Grms.	10·06	1·80	1·36	3·03	1·62	4·80	2·31	3·08	2·50	1·47	1·25	1·43	1·34
Sap. value	272·5	257·4	240·8	242·6	227·7	296·4	237·3	244·4	207·0	197·7	207·3	206·2	199·2
Acid value	7·9	54·5	74·1	46·2	25·8	80·5	31·2	62·4	48·4	59·9	61·1	61·8	35·9
Corrected weights of fully-saturated glycerides.													
(*a*) in "primary neutral"	38·28	44·79	35·28	32·47	18·59	18·07	15·62	16·43	16·70	7·74	8·27	4·76	4·49
(*b*) in "secondary neutral" ..	9·43	1·03	0·57	1·93	1·20	0·94	1·59	1·16	1·30	0·59	0·49	0·55	0·86
Total (grms.) ..	47·71	45·82	35·85	34·40	19·79	19·01	17·21	17·59	18·00	8·33	8·76	5·31	5·35
Per cent. fully-saturated glycerides.													
(i) observed	82·8	83·4	64·0	65·7	32·5	31·9	29·7	29·6	27·8	14·7	14·9	10·1	10·2
(ii) from previous detailed study ..	84		64		31		29		26	—		10	

The saponification values and iodine values of the fats employed in the above analyses were as follows:—

	Sap. value.	Iodine value.
Coconut fat	257·0	8·7
Palm-kernel fat	243·3	16·3
Butter-fat "A"	226·3	38·0
,, "B"	223·4	39·4
Mutton tallow	196·2	41·2
Beef tallow	198·0	49·5
Palm oil (Belgian Congo)	198·9	52·7

Table I shows that the present method of determination gives data for the content of fully-saturated glycerides which are moderately concordant, and which, in general, do not differ from the more elaborately estimated figures by more than 1–2 per cent. In its present state, therefore, it cannot be claimed that the order of accuracy approaches that of the more common operations in quantitative fat analysis (*e.g.* the determination of free acidity, saponification value, iodine value, etc.), but it is believed that observations by this process may have value in some aspects of the technical examination of fats, of which a few illustrations are given later.

In considering the application of the method to such problems, however, it is well to bear in mind not only the possible analytical limitations which have been duly pointed out, but also the extent to which it may be possible to fix limits for the characteristic fully-saturated glyceride contents of particular fats. This value will probably be found to be practically a constant figure in the case of the majority of the vegetable kernel fats. The seed fats of the *Palmae*, for example, have so far been observed to be remarkably constant in fatty acid composition, and it is very unlikely that any notable divergence from the figures given for coconut and palm-kernel fats will be encountered.

Again, the "soft oils" of the class embracing olive, arachis, cottonseed, sunflower-seed and similar fats, contain negligible amounts of fully-saturated glycerides, although a certain small amount of neutral (mainly non-fatty) material is usually isolated after oxidation. This amount (usually less than 1 per cent. of the fat), although insignificant in itself, tends to introduce further difficulties when attempts are made to apply the present method quantitatively to a mixture made up, for example, of a fairly large proportion of a soft oil with palm oil, oleo oil, or premier jus (all of which have low fully-saturated glyceride contents of the order of 8–20 per cent.).

Finally, butter-fats, tallows, palm oils and some other classes of fats are not so constant in fatty acid composition as most kernel fats, and in these cases it will be found that the fully-saturated glyceride content varies between certain limits: broadly speaking, this variation will be found to be in inverse ratio to the iodine value of the original fat. Although the maximum range of fully-saturated glyceride content in fats of variable iodine value of this type may well not exceed a few units per cent. of the whole fat, it is clear that no fixed value, for example, for butter-fats can be laid down at present.

EXAMINATION OF BUTTER-FAT FOR ADMIXED NUT OILS.—The detection of coconut fat in butter has received considerable attention recently, and methods depending ultimately on the proportion of lauric acid present have been suggested by Klostermann and Quast (*Z. Unters. Lebensm.*, 1927, **54**, 297), Atkinson and Azadian (*Ann. Falsif.*, 1927, **20**, 593), Grössfeld (*Z. Unters. Lebensm.*, 1928, **55**, 354; ANALYST, 1928, **54**, 600) and others. In view of the wide difference in fully-saturated glyceride content between coconut and palm-kernel fats on the one hand, and butter-fat on the other, a few trials were made of the present method in connection with this problem.

The butter-fat "A" (Table I) was mixed with various proportions of the coconut and palm-kernel fats used in this work, so that fat mixtures resulted containing respectively 5, 10 and 20 per cent. of coconut oil, and 10 per cent. of palm-kernel oil. These were tested by the procedure outlined in the earlier part of this paper, and the data given in Table II were obtained.

TABLE II.

Fat containing:	5 Per Cent. Coconut oil.	10 Per Cent. Coconut oil.	20 Per Cent. Coconut oil.	10 Per Cent. Palm-kernel oil.
Weight taken (grms.)	50·41	49·65	50·55	49·91
"Primary Neutral" (grms.)	15·90	17·92	20·86	18·19
Sap. value	240·3	247·8	248·4	243·0
Acid value	3·7	6·0	8·0	8·0
M.pt. (closed)	27–32°	28–33°	25–30°	30–36°
,, (open)	37–38°	37–38°	35°	38°
"Secondary Neutral" (grms.) ..	3·01	2·46	2·15	0·59
Sap. value	224·1	241·6	—	226·4
Acid value	53·4	21·5	20·4	51·2
Corrd. wts. of fully-saturated glycerides.				
(*a*) in "primary neutral"	15·31	16·84	19·20	16·73
(*b*) in "secondary neutral"	1·40	1·93	1·67	0·29
Total (grms.)	16·71	18·77	20·87	17·02
Fully-saturated glycerides, per cent. ..	33·2	37·8	41·3	34·1

The relevant data in the determination are the percentages of fully-saturated glycerides, the melting-points of the "primary neutral" products, and also the saponification values of the latter (these are affected to a minor extent by the small quantities of acidic material present, and it would, perhaps, be preferable to prepare the corresponding mixed fatty acids, remove the traces of azelaic acid present by repeated boiling-out with water, and determine the mean molecular weight of the purified acids). The corresponding data for the individual fats concerned are as follows (*cf.* Table I):

TABLE III.

	Butter-fat "A."	Coconut oil.	Palm kernel oil.
Fully-saturated glycerides, per cent. ..	31–32	83–84	64–65
"Primary neutral."			
M.pt. (closed)	34–36° C.	26–28° C.	30–32° C.
,, (open)	37–39° C.	25–26° C.	30–31° C.
Sap. value	244–245	265	256–257

The calculated and observed percentages of fully-saturated glycerides in various butter-fat nut-fat mixtures are given in Table IV.:

TABLE IV.

Butter-fat "A" containing nut-oil.	Coconut-oil. Fully-saturated glycerides.		Palm-kernel oil. Fully-saturated glycerides.	
Per Cent.	Calc. Per Cent.	Obsd. Per Cent.	Calc. Per Cent.	Obsd. Per Cent.
0	31	—	31	—
5	33·6	33·2	32·7	—
10	36·3	37·8	34·3	34·1
15	38·9	—	36·0	—
20	41·6	41·3	37·6	—
25	44·3	—	39·2	—
100	84	—	64	—

The presence of nut oils in butter is thus accompanied by an increase in the amount of fully-saturated glycerides, and it seems possible that the percentage of the latter, determined as described, is capable of indicating, to within about 5 per cent., the quantity of adulterant present, providing that it is possible to state whether coconut or palm-kernel oil has been employed. The addition of coconut oil has a marked effect on the melting-point of the fully-saturated components (observed in a closed tube), and it would appear that this is a very clear qualitative test for the presence of coconut oil in butter; the corresponding effect for palm-kernel oil as an adulterant is by no means so well-marked. Owing to the not very wide differences between the saponification values of the "primary neutral" products of oxidation of butter-fat and of the nut oils, and to the influence of traces of azelao-glyceride products on the values obtained, these figures are of no great utility in the present instance.

EXAMINATION OF BUTTER-FAT FOR ADMIXED CARCASE FATS.—Possible adulteration of butter-fats with other animal fats (tallows, etc.) is a problem which is encountered more especially in the case of ghee or Indian butter. A study of the fully-saturated glyceride contents and probable component glyceride structure of typical buffalo and cow ghee is being carried out in these laboratories, but, for the present purpose, we have examined mixtures of butter-fat and beef tallow by the oxidation method. The results show that the saponification value and melting point of the "primary neutral" portion of the fully-saturated glycerides afford a fairly clear indication of the presence of admixed tallow, especially if the latter forms 10 per cent. or more of the whole fat. So far as we know at present, the normal variations in butter-fat would affect the fully-saturated glyceride content (possibly to the extent of several per cent.), but would not upset the saponification value or melting points of the *primary neutral*. In the case of carcase fat adulteration, it is the latter which are significant, the percentage of F.S.G. is of little use, as stated after Table VI.

TABLE V.

MIXTURES OF BUTTER-FAT "A" AND BEEF TALLOW (samples used in Table I).

Beef tallow content:	5 Per cent.	10 Per cent.	20 Per cent.
Wt. taken (grms.)	46·53	40·07	52·44
"Primary neutral," grms.	13·55	11·95	15·92
Sap. value	243·5	239·7	231·8
Acid value	7·0	7·7	6·1
M.pt. (closed)	33–40° C.	33–40° C.	35–44° C.
,, (open)	41° C.	43° C.	44° C.
"Secondary neutral," grms.	4·48	1·72	3·68
Sap. value	242·5	231·2	229·0
Acid value	61·0	27·9	51·6
Corrd. wts. of fully-saturated glycerides.			
(*a*) in "primary neutral"	12·60	11·03	14·95
(*b*) in "secondary neutral"	1·75	1·24	1·78
Total (grms.)	14·35	12·27	16·73
Fully-saturated glycerides, per cent. ..	30·8	30·6	31·9

The next Table (VI) gives a summary of the calculated proportions of fully-saturated glycerides in various butter-fat—beef-tallow mixtures, together with the observed values for these figures and for the saponification values and melting-points of the "primary neutral" fraction of the fully-saturated glycerides.

TABLE VI.

BUTTER-FAT "A"—BEEF TALLOW MIXTURES.

Beef-tallow present. Per Cent.	Fully-saturated glycerides. Calc. Per Cent.	Fully-saturated glycerides. Observed. Per Cent.	"Primary neutral" fraction. Sap. value.	"Primary neutral" fraction. M.pt. (closed). °C.	"Primary neutral" fraction. M.pt. (open). °C.
0	31	(31)	244–245	34–36	37–39
5	30·2	30·8	243·5	33–40	41
10	29·3	30·6	239·7	33–40	43
15	28·6				
20	27·9	31·9	231·8	35–44	44
25	27·0				
100	15	(15)	204–206	50–53	52

Owing to the relatively small difference in fully-saturated glyceride content between butter-fat and tallow, and to the fact that the percentage for tallow is lower than that for butter-fat, the determined values for this figure are of little quantitative use in this instance, even when the proportion of admixed tallow is fairly high. On the other hand, the saponification values of the "primary neutral" portions show progressive diminution with increasing percentage of tallow in the fat, and the melting-points of these portions are still more definitely indicative of the presence of tallow. The "melting-points" determined by the open-tube method are especially useful indications of the presence of even small proportions of tallow in the butter-fat.

If the results of further examination of a wider range of pure butter-fats and ghee-fats show that the above values for such fats do not differ too widely among themselves, the present procedure will evidently give a clear qualitative indication of the presence of admixed carcase fats, although its quantitative value in regard to this particular problem is probably limited.

ATTEMPTED APPLICATION OF THE METHOD TO THE STUDY OF EDIBLE FAT MIXTURES.—Some tests were made, in conclusion, to determine how far examination of the fully-saturated glycerides present in mixtures of fats for edible use might be of service in defining the probable composition of such mixtures. The general conclusions arrived at were: (i) that, if the nature of the fats present is already known, it is frequently possible to give an approximate estimate of their proportions, and (ii) that in some cases it is possible to utilise the method to show the nature of the fats present. The utility of the method in the latter sense is limited by the fact that a number of the fats likely to be present—premier jus, oleo oils, palm oil, and similar materials—all contain a low percentage of fully-saturated glycerides, the saponification values of which are, moreover, nearly the same. Consequently, fully-saturated components of this class usually form a relatively small proportion of the total fully-saturated glycerides obtained, and interpretaion of the results becomes very difficult. It seems that, without independent evidence of some other kind for components of this type, the value of the oxidation process is at present uncertain, and even that, in some cases, little more information can be obtained by its use than from the usual characteristics of the original mixture. At the same time, it is believed that further examination of the method, in conjunction with other forms of analytical procedure, may lead to its useful application in this direction, and the results of our examination of four fat mixtures, typical of those used in margarine, are, therefore, given in brief, mainly in the hope that other workers interested in this field may be encouraged to consider its application to specific problems, and to assist in the development of the new procedure.

The fat mixtures in question were (i) a mixture containing 50 per cent. coconut oil, 25 per cent. of beef tallow, and 25 per cent. of arachis oil, made up by ourselves from the fats used in the earlier part of this work, and (ii) three mixtures of fats kindly supplied to us by Mr. A. Andersen, of Planters' Foods, Ltd., the composition of these being unknown to us at the time we carried out the experiments. The values obtained for these fats were as follows: (See Table VII.)

In the case of Mixture I, the components of which were known, an approximate estimate of the amounts present can be made on the assumption that the arachis oil present does not contribute any fully-saturated glycerides to those obtained. From the saponification values (which are only approximately accurate, *cf.* p. 80) obtained in Table I for the fully-saturated components of coconut oil and beef tallow, and from that observed for those present in Mixture I (Table VII), it follows that about 90 per cent. (or 41 per cent. of the original fat) of the fully-saturated glycerides is contributed by coconut oil, so that, since 84 parts of fully-saturated glycerides correspond to 100 parts of coconut oil, the amount of the latter

originally present is $\frac{4100}{84}$, or 49 per cent. The balance of 4·3 per cent., if ascribed to beef tallow, corresponds with $\frac{430}{15}$, or 28·5 per cent., present in the original mixture.

TABLE VII.

Actual composition:	I. 50 per cent. coconut oil. 25 per cent. beef tallow. 25 per cent. arachis oil.	II. 50 per cent. coconut oil. 25 per cent. premier jus. 25 per cent. cottonseed oil.	III. 15 per cent. coconut oil. 40 per cent. palm-kernel oil. 30 per cent. oleo oil. 15 per cent. arachis oil.	IV. 60 per cent. oleo oil. 20 per cent. premier jus. 20 per cent. arachis oil.
Original fat :				
Sap. value	224·2	224·5	224·0	193·9
Iodine value	39·7	41·7	37·5	57·2
Fully saturated glycerides, per cent.				
(i) Found	45·3	46·2	37·3	8·5
(ii) Calculated (from above composition and data in Table I)	45·7	45·7	41·8*	9·0*
"Primary neutral" fraction of F.S.G.				
Sap. value	255·8	254·2	254·6	197·6
M.pt. (closed)	26–29° C.	25–30° C.	27–30° C.	43–46° C.
,, (open)	31–32° C.	35° C.	29° C.	51–52° C.
Portion of "Primary neutral" least soluble in acetone.				
Sap. value		201·8	(trace only)	199·5
M.pt. (closed)		57–58° C.	53–54° C.	57–58° C.

In mixtures consisting simply of (i) a known nut oil, (ii) a known premier jus, palm or similar oil, and (iii) a soft oil, it is thus probably possible to estimate by this process the proportions of each component to within about 5 per cent.

When mixtures of fats, whose qualitative, as well as quantitative, composition is unknown, are considered, the interpretation of the results becomes much more difficult. As a further aid in differentiating between the possible components, the "primary neutral" portions from Mixtures II, III and IV were crystallised several times from acetone, and the saponification values and melting-points of the least-soluble fractions were determined, as shown in Table VII.

The general results obtained for Mixture II were, on the whole, closely similar to those for Mixture I (of known composition), and it was considered that the material was evidently made up of a nut fat, a fat similar in composition to beef tallow, and a soft oil, the iodine value of which was deduced to be above rather than below 100. From the melting-points of the fully-saturated glycerides, we considered it likely that the nut oil was palm-kernel oil, although we did not

* Assuming percentage of fully-saturated glycerides in oleo oil (not yet investigated) as 10 per cent.

exclude the possibility of coconut oil (which, in fact, was the nut oil present). The numerical data obtained led us to suggest that the fat mixture was made up alternatively of 60–65 per cent. of palm-kernel oil and 20–15 per cent. of premier jus, with about 20 per cent. of a soft oil (probably cottonseed), or of 45–50 per cent. coconut oil, 35 per cent. of premier jus, and 15–20 per cent. of cottonseed oil. The second alternative is not far from the correct solution, except that the suggested proportion of premier jus is too high. It is evident that the estimation of fats of fully-saturated glyceride content below about 15–20 per cent. tends to give too high results; this arises from the fact that, after deducting the large proportion of fully-saturated material due to nut oils, the percentage left is usually very small (*e.g.* 25 per cent. of beef tallow corresponds to about 3·5 per cent. of F.S.G.), and this includes (in addition to any slight error or variation in the F.S.G. of the nut oils) the small amounts of non-oxidisable impurities of a non-fatty nature which result on oxidation of a soft oil (*cf.* p. 80, arachis oil).

We were unable to furnish correct interpretations of the data obtained for Mixtures III and IV. In part, this was owing to the fact that we had no precise data for oleo oil (a material which will probably be found to be more variable in characteristics than most of the other fats dealt with); in part it was caused by the apparent inability of the data at our command to discriminate, for example, between coconut and palm-kernel oil, or between premier jus, oleo oil, and palm oil. The points on which application of the method gave a certain amount of positive evidence in regard to Mixtures III and IV were briefly:

(i) Mixture III (like nos. I and II) was evidently largely made up of nut oil (a conclusion which is, however, likely from the saponification value of the fat itself); the lower percentage of fully-saturated glycerides, as compared with nos. I and II, together with the observed saponification values, may be taken as indicative of the presence of palm-kernel oil in quantity. It is, perhaps, fair to add that this feature was overlooked until the actual composition of Mixture III had been placed before us.

(ii) In Mixture IV, nut oils were equally clearly absent; and the small proportion of fully-saturated glycerides, with their characteristically low saponification values and very high melting-points, pointed to the presence of animal fats in quantity; the soft oil present was, however, overlooked.

(iii) It appears that the fully-saturated glycerides of oleo oil yield only traces of compounds sparingly soluble in acetone and melting above 50° C. (Mixture III); whilst, when premier jus is present (Mixtures II and IV), appreciable, though small, quantities of least soluble glycerides which melt at 57–58° C. can be isolated (it should be noted, however, that the corresponding fractions of the glycerides of palm oil have about the same melting-point, with a somewhat higher saponification value).

The procedure described in this paper is, therefore, at present not so applicable to the examination of edible fat mixtures as to the detection of specific adulterants in butter-fats; nevertheless, in the case of margarine fats composed only of nut

oils, animal fats, and soft oils, it is capable, in its present state, of yielding definite qualitative and some sort of quantitative information. It is quite possible that accumulation of many more data on individual fats and on various ranges of mixtures of them will lead to increased scope in its potential use as an aid in the analysis of margarine and similar fats, although discrimination between fats which contain small but similar proportions of fully-saturated glycerides will probably remain a matter of difficulty.

SUMMARY.—1. A description is given of a method for the determination of the percentage of fully saturated glycerides present in a fat under conditions of time and quantity of material examined applicable to analytical practice. The inherent difficulties and limitations of the method are discussed, and conclusions are based not only on the observed percentage of the fully-saturated glycerides, but also on their mean molecular weights (saponification values) and melting points.

2. The fully-saturated glyceride contents of the nut oils, and some other fats, are probably almost constant for a given fat, but the possible limits of variability in this characteristic for fats such as butters, tallows, and oleo oils, palm oils and some others require further study over a wider range of samples than has been practicable in the course of the present work. Certain possible applications of the method are, however, indicated.

3. The detection of nut fats in butter-fats is a problem in which the method appears to be especially useful. The proportion of fully-saturated glycerides in the nut fats is so much higher than in butter fats that by this means an approximate estimate of the percentage of nut fat present can be made, if the nature of the latter is known (*e.g.* coconut oil).

4. The detection of carcase fats in butter or ghee is also possible, although not with the same degree of exactitude as in the preceding case. Here the differences are most marked by diminution in the saponification value of the fully-saturated glycerides and by definite increases in their melting-point, as compared with the corresponding figures for the fully-saturated glycerides of butter fat.

5. The utility of the process, when applied to the examination of margarine fat-mixtures, has also been considered, but this is, in general, a more difficult problem. If, however, the mixture consists only of a nut fat with premier jus and a soft (*e.g.* arachis or cottonseed) oil, it is possible to deduce both the qualitative nature of the components and, to an approximate degree, their quantitative proportions. In other cases, especially when mixtures of premier jus, oleo oil, palm oil or of both coconut and palm-kernel oils are present, the value of the method is at present uncertain. Nevertheless, it is considered that by accumulation of sufficient data for a large range of typical mixtures and by considering other factors in addition to those on which attention is focused in the present paper, the determination of fully-saturated glyceride content may be made a useful means of examination of this class of fats.

We wish to express our cordial appreciation of the kindness of Messrs. Lever Brothers, Ltd., who made it possible for one of us (B.C.C.) to co-operate in this investigation by the award to him of the Lever Research Studentship in the Department of Industrial Chemistry of this University.

THE UNIVERSITY,
LIVERPOOL.

A New Type of Mercury Cathode Cell for the Determination of Minute Quantities of Arsenic.

BY T. CALLAN, M.Sc., Ph.D., F.I.C., AND R. T. PARRY JONES.

IN a previously published paper (T. Callan, *J. Soc. Chem. Ind.*, **1924, 43, 168T**), in which the use of a lead cathode in the electrolytic method for the determination of minute quantities of arsenic was discussed, a simple type of lead cathode cell was described, and conditions were specified to ensure the maximum activity of the lead cathode. This type of cell has given satisfactory service over a number of years.

The superiority of mercury over other metallic cathodes has, however, been emphasised in recent years, and, in particular, F. S. Aumonier (*J. Soc. Chem. Ind.*, **1927, 46, 341T**) has discussed this matter in considerable detail, and has described a special type of cell suitable for use with this metal. This involves the use of a porous pot, the upper and lower parts of which are rendered impervious by impregnation with montan wax, or other suitable medium, leaving an annulus of porous material in the middle. Whilst this type of cell functions quite satisfactorily, it has certain disadvantages: the process of impregnation of the porous pot is troublesome; it is difficult to ensure absolute cleanliness of the pot, particularly when comparatively highly arsenical solutions have been previously encountered; the adjustment of the level of electrolyte in an opaque cell presents obvious difficulties, and the cell possesses an appreciable electrical resistance.

There still remains, therefore, the need for an efficient, simple, and practical type of mercury cathode cell, in which the disadvantages, mentioned above, are absent.

It occurred to us, therefore, that the lead cathode cell, to which reference has previously been made, might be modified in order to adapt it for use as a mercury cathode cell.

Accordingly, the type of cell shown in Fig. I was designed. It consists of a glass flat-bottomed tube, about 5 in. long and about $1\frac{3}{8}$ in. in diameter, having an aperture of about 1 in. in diameter blown in the side about $\frac{1}{4}$ in. from the bottom of the tube. The aperture is covered by a piece of parchmentised* paper which

* Parchmentised paper is conveniently prepared by rapidly immersing No. 1 Whatman filter paper in pure 80 per cent. sulphuric acid for a few seconds and then washing with water; the paper should be stored under water until required.

acts as an efficient diaphragm of very low electrical resistance between the anolyte and the catholyte. The aperture is flanged, as shown in the diagram, in order that the parchment may be held tightly in position by means of a rubber band.

MERCURY CATHODE CELLS FOR ELECTROLYTIC ARSENIC APPARATUS

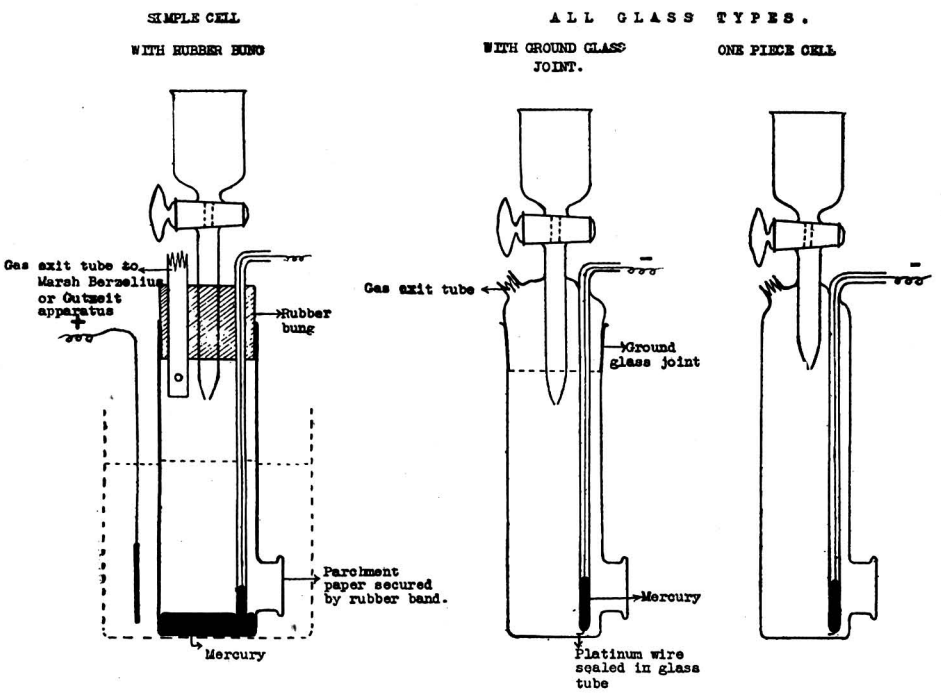


FIG. 1. FIG. 2. FIG. 3

The cell thus differs from the lead cathode cell previously described in having the diaphragm at the side, instead of at the bottom.

Mercury, previously purified by spraying through a long column of dilute nitric acid, is placed in the bottom of the tube, so that its surface is just below the level of the aperture in the side of the tube. Electrical connection is made with the mercury by means of a narrow glass tube, into the lower end of which is hermetically sealed a small piece of platinum wire (see Fig. 2). The whole of the exposed portion of the platinum wire dips below the surface of the mercury in the cell. A length of copper wire, dipping into this tube and making contact with the end of the platinum wire inside the tube by means of a short column of mercury, forms the negative terminal of the apparatus. The narrow tube, carrying the cathode lead, passes through a rubber stopper which closes the upper end of the cathode cell. This rubber stopper also carries (*a*) a glass tap funnel, of about 20 ml. capacity, through which the electrolyte or the test solution may be introduced, and (*b*) an exit tube, having a small side hole near the bottom, and

connecting directly with the purifying tube of the mercuric chloride or Marsh-Berzelius type of arsenic detector (not shown).

The cathode cell is immersed in the electrolyte of the anode cell, which is itself immersed in a cooling bath kept constantly at about room temperature. The anode consists of a sheet of platinum foil (about 2 in. × 1 in.) welded to a short length of stout platinum wire.

The electrolyte used is dilute (1:8) sulphuric acid, and a suitable current is 2 ampères for 15 minutes, followed by 4 ampères for a further 15 minutes.

More elaborate types of the same apparatus are shown in Figs. 2 and 3. These, being constructed entirely of glass, have the advantage of greater cleanliness, but can hardly be improvised in the average laboratory, as can the simple cell above described.

In Fig. 2 the apparatus is in two sections, the upper section (corresponding to the rubber stopper, tubes, and funnel in Fig. 1) being connected to the lower section by means of a ground glass joint.

Fig. 3 shows the same apparatus in one piece. This is a very convenient form, which avoids all danger of gas leakage, and obviates the need for a ground joint. Owing to the width of the aperture at the lower end of the tube, it can be as readily cleaned as the other forms.

The mercury cathode cell,* as described, has been in use in this laboratory over a considerable period, and has been found most efficient and reliable. In its simplest form, using a rubber stopper, it is easily constructed, and convenient to use. No poisoning of the mercury cathode has been observed, but, as a precautionary measure, the mercury is purified before use each day by spraying through dilute nitric acid.

When used in conjunction with mercuric chloride paper, the stains are invariably sharply defined and of good colour, whilst they are equal in intensity to those given using a lead cathode in its most sensitive condition.

CENTRAL ANALYTICAL LABORATORY,
BRITISH DYESTUFFS CORPORATION, LTD.

* The apparatus in either form can be obtained from Messrs. Baird and Tatlock (London), Ltd.

The Quantitative Analysis of Mixtures of Nickel and Cobalt.

By S. GLASSTONE, D.Sc., F.I.C., and J. C. SPEAKMAN.

During the course of an investigation of nickel-cobalt alloys it became necessary to have a rapid volumetric method for the analysis of small amounts, 0·02 grm. or less, of such alloys. The majority of methods proposed for the separation of nickel and cobalt are only applicable if one of the metals is present in a large excess; in the work contemplated, however, alloys varying in composition from 100 per cent. of nickel to 100 per cent. of cobalt were expected. From a preliminary study of the available methods for the quantitative analysis of mixtures of nickel and cobalt (*cf.* Singleton, *Industrial Chemist*, 1927, **3**, 406) it appeared that the most feasible procedure would be to determine the total nickel and cobalt by titration with potassium cyanide, and then to determine the cobalt separately by oxidation to hydrated cobaltic oxide.

For determining nickel, the volumetric cyanide method with silver iodide as indicator (Moore, *Chem. News*, 1895, **72**, 92; Beckurts, *Die Methoden der Massanalyse*, 1913, p. 1021 *et seq.*; Sutton, *Volumetric Analysis*, 1922, p. 284) is very accurate; if small amounts of cobalt are present the method is still applicable, but the cobalt is reckoned in with the nickel. In the course of the present work it was found that, if the cobalt formed more than about 10 per cent. of the total nickel and cobalt, the end-point of the titration was indistinct and unreliable. An attempt was made to remove the cobaltous ions in the form of a complex anion (*e.g.* by the addition of potassium tartrate, which is used if iron is present), but no satisfactory results could be obtained. Attention was, therefore, turned to the modified cyanide method used by Rupp and Pfennig (*Chem. Ztg.*, 1910, 321; *J. Soc. Chem. Ind.*, 1910, **29**, 518); according to these authors, the titration may be carried out, without the use of an additional indicator, by running nickel or cobalt solutions into a known volume of standard cyanide until a permanent precipitate is just formed. With nickel this precipitation occurs when the ratio of [Ni] to [CN] is 1 : 4, but with cobalt the ratio is 1 : 5, implying that the respective complexes are $Ni(CN)_4''$ and $Co(CN)_5'''$, or possibly $Co(CN)_5(H_2O)'''$. As soon as the amount of nickel or cobalt added exceeds that required for complex formation, the simple cyanide is precipitated, and hence acts as an indicator of the completion of a certain definite process. Rupp and Pfennig found that the end-point was only distinct if the solutions were not too dilute, and recommended the use of 0·5 *N* cyanide with a nickel solution of 0·07–0·17 *M*, or with a cobalt solution of 0·035–0·13 *M*. The limits set to the dilution of the solution are probably connected with the fact that the cyanides of nickel and cobalt are comparatively soluble; when the titration is carried out in dilute solutions supersaturation may readily occur and precipitation be delayed (*vide infra*, p. 96). For the investigations contemplated,

however, more dilute solutions than those suggested by Rupp and Pfennig would have to be used, and it was one of the objects of the present work to see if this were possible.

Among the conclusions reached by Müller and Schluttig (*Z. anorg. Chem.*, **1924, 134**, 327), as a result of the electrometric titration of a cobalt solution with potassium cyanide, using a silver indicator-electrode, was that cobalt formed two complex cyanide ions, *viz.* $Co(CN)_4''$ and $Co(CN)_6'''$. If this is the case, the theoretical basis of the Rupp and Pfennig method for determining cobalt is considerably weakened, and the accurate results obtained by them—and us—would appear to be fortuitous. A similar instance is the case studied by Glasstone (*J. Chem. Soc.*, 1929, 706): copper forms two complex ions, $Cu(CN)_2'$ and $Cu(CN)_3''$, and precipitation of cuprous cyanide occurs when the ratio [Cu] to [CN] is approximately 2·5. This result might at first sight be applied to the titration of copper solutions with cyanide, or *vice versa*, but the precipitation ratio is found to depend on the experimental conditions; the method is, therefore, useless for analytical purposes. It was desirable, in these circumstances, to attempt the potentiometric titration of nickel and cobalt solutions with potassium cyanide, using nickel and cobalt electrodes respectively, in order to avoid any errors which might arise from the use of a silver electrode. It appeared possible also that such a titration in a mixed solution, using perhaps a nickel-cobalt alloy electrode, might serve to determine both metals simultaneously.

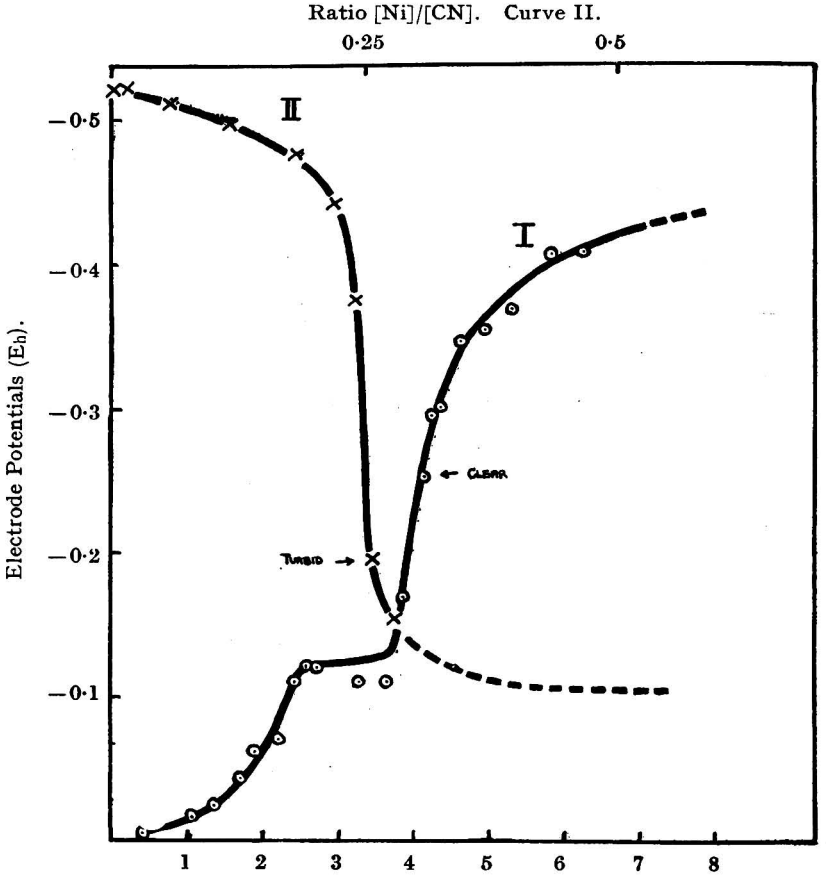
As the results of the electrometric titrations are of more fundamental importance they will be described first.

EXPERIMENTAL.—Reversible electrode potentials with nickel and cobalt are very difficult to obtain (see Haring and Vanden Bosche, *J. Phys. Chem.*, **1929, 33**, 165; Newbery, *J. Amer. Chem. Soc.*, **1929, 51**, 1430), but these were not essential for the present problem; it was quite sufficient to measure stable and, if possible, reproducible potentials under comparable conditions. When titrating a nickel solution with potassium cyanide the best results were obtained by working at 95° C. while hydrogen was passed through the solution; a subsidiary polarising current of about 2×10^{-4} amp. was used with the nickel indicator-electrode as cathode, in order to keep it in an active condition. For the reverse titration a polarising current was not used. With cobalt, polarising the electrode effected no improvement, and the bubbling of hydrogen caused serious disturbances of the electrode potential; the solutions used, therefore, were saturated with hydrogen, and an atmosphere of hydrogen was maintained in the titration vessel, but the gas was not bubbled during the measurement of potentials.

The electrodes were made by depositing nickel or cobalt electrolytically on a clean copper sheet; the exposed area was about 3×1 cm.^{2}. In every case the comparison electrode was a saturated potassium chloride calomel half-element, and the potential measurements were made by the Poggendorff compensation method, two resistance boxes instead of a bridge wire being used. The nickel and cobalt solutions were generally about 0·05 *M* at the commencement of the experiments, but naturally became more dilute as the titration proceeded.

The results of the electrometric titrations are given in Figs. 1, 2 and 3. It should be emphasised that the electrode potentials indicated are by no means reversible, nor are they exactly reproducible. The fact that nickel and cobalt begin to dissolve in cyanide solutions, with the evolution of hydrogen, at a potential of about $-0{\cdot}7$ volt further increases the instability of the electrodes. In spite of these factors, the general tendency of the results is, however, quite definite, as curves of the same type could be obtained repeatedly.

Fig. 1.

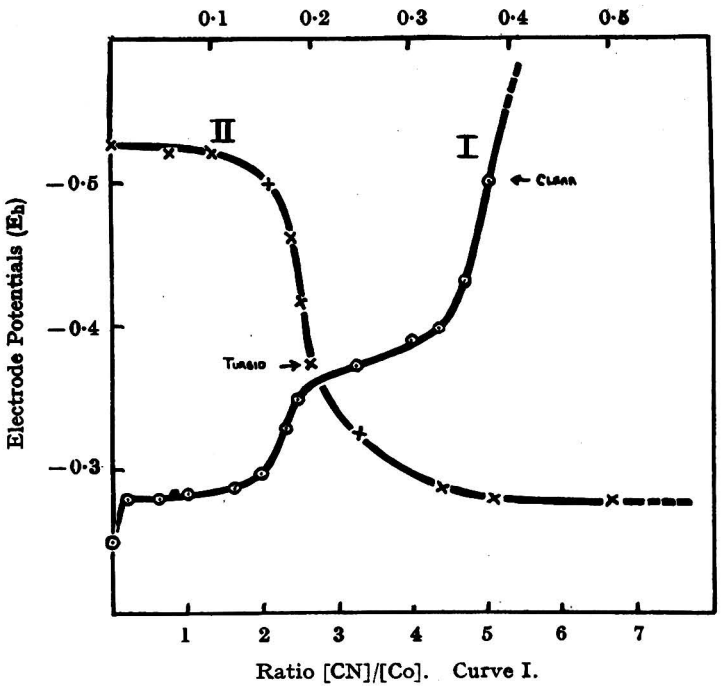


ELECTROMETRIC TITRATION OF NICKEL SOLUTION.

RESULTS.—Curve I (Fig. 1) shows the variation of electrode potential during the titration of nickel sulphate with potassium cyanide, and Curve II the variation when the cyanide is titrated with the nickel solution. The salient features of the curves are independent of temperature. In each case there is definite evidence of

the formation of the complex ion $Ni(CN)_4''$, since both curves show a clear break* at a [Ni]:[CN] ratio of 1:4. The appearance of turbidity at a definite stoicheiometric ratio corresponding to this break (Curve II) shows that the Rupp and Pfennig method for determining nickel has a sound theoretical basis. The break corresponding to the complete precipitation of $Ni(CN)_2$ may be seen on Curve I, but it is by no means sharp; it appears, therefore, that the solubility product of this cyanide is comparatively large. In dilute solutions it is natural to expect that the break would be even less distinct; and in Curve II, where the dilution is much

Fig. 2.



ELECTROMETRIC TITRATION OF COBALT SOLUTION.

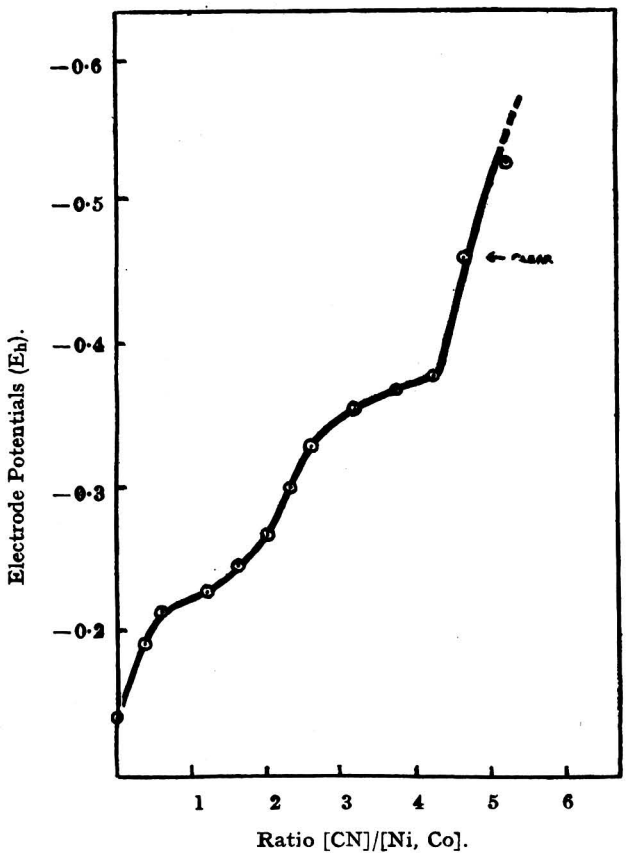
greater, the break does not appear. The absence of a corresponding inflexion from the titration curves of Müller and Schluttig (*loc. cit.*) may thus be attributed to the dilution of the solution, rather than to the slowness of the reaction $Ni(CN)_4'' + Ni^{\cdot\cdot} \rightarrow 2Ni(CN)_2$, as suggested by these authors.†

* The term "break" refers to a rapid change of electrode potential resulting from a small change in the metal/CN ratio; a "sharp" or distinct break is indicated by an almost vertical portion of the electrode potential curve, whereas an "indistinct" break is shown by an inflexion in the curve which has no vertical portion.

† We have observed the inflexion with Müller and Schluttig's method, using 0·03 *M* solutions.

Fig. 2 (Curve I) shows the results of the electrometric titration of cobalt sulphate solution with potassium cyanide, and Curve II those for the reverse titration. The break, although not so sharp as with nickel, definitely occurs in both cases at a ratio [Co]: [CN] of 1: 5, but there are no signs of the break at 1: 4 reported by Müller and Schluttig (*loc. cit.*) when using a silver electrode. The

Fig. 3.



ELECTROMETRIC TITRATION OF MIXTURE OF NICKEL AND COBALT.

appearance of turbidity in the titration of potassium cyanide with cobalt solution corresponds to the inflexion in the curve and to the completion of the formation of the complex ion $Co(CN)_5'''$. The absence of any inflexion corresponding to $Co(CN)_4''$ does not rule out the possibility of the presence of this ion; if it were formed to any appreciable extent, however, the production of a turbidity when titrating potassium cyanide with a cobalt solution, and the disappearance of the

turbidity in the reverse titration, would not always occur at the definite stoichiometric ratio of [Co]: [CN]=1: 5 (*cf.* the case of copper already quoted, p. 94). It is clear, therefore, that this value is not fortuitous, but is in accord with the existence of only one complex ion. The electrometric titration thus supplies strong theoretical evidence for the reliability of the Rupp and Pfennig method of estimating cobalt.

The results of titrating a mixed solution containing equivalent amounts of nickel and cobalt, using an alloy electrode, are shown in Fig. 3. The separate formation of $Ni(CN)_4''$ and $Co(CN)_5'''$ is not apparent, presumably because the electrode potentials of $Ni|Ni(CN)_4''$ and $Co|Co(CN)_5'''$ are almost identical. The marked break in the curve, and the disappearance of the precipitate, occurs, as would be expected under these conditions, when the solution contains 1[Ni, Co] to 4·5[CN]. Even though a curve of the type of Fig. 3 does not indicate the individual formation of $Ni(CN)_4''$ and $Co(CN)_5'''$, yet, theoretically, it offers a complete method of analysing a mixture of nickel and cobalt. The first break in the curve, corresponding to the complete formation of $Ni(CN)_2$ and $Co(CN)_2$ should occur at a ratio of 1[Ni, Co]: 2[CN], whereas the second break should appear when [Ni, Co] is to [CN] in the ratio of 1 to $\frac{4[Ni] + 5[Co]}{[Ni] + [Co]}$. If the titration values corresponding to these breaks are determined, the values of [Ni] and [Co] can be calculated. Unfortunately, the first break is very indistinct (see Fig. 1, Curve I; Fig. 2, Curve I; and Fig. 3) in the dilute solutions used, and its position cannot be judged with certainty; if the difficulty of obtaining stable potentials were overcome, it is possible that this method might prove applicable to the complete analysis of comparatively concentrated solutions containing both nickel and cobalt.

Ordinary Titration of Total Nickel and Cobalt.—The satisfactory nature of the Rupp and Pfennig method for simple and mixed solutions was confirmed further by the direct titration of solutions containing known amounts of nickel and cobalt. For the present purpose, however, the concentration limits set by the originators of the method were too high, and, hence, experiments were made with more dilute solutions. It was found that satisfactory end-points, within the limits of accuracy required, could be obtained with a $N/6$ cyanide solution and nickel or cobalt solutions down to 0·01 M. Under these conditions, however, the turbidity at the end-point was very slight, and was detected either by the Tyndall effect, with an electric light, or by viewing a sharply defined object through the solution. Free acid and free alkali, if present to any appreciable extent, interfere seriously with the cyanide titration in dilute solution; it was found advisable, therefore, to add ammonium hydroxide to the acid solution until it was just alkaline to brom-phenol-blue (pH about 5). Experiments showed that, provided the concentration of ammonium salts was less than about N, the results were not affected.

Table I contains some of the results obtained with separate nickel and cobalt solutions, and Table II with mixed solutions containing various proportions of the

two metals. For measuring out the small quantities of cyanide shown a micro-burette was used.

TABLE I.

Titration of 0·175 *N*-KCN with:

0·00965 *M*-$NiSO_4$

KCN. (c.c.).	$NiSO_4$. Found. c.c.	$NiSO_4$. Calcd. c.c.
1·73	7·84	7·85
2·37	11·04	10·75
2·55	11·59	11·56
6·94	31·23	31·40

0·0106 *M*-$CoSO_4$

KCN. (c.c.)	$CoSO_4$. Found. c.c.	$CoSO_4$. Calcd. c.c.
2·15	7·85	8·00
2·64	8·75	8·72
3·53	11·80	11·65
4·85	16·07	16·01
9·68	32·26	31·96

TABLE II.

Titration of 0·175 *N*-KCN with mixtures of $NiSO_4$ and $CoSO_4$ of total concentration about 0·01 *M*.

0·00915 *M*-$NiSO_4$
0·00053 *M*-$CoSO_4$

KCN (c.c.)	Mixture. Found. c.c.	Mixture. Calcd. c.c.
1·89	8·51	8·46
3·01	13·50	13·42
6·90	30·80	30·77

0·00820 *M*-$NiSO_4$
0·00159 *M*-$CoSO_4$

KCN (c.c.)	Mixture. Found. c.c.	Mixture. Calcd. c.c.
1·755	7·59	7·54
3·41	14·90	14·64
6·32	27·53	27·14

0·00483 *M*-$NiSO_4$
0·00530 *M*-$CoSO_4$

2·01	7·85	7·68
3·33	12·88	12·72
6·27	24·0	23·94

0·00144 *M*-$NiSO_4$
0·00901 *M*-$CoSO_4$

3·04	10·60	10·47
3·11	10·80	10·71
6·57	22·38	22·62

In view of the fact that the total nickel and cobalt used in these titrations did not exceed about 0·017 grm., the results are satisfactory, and indicate that the method is applicable to various mixtures of the two metals; it is, therefore, suitable for the determination of the total [Ni]+[Co] in small quantities of alloys.

DETERMINATION OF COBALT IN PRESENCE OF NICKEL.—The most suitable method for determining the cobalt was that involving oxidation to hydrated cobaltic oxide by sodium perborate in alkaline solution (Engle and Gustavson, *J. Ind. Eng. Chem.*, 1916, **8**, 901); the nickel is quite unaffected by this reagent. Willard and Hall (*J. Amer. Chem. Soc.*, 1922, **44**, 2237) studied four methods for reducing the cobaltic oxide and estimating the cobalt: (i) by ferrous sulphate,

(ii) by stannous chloride, (iii) by titanous chloride, and (iv) by potassium iodide; all in acid solution. The first method is inaccurate, and the second and third require rigid exclusion of air. In the fourth method Willard and Hall found that the dissolution of the cobaltic oxide was so slow that appreciable oxidation of the acid potassium iodide occurred, and elaborate precautions were taken to eliminate this source of error. This method, however, seemed the most promising for dealing with small amounts of cobalt, and, since the time of dissolution of the oxide also would be small, the error due to atmospheric oxidation would be decreased. The experimental method was consequently very much simplified, and the following procedure was adopted:

About 5 c.c. of a solution containing from 0·0005 to 0·005 grm. of cobalt were treated in a 300 c.c. flask with about 0·5 grm. of sodium perborate, 10 to 15 c.c. of 2 *N*-sodium hydroxide solution and some 15 c.c. of water. After boiling for ten minutes the flask was loosely corked and cooled quickly in a stream of running water. Four to ten c.c. of *N*/10 potassium iodide solution, a few crystals of sodium carbonate,* and 10 to 15 c.c. of 8 *N*-sulphuric acid were added, and the flask stoppered and allowed to stand until a clear solution was obtained. The iodine liberated was then titrated with *N*/50-sodium thiosulphate solution, starch being used as indicator towards the end of the titration. To eliminate errors arising from oxidation by the air of the strongly acid iodide solution (and perhaps from other uncertain causes), a blank titration was carried out with similar amounts of iodide and acid, but without cobalt; the thiosulphate required (about 0·04 c.c. of *N*/50) was subtracted from the total titration of the cobalt solution. It may be mentioned that the oxidation error has been found to increase rapidly with increasing acid concentration (see also Swift, *J. Amer. Chem. Soc.*, 1929, **51**, 2682).

Results obtained by this method in solutions containing cobalt salts only are given in Table III, and those obtained in solutions of different ratios of nickel and cobalt are quoted in Table IV.

TABLE III.

DETERMINATION OF COBALT.

Cobalt taken.	Sodium thiosulphate 0·0201 *N* (corrected for blank).	Cobalt found.
Grm.	c.c.	Grm.
0·00047	0·41	0·00049
0·000625	0·49	0·00058
0·00156	1·32	0·00157
0·00218	1·84	0·00218
0·00312	2·56	0·00304
0·00312	2·71	0·00322
0·00625	5·25	0·00622

* The object of the carbonate was to produce, on acidifying, a rush of carbon dioxide with which to drive the air out of the flask.

TABLE IV.

DETERMINATION OF COBALT IN PRESENCE OF NICKEL.

Cobalt taken. (Molar.)	Nickel taken. (Molar.)	Cobalt found. (Molar.)
0·00000	0·00965	0·00000
0·00052	0·00915	0·00055
0·00156	0·00820	0·00161
,,	,,	0·00162
0·00520	0·00483	0·00520
,,	,,	0·00521
0·00885	0·00144	0·00876
,,	,,	0·00895

It is clear from these results that the method described is reasonably accurate for the determination of small amounts of cobalt, and that the accuracy is not affected by the presence of nickel.

TEST OF THE COMPLETE ANALYTICAL METHOD.—The solution was divided into two parts; the total nickel and cobalt in one portion was determined by means of the modified Rupp and Pfennig method, and the cobalt was determined in the other by means of the method just described. The complete analyses for various solutions are given in Table V.

TABLE V.

ANALYSES OF MIXTURES OF NICKEL AND COBALT.

Solution as made up.			Solution as analysed.		
Cobalt. (Molar.)	Nickel. (Molar.)	Cobalt. Per Cent.	Cobalt. (Molar.)	Nickel. (Molar.)	Cobalt. Per Cent.
0·00093	0·00525	15·0	0·00102	0·00504	16·8
0·00465	0·00525	46·5	0·00465	0·00527	46·4
0·00093	0·00905	9·05	0·00097	0·00940	9·4
0·00837	0·00105	89·0	0·00826	0·00104	89

The agreement between the actual compositions of the mixtures and the analytical determinations is quite satisfactory and sufficient for general purposes, considering the small amounts of material analysed. It should be mentioned that the errors involved in the two parts of the analysis reinforce each other. The tendency in the Rupp and Pfennig titration is to overshoot the end-point, so that total nickel and cobalt is low, whereas in the iodine titration the cobalt may be high on account of atmospheric oxidation. The agreement obtained in such dilute solutions speaks highly for the potential accuracy of the method.

COLORIMETRIC METHOD.—An attempt was made to utilise the colorimetric method of Evans (ANALYST, 1925, **50**, 398) for the determination of cobalt in the presence of nickel. Unfortunately, this method was only found to be satisfactory for the analysis of solutions containing relatively little cobalt; if a large proportion of this metal were present, the colour *shade*, apart from the intensity, could not be matched with the standards.

SUMMARY.—(i) Electrometric titrations of nickel and cobalt solutions with potassium cyanide, with the use of nickel and cobalt indicator-electrodes, respectively, have supplied a sound theoretical basis for the Rupp and Pfennig method of determining these metals.

(ii) Ordinary titrations have confirmed the accuracy of the method, and shown its applicability to mixtures of nickel and cobalt, and to more dilute solutions than have previously been used.

(iii) A modified iodimetric method has been developed for the determination of small amounts of cobalt; it is applicable in presence of various amounts of nickel.

(iv) A method is described for the rapid volumetric analysis of small quantities of mixtures of nickel and cobalt.

We should like to express our thanks to the Department of Scientific and Industrial Research for a grant which enabled one of us (J. C. S.) to take part in this investigation.

THE UNIVERSITY, SHEFFIELD.

First Report of the Sub-Committee on the Determination of Arsenic, Lead, and other Poisonous Metals in Food Colouring Materials to the Standing Committee on the Uniformity of Analytical Methods.

I. THE DETERMINATION OF ARSENIC.

THE Sub-Committee was convened by the Standing Committee, and consists of the following members:

T. Callan, M.Sc., Ph.D., F.I.C. (Chairman), nominated by the British Dyestuffs Corporation.
S. G. Clifford, A.I.C. (Hon. Sec.), nominated by the Oil and Colour Chemists' Association.
H. Drake Law, D.Sc., F.I.C., and W. G. Messenger, B.Sc., A.I.C., nominated by the Society of Public Analysts and Other Analytical Chemists.
T. Macara, F.I.C., nominated by the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades.
J. R. Nicholls, B.Sc., F.I.C., nominated by the Government Chemist.

The terms of reference of the Sub-Committee from the Standing Committee were:—To investigate, formulate and recommend methods for the determination of arsenic, lead, copper, tin, zinc, and other metals in food-colouring materials.

The terms of reference do not include consideration of the permissible quantities* of such metallic impurities, and the Sub-Committee has been careful to consider, therefore, only methods for their determination.

It was decided to deal in the first place with the question of the determination of arsenic in synthetic dyestuffs. A general invitation to all interested was published in THE ANALYST and other journals to communicate relevant information to the Sub-Committee, and information as to the method they employed was solicited from a number of important firms and individuals. As a result communications were received from a number of dyestuffs manufacturers and important firms in the confectionery trades and from individual analysts.

DESTRUCTIVE WET OXIDATION.—The methods received from these sources were carefully considered, and it appeared that there was a preponderance of opinion conforming with the view of the Sub-Committee, that destructive wet oxidation was the best method of bringing the colouring matter into a suitable condition in which arsenic could be satisfactorily determined. This method has, therefore, been exhaustively studied by the Sub-Committee.

At the outset it was soon realised that, whilst it was comparatively simple to lay down specified conditions for the destructive wet oxidation of individual dyestuffs, it was a matter of very great difficulty to devise conditions which would be applicable to all, or nearly all, colours. In some cases violent initial reaction took place; in other cases excessive frothing caused difficulty, whilst in still other cases an initial controllable reaction was followed by violent reaction or even deflagration, probably due to the formation of explosive compounds. Many samples of dyestuffs were circulated and examined by members of the Sub-Committee, and eventually it was found possible to standardise the conditions of destructive wet oxidation so that practically all cases could be successfully dealt with.

Nitric acid was eventually selected as the most suitable oxidising agent, although other agents (*e.g.* potassium persulphate) were investigated.

DISTILLATION OF THE ARSENIC.—The Sub-Committee came to the conclusion that, to obtain strictly comparable results, it was necessary that the solution, after the process of destructive wet oxidation and before actually determining the arsenic contained therein, should be, as far as possible, freed from disturbing substances, and should be in a similar condition in all cases.

In order to arrive at this desirable state of affairs, it was considered essential that a distillation process should follow the process of destructive wet oxidation, so as to obtain a distillate containing all the arsenic and no disturbing impurities.

A full investigation of various distillation processes was carried out to discover the most suitable conditions, and the Sub-Committee would particularly stress the importance of using, in this stage of the process, the relative quantities which are specified in the detailed methods which follow hereafter. Using such quantities, there is a steady evolution of hydrochloric acid gas throughout the distillation, which is essential to the success of the method, whilst serious divergence from the recommended quantities is liable to lead either to too vigorous reaction in the early stages of the distillation, whereby arsenious chloride may be swept through the receiver, or to the evolution of water, resulting in the decomposition of arsenious chloride in the still head.

The best conditions for the distillation of the oxidised product having been ascertained, the possibility of shortening the process by distilling the original dyestuff with sulphuric and hydrochloric acids, without previous oxidation, was carefully investigated.

* See ANALYST, 1928, **53**, 217.

A large number of samples was examined by this direct distillation process under many conditions of experiment, and, as a result, the Sub-Committee has come definitely to the conclusion that, whilst in most cases the direct method gives satisfactory results provided two distillations are carried out, in certain cases, *e.g.* nigrosine, the results are low, and even after two distillations there may still be an appreciable quantity of arsenic remaining in the distilling flask, which is only slowly liberated and carried over.

The Sub-Committee is, therefore, unable to recommend this method as a general one, but it is so rapid and simple that full details for carrying it out are given, with the warning that, whilst it is very suitable as a rapid sorting-out test, it is not entirely reliable.

The Sub-Committee also investigated the official standard method, issued by the U.S. Dept. of Agriculture (*Bulletin*, No. 1390, 1927) for determining arsenic in certain specified dyestuffs, which consists in adding a large excess of sodium phosphate and ammonia to a solution of the dyestuff, either after or without previous partial oxidation with nitric acid, and then adding a slight excess of magnesia mixture and determining the arsenic brought down in the magnesium-ammonium-phosphate precipitate.

Whilst results obtained by this method on certain dyestuffs were satisfactory, it was considered that the method was not of general application to all classes of dyestuffs, and its investigation was not further proceeded with.

DETERMINATION OF ARSENIC* IN THE PREPARED SOLUTION.—The Sub-Committee considered the methods available for the actual determination of arsenic in the solution resulting from the oxidation and distillation processes, and came to the conclusion that the method eventually to be adopted should be the simplest attainable, and one, if possible, which did not require the provision of special apparatus.

It was appreciated that many refinements or modifications of the Marsh–Berzelius and Gutzeit methods were available to choose from, for example, the use of electrolytic methods. It was felt, however, that such methods often involved a special technique and special apparatus, and should not be resorted to, unless simpler methods were inadequate.

As the simplest method in general use is the "Gutzeit" method originally due to Mayençon and Bergeret,† this was carefully investigated, taking as a basis the standard official method prescribed by the British Pharmacopoeia, 1914, using zinc and acid. It was found that the Pharmacopoeia method, if modified in certain respects, was entirely satisfactory when applied to the solution free from all disturbing impurities as obtained after distillation, and this method is recommended by the Sub-Committee.

During the course of the investigation of a large number of samples of dyestuffs of all types circulated among the members of the Sub-Committee for analysis, quite serious discrepancies between the individual results were found in the early stages of the work, even when the same method and identical material were employed. Such discrepancies have been systematically traced to small variations in either manipulative details or unsuspected sources of error. As the work of the Sub-Committee has progressed, such sources of error have been gradually eliminated, and precautions for avoiding them have been incorporated in the text of the recommended methods where required.

* The term "arsenic" is used for the sake of brevity, though the results are invariably expressed as arsenious oxide.

† *Compt. rend.*, 1874, **79**, 118.

As illustrations of such sources of error might be mentioned: (*a*) the importance of not adding sulphuric acid in the *early* stages of method (A) until the initial reaction with nitric acid has subsided (in certain methods received from outside sources sulphuric acid was added at the *commencement* of the oxidation process, so that if the dyestuff contains a large amount of sodium chloride, as is often the case, there is real danger of loss of arsenic); (*b*) the necessity of adhering to the strengths of nitric acid recommended if explosion of easily oxidisable colours is to be avoided; (*c*) the importance of the relative quantities of reagents in the distillation process, referred to above.

In illustration of the importance of apparently trivial details, it might be mentioned that the substitution of rough-surfaced paper for the smooth-surfaced paper which is recommended for the preparation of mercuric chloride test papers led to serious discrepancies in the judgment of the intensity of the colour obtained.

Attention is also drawn to the necessity of "pickling" all glass apparatus by a preliminary boiling with dilute hydrochloric acid before use. Certain varieties of resistance glass contain arsenic as an essential constituent, and it is important to ascertain, therefore, that all glassware used does not yield traces of arsenic under the conditions of the test. The conditions are particularly severe in the oxidation and distillation processes.

The Sub-Committee considers that, provided the conditions laid down are strictly followed, there should be no difficulty in obtaining accurate and concordant results. The following table gives the actual results obtained by the individual members of the Sub-Committee, using method "A" on samples of five dyestuffs containing widely differing amounts of arsenic:

	Arsenious oxide parts per 10^6.					
	A.	B.	C.	D.	E.	F.
Acid yellow ..	5,5·5	4·5	5·2	5·8	5,5·5	5,5·6
Benzo-purpurine	1·,1·5	2	1·4	1·5,2	1·5	1·5
Ponceau ..	2·5	2	2·8	1·5	1·2	2
Tartrazine ..	1	1·5	1·5	0·9	0·8	0·8
Indigo carmine ..	9	8	8·5	9	9	8·5

These results do not, of course, necessarily prove that all the arsenic present was accounted for. To prove this is, actually, impossible. The Sub-Committee has, however, satisfied itself that when known amounts of arsenic are added to a dyestuff containing only a very small amount of arsenic, a satisfactory recovery of the arsenic actually added is obtained. Thus, arsenic in the form of a standard solution was added to the sample of tartrazine, referred to above, at the commencement of the analysis after weighing into the flask, and an analysis was made by each member of the Sub-Committee, the results obtained being as follows:

	Arsenious oxide, parts per 10^6.					
	A.	B.	C.	D.	E.	F.
Added	4·5	3·5	4	8	5	5
Recovered ..	4	3–3·5	3·8	7·5, 8	4·8, 5	4·5, 5

In considering the above results, which may be regarded as being typical of a very large number of analyses carried out by the Sub-Committee on different classes of dyestuffs, it is important to remember that the result obtained depends finally on an *estimation* rather than on a determination. This is true in all cases where extremely small amounts of arsenic are in question, whether the method be the Marsh–Berzelius method, involving estimation of the intensity of a mirror, or as in the method here recommended, the intensity of a stain on a test paper.

In the case of a stain on mercuric chloride paper corresponding to, say, 0·005 mgrm. As_2O_3, which is a very suitable intensity of stain to evaluate, it is a very moot point whether the average eye would detect with certainty the difference between a stain given by 0·0045 and 0·005 mgrm. of As_2O_3, or between 0·005 and 0·0055 mgrm. of As_2O_3, so that a stain which is estimated to be equal to 0·005 mgrm. of As_2O_3 may actually be equivalent to 0·0045 or 0·0055 mgrm., or a stain actually due to 0·0045 mgrm. may be estimated to be either 0·004 or 0·005 mgrm., according to the individual judgment. The effect of this inherent possibility of error of, at least, 0·0005 mgrm., is shown in the following table, it being assumed that aliquot portions of the test solutions are taken in each case to give a stain equal to 0·005 mgrm. of As_2O_3.

Parts of As_2O_3 per million present.	Effect of a difference of 0·0005 mgrm. on the results, expressed as parts As_2O_3 per million.
1	± 0·1
5	± 0·5
10	± 1
20	± 2

Thus, in the case of a sample containing 5 parts per million of As_2O_3, it would be possible for two analysts to differ by one part per million, attributable solely to the personal equation in the matter of judging the intensity of the coloration.

The methods, as finally adopted by the Sub-Committee, are as follows:

DIRECT DISTILLATION METHOD.—In order to obtain rapidly an approximate idea of the amount of arsenic in a dyestuff a direct distillation method may be employed. As a rule, this method gives results closely approximating to the true arsenic content. It is liable to give somewhat low results with certain colours, *e.g.* nigrosine. It is, however, very useful as a "sorting-out" test. The method is to be carried out as follows:

Five grms. of the dyestuff are weighed into a 100 ml. or 200 ml. Kjeldahl flask of resistance glass or silica, with a ground-in condenser, into which has been blown a tap funnel as shown in Fig. (i). A mixture of 20 ml. of concentrated sulphuric acid and 14 ml. of water, previously mixed and cooled to 5° C., is then added to the colour, and the whole is thoroughly mixed, the mixture meanwhile being cooled externally. While still cooling, 5 grms. of a mixture made up in the following proportions—5 grms. of sodium chloride, 0·5 grm. of hydrazine sulphate, 0·02 grm. of potassium bromide—are added, followed rapidly by 10 ml. of concentrated hydrochloric acid. Care should be taken that no solid material comes in contact with the ground-in portion of the flask. The mixture of chloride-hydrazine-bromide can be made up in bulk, and will keep indefinitely in a tightly-stoppered bottle.

The condenser is quickly fitted, and the mixture distilled* into 10 ml. of water and 2 ml. of nitric acid (sp. gr. 1·42), contained in a receiver which is cooled externally. The exit end should dip below the surface of the liquid. When all, or nearly all, of the hydrochloric acid has distilled over (indicated by the appearance of steam in the condenser, accompanied by strong tendency to suck back), the distillation is stopped, and the receiver changed. The contents of the Kjeldahl flask are then cooled to room temperature, a further quantity of 10 ml. of

* If difficulty due to frothing is experienced, 2 ml. of amyl alcohol may be added to the contents of the Kjeldahl flask at the commencement of the distillation, in which case the "end-point" is taken as that point at which amyl alcohol appears in the condenser and the bulb of the condenser is full of vapour.

concentrated hydrochloric acid is added slowly, through the tap funnel in the condenser, and the distillation continued into a further quantity of 10 ml. of water and 2 ml. of nitric acid (sp. gr. 1·42) until the hydrochloric acid has again been driven off. The first and second distillates are mixed and evaporated to dryness on the water-bath in a porcelain basin.

Five ml. of water are then added and evaporated to dryness, and this is repeated to make sure that all nitric acid has been eliminated. The residue is then dissolved by warming with 3 ml. of concentrated sulphuric acid, cooled and cautiously diluted to 25 ml. with water. The solution is then ready for transference to the arsenic apparatus, described later.

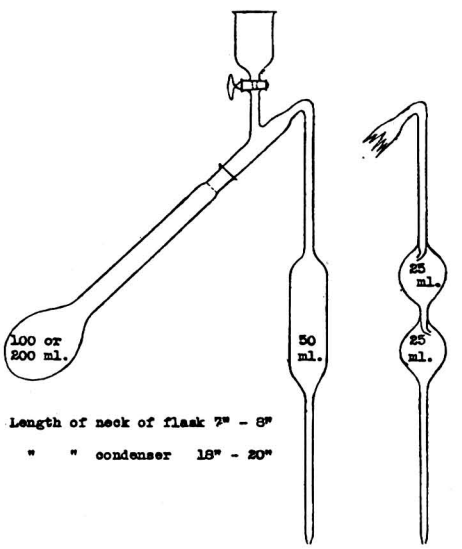


Fig. (i) Fig. (ii)

METHOD A.—The following method, whilst more tedious than the direct distillation method, must be used to obtain accurate results:

Five grms. of the sample are treated with 10 ml. of 30 per cent. nitric acid in a 100 ml. or 200 ml. resistance glass or silica Kjeldahl flask,* or in the distillation flask, already referred to. The mixture, after any initial vigorous reaction has subsided, is heated until any further vigorous reaction ceases, then cooled, and 10 ml. of concentrated sulphuric acid gradually added at such a rate as not to cause excessive frothing or heating (10 minutes is usually required), and the heating continued until the liquid appreciably darkens in colour. To the hot solution 5 ml. of nitric acid (1·42 sp. gr.) are added slowly in small portions, and the liquid boiled down until colourless or pale yellow.

If this amount of nitric acid is insufficient to bring about the desired decolorisation, further acid is added in small portions at a time, preferably by dropping in from a burette, note being kept of the amount. After cooling, the liquid is diluted with 50 ml. of water and transferred to the flask of the special distillation apparatus shown in Fig. (i) or to a similar apparatus without the tap funnel. The solution is first boiled down, without inserting the condensing arm, to 10 ml.,

* If excessive frothing is experienced in the early stages the preliminary treatment may be carried out in a 500 ml. beaker until frothing has been brought well under control. The mixture is then transferred to the Kjeldahl flask, the subsequent procedure being as above.

or until white fumes appear, cooled, diluted, and again boiled down to 10 ml., cooled, and 7·0 ml. water added.

The liquid is well cooled, and 5 grms. of the chloride-hydrazine-bromide mixture referred to in the direct distillation method are introduced through a short-stemmed funnel (avoiding contamination of the ground portion of the neck of the flask), followed immediately by 10 ml. of concentrated hydrochloric acid. The condenser* is quickly fitted, and the liquid distilled into an externally cooled mixture of 10 ml. of water and 2 ml. of nitric acid,† until its volume is reduced by about half, or until about 5 minutes after the condenser is full of steam, the exit end dipping below the surface of the liquid. The distillate is then evaporated to dryness on the water-bath, the residue twice evaporated to dryness with 5 ml. of water to remove nitric acid, dissolved by warming in 3 ml. of concentrated sulphuric acid, and diluted to 25 ml. with water, as described under the direct distillation method.

METHOD B.—Certain colours—particularly basic dyestuffs—give trouble in the oxidation process, owing to their liability to violent deflagration. In such cases the following modification of method "A" may be employed:

Five grms. of the dyestuff are placed in a resistance glass or silica Kjeldahl flask, and 25–30 ml. 30 per cent. nitric acid are added. The mixture is warmed until the initial vigorous reaction is over. At this point the formation of a spongy or tarry cake is usually observed. The mixture is cooled and the acid liquor poured off. The tarry material in the flask is washed with the smallest possible quantity of water, and the washings added to the acid liquor. To the tarry residue are added 10 ml. of concentrated sulphuric acid, and the contents of the flask are agitated until the residue is dispersed.‡ Concentrated nitric acid is then added, drop by drop, until vigorous oxidation is over, the mixture being warmed if necessary. The poured-off acid liquor is returned to the flask and the mixture is boiled down until the colour just commences to darken. Destruction is completed as in method "A."

DETERMINATION OF ARSENIC IN THE PREPARED SOLUTION.—The arsenic in the prepared solution from the direct distillation process, or from the distillation process following the oxidation process, is to be determined by the following method:

The apparatus for carrying out the test shall be exactly as defined in the British Pharmacopoeia, 1914, with the following exception:

> The gas exit tube shall have an internal diameter of 4 mm., and shall not be widened out at the gas exit end, but shall be ground smooth at right angles to the tube.

The test papers are to be prepared by soaking smooth-surfaced filter paper, similar in substance and texture to No. 1 Whatman filter paper, in a saturated aqueous solution of mercuric chloride, and are to be dried in a warm atmosphere without exposure to bright daylight. The edges of the dry papers are to be rejected, and the paper must be kept in the dark until required. The mercuric chloride paper

* If the form of condenser shown in Fig. (i) does not effectively prevent sucking back, the type shown in Fig. (ii) may be substituted.

† The process may be appreciably shortened by distilling into 10 ml. of saturated bromine water instead of into dilute nitric acid. In this case care must be taken that there is an excess of bromine water in the receiver at the end of the distillation. No attempt should be made to boil off the excess of bromine from the distillate, otherwise loss of arsenic may ensue. The distillate is made up directly to 25 ml. with water and a suitable aliquot taken for transference to the apparatus, subsequently described.

‡ If it is found that the tarry matter cannot be satisfactorily treated in the Kjeldahl flask, it may be transferred to a 600 ml. beaker. In this case it may be necessary to use rather more than 10 ml. H_2SO for the subsequent treatment. If more than 10 ml. sulphuric acid are used the solution is boiled down to the corresponding volume, and water in the proportion already described, that is, 7·0 ml. of water, for each 10 ml. of acid, is added.

shall be used in the test in the form of a disc held flatly and firmly against the ground end of the tube by any suitable means. The method recommended by the British Pharmacopoeia, 1914, of securing the test paper at the end of the tube by means of a rubber band is not a satisfactory method; more effective methods are those to which reference is made.*

In carrying out the test, either the whole of the solution (corresponding to 5 grms. of dyestuff) or, if the arsenic content is more than 1 part per million, such an aliquot portion as would contain from 0·004 to 0·010 mgrm. arsenic, is mixed with sulphuric acid, stannated hydrochloric acid and water in the following proportions:

x ml. of prepared distillate,
$25-x$ ml. of 1:8 sulphuric acid,
8 ml. of stannated hydrochloric acid B.P.,
and water to make up to 60 ml.

The mixture is transferred to the apparatus, 10 grms. of granulated zinc are added, and the apparatus immediately assembled. It is essential that the zinc used should not only be free from arsenic, but that it should also be "active." A blank determination should give no stain on the test paper under the conditions of the test, but should give a definite stain when 0·001 mgrm. of As_2O_3 is present.

The reaction is allowed to proceed without the application of external heat for 15 minutes, the mercuric chloride paper being protected from strong light, and the reacting vessel is then transferred to a water-bath maintained at 35–40° C. for 30 minutes. The stain so produced on the paper is compared with a series of freshly made standard stains prepared by adding a known amount of arsenic to tests carried out under the conditions as standardised above, a suitable range being 0·001 to 0·010 mgrm., the stains being examined under normal daylight conditions. Adequate precautions are to be taken and blank tests made to ensure that the apparatus and all reagents used give no visible stain.

For, and on behalf of, the Sub-Committee,

(*Signed*) T. CALLAN (*Chairman*).
S. G. CLIFFORD (*Hon. Sec.*).

DECEMBER, 1929.

* ANALYST, 1927, **52**, 700–701.

Sixth Report of the Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods.

THE DETERMINATION OF CITRAL IN LEMON OIL.

THE Essential Oil Sub-Committee has found that the method official in the British Pharmacopoeia, 1914, for the determination of citral in lemon oil is open to objection on account of the large variation in the results obtained.

Numerous experiments have been carried out, and, as a result, the Sub-Committee unanimously recommends the method given below. To ensure a reasonably sharp end-point being obtained, it is essential that strict attention be paid to the relative proportions of the reacting substances.

The following solutions are required:

Indicator Solution.—A 0·2 per cent. solution of pure methyl orange in 60 per cent. alcohol.

N/2 *Alcoholic Potash.*—Prepared with pure 60 per cent. (by volume) alcohol and standardised against *N*/2 hydrochloric acid, using methyl orange as indicator, and running the alkali *into* the acid until the full yellow colour is obtained.

N/2 *Hydroxylamine Hydrochloride.*—Dissolve 3·475 grms. of pure hydroxylamine hydrochloride in 95 c.c. of pure 60 per cent. (by volume) alcohol; add 10 drops of the indicator solution, and adjust to the full yellow colour of the indicator with the *N*/2 alcoholic potash, and make up to 100 c.c. with 60 per cent. (by volume) alcohol.

METHOD OF DETERMINATION.—Weigh out exactly, into a stoppered tube—approximately 150 mm. long by 25 m/m. diameter—about 10 grms. of the lemon oil, add 7 c.c. of *N*/2 hydroxylamine hydrochloride and 1 drop of the indicator: shake and titrate with the *N*/2 alcoholic potash until the red colour changes to yellow. Continue the shaking and titrating until the full yellow colour of the indicator is permanent in the lower layer after shaking vigorously for two minutes, and then allowing to stand to separate. The reaction is slow towards the end, but should be complete in about 15 minutes.

The completed titration liquid, plus a slight excess of alcoholic potash (1 or 2 drops), may be used as colour standard for the end-point of a subsequent determination.

The number of c.c. of *N*/2 alcoholic potash required, multiplied by the correcting factor 1·008, by the factor 0·076 for citral, and by 100, and divided by the weight of the oil taken, will give the percentage of aldehydes, calculated as citral.

The correcting factor is necessary, owing to the fact that the end-point of the titration, as carried out above, occurs at a *p*H different from that of neutral hydroxylamine hydrochloride.

The volume of the *N*/2 hydroxylamine hydrochloride used should be varied according to the citral content of the oil, the excess of hydroxylamine hydrochloride over the alcoholic potash required, being from 1 c.c. to 2 c.c. in all cases.

Determinations made by members of the Sub-Committee lead us to the opinion that the maximum variation with this process is within ±0·1 per cent.

RESULTS OF THE DETERMINATION OF CITRAL IN A SAMPLE OF LEMON OIL BY MEMBERS OF THE SUB-COMMITTEE.

Member.		Member.	
1	4·04 per cent.	7	4·03 per cent.
	4·05 ,,		3·96 ,,
2	3·89 ,,	8	4·05 ,,
3	3·95 ,,	9	4·05 ,,
4	4·03 ,,		4·05 ,,
	3·95 ,,		4·05 ,,
5	4·08 ,,	10	3·94 ,,
6	4·07 ,,		4·01 ,,

Maximum variation 0·19 per cent.

(*Signed*)

John Allan (Chairman), C. T. Bennett, S. W. Bradley, E. Theodore Brewis, L. E. Campbell, Thos. H. Durrans, T. W. Harrison, Ernest J. Parry, C. Edward Sage, W. H. Simmons.

T. Tusting Cocking (Hon. Sec.).

NOVEMBER 30th, 1929.

Report of the Milk Products Sub-Committee to the Standing Committee on Uniformity of Analytical Methods.

MILK PRODUCTS. REPORT No. 2.

THE DETERMINATION OF SUCROSE IN SWEETENED CONDENSED MILK.

THIS Sub-Committee was convened by the Standing Committee, and consists of the following members:—

Nominated by the Government Chemist: A. More, A.R.C.S., F.I.C.

Nominated by the London Chamber of Commerce: E. R. Bolton, F.I.C., H. Jephcott, M.Sc., F.I.C., *Ir.* W. J. P. Pelle, and J. Tavroges, B.Sc., A.I.C.

Nominated by the Manufacturing Confectioners' Alliance: T. Macara, F.I.C.

Nominated by the Society of Public Analysts and Other Analytical Chemists: G. D. Elsdon, B.Sc., F.I.C., E. Hinks, M.B.E., B.Sc., F.I.C. (*Chairman*), E. B. Hughes, M.Sc., F.I.C. (*Hon. Sec.*), A. E. Parkes, F.I.C., and J. D. Roberts, B.Sc.

The report now submitted deals with the determination of sucrose in sweetened condensed milk.

It may be recalled that the Sub-Committee was of opinion that the determination of total milk solids would be best attained by the subtraction of the sucrose present from the total solids (see *Report No.* 1, p. 2; THE ANALYST, 1927, **52**, 403) provided that no significant decomposition of the milk solids or of the sucrose had occurred. The determination of lactose, therefore, is not of immediate importance, and accordingly this determination has been left for consideration later, though a considerable amount of work was carried out thereon during the earlier stages of this enquiry.

For the determination of sucrose, which, in the case of condensed milk, necessarily involves a sugar determination before and after inversion, the following methods in particular have been studied in detail:

1. COPPER REDUCTION METHODS.
2. IODIMETRIC AND CHLORAMINE-T METHODS.
3. POLARIMETRIC METHODS.

1. COPPER REDUCTION METHODS.

These were investigated at considerable length, some 460 analyses having been made. They were, for the determination of sucrose, finally abandoned, because it was found that there were too many factors influencing the uniformity of results, and that the desired accuracy of within $\pm 0{\cdot}5$ per cent. of the sucrose

present (equivalent to $\pm 0{\cdot}2$ per cent. on a normal condensed milk) could not be consistently obtained.

Copper reduction methods are fundamentally empirical and depend on careful observance of specified conditions of experiment, and, to some extent, upon the personal factor of the worker. These considerations indicate that the most satisfactory procedure would be for each laboratory to establish its own tables for the relation of sugar to reduced copper for any specified method. If possible, such a procedure is to be avoided, particularly where the method may be employed in cases of dispute, a circumstance which has throughout been borne in mind by the Committee.

Another objection to any copper reduction method is that the reducing power of a mixture of reducing sugars is not necessarily the sum of those of the constituent sugars. Similarly, there is the effect of sucrose upon the reducing power of the reducing sugars, which is recognised and, to some extent, allowed for in certain published tables, *e.g.* those of Munson and Walker, Lane and Eynon, and Jessen-Hansen.[1]

The precipitants used in preparing the sugar solution for the reduction process, and the salts of the milk itself, are also potential factors in influencing the course of the reduction.

The methods investigated were:—

(*a*) *Brown, Morris and Millar.*[2]—This method did not receive such full consideration as the others, it being found that it did not give the accuracy or uniformity required. This is probably due to the maintenance of the reacting solutions for a relatively long time at 100° C.

(*b*) *Quisumbing and Thomas.*[3]—This was found to be a method capable of giving excellent results, and satisfactory particularly in respect of the avoidance of appreciable effect of the sucrose on the reduction before inversion. It was found, however, that the method is highly susceptible to slight departures from specified conditions, such as small variations in temperature, size of beaker, thickness of beaker wall, and so on.

(*c*) *Munson and Walker.*[4]—Better results were obtained by this method than by (*a*), probably owing to the shorter period of heating, but the process is nevertheless sufficiently free from the personal factor to ensure consistently the desired accuracy.

(*d*) *Lane and Eynon.*[5]—Comparable conditions are readily obtained by the Lane and Eynon form of the volumetric Fehling method, and considerable success was achieved by its use. The results indicated that, for the requisite degree of accuracy, it would nevertheless be necessary for each analyst to prepare his own tables.

2. IODIMETRIC AND CHLORAMINE-T METHODS.

The iodimetric method of determining aldoses was applied to the determination of lactose in milk by Kolthoff[6] and by Tilmans and Strohecker[7]. The behaviour of sucrose in the iodimetric titration was investigated by Hinton and Macara[8]

[1] *Meddelelser fra Carlsberg Lab.*, 1923, **15**, No. 3.
[2] *J. Chem. Soc.*, 1897, **71**, 100 and 281.
[3] *J.A.C.S.*, 1921, **43**, 1503; A. L. Bacharach, ANALYST, 1923, **48**, 521.
[4] *Methods of Analysis of the A.O.A.C.*, 2nd edition, p. 190.
[5] *J. Soc. Chem. Ind.*, 1923, **42**, 32T, 143T, and 463T; and 1927, **46**, 434–435T.
[6] *Z. Untersuch. Nahr. Genuss.*, 1923, **45**, 141.
[7] *Z. Untersuch. Nahr. Genuss.*, 1924, **47**, 377.
[8] ANALYST, 1924, **49**, 2.

and, for the determination of sucrose in the presence of milk, it was in the form recommended by these authors that the method was examined by this Committee.

The chloramine-T method used was that described by Hinton and Macara.[9]

Over 500 analyses by these methods were made by the Committee, and the chloramine-T method was found to be definitely superior to the direct iodimetric. The reaction allows of better control, the absorption of iodine by non-sugars is minimised, and the corrections necessary for variations in the sugar concentration are definitely established.

It is considered that for the determination of lactose the chloramine-T method is promising, but, in the determination of sucrose it did not yield, in the hands of the Committee, the desired degree of accuracy, partly owing to the high dilution of the inverted sugar solutions necessitated by the process.

3. POLARIMETRIC METHODS.

The Committee considered that whatever process might be adopted for the determination of sucrose, ultimately the application of a polarimetric method would almost certainly be involved, in view of the possible presence of invert sugar in certain cases. Accordingly polarimetric methods were studied, and in the course of the work it was found that the desired degree of accuracy and concordance were more readily obtained by the procedure described below, than by any of the methods referred to above.

All the polarimetric methods examined are based on the same principle, namely, that of the Clerget inversion. Detailed investigation has been made of the following factors necessarily involved in any such process:

(*a*) Clarification.

(*b*) Muta-rotation.

(*c*) Effect of non-sugars: "neutral" and "acid" polarisation.

(*d*) The inversion.

(*e*) The inversion divisor factor.

(*f*) The correction for the volume of the precipitate produced in the clarification.

CLARIFICATION.—The employment of acid mercuric nitrate precipitant was rejected owing to the inversion effect on the sucrose before the direct reading could be taken, and also on account of incomplete precipitation of protein.

The use of neutral mercuric nitrate as prescribed by the Association of Official Agricultural Chemists[10] was studied. This reagent is a good precipitant, but the neutralisation and necessary shaking before making to volume, introduce experimental difficulty and liability to error of volume.

The Committee found a phosphotungstic reagent, described later, as modified from that of Hinton and Macara,[11] to be generally an excellent precipitant. It ensures, as a rule, complete precipitation of protein, rapid filtration and a clear bright filtrate. It has been established that there is no significant inversion of the sucrose by the reagent, provided that the polarimetric reading is taken within 40 minutes of the precipitation. A certain acidity during clarification, as determined by examination of the filtrate, is required to ensure the removal of the protein. On the other hand, too great an acidity in the filtrate may cause inversion of the sucrose before the direct rotation can be read. Further, in the case

[9] ANALYST, 1927, **52**, 668.
[10] *Methods of Analysis of the A.O.A.C.*, 2nd edition, p. 274.
[11] ANALYST, 1927, **52**, 676.

of certain milks, it was found that the matter became complicated by the use of ammonia (followed by acid) to destroy muta-rotation. This ammonia is removed to a greater or less extent by the reagent, resulting in a more acid solution, and thus involving the danger of inversion. Attempts to obviate this by reducing the amount of acid used for neutralising the ammonia resulted, in some cases, in incomplete precipitation. On account of these defects, the method has not been recommended, although the process normally gave good results.

The Dutch Codex[12] [13] specifies the use of zinc acetate and potassium ferrocyanide for the clarification of milk. This method of clarification was studied by the Committee, and was found to give an excellent filtrate. Though free acetic acid is present in this filtrate, its acidity is such (about *p*H 5) that no danger of inversion of sucrose can arise, even if left for several hours. The method recommended (p. 121) employs this process of clarification.

The phosphotungstic acid method is, nevertheless, given in full detail in an appendix to this report, in case analysts may find it more convenient in certain circumstances.

(*b*) MUTA-ROTATION.—It is well known that freshly dissolved lactose hydrate exhibits muta-rotation, and, as sweetened condensed milks generally contain crystallised lactose, solutions of these milks might have been expected to exhibit muta-rotation effects.

No such effects were, however, met with in solutions prepared by dissolving the samples in hot water (80° C. to 90° C.) and clarifying with one of the reagents specified above, except in the case of a sample of unsweetened evaporated milk which was stated to be eight years old. In this case the reading of a 20 per cent. solution in a 2 dm. tube fell only 0·05° in three days.

On the other hand, the filtrates of ordinary samples dissolved in lukewarm water did exhibit muta-rotation to a significant extent. From these results it would appear that the use of water at a temperature of 80–90° C. in dissolving the sample is sufficient to destroy these effects. The Committee recommend, however, that the possibility of errors arising from this cause should be guarded against, and that for this purpose ammonia should be added to the solution of the milk, followed by neutralisation with acid as described below.

(*c*) EFFECT OF NON-SUGARS: "NEUTRAL" AND "ACID" POLARISATION.—It is well known that the rotation of sugars is affected to a greater or less extent by other substances in solution. Jackson and Gillis[14] have shown that salts and acids have a marked effect on the rotation of sucrose and invert sugar, but particularly so in the case of the latter. They called attention especially to the fact that invert sugar had markedly different rotations in neutral and acid solutions. In determining sugar by the Clerget process, therefore, it is necessary to consider these influences. Obviously, any non-sugars which are capable of producing an optical rotation themselves must also be considered. This becomes particularly necessary when the optical activity of the substances, *e.g.* proteins and amino acids, varies with the degree of acidity of the solution.

As a clarified milk filtrate must contain various salts, and might conceivably contain small amounts of optically active nitrogenous substances, the Committee at first decided to investigate the neutral polarisation process of Jackson and Gillis (*loc. cit.*), as by this process any error arising from the variation in rotation of invert sugar and of the optically active non-sugar bodies due to change of acidity is obviated. Later, however, it was found that the clarifiers used removed

[12] *Codex Alimentarius (Holland)*, No. 1, Melk, 3rd edition, 1920.
[13] Carrez, *Ann. Chim. anal.*, 1909, **14**, 187.
[14] *Bureau of Chemistry Sci. Paper*, No. 375, 1920.

all the proteins, only minute amounts of other nitrogenous bodies remaining in the clarified filtrate, and these had no discernible effect on the rotation.

Again, invert sugar is seldom present in any appreciable quantity in condensed milks. Further, it was shown by Hinton and Macara[15] that various muta-rotation effects may result when acid solutions containing lactose, dextrose and laevulose are neutralised with soda or ammonia, and equilibrium is sometimes reached only after several hours. In the circumstances it seemed to the Committee that the neutralisation process offered no particular advantage over the acid method usually employed, since the effect of the salts and acid on the rotation of the sugars before and after inversion could be equally well allowed for by determining the inversion factor for sucrose under the actual conditions obtaining in any specified method of determining sucrose in a condensed milk. The Committee recommend, therefore, that the direct reading should be taken on the clarified filtrate without further treatment, and the invert reading without neutralisation.

(*d*) THE INVERSION PROCESS.—The principle of the inversion process described by Jackson and Gillis[15] has been adopted, but the details have been modified to meet the special case of a milk filtrate prepared as specified later. For example, the volumes of the sugar solution and acid used with the phosphotungstic method of clarification are somewhat different from those prescribed by Jackson and Gillis. As these modifications resulted in a slightly less acid solution during inversion, the time of heating had to be extended to 12 minutes. When zinc acetate and potassium ferrocyanide were finally adopted as the clarifying agents, it was found that the amount of acid used for inversion had to be increased by 20 per cent., presumably on account of the buffering effect caused by residual acetates in the filtrate. The Committee has proved that the sucrose is completely inverted by the modified procedures.

(*e*) THE INVERSION DIVISOR FACTOR: (Q)*.—It has been pointed out in paragraph (*c*) that the rotation of the sugars is affected by salts and acids, and it is also influenced by the concentration of the sugars in solution. Since, in milk filtrates prepared as recommended by the Committee, there are present both the natural salts of the milk and those resulting from the clarification process, it became necessary to determine the actual change in rotation which takes place when sucrose is inverted under these conditions. This was done by adding a known amount of sucrose to a milk filtrate prepared in the prescribed manner, after which the rotations of the solution were taken before and after inversion by the modified process, *i.e.* the change of rotation of the sucrose on inversion was taken in a solution having as nearly as possible the same composition as that of the clarified filtrate of a sweetened condensed milk. In this way an inversion divisor factor was obtained which is applicable to filtrates having the sugar concentrations ordinarily obtained in applying the process to sweetened condensed milks. The divisor factors so obtained under the conditions of the processes described later, all measurements being taken at 20° C., are:

	Zinc acetate-potassium ferrocyanide precipitant. Q.	Phosphotungstic acid precipitant. Q.
Sodium light	(i) 0·8825	(iv) 0·8865
Mercury green line (prism or special Wratten screen, No. 77A)	(ii) 1·0392	(v) 1·0439
International sugar scale (j) light ..	(iii) 2·549	(vi) 2·561

* "Q" is the change in specific rotation of sucrose on inversion, divided by 100.

[15] ANALYST, 1927, **52**, 687.

Correction of Inversion Divisor Factor for Concentration and Temperature.—Should a condensed milk not yield a filtrate having the sugars in a concentration approximating to that usually present, then a correction must be applied to these inversion factors. Vosburgh[16] showed that the effect of concentration of sugars on the specific rotation of mixtures of dextrose and laevulose was dependent on the total sugar concentration. Monier–Williams[17], extending this work to include mixtures of sucrose, lactose and laevulose, proved that the principle still held good, and deduced a simplified expression for this concentration effect. Similarly, the above factors, which apply to solutions having a total concentration of about 9 per cent. of sugars, may be corrected for concentration, when they become:

(i) 0·8825 + 0·0006 (C–9)
(ii) 1·0392 + 0·0007 (C–9)
(iii) 2·549 + 0·0017 (C–9)
(iv) 0·8865 + 0·0006 (C–9)
(v) 1·0439 + 0·0007 (C–9)
(vi) 2·561 + 0·0017 (C–9),

where C is the percentage of total sugars in the inverted solution as polarised.* Only in exceptional circumstances need the correction be applied.

All volumes have been measured at 20° C., and polarimetric readings taken at that temperature. A variation of ±1° C. makes little significant difference in the direct reading, but in the case of the "invert" reading a variation of over ±0·2° C. will certainly necessitate a correction for temperature. The Committee determined the correction to be applied to the factor, if the readings are taken at between 18° C. and 22° C. for milk filtrates prepared according to the method recommended, to be —0·0033 (T–20) for sodium light, and —0·0039 (T–20) for the mercury green line.

Combining corrections for concentration (where required), and temperature, the inversion divisor factor (Q) becomes:

(i) 0·8825 + 0·0006 (C–9) — 0·0033 (T–20)
(ii) 1·0392 + 0·0007 (C–9) — 0·0039 (T–20)
(iii) 2·549 + 0·0017 (C–9) — 0·0095 (T–20)
(iv) 0·8865 + 0·0006 (C–9) — 0·0033 (T–20)
(v) 1·0439 + 0·0007 (C–9) — 0·0039 (T–20)
(vi) 2·561 + 0·0017 (C–9) — 0·0095 (T–20)

(*f*) CORRECTION FOR VOLUME OF PRECIPITATE.—The determination of the correction to be allowed for the volume of precipitate has been the source of much trouble; indeed, it is the main difficulty of the polarisation method.

Having decided upon the nature of the precipitant to be used, and having determined the inversion divisor factor for sucrose in a milk filtrate, the Committee made investigations to establish the correction required for the volume of precipitate formed in clarification of the milk solution.

Firstly, it was found that the concentration of sucrose in the filtrate after such clarification and filtration was always higher than could be accounted for when calculating the volume of the precipitate from the specific volumes for the fat (1·08) and protein (0·74).

* The approximate concentration of the total sugars in the inverted solution may be calculated from the direct reading and the change on inversion in the usual manner, using the usual values for the specific rotations of sucrose and lactose and for the change on inversion.

[16] *J. Amer. Chem. Soc.*, **1920**, **42**, 1696.
[17] ANALYST, **1928**, **53**, 574.

Modifying the manner of adding the precipitant proved that this higher concentration was not thereby affected, but the correction necessary for a given sample of milk was found always to be the same in the case of the particular precipitant under test. The Committee prefer, in speaking of this correction, to refer to it as correction for volume of precipitate, rather than as an actual volume.

Ordinary dilution methods for avoiding direct correction for the volume of precipitate, such as, for example, that of Carrez,[18] introduce errors which become exaggerated in the calculation of results. Efforts to determine the actual volume of precipitate were made, employing a method based on that given by Lampitt and Hughes[19] for the determination of insoluble matter in milk powder. Results showed that the volume of precipitate itself was definitely greater than that calculated from the specific volumes of the fat and protein, and, in the case of the phosphotungstic acid precipitant, by an amount corresponding to that to be expected from the amount of phosphotungstic acid added, practically all of which is precipitated with the protein. The most satisfactory method of arriving at this correction was considered, however, to be a polarimetric one, by determining the rotation due to sucrose dissolved in a milk filtrate after clarification, and applying this to find the concentration of the sucrose in a milk which has been clarified after addition of the sucrose. From the increase in concentration in the latter case the correction for the volume of precipitate can be calculated.

The following outline of the procedure adopted will make the principle clear:

1. Weigh 100 grms. of milk into a 200 ml. flask. Add precipitating reagents, make up to the mark at 20° C., filter and polarise at 20° C.

   Reading = A.

2. Weigh 100 grms. of the same milk and 16·8 grms. of sucrose into a 200 ml. flask, and dissolve the sugar, add precipitating reagents, make up to the mark at 20° C., filter and polarise at 20° C.

   Reading = B.

3. Weigh 140 grms. of the same milk into a 200 ml. flask; add 7/5ths of the previous quantities of precipitating reagents. Make up to the mark at 20° C. and filter. Take 70 ml. of the filtrate, make up to 100 ml. and polarise at 20° C.

   Reading = C.

4. Measure 70 ml. of the filtrate from No. 3 into a 100 ml. flask, add 8·4 grms. sucrose, dissolve, make up to the mark at 20° C., and polarise at 20° C.

   Reading = D.

The correction for volume of precipitate for 100 grms. of milk

$$= 200\left(1 - \frac{D-C}{B-A}\right) \text{ ml.}$$

Values so obtained gave satisfactory results when applied to the analysis of mixtures of milk with known amounts of sucrose. In the case of the phosphotungstic acid method described in the appendix to this report, the correction was found to be the calculated theoretical volume of precipitate (fat × 1·08 + protein × 0·74),plus 1·5 ml., for 40 grms. of sweetened condensed milk.

In the zinc acetate-potassium ferrocyanide method, which is the method recommended by the Committee, it was found that this additional correction was

[18] Carrez, *Ann. Chim. anal.*, 1908, **13**, 17.
[19] ANALYST, 1924, **49**, 176–177.

about double that for the phosphotungstic acid precipitant. Further investigation showed that the additive correction does not meet all cases, but varies definitely with the percentage of protein in the milk. This additive factor can only be applied, therefore, to condensed milk of normal composition.

It was found in the case of the zinc ferrocyanide precipitant that the total correction for the volume of precipitate can be satisfactorily arrived at by applying constant volume factors to the amounts of fat and protein present. A large number of determinations (using, fresh milk of varying fat content; condensed full cream milk, condensed skim milk; skim milk powder, full cream milk powder, and mixtures of these) have shown that the apparent volume in millilitres of the precipitate due to one grm. of protein and the ferrocyanide associated with it, varies between 1·49 and 1·63, with a mean value of 1·55, the specific volume of the fat not being affected by the precipitant. No variation in this factor (1·55) was found to occur when fresh milk was concentrated in the presence of sucrose to the condition of ordinary sweetened condensed milk, as shown by the preparation of a sweetened condensed milk in the laboratory from known quantities of fresh milk and sucrose and analysis of the product.

Analysis of milk-sucrose mixtures of known composition (including full-cream, skimmed or partly-skimmed milk), in accordance with the method prescribed and using the correction as determined above, gave results in close agreement with the actual amount present. (See Table 1, page 119.)

Monier-Williams[20] adopts a procedure which avoids the use of any correction for the volume of precipitate. This necessitates the use of solid precipitants and the determination of total solids in the filtrate. Certain assumptions have to be made, also, as to the state of hydration of the phosphotungstic acid and of the proteins in the original milk-sucrose mixture, and in the total solids of the filtrate. The Committee considers that an actual determination of the volume correction is preferable.

THE POLARIMETER.

Members of the Committee have been using various types of polarimeter. In order to compare these, readings were taken on the same solutions, in different instruments, with different sources of light, using the same polarimeter tube, and the temperature being carefully controlled at 20° C. The results were as follows:

Saccharimeter: International sugar scale, white light with dichromate screen; factor to convert to angular degrees D line, 0·3462.

Change on inversion .. $14{\cdot}84_0°$.

Polarimeter: Sodium light.

Change on inversion .. $14{\cdot}83_6°$.

Polarimeter: Mercury vapour lamp; green line used (with prism); factor to convert to D line, 0·8492.

Change on inversion .. $14{\cdot}84_4°$.

In another laboratory a member, using the green line of the mercury vapour lamp, found exactly the same change on inversion when using either a prism or the special Wratten screen No. 77A.

RESULTS OF ANALYSES BY MEMBERS OF THE COMMITTEE.—In the tables which follow there are given the results of analyses using the method recommended by the Committee (zinc acetate-potassium ferrocyanide precipitant), and in the

[20] ANALYST, 1928, **53**, 569.

appendix similar tables are given of analyses with the phosphotungstic acid precipitant.

In each table the separate results are those reported by individual members of the Committee making the analyses, the results being, as a rule, the means of duplicate determinations. In each set of results there is at least one obtained by the use of each of the three types of polarimeter referred to above.

In the case of the condensed milk analyses, a bulk of condensed milk was prepared in a condensery, filled into tins, and one tin from the batch was sent for analysis to each of the eight or nine members of the Committee taking part in the trial. All analyses are reported.

In the case of the trials reported in Tables II and III there were available factory data as to the amount and composition of the milk condensed, the amount of sugar added, and the weight of the product obtained; from these the anticipated composition of the condensed milk could be calculated, and this anticipated figure is reported at the foot of the tables.

TABLE I.

MILK SUCROSE MIXTURES OF KNOWN COMPOSITION.

Mixtures of fresh milk and sucrose were made to correspond to a solution of condensed milk containing 42 per cent. of sucrose.

Sucrose (calculated as for a condensed milk), actual 42·0 per cent.

Sucrose found:

Using whole milk. Per Cent.		Using skim milk. Per Cent.	
42·01		42·02	
42·00		42·07	
41·93		41·99	
41·98		41·99	
41·97		42·16	
Mean 41·98		Mean 42·05	
Maximum deviation from actual	0·07	Maximum deviation from actual	0·16
Mean deviation from actual	0·02	Mean deviation from actual	0·05

TABLE II.

SWEETENED FULL CREAM CONDENSED MILK.

Total solids, 73·81 per cent.; fat, 8·54 per cent.; protein (N × 6·38), 9·15 per cent.

Sucrose found:

Per Cent.	
42·55	
42·59	
42·61	
42·64	
42·48	
42·55	
42·45	
42·45	
Mean 42·54	
Maximum deviation from ,,	0·10
Mean ,, ,, ,,	0·06

Factory data indicated that the condensed milk should contain 42·65 per cent. of sucrose.

TABLE III.

SWEETENED CONDENSED SKIMMED MILK.

Total solids, 71·82 per cent.; fat, 0·36 per cent.; protein (N × 6·38), 10·17 per cent.

Sucrose found:

	Per Cent.
	44·28
	44·54
	44·58
	44·53
	44·61
	44·68
	44·49
	44·42
Mean	44·52
Maximum deviation from ,,	0·24
Mean ,, ,, ,,	0·09

Factory data indicated that the condensed milk should contain 44·50 per cent. sucrose.

RECOMMENDATIONS.

The Committee recommend:

(*a*) That for the determination of sucrose in sweetened condensed milk the polarimeter method, with zinc acetate-potassium ferrocyanide clarification as described below, should be employed.

(*b*) That, for the purpose of the Public Health (Condensed Milk) Regulations, the percentage of total milk solids should be determined by subtracting the percentage of sucrose found by this method from the percentage of total solids as determined by the process described in their Report No. 1, it being understood that the sample is a product prepared from milk and sucrose only, and that it is in normal sound condition.

METHOD OF DETERMINATION OF SUCROSE IN SWEETENED CONDENSED MILK.

REAGENTS.—Zinc acetate solution: 21·9 grms. of crystallised zinc acetate, $Zn(C_2H_3O_2)_2$, $2H_2O$, and 3 ml. of glacial acetic acid, in water, made up to 100 ml.

Potassium ferrocyanide solution: 10·6 grm. of crystallised potassium ferrocyanide in water made up to 100 ml.

Hydrochloric acid solution = 6·34 times normal.

Concentrated ammonia solution, nominal 0·880.

Dilute ammonia solution: 10 ml. of concentrated ammonia solution diluted with water to 100 ml.

Dilute acetic acid solution approximately equivalent to the dilute ammonia solution.

APPARATUS.—The instrument used for measuring the optical rotation may be either a polarimeter or a saccharimeter, using, for the polarimeter, sodium light, or the green line of the mercury spectrum separated by means of a prism or by the use of a special Wratten screen No. 77*a*, and for the saccharimeter white light from an incandescent electric lamp after passing through 15 mm. of a 6 per cent. solution of potassium bichromate.

Tubes, of not less than 2 dm., exactly calibrated for length.

Flasks and pipettes accurately calibrated in millilitres.

A standardised thermometer, reading to 0·1° C.

PREPARATION OF THE SAMPLE.—Mix the sample in the manner prescribed in Report No. 1, page 1 (ANALYST, 1927, **52**, 402).

PROCEDURE.—Transfer to a 100 ml. beaker an accurately weighed quantity, approximately 40 grms., of the well-mixed sample; add 50 ml. of hot distilled water (80° C.–90° C.), mix, transfer to a 200 ml. measuring flask, washing in with successive quantities of distilled water at 60° C., until the total volume is from 120 to 150 ml. Mix, cool to air temperature, and then add 5 ml. of the dilute ammonia solution. Again mix, and allow to stand for 15 minutes. Add a sufficient quantity of the dilute acetic acid solution to neutralise the ammonia added (the exact equivalent is determined beforehand by titration), and again mix. Add, with gentle mixing, 12·5 ml. of zinc acetate solution and mix, followed in the same manner by 12·5 ml. of potassium ferrocyanide solution. Bring the contents of the flask to 20° C. and add distilled water at 20° C. up to the 200 ml. mark.

> Up to this stage all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles, and, with the same object in view, all mixings should be made by rotation of the flask rather than by shaking. If bubbles are found to be present before completion of dilution to 200 ml., their removal can be assisted by temporary attachment of the flask to a vacuum pump, and rotation of the flask.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes and then filter through a dry filter paper, rejecting the first 25 ml. of filtrate.

Direct Polarisation.—Determine the rotation of the filtrate at 20·0° C.*

Inversion.—Pipette 40 ml. of the filtrate obtained as above into a 50 ml. flask; add 6 ml. of 6·34 normal hydrochloric acid. Immerse for 12 minutes the entire bulb of the flask in a water-bath maintained at 60° C., mixing by rotatory movement during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, dilute to 50 ml. at 20° C. with distilled water, mix and allow to stand for one hour.

Invert Polarisation.—Determine the rotation at 20·0° C.*

* For the values for Q for various sources of light, and for the corrections to be applied, where necessary, for concentration and temperature, see (e) inversion divisor factor above (pp. 115, 116).

Calculation.— W = Weight of sample taken, in grms.
F = Percentage of fat in the sample.
P = Percentage of protein (N × 6·38) in the sample.
V = Volume to which the sample is diluted before filtration.
v = correction in ml. for volume of precipitate produced during clarification.
D = observed direct polarimeter reading.
I = observed invert polarimeter reading.
l = length in dm. of polarimeter tube.
*Q = Inversion divisor factor.

Then $$v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 1{\cdot}55)]$$

and the percentage of sucrose in the sample

$$= \frac{D - (\frac{5}{4} \times I)}{Q} \times \frac{V - v}{V} \times \frac{V}{l \times W}.$$

For and on behalf of the Sub-Committee,

(*Signed*) E. HINKS (*Chairman*).
E. B. HUGHES (*Hon. Secretary*).

APPENDIX.

As stated under Section 3(*a*), p. 113, the phosphotungstic reagent was found to be an excellent precipitant, and its application was investigated at considerable length. The method is of sufficient value to warrant its inclusion as an appendix to this report.

PHOSPHOTUNGSTIC ACID PRECIPITANT.—Fifty grms. of crystalline sodium tungstate, $Na_2WO_4.2H_2O$, and 6 grms. of crystallised disodium phosphate are dissolved in about 200 ml. of distilled water, and 220 ml. of twice-normal hydrochloric acid solution (or the equivalent amount of acid of other normality) are added slowly with stirring. The solution is diluted to 500 ml. and filtered. The acidity of the reagent should be so adjusted that 20 ml. require approximately 16·0 ml. of half-normal sodium hydroxide solution when titrated with methyl orange as indicator, and the *p*H of the reagent, diluted to five times its volume with water, is approximately 1·3.

PROCEDURE.—The procedure for the determination of sucrose with this precipitant is similar to the process described above with zinc acetate and potassium ferrocyanide as precipitant, but differs from that process in the following details:

Three-fourths only of the ammonia used to destroy muta-rotation is neutralised with dilute hydrochloric acid, 40 ml. of the reagent are added, in two portions of 20 ml. each by means of a pipette, mixing after each addition by rotation of the flask; the liquid should stand for 10 minutes before filtration; and direct polarisation must be read as soon as possible, and, in any case, within thirty minutes of

the commencement of filtration. For inversion only 5 ml. of 6·34 normal hydrochloric acid solution are used.

Calculation.—This is similar to that given on p. 122, except that

$$v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 0{\cdot}74)] + 1{\cdot}50 \text{ ml.}$$

RESULTS OF ANALYSES BY MEMBERS OF THE COMMITTEE.

TABLE IV.

Mixtures of fresh milk and sucrose were made to correspond to a solution of condensed milk containing 42 per cent. of sucrose.

Sucrose (calculated as for a condensed milk), actual 42·0 per cent.

Found:

	Per Cent.
	42·06
	41·95
	41·97
	41·96
	41·96
	42·10
	41·98
Mean	42·00
Maximum deviation from actual	0·10
Mean ,, ,, ,,	0·05

TABLE V.

SWEETENED FULL CREAM CONDENSED MILK.

Fat, 9·50 per cent.; protein (N × 6·38), 8·80 per cent.

Sucrose found:

	Per Cent.
	41·94
	41·70
	41·80
	41·68
	41·70
	41·90
	41·90
	41·93
Mean	41·82
Maximum deviation from ,,	0·14
Mean ,, ,, ,,	0·10

TABLE VI.

SWEETENED CONDENSED SKIMMED MILK.

Total solids, 71·84 per cent.; fat, 0·73 per cent.; protein (N × 6·38), 9·75 per cent.

Sucrose found:

	Per Cent.
	45·63
	45·39
	45·60
	45·25
	45·60
	45·25
	45·64
	45·50
	45·66
Mean	45·50
Maximum deviation from ,,	0·25
Mean ,, ,, ,,	0·14

Notes.

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

THE RISING OF FAT IN MILK. THE PERCENTAGE OF FAT IN CREAM.

(*Read at the Meeting of the North of England Section, November* 30, 1929.)

IN many cases of fat deficiency in milk the rate at which the cream rises has been of importance. We have carried out a number of experiments, on samples taken under the Food and Drugs Acts, by allowing milk to stand in a separator for varying lengths of time, drawing off the separated milk, and examining the cream layer. The results obtained are set out in the following table:

Fat in original milk. Per Cent.	Time of standing. Hours.	Fat in cream. Per Cent.	Fat in original milk. Per Cent.	Time of standing. Hours.	Fat in cream. Per Cent.
3·3	23	15·9	3·5	2½	14·2
4·4	11½	38·2	4·4	2½	14·4
3·5	5	18·6	3·6	2½	18·9
3·7	5	19·8	4·3	1½	19·2
3·6	4½	15·3	3·6	1½	13·4
3·6	4	20·7	3·4	1½	18·4
3·6	4	21·7	3·4	1½	14·4
3·5	3½	16·5	3·4	1½	15·6
3·2	3½	16·8	3·3	¾	11·7
3·2	3½	18·5	3·5	¾	13·5

These results, though not sufficient in number to enable definite conclusions to be drawn, show that very considerable separation of fat, in milks that have once been separated, can take place in a comparatively short time. A definite cream line not infrequently appears on standing for half-an-hour, and some separation of fat must, of course, take place before this time.

It has been held that, although milk in which the fat has once risen and again been incorporated more quickly separates, yet milk freshly drawn may remain standing for one to two hours without material separation of fat. In order to test this point, the freshly drawn milk of a herd was sampled at the farm by ourselves from the top of a churn immediately after milking and again after standing half-an-hour. The results obtained were as follows:

FAT IN UPPER LAYER.

Uncooled milk.		Cooled milk.	
Immediately. Per Cent.	After ½ hour. Per Cent.	Immediately. Per Cent.	After ½ hour. Per Cent.
4·2	6·2	5·0	5·6
4·5	8·7		

These results show that, in the particular milks examined, the separation of fat is noticeable in a quarter-of-an-hour, and material in half-an-hour.

Cream has been defined as "that portion of milk rich in milk fat which has risen to the surface of milk on standing and has then been removed or . . . ." From the experiments given above in Table I it would appear that cream, according to this portion of this particular definition, must contain at least more than 10 per cent. of fat, and that about 15 per cent. would be a reasonable proportion for a "hand-skimmed" cream. The definition goes on to say," or has been separated from milk by centrifugal force," but such a standard would be quite unsuitable for a cream so prepared; neither would the definition appear to admit of the dilution of a separated cream to the consistence of a hand-skimmed cream. There is, of course, very little, if any, hand-skimmed cream now on sale, so that a standard of 30 per cent. of fat for cream, and 45 to 50 per cent. of fat for thick cream, would appear to be desirable.

The analytical results recorded in this note have been obtained by our colleague, Miss C. Mayne, B.Sc., to whom our thanks are due.

G. D. ELSDON.
J. R. STUBBS.

THE LANCASHIRE COUNTY COUNCIL LABORATORY,
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THE STERILISATION OF SEA WATER BY MEANS OF CHLORINE.

THIS little investigation was made in connection with the proposal to chlorinate the water of a swimming bath in which sea-water is used. The question was raised whether, owing to the presence of bromides and consequent displacement of bromine by chlorine, the taste and effect upon the eyes would be increased and the sterilisation properties impaired. It appeared unlikely that either of these eventualities would result; one was inclined to assume that taste, irritant effect and sterilising action would be of the same order, equivalent for equivalent, but, so far as we are aware, the matter has never been set on an experimental basis. Since the net result of chlorinating sea-water is the same as that of brominating fresh water, the latter was employed, as enabling one to judge the taste more readily.

Solutions of bromine were made up with tap water, in litre bottles, equivalent to 0·15, 0·25, 0·35, and 0·45 part per million of chlorine. The presence of bromine could not be detected by taste on sipping the water, even in the strongest solution, *viz.* the equivalent of 0·45 part per million of chlorine, but, if a good draught was taken, the bromine could just be tasted, even in the highest dilution but one. The effect on the eyes was tested with the equivalent of 0·35 part per million, an eye-bath being intermittently used for 15 minutes; no effect was noticed. The sterilisation effect was tested by adding *B. coli* to the four dilutions and withdrawing 1 c.c. for cultivation after 5, 10, 15, and 20 minutes. Two experiments were made: in the first, all the 16 cultures from the treated waters were sterile as regards *B. coli* after 5 minutes, an untreated control showing 14 *B. coli* per c.c.; in the second, all 16 cultures from the treated waters were again sterile, the untreated control showing 720 *B. coli* per c.c. The sterilisation effect was more marked than we anticipated, for we quite expected to find some *B. coli* living in the highest dilution after only 5 minutes' exposure. The tap water used is very free from organic matter, and probably the highest dilution would not be so effective in local sea-water, which is muddy.

From the above experiments it is evident that bromine is at least as efficient for sterilisation as its equivalent of chlorine, and, as regards taste and effect on the eyes, it is not perceptibly more objectionable.

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E. T. Illing.

Somerset County Laboratory.

Legal Notes.

Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.

THE STRENGTH OF RUM.

On December 28, 1929, a firm of wine and spirit merchants was fined £10 and costs at Stockport, for selling rum which was not of the nature, substance and quality demanded.

Mr. F. Knowles, for the prosecution, said that the rum sold had been found to contain 9·7 per cent. of added water, and to be 41·48 degrees under proof. There was nothing to prevent the defendant selling it at that strength, provided notice was given, by label, to the purchaser.

The defendants admitted a technical defence, but pleaded that the rum was only intended for use as rum sauce for Christmas puddings, and was advertised for that purpose.

LABELLING OF PRESERVED SAUSAGES.

ON December 13, 1929, a shopkeeper was summoned at Bow Street Police Court, for selling sausages preserved with sulphite without having them labelled in accordance with the Preservatives Regulations. The sausages contained 370 parts of sulphur dioxide per million.

The inspector admitted that the other packets of sausages in the shop window were all duly labelled, but he did not see a notice to that effect on the cash register. He agreed that there was another half-pound of sausages lying on the counter. The manager had said to him that the sausages were of their own make and did not contain preservatives.

The solicitor for the defence contended that the Regulations did not require each individual sausage to be labelled. He submitted that, if a pound packet of sausages was divided at the request of the customer, as in this case, it was not necessary to label each portion sold; it was sufficient compliance with the Regulations if the original packet bore a label stating that a preservative was used.

An assistant in the shop proved that he took a pound packet of sausages from the window and divided it into two parts. The pound packet bore a label stating that the sausages contained preservative, but he did not put a label on the half-pound purchased.

The manager denied that he had told the inspector that the sausages were their own make and contained no preservative. Proprietary sausages were not supplied with two labels, although since this case the manufacturers, at his request, had supplied him with two labels for each pound.

The Magistrate (Mr. Graham Campbell) said that he did not think that there was sufficient evidence on which to convict, and dismissed the case.

"CHEDDAR CHEESE."

ON December 18, 1929, two men were summoned at Aylesbury for obtaining from two grocers by false pretences the sum of £3 18s. and £4 17s. 6d. for cheese falsely represented to be Cheddar cheese.

Mr. Eric Voelcker, Public Analyst, said that the sample had an unpleasant smell and contained skimmed milk and fat that was foreign to butter fat. The amount of fat in one portion was only half what one would expect to find in genuine Cheddar cheese. A second sample, taken from the centre of the cheese, was sound and of good quality, and was genuine Cheddar cheese.

When the case was heard at the Court of Quarter Sessions on December 30, evidence was given that the cheese was a margarine cheese, into the top of which had been inserted a piece of Cheddar cheese of good quality, from which samples to submit to prospective purchasers had been scooped.

Mr. Eric Voelcker repeated his evidence given at the Police Court, and said that this cheese was made up of vegetable oils, and contained only 16 per cent. of fat, whereas a Cheddar cheese should contain 30 to 33 per cent. of milk fat.

Dr. Bernard Dyer described the substance as a very poor margarine cheese; originally it was of a texture resembling india rubber, but became later of the consistence of horn.

The jury found one of the defendants guilty, and he was sentenced to nine months' imprisonment.

Ceylon.

REPORT OF THE AGRICULTURAL CHEMIST FOR THE YEAR 1928.

THE Agricultural Chemist, Mr. A. W. R. Joachim, in his annual report, states that during the year there was a marked increase in correspondence of a purely technical nature, as compared with previous years. The following were among the numerous subjects on which advice was sought and given:—Green manures—analyses, methods of treatment, uses, decomposition; cattle manure; artificial farmyard manure; composition of wood and other ashes; manures and fertilisers; manuring of tea, rubber, coconuts, fruits, vegetables; agricultural products—essential oils, sugar cane, tobacco, papain, annatto; analyses of fruits and vegetables; soils—treatment, cultivation, conservation of moisture, analyses; fodder grasses, feeding stuffs and rations for livestock; insecticides and weed-killers.

The total number of samples examined analytically during the year for advisory purposes, exclusive of those undertaken in connection with research work, was 358 as against 340 during 1927.

Among the investigations completed during the year were the following:

STUDIES OF CEYLON PADDIES, RICES, AND MILLING PRODUCTS.—This investigation, started in 1927, was completed early in 1928. Altogether, 35 samples of paddies, rices, and milling products were analysed. The more important results obtained were as follows:—

(1) The Ceylon paddies examined are similar in composition to those of other rice-growing countries. They appear to be richer in protein and minerals than the latter. Hill paddy was found to be richer in organic constituents, but poorer in minerals than irrigated paddies.

(2) The pounding and polishing of rices result in a marked decrease in the fat, phosphoric acid, and fibre contents of the grain. Greater losses of fat and phosphoric acid occur as a result of machine-polishing than by the village method of polishing.

(3) Parboiling affects inappreciably the chemical composition of rices.

(4) More than half the phosphoric acid, three-fourths of the fat, and one-fifth of the protein of *murungan* paddy are found in the bran.

At the request of the Economic Botanist an experiment to determine the changes in acidity on keeping cooked rice (raw and parboiled) was carried out. Cooked raw rice developed slightly greater acidity at all stages of keeping than cooked parboiled rice.

THE EFFECTS OF REFUSE WATER FROM COCONUT FIBRE MILLS ON PADDY YIELDS.—The pot experiment, started in 1927, and designed to ascertain the effects on the germination and yield of paddy of a 10 per cent. dilution of refuse water from coconut fibre mills, showed that, while the germination of the seedlings was apparently in no way influenced by the treatment, the yields of both paddy and straw were adversely influenced, although the vegetative growth of the paddy appeared to have been stimulated by the refuse water.

Connecticut Agricultural Experiment Station.

ANNUAL REPORTS ON FOOD PRODUCTS AND DRUG PRODUCTS.*

In his Reports for the year 1928 the Chemist in charge of the Station, Dr. E. M. Bailey, deals with the work done in connection with the inspection of food and drugs. In addition, the department is responsible, by special statutes, for the certification of Babcock glassware and dairy thermometers, and for co-operation with the State Water Commission. The following particulars of certain special investigations (*inter alia*) are given:—

Coffee Deprived of Caffeine.—Three samples of one brand were found to contain 0·05, 0·07 and 0·03 per cent. of caffeine, respectively, so that the manufacturer's claim, that 94 to 97·5 per cent. of caffeine had been removed, was fairly substantiated. One of these samples had the following composition:—Ash, 4·20; water-soluble ash, 3·48; water-insol. ash, 0·72; soluble solids, 20·68; petroleum spirit extract, 15·09; and caffeine, 0·05 per cent.

The product of another manufacturer contained 0·07 per cent. of caffeine.

Special Foods.—The Energen products are labelled showing the amounts of protein, fat and carbohydrate per unit of food, and these statements are substantially correct, as shown by analysis. The products in general show large amounts of carbohydrate which must be classed as "available" (starch plus soluble carbohydate). Agar-Bran Biscuits are relatively low in protein, and the carbohydrate is probably very largely unassimilable. Energen Protein Food is low in carbohydrate, but the high protein is a potential source of a considerable amount of glucose in digestion.

Jeru Artichoke Flakes are prepared from Jerusalem artichokes by slicing, and cooking in oil. The carbohydrate is chiefly inulin, and the result reported as inulin in the analysis is based upon the reducing sugar obtained upon a 15-minute hydrolysis, at which time experience has shown the maximum reducing power is reached. On longer hydrolysis, reducing sugars diminish, probably due to the destruction of laevulose.

The advantage of inulin-feeding in cases of diabetes has been the subject of extended inquiry on the part of students of this disease. A recent paper by Carpenter and Root (*Arch. Intern. Med.*, 1928, **42**, 64) describes some convincing experiments.

The flakes had the following composition:—Water, 6·91; ash, 3·30; protein, 3·79; fibre, 2·01; reducing sugar (calculated as inulin after 15 minutes' hydrolysis), 35·86; undetermined carbohydrate, 1·70; and fat, 1·20 per cent.

Bake-Apple Berry.—A sample of canned fruit (in tins) contained the fruit variously known as "Bakapple," "Bake-apple Berry" or "Cloudberry." It resembles the American raspberry, and its botanical name is *Rubus chamaemorus*. The plant is found in the peat bogs of the far north, even within the Arctic Circle. It is found in northern Europe, Asia and North America. In the United States it occurs only as far south as Maine, in the east, and British Columbia in the west.

The composition of the sample was as follows:—Water, 84·24; ash, 0·52; protein, 1·94; fibre, 3·33; total sugar (calculated as invert sugar), 4·70; undetermined carbohydrate, 4·99; and fat, 0·28 per cent.

Sulphur Dioxide in Dried Fruits.—The limit beyond which sulphur dioxide should not be allowed in food products is a debated question. The regulations in the State of Connecticut raise no objection to this substance in products which, by long usage, have been prepared with sulphur dioxide, provided it is not used to

* *Bulletin* 307, 1929.

conceal damage or inferiority, such as the marketing of excessive water; and provided the proper label declaration is made. What quantity may be regarded as a menace to health has not been determined, and no official limit for sulphur dioxide in foods has been fixed. At one time a limit of 350 mgrms. per kilo was proposed.

Thirteen samples examined during the year contained from 47·2 to 2152 mgrms. per kilo.

MAPLE SUGAR.—A sample contained:—Moisture, 13·28; ash, 0·97; insoluble ash, 0·38; lead number (Winton), 2·38; and malic acid, 0·58 per cent.

SAUSAGES.—With proper declaration, cereal or starchy material may be used in sausage, provided the amount does not exceed 3·5 per cent.

Two samples were examined for moisture and nitrogen. From these values excess water in sausage can be estimated. It is permissible to use 3 per cent. of water or ice in the manufacture of sausage, and somewhat more in the case of those types of sausage which are smoked or cooked, but in no case should more water be added than is required to make the products palatable. The ratio of water to protein in the usual cuts of meat which are used in sausage-making is about 4 to 1. In one of the samples, moisture was found to be 54·7 per cent., and protein (nitrogen × 6·25), 13·63 per cent., and no added water was indicated. In another sample, 54·5 per cent. of water and 14·13 per cent. of protein were found which would indicate that not more than 2 per cent. of water had been added.

Skimmed milk powder, if used in the manufacture of sausage, requires a declaration. A sample said to contain about 5 per cent. of such powder, and so labelled, was examined; and also a sample of the milk-product used. The powder contained 49·5 per cent. of sugar, calculated as lactose. The sausage, on microscopic examination, showed the presence of the milk product, and direct reducing sugar was present to the extent of 1·9 per cent., which would indicate approximately 4 per cent. of milk product in the sausage.

TEA.—The following table summarises the results of analyses of experimental package teas:

	Water	ASH						Nitrogen	Caffeine, from N	Fibre	Tannin	Pet. Spt. Extract 40 hrs. extraction	Vol. oil; Loss at 110° C., basis of 40 hrs. extraction.
		Total	Water-soluble	Water-insoluble	Acid-insoluble	Alkalinity water-sol., c.c. N acid /100 grms.	Alkalinity water-insol., c.c. N acid /100 grms.						
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1926													
Black	5·03	5·21	2·96	2·25	0·07	29·5	34·5	4·20	2·78	9·89	6·16	1·08	0·10
1927													
Black—Maximum..	6·28	5·26	3·18	2·35	0·25	32·0	37·5	4·21	2·84	10·48	8·01	1·01	0·20
Minimum ..	5·68	5·00	2·89	1·98	0·05	30·0	34·0	4·15	2·71	9·06	6·65	0·81	0·14
Average ..	6·07	5·17	3·04	2·13	0·10	31·3	34·9	4·18	2·76	9·76	7·29	0·90	0·17
1928													
Black—Maximum..	7·23	5·83	3·14	2·69	0·14	32·8	40·5	4·22	2·84	10·15	9·35	0·90	0·27
Minimum ..	6·01	4·99	2·93	2·06	0·05	29·0	35·0	4·06	2·74	9·73	7·97	0·70	0·20
Average ..	6·61	5·21	3·00	2·22	0·09	30·9	36·9	4·16	2·80	9·93	8·46	0·82	0·22
1926													
Green	3·23	5·53	3·27	2·26	0·20	32·8	31·8	4·37	2·15	11·0	5·87	2·82	0·10
1927													
Green—Maximum..	5·65	5·58	3·66	2·18	0·28	36·0	28·0	4·44	2·45	10·61	7·54	1·79	0·24
Minimum ..	4·11	5·35	3·37	1·92	0·19	34·5	26·5	4·31	2·12	9·77	6·61	1·49	0·20
Average ..	4·71	5·50	3·50	2·01	0·24	35·4	27·3	4·34	2·21	10·20	7·03	1·66	0·22
1928													
Green—Maximum..	5·74	5·43	3·39	2·13	0·32	35·5	30·5	4·38	2·19	11·10	9·11	1·89	0·28
Minimum ..	4·50	5·32	3·25	1·97	0·17	34·3	27·8	4·26	2·13	10·57	7·20	1·11	0·21
Average ..	5·01	5·38	3·32	2·05	0·23	35·0	29·1	4·31	2·16	10·78	8·26	1·42	0·25

It will be seen that total nitrogen and caffeine remained remarkably constant. After making allowance for defects in the method of determination, the results for tannin show a consistent increase as the teas age. The observations of tea-tasters that old teas are decidedly astringent, suggest a possible relationship between this quality and the tannin increase. Substances soluble in petroleum spirit decrease somewhat in teas kept for one or two years.

An attempt to determine volatile oil by the tentative A.O.A.C. method (*Methods of Analysis*, p. 340) was unsatisfactory, only minute quantities being obtained even on prolonged distillation.

SOLUTION OF MAGNESIUM CITRATE.—In several instances the inspection samples bore labels showing that they were made according to the 9th revision of the Pharmacopoeia instead of the 10th, to which there is no objection if the composition of the product conforms to the standard indicated by the label.

One product was labelled as "Aperient Magnesia." The Connecticut State law permits sub-standard drugs to be sold, provided their sub-standard character or true strength is indicated. Since "aperient" means "laxative" or "purgative," and applies to a standard product as well as to a sub-standard one, the term is not sufficiently descriptive. The label should bear the further statement, "not a U.S.P. product," or words of similar effect.

WITCH HAZEL.—Distilled extract of witch hazel or witch hazel water should contain not less than 14 per cent. of alcohol (by vol.), and should not contain denaturing substances, such as wood alcohol, diethylphthalate, etc. Of 14 official samples, 5 were considerably below the required alcoholic strength, and 1 contained a trace of diethylphthalate.

"DIABETIC WINE."—A proprietary article thus described gave the following analytical results:—Sp. gr. at 20° C., 1·0106; total solids (grms. per 100 c.c.), 10·92; ash, 1·07; extract (calc.), 9·46; acidity as tartaric acid, 0·74; invert sugar, 0·38; sucrose, trace; iron and aluminium, trace; calcium oxide, 0·26; phosphoric acid (P_2O_5), 0·56; P_2O_5 in ash, 0·40; total nitrogen, 0·02; alcohol (by vol.), 17·74 per cent. Sodium glycerophosphate (calculated from P_2O_5), 2·48 per cent. Aloes, quinine, glycerophosphates and saccharin present.

The label on the product stated, in part, that this wine "is a powerful tonic and nerve restorative highly recommended by leading physicians for diabetes, wasting disease," etc.; and further, in part, "a valuable auxiliary in the treatment of diabetes and an aid in the disappearance of sugar in the urine."

Direct claims of curative properties were not made, but the language was such as to convey the impression to the consumer that curative or mitigative effects were to be expected in cases of the disorders mentioned, whereas the substances contained in the wine are not recognised by authoritative opinion as likely to produce such results. The manufacturers of the article, at a hearing before the Dairy and Food Commissioner, readily agreed to revise their label and to omit false and misleading declarations, and this has now been done.

United States Department of Commerce.

REPORT OF THE BUREAU OF STANDARDS FOR THE YEAR ENDING JUNE 30, 1929.*

THE work of the Bureau of Standards is divided into three main sections: (*a*) Research and Testing; (*b*) Office Operation; and (*c*) Construction and Commercial Standardisation. The section (*a*) is sub-divided under electricity; weights and

* Miscellaneous Publication No. 102.

measures; heat and power; optics; chemistry; mechanics and sound; organic and fibrous materials; metallurgy; and clay and silicate products. The section (*c*) is sub-divided into simplified practice; trade standards; specifications; and building and housing. The work is made effective through voluntary co-operation of the State and municipal governments; scientific and professional societies; trade associations; manufacturers, and individuals who incorporate findings into State law, municipal ordinance or a standard, and also through the research associate plan under which 98 associates representing 48 industries and associations were working at the bureau in the current year.

INVESTIGATION OF PUBLIC UTILITY STANDARDS.—Methods for the analytical separation of natural gases by fractional distillation were completed; an improved balance for specific gravity and density determinations of gases was designed, and also one for direct determination of density of gases; corrosion of commercial oven linings under service conditions was determined; efficiency of storage water heaters was measured; performance standards recommended, and a study of floor temperatures under radiant heaters made; laboratory burners for propane and butane were constructed.

COLOUR STANDARDISATION.—The selection of a "white" standard has been studied from the point of view of a definition in terms of the sun's radiation (outside the atmosphere); of a completely overcast sky; or of a "black" body when the ratio of its luminosity to its total radiation is a maximum, about 0·14. An inter-laboratory comparison of several calibrated optical filters used for equalising colour has shown that consistent values may be obtained by the spectro-photometric method in the measurement of the intensities of deeply coloured light, and such values agree more or less satisfactorily with the so-called flicker and equality of brightness methods. Blue filters are being evaluated by the national laboratories in England, France, Germany, and U.S.A., so that the relative efficiencies of vacuum and gas-filled lamps may be expressed on a common basis. The calibration of 65 of the "35 yellow" Lovibond glasses used for colour grading of edible oils has been completed, and the effect of temperature on readings with red and yellow glasses was found to be small.

INVESTIGATION OF TEXTILES, ETC.—Chemical and physical tests of commercial writing and book papers are being made which indicate that the processing of the fibre, irrespective of its source, is the most important factor in its rate of deterioration. A method for determining the bulk of paper was developed, and tests for gloss and opacity completed. The use of sawdust and waste papers for roofing felts has been found possible, but rayon has no paper-making value. Investigations on building board from compressed wood, flax from New Zealand, insulating board from liquorice root, and a very strong paper from Japanese mitsumata fibre were made.

SUGAR STANDARDISATION.—The problem of washing and cleaning artichoke tubers from foreign material, has been solved. About 10 tons of the cleaned tubers were worked, and a study made of the 8 per cent. of substance which is not converted into laevulose, showed the presence of a group of new disaccharides; one, recovered in crystalline form, was composed of 2 mols. of laevulose. This has been called difructose anhydride. The analysis of products containing laevulose has been brought to such a point that it is available for plant control. The transparency and optical activity of sugar solutions for spectrophotometric analysis have been studied; asbestos fibre used for filtration may now be prepared in 2 hours instead of several days. A table of weights per gal. of sugar solution at the standard temperature of 20° C., has been drawn up, with a supplementary table of weights at different temperatures.

HIGH TEMPERATURE INVESTIGATION.—A comparison of the thermoelectric portion of the International Temperature Scale with the 3 other thermoelectric scales used since 1912 was made, and none of these differed from it by more than 0·3° C. The freezing point of copper was determined on the International Scale as 1083·0° C., and of the copper-silver eutectic as 779·4° C. The E.M.F. against platinum of a series of these alloys with 1 to 100 per cent. rhodium was determined over the range of 0° to 1200° C. The average freezing point of two separate lots of nickel was 1454·9° C., and when taken as 1455° C. is not believed to be more than $\pm 1°$ in error, and is a convenient point for calibration of the standard optical pyrometer.

INDUSTRIAL RESEARCH.—New descriptions have been prepared of the arc and spark spectra of lanthanum, chlorine, bromine, iodine, arsenic, krypton and xenon. A classification has been made of nearly all the lines of the arc spectra, and information obtained as to the most sensitive lines for spectrochemical detection and quantitative determination of small quantities. The main features (except for arsenic) of the spark spectra have been found and the strongest lines classified. Impurities were identified in proof gold of the Bureau of the Mint, and a higher standard is now being maintained.

RADIO RESEARCH.—The frequency standard has been improved by means of piezo oscillators, of which the error in constancy is less than 5 parts per 1,000,000; a theoretical and experimental study of the operation of the quartz plate in a piezo oscillator was made; the accuracy of testing piezo oscillators used by broadcasting stations was increased about 10 times, the vagaries of radio wave transmission and diurnal variations in signal strength were studied, and data as to the properties of the Kenelly Heaviside layer are being collected.

INVESTIGATION OF RADIO-ACTIVE SUBSTANCES AND X-RAYS.—Measurement of X-ray dosage by the agreed International Unit has shown that discrepancies in the measurements of the same radiation by different laboratories is due to insufficient precautions to ensure that experimental conditions fulfil the requirements of the theory. During the year, 1050 radio-active preparations were tested —a total of 11,300 mgrms. of radium. D. G. H.

Ministry of Health.

THE following letter, signed by the Assistant Secretary, has been sent to the clerks of authorities administering the Food and Drugs Act in England and Wales:

CIRCULAR 1059.

FOOD AND DRUGS (ADULTERATION) ACT, 1928.

(DAMAGED TEA AND TEA SWEEPINGS.)

1. I am directed by the Minister of Health to request that a copy of the Report of the Public Analyst for the fourth quarter of the present year may be transmitted to this Department during the month of January.

2. The Minister has been informed by the Commissioners of Customs and Excise that in consequence of the abolition of the tea duty it is no longer possible for their officers to exercise the same control as heretofore in preventing the delivery of damaged tea and tea sweepings. The attention of the appropriate Port and Riparian Sanitary Authorities has already been drawn to the matter with a view to the exercise of their powers under the Public Health (Imported Food) Regulations, 1925, but the Minister thinks that Food and Drugs Authorities should also be aware of the possibility of the relaxation of Customs control resulting in a slight increase in the quantity of contaminated tea offered for sale.

3. Copies of this Circular are being sent to the Medical Officer of Health and the Public Analyst.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Determination of Nitrates and Nitrites in Whey. E. Ohlsson and H. Fredholm. (*J. Assoc. Off. Agric. Chem.*, 1929, **12**, 429–434.)—For the determination of nitrates in aqueous solution free from nitrite, the solution (not more than 50 c.c.) is poured into one-half of Widmark's extraction apparatus (*Bull. Soc. Chim. Biol.*, 1928, **10**, 669), which consists of two extraction funnels joined some distance above the taps by a short wide glass tube, 10 c.c. of 0·1 *N* sulphuric acid being added. The other half of the apparatus is charged with 20 c.c. of 0·5 *N* sodium hydroxide and a suitable quantity (400 c.c.) of ether. After extracting for 48 hrs. (60–70 hrs. if precipitated protein or other sediment is present), the sodium hydroxide solution is drawn off, and that part of the apparatus is washed thrice with about 10 c.c. of water. The alkaline liquid, together with the washings, is warmed on a water-bath to expel the ether present, cooled, and neutralised with sulphuric acid, and used for the determination of nitric acid by Scales and Harrison's method (ANALYST, 1920, **45**, 223). With whey, the determination of nitrates is carried out similarly, but it is advisable, during the extraction, to stir up once or twice with a glass rod the precipitate of protein formed on addition of sulphuric acid.

When nitrates are to be determined in presence of nitrites, the latter must first be destroyed. This is best done in neutral solution by Hahn's method (*Ber.*, 1917, **50**, 705), making use of 2: 4-diamino-6-oxypyrimidine sulphate. Of this reagent, which may be prepared by Traube's method (*Ber.*, 1900, **33**, 1371; 1913, **46**, 3839), about 0·5 grm. is added to the solution, which is left for 30 minutes with occasional stirring, and is then introduced, together with sulphuric acid, into the extraction apparatus without filtering. The subsequent procedure is that described above.

To determine nitrates and nitrites together, 30 c.c. of the solution, mixed with 5 c.c. of 5 per cent. potassium permanganate solution, are treated with 10 c.c. of 0·1 *N* sulphuric acid, added, drop by drop, so that the liquid may not become too acid before the nitrous acid is oxidised. After several minutes, a few drops of saturated oxalic acid solution are added to destroy the excess of the oxidising agent, the solution being then extracted as usual. With whey, this determination is carried out in the same manner, except that addition of oxalic acid is mostly unnecessary, as the whey contains sufficient organic matter to reduce the excess of permanganate. T. H. P.

Report on Eggs and Egg Products. J. C. Palmer. (*J. Assoc. Off. Agric. Chem.*, 1929, **12**, 343–346.)—In the determination of lipoid phosphoric anhydride and total phosphoric anhydride in eggs and egg products, alcoholic potassium hydroxide forms a satisfactory fixing agent for it. It is not necessary

to ash the material, since complete charring at 550° C. leads to the same results. Boiling the charred residue with concentrated nitric acid fails to increase the yield of phosphoric anhydride, so that no pyro- or meta-phosphate is formed during the heating. Ageing of dried eggs is accompanied by a drop in the quantities of lipoids, lipoid phosphoric acid and water-soluble nitrogen. Contrary to the general view, old egg products do not always show a lipoid: fat ratio less than unity. A high value for the lipoid: lipoid phosphoric anhydride ratio of noodles may indicate either old noodles or an incorrect value for the lipoid phosphoric anhydride owing to the use of insufficient fixing agent, etc. In such cases the total phosphoric anhydride should always be determined. T. H. P.

Action of Aldehydes on Wheaten Bread. L. Karácsonyi. (*Z. Unters. Lebensm.*, 1929, **58**, 517–524.)—Katz (*Z. physiol. Chem.*, 1915–1916, **96**, 314) observed that the rapidity with which bread becomes stale is greatly diminished by the presence of an aldehyde. The extent of this effect is found to be proportional to the volatility and solubility in water of the particular aldehyde, which must be present in sufficient concentration to exert any influence. Some aldehydes alter the consistency of the crumb, and others change its colour, light playing no part in such coloration. The action of the aldehydes is mainly on the proteins, since starch and fat are not coloured, whereas aldehydes impart to bread-protein preparations and to gluten the characteristic coloration produced in the crumb itself. T. H. P.

Detection and Determination of Oxymethylfurfural in Honey and Artificial Honey. F. Weiss. (*Z. Unters. Lebensm.*, 1929, **58**, 320–331.)—Ten grms. of sample are extracted five times with 5 c.c. portions of ethyl acetate, and the residue left after gentle evaporation on the water-bath to 1 c.c. dissolved in 0·5 c.c. of water and filtered. Five c.c. of a saturated solution of *p*-nitrobenzhydrazide in 30 per cent. acetic acid and 0·5 c.c. of water are added, and in a few minutes lemon-yellow crystals of oxymethylfurfural-*p*-nitrobenzhydrazone ($C_{13}H_{11}N_3O_5$) appear, which are filtered in a weighed crucible after 1 hour, dried for 2 hours at 105° C. and weighed. The reagent, $C_6H_4.NO_2.CO.NH.NH_2$, is prepared by heating *p*-nitrobenzoic ester with a slight excess of hydrazine hydrate under a reflux condenser for 3 hours, and is recrystallised as yellow needles, m.pt. 207° C., slightly soluble in water or alcohol, and sparingly soluble in ether. It gives precipitates with aldehydes and ketones, but not with glucose or fructose. The precipitate, m.pt. 206° to 208° C. (decomp.), is insoluble in water or petroleum spirit, and slightly soluble in ether or alcohol. It is decomposed by strong hydrochloric acid into its constituents, which may be tested for, *e.g.* by the blue colour produced with a warm 20 per cent. alcoholic solution of diphenylamine. The factor 0·435 gives the oxymethylfurfural, 0·0322 to 0·2255 per cent. of which was found in 6 artificial honeys, whilst pure bee-honey gave no reaction. Honey heated at 95° C. gave a slight reaction. Extraction with ether may give anomalous results (*cf.* Fiehe and Kordatzki, ANALYST, 1929, **54**, 108, 241, 748; Nelson, *id.*, 603). J. G.

Study of Wine from Dried Grapes. R. Moredod. (*Ann. Falsif.*, 1929, **22**, 524–542.)—Swiss legislation forbids the importation, manufacture, storage, or sale of wine made from dried grapes, whilst in Greece, although the manufacture is permitted, all such wine must be exported. In order to conform to the definition of wine adopted by Switzerland and by the Office International du Vin, all ordinary wines, that is, completely fermented wines containing only a few grms. of sugar per litre, must answer the following tests: (1) They must give no fluorescence under the influence of ultra-violet light; (2) they must give no deep red coloration with resorcinol and hydrochloric acid; a faint pink or orange-pink colour may be neglected; (3) the reducing matters formed on inversion or saccharification should be less in amount than 0·5 grm. of invert sugar per litre, although certain heavy red wines give as much as 0·6 grm.; (4) they should give no persistent deep blue coloration, but at most a green coloration, with acid ammonium molybdate. To prepare this reagent, 15 grms. of ammonium molybdate are dissolved in 70 c.c. of water, the solution being poured into 130 c.c. of nitric acid (1·151). The liquid is decanted after standing for 24 hours at about 35° C., and should then yield no precipitate when heated to 50° C. The reagent gradually loses its sensitiveness. Before applying the test, 50 c.c. of the white (red) wine are mixed with 0·5–1(2–5) grms. of pure animal charcoal, left for 5–10 minutes and filtered, with addition of a little infusorial earth, through a moist pleated filter. If difficult to obtain perfectly clear and colourless in this way, the filtrate may be treated with 0·5–1 c.c. of a solution of egg-white in an equal weight of water. Seventeen c.c. of the liquid are mixed, without shaking, with 2 c.c. of the reagent in a test-tube, which is immersed in a gently boiling bath for 10 minutes. In doubtful cases the resulting liquid may be diluted to 100 c.c. with water, the difference between blue and green being thus emphasised. Examination of 132 white and 54 red wines from France, Italy, Spain, Algeria, Greece, Hungary, and Switzerland has been made in accordance with the above tests. Of the Greek wines, all but two gave the same reactions as wines prepared in the laboratory from dried grapes, and have, therefore, been derived to some extent from these. T. H. P.

Rapid Determination of Iron in White Wines. J. Ribéreau-Gayon. (*Ann. Falsif.*, 1929, **22**, 522–524.)—The method described is based on the blue coloration given by the wines when treated with potassium ferrocyanide and hydrogen peroxide. The six standard solutions, containing from 3 to 18 mgrms. of iron per litre, are prepared from a ferric chloride solution obtained by dissolving 1 grm of pure iron wire in hydrochloric acid and a little nitric acid and diluting to 1 litre. Ten c.c. of each of these liquids are placed in a test-tube, one drop of a 1 per cent. potassium ferrocyanide solution being introduced into each of the first three, and 2 drops into each of the last three tubes; 3 drops of 12 vol. hydrogen peroxide solution are then added to each tube to oxidise any ferrous chloride which may be formed in the ferric chloride solutions owing to the reducing action of mould growth. Ten c.c. of the wine are mixed, in a test-tube of similar size, with 3 drops of the ferrocyanide solution and then treated with 3 drops of the hydrogen peroxide

solution, the colour developed being compared with the colours of the standard tubes.

Wine poor in iron sometimes gives a slightly green tint difficult to compare with the blue standard tubes. Tubes containing the wine should then be placed behind the standards and a tube containing water behind the actual test; if this device fails, a new test with 10 c.c. of the wine and 1 drop of the ferrocyanide should be prepared. The method gives results in good agreement with those obtained by the thiocyanate method. T. H. P.

Preservation of Beer. R. de Fazi. (*Giorn. Chim. Ind. Appl.*, 1929, **11**, 499.)—Beer, to which 0·01–0·03 per cent. of hexamethylenetetramine has been added, remains unchanged for 14 days at the constant temperature 40°, for 49 days at 30°, or for 77 days at 20° C. There is, however, a risk of change of flavour owing to the presence of formaldehyde. T. H. P.

Egg-yolk Margarine. E. Vollhase, H. J. Steinbeck and E. Danielsen. (*Z. Unters. Lebensm.*, 1929, **58**, 342–352.)—Fendler's test for yolk in the liquid state (*Z. Hyg.*, 1904, **47**, 144) is modified, 200 grms. of the melted margarine being shaken with 100 c.c. of salt solution for 15 minutes at 50° to 60° C., and the separated aqueous layer cooled in ice and filtered clear. A yellow colour in the ethereal layer, after thoroughly shaking 50 c.c. of filtrate with 8 c.c. of ether, indicates egg-yolk, and a 0·25 per cent. standard solution may be used to match it approximately. The precipitin reaction is used for the dry yolk, 50 to 100 grms. of melted margarine being shaken with an equal volume of water at 50° C. for 15 minutes, and the aqueous layer cooled in ice and filtered clear. This usually contains about 1 per cent. of chloride ion, and is, therefore, diluted to a concentration equivalent to 0·85 per cent. of sodium chloride, after a test-titration of 5 c.c. with 0·1 *N* silver nitrate and a potassium chromate indicator. Antisera were obtained by the injection of Uhlenhuth's egg-yolk solution into the aural veins of dogs and titration of the resulting blood serum with this solution, a titre of at least 1: 10,000 being essential. Comparative titrations with the extract from the sample, under the conditions of the precipitin test, were then used to show the presence of egg-yolk. J. G.

Setting-Point Curve of Cocoa Butter. J. Straub and R. N. M. A. Malotaux. (*Chem. Weekblad*, 1929, **26**, 596–599.)—The temperature-time curve of a fat is determined on 25 to 35 grms. of the melted sample, placed in Shukoff's modified Dewar flask with a thermometer but no stirrer, and cooled under water at $10 \pm 0{\cdot}1°$ C., the temperature being read every 5 minutes. In the case of cocoa butter two types of curve were obtained for the same sample before and after treatment with hydrochloric acid (*cf.* Pichard, ANALYST, 1923, **48**, 556), and these are attributed to a non-fatty constituent which retards crystallisation, but which is removed by the acid. J. G.

Determination of the Molecular Weights of the Higher Saturated Fatty Acids and its use for the Determination of Lignoceric Acid in Hardened Arachis Oil Mixtures. J. Grossfeld. (*Z. Unters. Lebensm.*, 1929, **58**, 209–261.)—The literature shows that, in spite of the great variation in the amounts of saturated and unsaturated acids in arachis oil, the ratio of lignoceric to arachidic acid is approximately constant, and has the average value 44·1 : 55·9. Heiduschka and Felser's titration method for arachidic acid (*id.*, 1922, **43**, 381) fails in the presence of hardened fats, since the excess of stearic acid produces a precipitate in the presence of alcoholic potassium hydroxide solution, and the potassium salts prepared by their method are contaminated with free fatty acids. The sources of error in the determination of lignoceric acid by separation of the slightly-soluble fatty-acid fraction and calculation from the mean molecular weight are discussed, and it is considered preferable to obtain the molecular weight from the potassium content of the neutral potassium salt. This is prepared by saponification of 10 grms. of fat with 10 c.c. of 95 per cent. alcohol and 4 c.c. of 50 per cent. potassium hydroxide solution heated under a reflux condenser, sufficient stearic acid being added to make the total quantity present at least 1·5 grms. The hot solution is shaken with 150 c.c. of 95 per cent. alcohol, 4 c.c. of 96 per cent. acetic acid and 30 c.c. of a 1·5 per cent. solution of lead acetate in alcohol and acetic acid, and after 1½ hours the cold solution is filtered, and the residue dissolved in 200 c.c. of hot alcohol and acetic acid. The solution is concentrated to 20 c.c. boiled with 100 c.c. of hot water, 5 c.c. of dilute nitric acid, and the layer of fatty acids removed and saponified with 1 c.c. of 50 per cent. potassium hydroxide solution (*cf.* Kreis and Roth, *id.*, 1913, **25**, 81). The acids are then re-precipitated from the diluted soap-solution with 2 c.c. of 25 per cent. hydrochloric acid, washed, and neutralised by titration of a filtered solution in neutral 95 per cent. alcohol with 0·5 *N* alcoholic potassium hydroxide till a weak red colour is obtained in the presence of phenolphthalein. This is just removed by a few drops of a 0·5 per cent. solution of stearic acid in alcohol, and the solution warmed with 50 c.c. of neutral ether and cooled. Fine crystals in the cool solution indicate a small quantity of stearic acid, whilst a gelatinous precipitate denotes a large amount. After 2 hours, 150 c.c. of ether are added, and the next day the neutral potassium salt is filtered off, washed, and dried in air below 60° C., till constant in weight. Since incineration gives low results, the potassium is determined as perchlorate by addition of 12 c.c. of chloroform (to inhibit precipitation of the fatty acids), 0·5 c.c. of acetic acid and 2·4 c.c. of 20 per cent. perchloric acid to a solution of the potassium salt in 20 c.c. of 96 per cent. alcohol. The potassium perchlorate is filtered off the next day, washed, dried and weighed and, if a correction of 0·14 mgrm./c.c. is added to the result, an accuracy of 0·5 mgrm. is obtainable. Then if k is the potassium perchlorate value (percentage of $KClO_4$ obtained from the potassium salt), the mean molecular weight is $13856/k-38{\cdot}09$. The lignoceric acid content of the sample may then be found from the formula $16{\cdot}63(39{\cdot}54-k)$ for $k=34{\cdot}00$ to 37·16 (absence of stearic acid), or $6{\cdot}81\ (42{\cdot}98-k)$ for $k=37{\cdot}16$ to 42·98 (presence of stearic acid). Tables and graphs are provided to assist these calculations. The

method is applied to the detection and determination of hardened arachis oil in cocoa butter, and to the determination of stearic in the presence of palmitic acid. High values of k are obtained if potassium hydroxide containing carbonate is used, and it is advisable to standardise the method in the presence of pure stearic acid, when $\pm$ 0·04 per cent. of lignoceric acid, corresponding with 2 to 4 per cent. of arachis oil, may be determined. Six samples of hardened arachis oil offered as cocoa butter contained 1·2 to 1·64 per cent. of this acid, and these low figures, compared with the normal value of 2·5 to 3 per cent., are due probably to the presence of other fats. In lard, beef fat, butter fat, hazel nut, walnut, almond and apricot oils, illipé butter and karité fat, stearic acid was found to be the fatty acid of highest molecular weight. Behenic acid in hardened train oil may be determined by the same method from the formula 14·99 (42·98–k), and 2 samples were found to contain 4·46 and 4·65 per cent. J. G.

Constituents of Hydnocarpus Wightiana Oil. I. H. I. Cole. (*Phil. J. Sci.*, 1929, **40**, 499–502.)—In the fractional distillation of the ethyl esters of hydnocarpus and chaulmoogric acids (the main constituents of *Hydnocarpus Wightiana* oil) an optically active, low boiling, fraction is obtained. Fractions boiling between 151 and 163° C. were collected after 5 re-fractionations, and from this lauric acid was separated. From 2 kilos of esterified oil 1·12 per cent. of lauric acid was separated. An optically active liquid fatty acid was separated from the lauric acid mother liquors, probably a lower homologue of hydnocarpic acid, but the composition is not yet determined. D. G. H.

Seguidillas Beans and Oil. F. Agcaoili. (*Phil. J. Sci.*, 1929, **40**, 513–514.)—The bean of *Psophocarpus tetragonolobus*, the seguidillas, pal-lang, calamismis or asparagus bean, is edible when cooked. It has 12 per cent. of a skin resembling that of the soya bean, smooth and shiny, and is rounded, laterally compressed, about 10 mm. long, 8 wide and 7 thick. The composition of the sample examined was moisture, 9·74; fat, 17·04; protein, 32·81; starch, 12·5; carbohydrates not starch (by diff.), 18·70; ash, 4·01; and crude fibre, 5·20 per cent. The oil had the following characteristics: $n_D^{30°}$, 1·4666; Zeiss number, 62·8; sp. gr., 0·9284; saponification value, 175·6; and iodine value, 82·1. The bean is regarded as suitable for cultivation for the production of edible oil and foodstuffs (no prussic acid was detected), and bears a close resemblance to soya bean.

D. G. H.

Composition of Philippine Coffee. A. Valenzuela. (*Phil. J. Sci.*, 1929, **40**, 349–351.)—An analysis of seven varieties of raw Philippine coffees, *Caffea robusta*, Linn.; *liberica*, Heim.; *canephora*, Peirre; *abeocuta* Cr.; *exelsa*, A. Chev.; *ugandae* Cr.; and *arabica*, Linn. showed the composition to be:—Moisture, 8·11 (*robusta*) to 11·72 (*canephora*), average 9·86; caffeine, 1·62 (*arabica*) to 2·42 (*canephora*), average 1·87; fat, 7·39 (*canephora*) to 9·69 (*liberica*), average 8·71; reducing sugar, 3·98 (*arabica*) to 6·85 (*abeota*), average 5·22; crude fibre, 16·52 (*arabica*) to 25·80 (*canephora*), average 19·65; nitrogenous substances, 11·25 (*canephora*) to 15·01 (*liberica*), average 12·95; ash, 3·33 (*canephora*) to 4·57 (*robusta*), average

4·10; other nitrogenous substances by difference, 30·73 (*canephora*) to 41·17 (*arabica*), average 37·64 per cent. The caffeine percentage is slightly higher than that of foreign raw coffees, and the fat and reducing sugar slightly lower. The compositions appear to compare favourably with those of foreign coffees.

D. G. H.

Use of Active Charcoal for the Adsorption of Caffeine, particularly from Coffee Infusions. F. Sartorius and W. Ottemeyer. (*Z. Unters. Lebensm.*, 1929, **58**, 353–362.)—Comparative measurements, under varying conditions of the adsorptive powers of 8 carbons and 2 other substances towards solutions of caffeine and coffee extracts, showed that the amount adsorbed usually rises to a maximum after about 5 hours' contact, and then falls off to a value which remains constant for the period studied (20 hours). The maximum percentage adsorbed by 0·5 grm. of substance from 25 c.c. of 2 per cent. caffeine solution varied from 70 per cent. for Merck's carbon, to 4 per cent. for fluorite and silargel. With 4·5 grms. of carbon and 225 c.c. of 0·05 per cent. solution A.K.T. carbon was shown to be superior, and adsorbed 98·1 per cent. of the caffeine in 1 hour, and, in general, the adsorption increases with the proportion of carbon, there being a smallest optimum quantity for each product. Uninterrupted flow of a 0·05 per cent. solution through a tube of adsorbent showed that the maximum adsorption obtained at 80° C. is 96 per cent., falling to 68 per cent., whilst for cold solutions the corresponding figures are 87 and 39 per cent. Similar effects were obtained with coffee extracts, the colour and aroma being unimpaired. The use of carbon is recommended for household purposes, but not as a clearing agent in the analysis of coffee, even when extraction with chloroform is employed, as the loss of caffeine under the conditions described may exceed 90 per cent. The sample (500 c.c.) is best precipitated with 50 c.c. of (German Pharmacopoeia) aluminium acetate solution and 1·5 grms. of sodium carbonate, the clear liquid decanted, evaporated with alumina, and extracted with 50 c.c. each of hot and cold chloroform. The residue left on evaporation is dissolved in 100 c.c. of water, 25 c.c. of 1 per Cent. potassium permanganate solution added, and then a drop of hydrogen peroxide and acetic acid. The solution is filtered, 100 c.c. evaporated, and the caffeine determined by precipitation as periodide with excess of a solution of iodine and potassium iodide (Gomberg). The residual iodine is titrated with sodium thiosulphate solution, and should be approximately 25 c.c. of 0·02 *N* solution for 100 mgrms. of caffeine.

J. G.

Commercial Glyzines (Ammonium Glycyrrhizates). A. Bonis. (*Ann. Falsif.*, 1929, **22**, 518–522.)—The content of ammonium glycyrrhizate in the commercial product cannot be judged from the proportion of ammoniacal nitrogen present. The method of determination based on removal of the gums and dextrins, precipitation of glycyrrhizic acid and conversion of this into the ammonium salt, does not give exact results, but the values obtained are sensibly proportional to the true contents of ammonium glycyrrhizate. This method gave for a number of the commercial products the percentages: 43·52 (German), 50·16, 81·28 (Belgian),

81·44 (French), and 82·50. After precipitation of the glycyrrhizic acid, the first two of these yielded residual liquors of high optical rotation and low copper-reducing power, so that they probably contain carbohydrates of the dextrin type. Whether such compounds were added fraudulently or formed from the starch of the liquorice root during the process of manufacture cannot be decided.

T. H. P.

Aldehyde-Oxidation Reactions for Phenols, particularly the Opium Alkaloids. C. C. Fulton. (*J. Assoc. Off. Agric. Chem.*, 1929, **12**, 434–441.)—The colour reaction given by a phenol with an aldehyde in presence of strong acid and an oxidising agent appears to be a general phenolic reaction, and is due essentially to oxidation of the phenol-aldehyde compound. The reaction serves as a means of (1) identifying the opium alkaloids, (2) identifying other phenols and phenolic compounds, (3) detecting and identifying formaldehyde, and (4) identifying a substance as an aldehyde. Some of the reactions, although taking place in fairly concentrated acid, are able to detect 1 part of formaldehyde in 1,000,000 parts of aqueous solution, especially if a distillate is used for the test, and if this is tried with several of the suitable alkaloids. Pseudomorphine, in particular, is almost a general reagent for aldehydes, giving a green or blue colour when used with concentrated sulphuric acid and the nitric acid oxidising agent. A non-aldehyde may, however, be decomposed by the sulphuric acid, with formation of one or more aldehydes.

The tests on the opium alkaloids are made by placing a little of the alkaloid on a spot plate, adding 0·4–0·5 c.c. of the solution of formaldehyde in sulphuric acid so as practically to fill the spot, stirring with a glass rod, adding a single drop of one of the oxidising solutions (see below), and again stirring. The alkaloid should be either free or as sulphate. A strong bright colour is usually produced at once, but 15 to 30 minutes should be allowed. The following oxidising agents may be used, the ferric solution being best with concentrated sulphuric acid, and the nitric acid solution with acid not of full strength: 0·5 c.c. of bromine water mixed, while cooling under running water, with 3·5 c.c. of concentrated sulphuric acid; 1 c.c. of 10 per cent. aqueous ferric sulphate solution mixed, while cooling, with 3 c.c. of concentrated sulphuric acid; a mixture of 1 c.c. of a solution of 5 drops of concentrated nitric acid in 50 c.c. of water with 3 c.c. of concentrated sulphuric acid; a mixture of 1 c.c. of a 1: 30 nitric acid solution with 3 c.c. of concentrated sulphuric acid. As aldehyde solution use is made of Marquis's reagent, which is made by adding 2 drops of 37 per cent. formaldehyde solution to 3 c.c. of concentrated sulphuric acid, and should give no coloration with pure brucine. This alone gives the following colour reactions: with morphine, crimson changing to purple; codeine, purple; pseudomorphine, red; apomorphine, purple changing to dark green; papaverine, delayed purple-red; narcotine, purple changing to brown and yellow; narceine, orange changing to red; thebaine, brown-red changing to orange. If the reagent is followed by the bromine reagent, the pseudomorphine red changes to purple and gradually to deep blue, while morphine gives violet changing to purple red, and codeine deep blue, the other colours being but little

affected. The Marquis reagent, followed by the nitric acid oxidising solution, gives with a small quantity of thebaine a deep bluish green, changing to a persistent deep bright green, whereas, if the stronger nitric acid solution is used, a deep green fading to pale orange is observed. These reactions are modified in various ways if the Marquis reagent is diluted to different extents with concentrated sulphuric acid, or if the sulphuric acid used is diluted with water. T. H. P.

Chemical Characteristics of Herba Lobelia. L. Rosenthaler. (*Amer. J. Pharm.*, 1929, **101**, 786–787.)—Five grms. of Lobelia are distilled with 50 grms. of water and 5 grms. of sodium hydroxide, and 10 c.c. of distillate collected. With sodium nitroprusside and sodium hydroxide the distillate will show a deep orange to dark purple colour, turning to purple violet, and then dirty violet, on addition of acetic acid. With phenylhydrazine hydrochloride or with 1–4 nitrophenylhydrazine (in dilute acetic acid) a strong turbidity results. The distillate obtained without sodium hydroxide gives no characteristic reaction with sodium nitroprusside, and only faint turbidities with the hydrazines. Controls with leaves of *Belladonna, Coca, Jaborandi, Stramonium,* and *Uvae Ursi* were negative. The reaction of ferric chloride, and some of the alkaloid precipitating agents on aqueous extracts of Lobelia, are not specific. D. G. H.

Ephedra Alkaloids. T. and H. Smith. (*Pharm. J.*, 1929, **123**, 606.)—Racemic ephedrine (ephetonin) has a m.pt. 73–74° C., ephedrine hydrochloride (pure crystalline) 218° C., and $[\alpha]_D$ 36·6° in water; ephedrine sulphate crystallised from alcohol, 243° C. and $[\alpha]_D - 30°$. The relative insolubility of *l*-ephedrine oxalate allows of the separation of the alkaloid from its associated isomer, *d*-pseudo-ephedrine. Ephedrine salts are stable, *l*-ephedrine has a m.pt. of 40° C., and a water solution an $[\alpha]_D$ of +13·75° C., but an alcoholic solution is laevorotatory, −6·3°; *d*-pseudo ephedrine has a m.pt. 118° C. and $[\alpha]_D$ 50°. Ephedrine can exist in 6 forms:—*l, d, dl,* ephedrine; and *l, d,* and *dl* pseudo-ephedrine; and all have been prepared synthetically. The value of the herb Ma Huang depends on the *l*-ephedrine content. On evaporating a chloroform solution of *l*-ephedrine, if heat be applied, a somewhat violent reaction results in conversion of the alkaloid to the hydrochloride. After separation of the *l*-ephedrine and *d*-pseudo-ephedrine from the Ma Huang the remaining small oily residue deposits crystals from which *l*-methyl ephedrine m.pt. 88° C. and $[\alpha]_D - 29·2$ may be obtained. Nor-*d*-pseudo ephedrine may also be obtained from the crude crystalline mass, and has a m.pt. 77–78° C. and $[\alpha]_D + 32°$. In dilute alcohol *nd*-pseudo-ephedrine forms supersaturated solutions. *l*-ephedrine and *d*-pseudo-ephedrine (the alkaloids present in the herb in the largest quantities) are not particularly sensitive to potassium mercuric iodide solution, but *l*-methyl and *nd*-pseudo ephedrine are readily precipitated by it from a 1 per cent. solution of their sulphates. D. G. H.

Commercial Nicotine-free, Nicotine-poor and Nicotine-harmless Tobacco Preparations. K. Baumann and J. Kuhlmann. (*Z. Unters. Lebensm.*, 1929, **58**, 524–529.)—Of 43 samples of tobacco, cigars, etc., sold as either

of low or of non-injurious nicotine-content, 14 contained between 0·76 and 1, 14 between 1·01 and 1·25, 7 between 1·26 and 1·5, 6 between 1·51 and 1·75, and 2 over 1·76 per cent. of the alkaloid. The necessity for legislation on this matter is emphasised. (*Cf.* Pfyl and Schmitt, ANALYST, 1927, **52**, 728.) T. H. P.

Marbling and Corrosion of the Interior of Preserve Tins. W. D. Bogatsky, W. A. Biber and L. G. Kischinewskaja. (*Z. Unters. Lebensm.*, 1929, **58**, 506–517.)—The appearance of spots giving a marble effect to the inside of tins is independent of the character of the preserved material, and is due to the action of organic sulphur compounds. The phenomenon is not caused by high temperature, pressure, the presence of lead in the tin used, or the presence of copper, but is due to the fact that, with light and irregular tinning, the metal may in some parts be either wholly or almost devoid of tin. The spots consist either of mixed tin and iron sulphides, or of iron sulphide showing through the thin layer of tin. Corrosion, on the other hand, is accompanied by the solution of appreciable quantities of tin, and is retarded by a number of circumstances, among them the influence of the oil used in the preparations. Tin plate protected by a film of tin sulphide resists the action, in the cold, of many acid juices and of sulphur-containing organic compounds. T. H. P.

Biochemical.

Enzymic Method for Detection and Determination of Tyrosine in Urine. S. S. Lichtman and H. Sobotka. (*J. Biol. Chem.*, 1929, **85**, 261–273.)—The crystalloscopic method commonly used for the detection of tyrosine in urine concentrates has been replaced by an enzymic method. The enzyme tyrosinase is found in certain fungi, in the meal-worm, in potatoes, etc. A method is now described for obtaining an enzymic preparation in dry stable form from potato juice. The method is far from efficient in regard to purity and yield, as the elimination of inert material is offset by loss of enzymic activity during the various steps of the preparative process. A practical method for the standardisation and determination of tyrosinase is outlined. The authors suggest, as a practical tyrosinase unit, that minimum amount of enzyme which will form a visible amount of melanin within 24 hours when reacting with 0·2 mgrm. of tyrosine in 5 c.c. of a phosphate buffer solution of *p*H 6·8 at room temperature. A unit has been found in approximately 12·5 mgrms. of an active crude preparation. Up to 70 units were yielded by 100 c.c. of active potato juice. The tyrosinase value of a preparation is defined as the number of units contained in 100 grms. of the preparation; thus the tyrosinase value of an active enzyme has been found to be about 8000. A biochemical method is described for the detection of free tyrosine in untreated urine, based on the enzymic oxidation of this amino acid. The primary phase of the reaction, the formation of a rose-coloured substance, is entirely dependent on enzymic activity; the subsequent non-enzymic formation of melanin adds to the sensitivity and specificity of the reaction. The principle of the method is the comparison of the primary red and secondary brown and black phases of the

reaction, and the recognition of a melanin threshold when an adequate amount of enzyme interacts with a minimum concentration of 0·0125 per cent. tyrosine in urine. The violet end-stage permits the identification and colorimetric comparison even in dark urines. The presence of an inhibitory factor in urine, which reduces the sensitivity of the test, is demonstrated. Under standard conditions, in the presence of an excess of oxygen and enzyme, tyrosine can be detected in solutions of free tyrosine in a minimum concentration between 0·004 and 0·002 per cent. Substances giving atypical reactions are enumerated, and the criteria of their differentiation, especially that of *p*-cresol, stated. The applicability of the method to the detection of tyrosine in other pathological body fluids is mentioned. The advantages of the biochemical over the crystalloscopic method as a qualitative method involve economy of time and labour, higher specificity, sensitivity and dependability, and its application directly to untreated urine. In addition, it permits the quantitative evaluation of tyrosine. P. H. P.

Determination of Isopropyl Alcohol in the Presence of Acetone in the Urine. C. A. Cook and A. H. Smith. (*J. Biol. Chem.*, 1929, **85**, 251–260.)—A method for the determination of isopropyl alcohol alone, or in the presence of acetone, has been developed, and has been applied to the analysis of urine. Under the conditions of the method, isopropyl alcohol gives a maximum yield of acetone, but it is realised that other compounds containing the isopropyl group would yield some acetone. The method consists in the oxidation of the isopropyl alcohol to acetone by heating under a reflux condenser with dichromate and sulphuric acid, and the measurement of the resulting acetone as the mercuric sulphate complex of Denigès (*Compt. rend.*, 1898, **126**, 1868; **127**, 963). The pre-formed acetone is determined on a separate sample by distillation into hydroxylamine hydrochloride and titration of the acid liberated in the condensation of the acetone with the hydroxylamine hydrochloride. The procedure for the total acetone is essentially like that of Van Slyke (*J. Biol. Chem.*, 1917, **32**, 455) for acetone bodies in the urine. It is necessary to remove extraneous substances which might also yield a precipitate with Denigès' reagent. The whole procedure is described in detail, and certain requirements as to the concentration of alcohol and acetone and the optimum experimental conditions are discussed. The reaction of hydroxylamine hydrochloride with acetone has long been known, and is generally regarded as a promising method for the accurate determination of the acetone present in commercial mixtures, but actually it has been little used either for acetone or for higher ketones, owing partly to certain inherent difficulties of the method, but largely to lack of investigation of its application and possibilities. The hydrochloric acid liberated on formation of the acetoxime is titrated with 0·1 *N* alkali, with the use of methyl orange as indicator. Hydroxylamine sulphate may be used instead of the hydrochloride. A correction factor $\frac{100}{94{\cdot}4}$ or 1·057 is used; this takes into account the fact that under optimum conditions the oxime reaction only reaches 94·4 per cent. completion. The results obtained in a determination are given, and show the percentage recovery with controlled acetone and alcohol concentrations. P. H. P.

Determination of Glycogen in Small Amounts of Tissue. A. E. Osterberg. (*J. Biol. Chem.*, 1929, **85**, 97–108.)—Studies on the glycogen content of biological material have been handicapped by the fact that the more reliable chemical methods for analysis necessitate the use of considerable amounts of tissue; consequently, experiments which involve the use of small animals, such as tadpoles, or the isolation of a small specific tissue, such as a branch of a nerve, or even repeated determinations of the glycogen content of an organ under observation, can only be carried out by methods designed for macro determinations. Micro determinations by histological staining procedures are unsatisfactory. However, a technique is now described with which the glycogen content of a specimen of tissue weighing from 5 to 15 mgrms. can be accurately determined. The technique is based on the principles of the method of Pflüger for the determination of glycogen in biological material, namely, the destruction of the tissue by means of concentrated alkali, and the isolation and purification of the glycogen by means of alcoholic precipitation, followed by the inversion of the glycogen to glucose. The glucose so obtained is determined by the new ferricyanide procedure of Folin (*J. Biol. Chem.*, 1928, **77**, 421). The details of technique found to be essential in handling minute amounts of tissue, and the apparatus with which glycogen can be isolated and converted to glucose, are described. Glycogen, when added to such tissue, may be recovered with a degree of accuracy sufficient for purposes of studies of tissue glycogen. It has been definitely established that 60 per cent. potassium hydroxide at 100° C. does not destroy glycogen. Data are presented on the application of the technique to the determination of glycogen in hepatic and cardiac tissue, showing the reproducibility of the results and the satisfactory recovery of added glycogen. P. H. P.

Nature and Identity of Wheat Glutenin. M. J. Blish and R. M. Sandstedt. (*J. Biol. Chem.*, 1929, **85**, 195–206.)—For many years the gluten of wheat flour has been thought to consist primarily of two distinct and individual proteins, glutenin and gliadin. Both proteins have been frequently isolated and purified by various investigators, and their constitution and properties have been studied by such methods as have been available. On comparison of different analyses of the same protein, it is seen that there is greater concordance among the results of analyses of gliadin than is correspondingly true with glutenin; therefore, the chemical identity of the former seems to be far more definitely established than that of the latter. The data for gliadins indicate that gliadins from different wheats are identical, whereas the data for glutenins indicate either (1) glutenins from different wheats are not identical, or (2) methods for the preparation and purification of glutenin are uncertain and unreliable, and the true chemical nature, identity, and individuality of this protein have not been satisfactorily established. A preliminary report is given of certain experiments bearing upon the latter possibility. The results show that glutenin, as prepared by customary methods which involve extraction with or temporary solution in alkali, is a product resulting from an irreversible alteration by the action of alkali on a more

complex protein body. Both yield and chemical constitution of glutenin prepared by the usual methods will vary with the concentration of alkali. A new "glutenin" has been prepared by a procedure in which exposure to alkali is avoided at all stages. It differs from the usual glutenin both in physical properties and in chemical constitution. Nine or ten samples of it which were prepared agree within a reasonable factor of error both as to per cent. of amide nitrogen (averaging about 22 per cent. of the total nitrogen) and arginine nitrogen (averaging about 9 per cent.). It is probable that some irreversible alteration occurs when any protein material is dispersed in alkaline solution, regardless of the concentration of alkali. The authors conclude that there is occasion for further intensive investigation of the nature of the nitrogenous material of wheat and flour, and of the other cereals as well, and that such investigation will doubtless lead to a substantial revision of present-day ideas as to the true character of this protein, or group of proteins, as the case may be. This situation applies with special force to the so-called cereal "glutelins." P. H. P.

Methods for Determination of Nitrogenous Constituents of a Cyanophoric Plant; *Prunus laurocerasus*. M. E. Robinson. (*Biochem. J.*, 1929, **23**, 1099–1113.)—The technique usually employed in determination of the nitrogen distribution in plants entails maceration of the tissues with water and some plasmolysing agent, and subsequent separation of the heat-coagulable and water-soluble substances. A method of extraction which involves maceration of the tissues is unsuited to investigations of cyanophoric plants, as serious losses of cyanide-nitrogen would be thereby unavoidable. Methods have now been described for the determination of the following nitrogenous constituents of cyanophoric plants:—Total, insoluble, non-coagulable, proteose-, cyanide-, ammonia-, amide-, and nitrate-nitrogen, and examples of their application to leaves of *Prunus laurocerasus* and seedlings of *Sorghum vulgare* have been given. The methods have been adapted to the use of such quantities of material as can be conveniently utilised in a respiration experiment, namely, 10–20 grms. of fresh weight of fully-grown leaves, quantities considerably smaller than those generally used in the standard methods for the quantitative determination of the distribution of plant-nitrogen. Full details of the methods are given. P. H. P.

The Indophenol Reaction in Biological Oxidations. D. C. Harrison. (*Biochem. J.*, 1929, **23**, 982–999.)—During the course of some experiments on the effect of thyroid on tissue oxidations it was found that a marked increase in the indophenol reaction given by the tissues of rats was brought about by feeding with thyroid. This result was difficult to explain, since the factors concerned in the indophenol reaction are as yet only partly understood. Experiments were therefore designed to throw some light on the mechanism of this reaction. The indophenol reaction is readily given by hydrogen peroxide in the presence of peroxidase, and it occurred to the author that the indophenol reaction in tissues might be due, at least in part, to secondary oxidation in the presence of peroxidase by hydrogen peroxide formed during the oxidation of other oxidising systems. The results

obtained show that the secondary oxidation of dimethyl-*p*-phenylenediamine, leading to a positive indophenol reaction, can be brought about during the oxidation of hypoxanthine by xanthine oxidase (from milk or liver) in presence of peroxidase. The indophenol reaction can, therefore, be brought about without the agency of a specific indophenol oxidase. A method is described for the preparation of an active solution of xanthine oxidase from liver. *p*-Phenylenediamine increases the oxygen uptake of unwarmed yeast, which does not give an indophenol reaction. The methylene blue technique is shown to be unreliable as a means of measuring the reducing systems in the yeast cell. A scheme of reactions is described to explain the significance of the indophenol reaction in cell oxidations, and it is suggested that cytochrome may be involved in a similar series of reactions. The indophenol reaction is no criterion for the presence of an activator of oxygen. The author considers that the explanation of the indophenol reaction and its significance in cell oxidations is best accounted for by the assumption of a combined action of an indophenol oxidase, as suggested by Keilin (*Proc. Roy. Soc.*, 1929, B, **104**, 206), and the secondary oxidation mechanism suggested by him. P. H. P.

New Method for Determination of the Activity of Certain Oxidases with Preliminary Study of the Potato Oxidase. A. E. Stearn and A. A. Day. (*J. Biol. Chem.*, 1929, **85**, 299–306.)—The presence of oxidase has ordinarily been detected and its activity measured either by determination of the rate of disappearance of gaseous oxygen, or by the formation of a coloured oxidation product from a colourless reduced agent. A method which is now proposed is, where applicable, easier than the first method, and has the advantage over the second that colour or turbidity does not interfere with its use. In connection with some electrometric determinations of the *p*H of blood with a quinhydrone electrode, a very slow drift toward a more positive potential was noted, especially when small amounts of quinhydrone were used; this was accelerated by the bubbling of air through the liquid, and the addition of more quinhydrone tended to restore the original potential. The drift appeared to be due to oxidation of the hydroquinone in the quinhydrone, to quinone. It was therefore thought that observations of the change of potential of such an electrode might offer a simple method for detecting the presence of certain sufficiently active oxidases or oxygenases, for comparing the activities of preparations of these enzymes, and possibly, for studying such catalysed oxidation reactions themselves. The principle of the method depends on the fact that the potential of a reversible system consisting of any substance and its oxidation product is determined, other factors remaining constant, by the ratio of the concentrations of material in the oxidised state to that in the reduced state. Quinhydrone furnishes such a reversible oxidation-reduction system, since it dissolves and gives equimolar concentrations of quinone, the oxidised form, and hydroquinone, the reduced form. Thus, if other factors are constant, any oxidation of hydroquinone is shown by the potential rise, inasmuch as such oxidation alters the 1 : 1 concentration ratio of quinone to hydroquinone, which obtains in quinhydrone, to some higher value. Indicative experiments which are reported were

carried out with potato oxidase, and an ordinary potentiometer with a saturated calomel half-cell as reference electrode. The results show that, although the times of oxidation through the same potential change are not proportional to the enzyme concentrations, nevertheless there is a rough proportionality, through the range of conditions studied, between time of oxidation and quantity of quinhydrone substrate, and also if a constant ratio of enzyme to substrate is maintained, the times of oxidation through a given potential change are, although not equal, at least of the same order. There seems to be a rapid slowing down of the oxidation for enzyme concentrations somewhat smaller than those reported. Enzymes are usually measured by activity rather than concentration, and these two magnitudes may not be proportional. A point to be considered is the fact that the oxidised component accumulates and builds up an increasing oxidation potential, against which the reaction must be forced; thus the conditions are not the same at the end of a 30 millivolt rise as at the initial potential. After oxidation, causing a rise of 30 mvs., and addition of a second amount of quinhydrone, the measured E.M.F. always agreed very closely with the calculated E.M.F. The authors hope to study the method, extend the limits of various concentration factors, and poise the solution at various oxidation potentials. P. H. P.

Observations on the Concentration of Vitamin *B*. B. C. Guha and J. C. Drummond. (*Biochem. J.*, 1929, **23**, 880–897.)—A repetition and revision of much of the earlier work on vitamin *B* is necessitated by the discovery in recent years of its complex nature. It consists of at least two factors, vitamin B_1 and vitamin B_2. Vitamin B_1 is the factor, a deficiency of which is apparently related specifically to the typical convulsive symptoms associated with vitamin *B*-deficiency, and it differs from vitamin B_2 by its relative instability to heat and alkali. The results of certain attempts made by the authors during the last two-and-a-half years to concentrate vitamin B_1 are now recorded. The activity of the preparations made was studied by tests on both rats and pigeons. Wheat embryo was selected as the starting material, since it is very rich in vitamin B_1 and relatively poor in vitamin B_2. Two different processes of fractionation were investigated; one consisted of fractionation by lead acetate, charcoal adsorption at various hydrogen ion concentrations, fractionation with phosphotungstic acid, adsorption on silver oxide, fractionation with alcohol, and treatment with picrolonic acid, and the other consisted of adsorption by fuller's earth, fractionation with silver nitrate and baryta, fractionation by phosphotungstic acid, fractionation with platinic chloride, and fractionation with gold chloride. A concentrate was obtained after fractionation with platinic chloride, of which the pigeon-curative day-dose is 0·005 mgrm., and which promotes good growth in rats in daily doses of 0·015 mgrm., when supplemented by vitamin B_2. From the experiments in connection with fractionation by gold chloride it appears reasonable to infer that the activity of vitamin B_1 is probably to be ascribed to more than one factor. In this respect, the rat-experiments and pigeon-experiments appear to corroborate each other. However, conclusive evidence about the multiple nature of vitamin B_1 can only be

obtained when a more complete separation of the two (or more) components is effected by fractional crystallisation of the gold salt or by other means. Certain properties and reactions of the concentrates are described. As regards the properties of vitamin B_1, its behaviour appears to be determined to an extraordinary extent by the presence of other substances and by the previous treatment of a given preparation. Certain substances which have been claimed to possess antineuritic or growth-promoting properties, before the multiple nature of vitamin *B* was recognised, were re-tested for vitamin B_1. Of these, yeast nucleic acid, nicotinic acid, betaine and the substance of M.P. 234–5° C., described by Drummond and Funk (*Biochem. J.*, 1914, **8**, 598), gave negative results. The volatile bases liberated from marmite when it is boiled with 20 per cent. sodium hydroxide solution also gave a negative result. The results show no fixed ratio between the rat-dose and the pigeon-dose of the various concentrates investigated. Whether this indicates a difference between the rat-factor and the pigeon-factor, or whether it reflects on the accuracy of the present biological methods of assay, is uncertain, but probably the latter is the more likely explanation. P. H. P.

Distribution of Vitamin *A* in some Maize Milling Products. C. R. Meyer and R. A. Hetler. (*J. Agric. Res.*, 1929, **39**, 767–780.)—Various milling products obtained in treating whole yellow maize were used as supplements to vitamin *A*-deficient basal diets given to rats which showed typical vitamin *A* depletion. The whole maize was found to be rich in vitamin *A* (rapid growth being obtained when 1·5 grm. per animal was given daily, and ophthalmia was cured with 1 grm.); gluten (0·25 grm.) also cured ophthalmia and produced normal growth, but steep water, reel slop and grits, even when fed in large proportions, effected no cure. Vitamin *A* is concentrated in the endosperm, and is in greatest proportion nearest to the seed coats. It is also present in the crude oil, but germs and germ meal were lacking in it. The distribution of vitamin *A* is regarded as associated with yellow pigmentation. D. G. H.

Hypervitaminosis and Vitamin Balance. IV. An Instance of Vitamin Balance. L. J. Harris and T. Moore. (*Biochem. J.*, 1929, **23**, 1114–1121.)—Harris and Moore (*Biochem. J.*, 1928, **22**, 1461; ANALYST, 1929, **54**, 249) published details of investigations in which it was shown that the substitution of cod-liver oil for arachis oil in rations which contained various restricted allowances of vitamin *B* resulted in a further lowering of growth rates. Comparatively large quantities of cod-liver oil were administered, and, since there are many substances in cod-liver oil of whose chemical nature and properties little is known, there was no necessity to assume that the ill-effects observed were necessarily due to vitamins. The experiments have now been repeated with the use of vitamin concentrates instead of cod-liver oil, so as to eliminate at least the materials present in the saponifiable fraction of the oil. Marmite extract supplied the vitamin *B*. The effects were compared upon the animals of variations either (1) in the marmite allowance at each level of concentrate, or (2) in the concentrate allowance at each level of marmite. The results showed a remarkable regularity in the retarding

action of the vitamins *A* and *D* concentrate upon growth rates; in fact, the action was no less regular than the inverse accelerating action of marmite. The results, which are shown by graphs, are discussed. The following conclusions are summarised:—The need of the rat for marmite (vitamin *B* complex) is increased *pro rata* when increasing excess of cod-liver oil concentrate (vitamins *A* and *D*) is administered concurrently. Thus, in order to produce any given rate of submaximal growth, more and more marmite must be given as more and more of the excess of concentrate accompanies it in the diet. Again, a restricted amount of marmite which is normally adequate for prolonged maintenance becomes inadequate to prevent death when excessive concentrate is given. Rats dying under these conditions could then be cured by administration of additional vitamin *B* (as wheat-germ extract or marmite) without removal of the extra concentrate. Hence, an amount of concentrate which is harmful when given with a moderate marmite allowance is no longer harmful when given with a sufficiently augmented marmite allowance. It is considered most probable that this antagonistic effect is exerted between the vitamin *B* complex of the marmite, etc., and the vitamin *A* of the concentrate, or cod-liver oil; but the possible action of other unidentified substances is not excluded. P. H. P.

Toxicological.

Tremetol, the Compound that produces "Trembles" (Milk Sickness). **J. F. Couch.** (*J. Amer. Chem. Soc.*, 1929, **51**, 3617–3619.)—The active principle of *Eupatorium urticaefolium* (richweed or white snakeroot of the Central States), or of *Aplopappus heterophyllus* (rayless golden rod or jimmy weed of the South-Western section) is tremetol, which may be prepared by extracting the bruised plant (not old and dried in the case of richweed) with alcohol under reduced pressure, and extracting the greenish fatty residue with boiling water. The insoluble material is extracted with boiling 50 per cent. alcohol, the solvent removed, and, after cooling and hardening of the residue, the water is poured off. The resinous mass is extracted with 30 per cent. boiling alcohol, and the solution filtered hot, and, on cooling, crude tremetol ester separates from the filtrate. A further crop of crystals may be obtained from the mother liquors. The crystals are re-hydrolysed by boiling with 5 per cent. alcoholic potash for 4 hours, the alcohol is distilled off, and the residue dissolved in water, and the free tremetol extracted with successive portions of ether. The ethereal solutions are united, concentrated, washed with dilute sodium hydroxide, and then with water, mixed with 4 vols. of petroleum spirit, filtered and allowed to evaporate. Solution in ether and reprecipitation with petroleum spirit is repeated twice, and, on removal of the solvent, the tremetol is left as a thick yellow oil of pleasant aromatic odour. It decomposes on distillation, is slowly volatile in steam, is insoluble in water, acids and alkalis, readily soluble in organic solvents, and oxidises readily in air and is laevorotatory. Either $C_{16}H_{22}O_3$ or $C_{17}H_{24}O_3$ is indicated as the formula. Tremetol absorbs 4 atoms of bromine per mol. at room temperature, and contains a phenyl nucleus and a side

chain with 2 double bonds. Phenolic hydroxyls and alkoxyl groups do not appear to be present, and no crystalline oxime or hydrazone has been obtained. Aldehyde and carboxyl groups are absent. D. G. H.

Agricultural.

Relation of Picking Time to Acetaldehyde Content and Core Breakdown in Bartlett Pears. C. P. Harley. (*J. Agric. Res.*, 1929, **39**, 483–493.)—The Bartlett pears were picked from 2 trees on four dates, and the pears of each picking divided in 3 equal parts, one being immediately ripened at 22–24° C., the next being stored at 0° C. for 1 month before ripening, and the third portion stored for 2 months. No acetaldehyde was found in pears analysed immediately after picking, but it was present after two days of cold storage, increasing until the highest concentration was reached about the time breakdown occurred. The accumulation of acetaldehyde was more rapid in the late picked pears. In the early picked pears the initial percentages of carbon dioxide in the intercellular gases were lower than in the late picked fruit, and rate of accumulation less rapid, the maximum percentage being reached some time before the first visible sign of breakdown. In general, a corresponding decrease of oxygen occurred, but not always, indicating that respiration may be in part intramolecular. The relation of carbon dioxide to production of core breakdown in Bartlett pears is in the establishment of optimum conditions for acetaldehyde production. D. G. H.

Lead Content of Grape Must and Wine Treated with Insecticides containing Lead. E. Kielhöfer. (*Z. Unters. Lebensm.*, 1929, **58**, 382–386.)—The use on grapes of sprays containing lead compounds of arsenic resulted in lead contents (in mgrms. per litre) of 1·4 in the must, 1·0 in the first runnings of the wine, and 0·5 in the second. The yeast sediment from 1 litre contained 15 mgrms., and fining experiments with potassium ferrocyanide removed only 0·3 out of 2·9 mgrms. per litre. The use of lead sprays is considered inadvisable, especially in dry seasons. J. G.

Water Analysis.

New Reagent for the Detection of Nitrites in Water. M. S. Vergnoux. (*Ann. Chim. anal.*, 1929, **11**, 366.)—The reaction depends on the formation of a clear blue coloration when water containing the NO_2 group is treated with neutral red (dimethylamino-toluophenazine hydrochloride). The reagent is prepared by grinding in a mortar 2 grms. of neutral red (Grübler) in 18 c.c. of water and filtering; to the filtrate, cooled in a small flask, are added 80 grms. of sulphuric acid at sp.gr. 1·842. (The reagent becomes dark green and remains unchanged for a considerable time.) One hundred to 150 c.c. of the water (filtered if necessary) are treated with 5 drops of the reagent and mixed rapidly. The presence of nitrites in a quantity equal to, or greater than, 0·5 mgrm. of nitrogen produces an immediate blue tint throughout the solution. When the nitrites are less than this amount, the blue colour appears

after a few minutes, passing though an intermediate violet stage. The advantages of the method are its sensitiveness and simplicity, and the fact that it is independent of ordinary temperature changes, and also is uninfluenced by the presence of the Na, Ca, Mg, Cl, NO_3 and SO_4 ions. R. F. I.

Organic Analysis.

Head and Blubber Oils of the Sperm Whale. T. P. Hilditch and J. A. Lovern. (*J. Soc. Chem. Ind.*, 1929, **48**, 359–368T.) **II. Investigation of the Component Wax Esters and General Structure of the Oils.**—The products of oxidation of sperm head oil (obtained by oxidising with potassium permanganate in acetone solution) have been examined by separating into neutral and acidic products and fractional distillation *in vacuo* of the respective saturated alcohols and acids. The data thus obtained are regarded as more reliable than those resulting from the analysis of the mixed saturated and unsaturated substances originally present. The head oil contained 29 per cent. of fully-saturated components, and in 100 parts of original oil there were present about 2 parts of fully-saturated glycerides, and 27 parts of wax esters built up entirely from saturated acids and alcohols. The fatty acids of lower molecular weight tend to associate with tetradecyl, octadecyl and in greatest proportion with cetyl alcohol. About 24 parts of oil were composed of esters of saturated acids with unsaturated alcohols, about 18 parts of esters of unsaturated acids with saturated alcohols, the remainder consisting of esters of unsaturated acids and alcohols and of mixed saturated unsaturated glycerides. The oil is of heterogeneous character; no marked proportion of cetyl palmitate is present, and the chief saturated wax esters are cetyl laurate and myristate. The blubber oil only contained 1–2 per cent. of fully-saturated components, and is not suited for detailed examination on the same lines, but it is of similar general structure to the head oil, and is a heterogenous mixture of wax esters and mixed glycerides with oleyl alcohol as chief alcoholic component, and oleic and palmitic acids comprising the greater part of the fatty acids. Oleyl oleate and palmitoleate are present in appreciable amounts, and cetyloleate, palmitoleate and perhaps palmitate may also be expected. Complete hydrogenation of sperm head oil yielded a white close-grained wax, melting at 43° C., with little apparent greasiness, and the blubber oil gave a hard lustrous white wax, melting at about 54° C.

III. Quantitative Determination of the Higher Fatty Alcohols Present.—The same general procedure that was used for the elucidation of the composition of the mixed fatty acids is utilised with slight modifications for the determination of the composition of the higher fatty alcohols, but the process is more complicated, and the results must be regarded as indicative only to within a few units per cent. The sperm head compounds consist mainly of cetyl (54 per cent.) and oleyl (27–30) alcohols with smaller quantities of tetradecyl (8), hexadecyl (4), octadecyl (6), and unsaturated C_{20} alcohols (10 per cent.). The blubber alcohols are mostly unsaturated C_{18} alcohols (66–70), mainly oleyl, with cetyl (25–27), and minor

amounts of octadecyl (1) and unsaturated C_{20} (8) alcohols. As in the case of the oils, the head alcohols are less unsaturated than the blubber alcohols, and contain substances with a pronounced tendency to lower molecular weights. It is probable that the alcoholic components are produced by the sperm whale itself. D. G. H.

Unsaponifiable Matter of Ego Oil. M. Tsujimoto. (*J. Soc. Chem. Ind. Japan*, 1929, **32**, 324B.)—Ego oil is obtained from the seeds of "Egonoki," *Styrax japonica*, a tree indigenous to Japan. The oil used in this work was obtained by extracting Tokyo seeds with ether, and was a dark greenish yellow liquid, with the following constants:—Sp.gr. at 40°/15° C., 0·9387; iodine value (Hanus), 116·5; acid value, 3·2; n_D^{20}, 1·4814; saponification value, 179, and unsaponifiable matter, 7·10 per cent. The unsaponifiable matter formed nearly white crystals of m.pt. above 100°, with an iodine value of 71. It contained only 1·7 per cent. of sterols, as determined by the digitonin method. After being twice recrystallised from methanol, the product melted at 116° C., and under the microscope was seen to consist of thin long prisms and needles. It was soluble in organic solvents except petroleum spirit, had an iodine value of 88·5, showed no optical activity, and was free from nitrogen. Analysis by combustion gave 69·21, 69·44 and 69·52 per cent. of carbon, and 5·72, 5·77 and 5·63 per cent. hydrogen, from which the molecular weight was calculated to be 323, the formula approaching that of $C_{19}H_{18}O_5$. It was found to take up one acetyl group and to contain one methoxyl group. The bromine addition compound melted at 164°, and contained 23·19 per cent. of bromine, which figure, however, is considerably below that contained in the compound $C_{19}H_{18}O_5Br_2$. Practically no hydrogen was absorbed on hydrogenation. It was concluded that the formula of the unsaponifiable matter corresponded to $C_{19}H_{18}O_5$. The compound, for which the name "egonol" is suggested, is similar in composition to a liquid compound found in nutmeg butter by Power and Salway (*J. Chem. Soc.*, 1908, **93**, 1653), and to the substance otobite (m.pt. 137°–138° C.), isolated by Baughman, Jamieson and Brauns from otoba butter (ANALYST, 1921, **46**, 138). R. F. I.

Inorganic Analysis.

Application of Ammonium Oxalate in Systematic Qualitative Analysis. M. O. Charmandarjan. (*Z. anal. Chem.*, 1929, **79**, 90–94.)—The scheme presented is applicable whether phosphoric acid is present or not. At the outset, ammonium, ferrous and ferric iron, and phosphoric acid are tested for. The boiling filtrate from the hydrogen sulphide precipitate (approximately neutralised, but still sufficiently acid to prevent precipitation of phosphates) is treated with ammonium sulphate, followed by excess of ammonium oxalate; the precipitate contains barium sulphate and the oxalates of strontium and calcium; after cooling it is collected, washed with dilute ammonium oxalate solution, ignited, and strontia and lime extracted with dilute hydrochloric acid. The filtrate is evaporated to dryness in porcelain and the ammonium salts volatilised; the residue is dissolved

in a minimum of strong hydrochloric acid, and the solution precipitated with a slight excess of barium hydroxide. The precipitate is collected (the filtrate being used for the detection of the alkalis), dissolved in 2 *N* sulphuric acid, and barium sulphate filtered off; the filtrate is treated with excess of 12 per cent. sodium hydroxide and bromine water or, better, hydrogen peroxide. The treatment gives a precipitate of iron, cobalt, nickel, manganese, and magnesium, and a solution containing aluminium, zinc, phosphoric and chromic acids. The alkaline filtrate is neutralised with phosphoric acid; the phosphates of aluminium and zinc are thus precipitated. They are separated from each other by ammonia, which dissolves zinc phosphate. W. R. S.

Separation of Iron and Aluminium by Chancel's Method. P. L. L. Robinson and W. E. Scott. (*Proc. Univ. Durham Phil. Soc.*, 1929, **8**, 155–157.)—Chancel's process (hydrolysis of aluminium salt by thiosulphate alone in neutral solution, ferrous salt remaining unaffected) has been adversely criticised. The authors, as the result of a series of check-tests, conclude that the time of boiling originally prescribed (15 minutes) is inadequate. They found it necessary to maintain ebullition for 10 hours to obtain quantitative precipitation, with 10 grms. of sodium thiosulphate for 0·3 grm. of alumina. W. R. S.

Determination of Alumina in Borosilicates. O. W. Krasnowsky. (*Z. anal. Chem.*, 1929, **79**, 175–183.)—An investigation was carried out in order to ascertain whether alumina can be determined by the usual method (ammonia precipitation, Blum's directions) in the course of a silicate analysis, without regard to the presence of boric acid. In the synthetic tests, aluminium was precipitated, first by itself, then in presence of sodium borate and of calcium, magnesium, and large quantities of sodium, chloride. No interference of the borate with the accuracy of the determination could be observed. As alumina must in any case be submitted to re-precipitation, and boric acid is more or less volatilised in the evaporation for silica, it is concluded that no steps need be taken to ensure the elimination of boric acid in the determination of the bases in borosilicates.

W. R. S.

Colorimetric and Gravimetric Determination of Uranium. P. N. Das-Gupta. (*J. Indian Chem. Soc.*, 1929, **6**, 763–779.)—Uranium is precipitated from solution by tannin as a bulky chocolate-brown complex. Acid solutions must first be neutralised with ammonia. The neutral or faintly acid liquid is treated with fresh 2 per cent. tannin solution (2 c.c. per 0·012 grm. U.), boiled, stirred, and ammoniacal 10 per cent. ammonium acetate solution added till the precipitate flocculates and the liquid clears. Liberal addition of ammonium salt favours coagulation. The precipitate is collected, and washed with slightly ammoniacal 2 per cent. ammonium nitrate solution; if fixed alkali salts are present, it is washed by decantation with several portions of the same wash-liquor. The wet precipitate is ignited and weighed as U_3O_8. The tannin precipitate is not soluble in alkaline carbonate, but soluble in dilute mineral, as well as excess of

acetic, acid. For small amounts of uranium, the method is considered more reliable than the usual precipitation method by ammonia, in which the presence of carbonate in the precipitant leads to low results. The brown coloration produced by tannin in very dilute uranium solutions (concentrations of the order of 0·02 grm. per litre) is made the basis of a colorimetric process, in which 2 c.c. of fresh 1 per cent. tannin, and 3 of 5 per cent. sodium acetate, solution are used to produce the colour. Gallic or resorcylic acid may be used instead of tannin for the colorimetric, but not for the gravimetric, determination. W. R. S.

Rapid Determination of Tungsten, Chromium, and Vanadium in High-Speed Tool Steel. W. Brüggemann. (*Chem. Zeit.*, 1929, **53**, 927–928, 947–950.)—The scheme given is stated to permit of the determination of the three metals in one and the same portion within an hour. Steels containing much chromium carbide require special processes for their solution, and are excluded from this scheme. *Tungsten.*—The turnings, which should be as fine as possible or pounded to fine powder, are weighed into a 400 c.c. beaker (1 grm. for 8 per cent. W, to 5 grm. for 0·5 per cent.), and boiled with hydrochloric acid (200 c.c. of 1: 4 acid for 2, and 1: 3 acid for 7, per cent. W); when solution is attained, the liquid is boiled down to small bulk, but not far enough to cause precipitation of silica; addition of a few drops of hydrofluoric acid may be advantageous. The hot liquid is stirred and slowly oxidised by, drop by drop, addition of nitric acid. Sudden foaming and precipitation of yellow tungstic acid prove the oxidation to have been accomplished. Great care should be taken not to add more nitric acid than is strictly necessary, especially with low tungsten contents, in order to ensure quantitative precipitation. The liquid is boiled a few minutes and left on a hot plate till the greenish-yellow precipitate is pure yellow, diluted with 2 to 3 volumes of water, and again boiled. The precipitate is collected and washed with very dilute hydrochloric acid till iron-free, twice with water, and dried and ignited to WO_3 in a porcelain crucible. Unless the tungsten content is very small (in which case longer settling is required) the determination may be carried out in 30 minutes. If discoloured, the tungstic oxide may contain chromium carbide; purification involves fusion at very low temperature with sodium hydroxide, the insoluble residue from the extraction of the sodium tungstate being fused with sodium peroxide, and the acidified solution added to the main filtrate. *Chromium.*—The filtrate from the tungsten precipitation is evaporated with 10 to 20 c.c. of strong sulphuric and the same volume of syrupy phosphoric acid to the incipient fuming stage in a 750 c.c. conical flask. (Tungsten-free chrome-steel—0·5 grm. is directly dissolved in sulphuric and phosphoric acids and 150 c.c. of water, oxidised with nitric acid as before, and evaporated to the fuming stage; insoluble chromium carbide is dealt with as explained above.) The assay is diluted to 500 c.c., treated with 5 c.c. of 0·5 per cent. silver nitrate solution and 10 grms. of ammonium persulphate, and heated to boiling. The elements present are oxidised in the order Cr: V: Mn: C; hence the permanganate colour is an indicator for complete oxidation, and if the manganese content of the steel is small, it is advantageous to add a

little manganous sulphate. The excess persulphate is destroyed by about 30 minutes' boiling; the boiling is then continued after addition of a few c.c. of hydrochloric acid (1: 1), which decomposes the permanganic acid. The flask is cooled in running water and titrated with acid ferrous sulphate solution (30 grms. per litre), standardised against permanganate, till the colour changes to green; an excess of 1 to 2 c.c. should not be exceeded. Decinormal permanganate is slowly added to the cold solution: the vanadyl as well as the ferrous salt is now re-oxidised. The pink coloration must persist for one minute before the oxidation of the vanadium can be considered complete. The ferrous sulphate consumed in the reduction of the chromic acid is found by difference. *Vanadium.*—The titrated liquid is stirred and again treated with the ferrous sulphate solution in indefinite excess with respect to the vanadium content; vanadyl salt is once more formed. Ammonium persulphate (10 grms.) is now added in the cold, whereby the chromium is re-converted into chromic acid, and the excess ferrous sulphate oxidised, whilst the vanadyl salt remains unaffected. The cold liquid is titrated very slowly, as before, with 0·1 *N* permanganate; the end-point should persist for one minute. This titration registers the vanadium only. W. R. S.

Microchemical.

Microchemical Test for Barium with Sodium Tungstate. G. Denigès. (*Ann. Chim. anal.*, 1929, **11**, 365.)—When to a drop of a 1 per cent. solution of sodium tungstate is added a droplet of barium chloride of the same titre, characteristic crystals of barium tungstate are formed which, under high magnification, appear as octahedra, more or less elongated, often truncated at their ends and markedly refractive at their centre. They are often grouped in the form of sheaves, also refractive at their centre. The reaction is positive with solutions containing 1 or even 0·5 grm. of barium per litre. Under the same conditions calcium and strontium are only precipitated when their concentration is 4 or 5 times greater, and give spheroidal crystals unlike those of the barium compound. Furthermore, the latter adheres to the glass in a characteristic manner.

To identify barium in barium sulphate, a platinum loop is wetted and dipped in a little of the powdered sulphate. The loop is dried, held in the blue cone of a Bunsen flame, where the sulphate is reduced to sulphide, and then placed in contact with a drop of sodium tungstate solution on a glass slide. After 1 or 2 minutes the characteristic crystals appear. The presence of sulphide can be proved by adding to the drop on the slide a droplet of a 5 per cent. solution of sodium nitroprusside when the violet colour will develop. R. F. I.

Reviews.

ANALYTICAL PROCESSES: A PHYSICO-CHEMICAL INTERPRETATION. T. B. SMITH, A.R.C.S., B.Sc. Pp. viii+373. London: Edward Arnold & Co. 1929. Price 12s. 6d. net.

This excellent and stimulating book presents a critical examination of typical

analytical processes in the light of modern theories, the author taking the view that analysis is a branch of applied physical chemistry. The book is subdivided into two parts. Part I discusses the theoretical foundations of some typical processes, *e.g.* the precipitation of barium and lead sulphates, ferric hydroxide, zinc sulphide, and silver halides; electro-analysis; and volumetric methods (determination of chlorides and cyanides by silver nitrate, acid-alkali titrations, and oxidation-reduction processes). Part II gives a critical examination of the more important theories employed, namely, those relating to solubility, supersaturation, crystallisation, colloidal phenomena, complex ions, and oxidation-reduction reactions.

It will be apparent from the foregoing remarks that this is essentially a book for the study, not the laboratory. By its presentation and very full discussion of theoretical analytical chemistry, the volume is in refreshing contrast to a type of book which makes up a certain proportion of contemporary analytical literature. I refer to certain manuals intended for laboratory use, wholly, or almost wholly, devoted to manipulative details (not always reliable), and as likely as not in laboratory slang; the rule-of-thumb treatment of the subject would almost justify the trite appellative "cookery book."

In Mellor's *Treatise on Quantitative Inorganic Analysis* (London, 1913, p. xxix) may be found the quaintly-worded statement: "the need for humouring the different analytical processes is seldom taught in the schools." This passage may serve to remind us of the change that is coming over the teaching of analytical chemistry; it is well within the memory of the older professional man of to-day that his rather slender college training in the analytical art ran along more or less empirical lines, a high standard of work being attained only by a few specialists who had devoted much individual study to the subject. *Tempora mutantur*, and the average is now being raised through the dissemination, by books such as the one under review, of our progressive knowledge of the physico-chemical phenomena intervening in analytical operations.

The style is precise and lucid, though a rather common minor confusion in nomenclature occurs: $[Ag(NH_3)_2]$ is described as the "silver *ammonium* ion" (p. 175), or the "silver *ammonium* complex" (p. 323); "silver *ammonium* nitrate" is prepared from silver nitrate and ammonia (p. 311). Elsewhere the commonly accepted terms are used, *e,g.* the "*ammine*-forming capacity of aqueous ammonia" (p. 347), and "the concentration of an *ammine*" (p. 341). [Reviewer's italics.]

W. R. SCHOELLER.

CHEMISTRY IN DAILY LIFE. By S. GLASSTONE, D.Sc., F.I.C. Pp. 250, with 21 illustrations. London: Methuen & Co. 1929. Price 6s.

Interest in the modern applications of science to the problems of life and industry is steadily on the increase among the non-scientific public, and the present volume is intended to provide information and instruction, suited to the needs of the layman and the student, in the service rendered by chemistry to our daily life.

The comprehensive nature of the text may be gathered from the titles of a few

of the twenty chapters into which this is divided, these including "The Beginnings of Chemistry; Air and its Gases; Glass, Soap and Hydrocarbons; Our Daily Food; Catalysis and Enzymes; Fuels; Artificial Food and Clothing"; etc. The author has wisely refrained from limiting the reading matter to the province of chemistry only, and has devoted sections to the highly important subjects of the vitamins and insulin.

The subject-matter throughout is carefully selected, thoroughly up-to-date and sound, and consists of a pleasingly interwoven continuous narrative which is lucid and concise, whilst at the end of each chapter are given lists of questions relating to the contents, suggestions for essays and further study, reference books, and suggestions for experiments; but it is curious among the last-named to find visits to waterworks, iron foundries, dyeworks, etc., given. Whilst containing everything necessary for full comprehension by the reader, the text is free from redundancy, and the frequent references to other pages are in all cases correct. The illustrations, all of which are diagrammatic, are clear and free from unnecessary detail; and, although but little direct reference is made to some of them in the text, the description below or on the diagram is sufficient.

The text is unusually free from errors, typographic and otherwise, but on page 158 a small o occurs in the formula for carbon dioxide. The index, in spite of its numerous references, amounting to considerably over 700, is unfortunately inadequate to cover the wide range of subjects dealt with in the text. Several items, such as "thyroid gland," "ptyalin," "barley," "fire-fly," etc., are not included, and such omissions may cause a little difficulty to the less experienced reader. The care with which the proof-reading has been carried out is reflected in the high degree of accuracy shown by the text, and the page numbers indicated in the index, and the author has achieved commendable success in his difficult task of producing a volume adapted both to the requirements of the general reader and as a valuable aid in the teaching of chemistry to students. The resulting work is undoubtedly the best of its kind that has been published up to the present, and well deserves the attention of all interested in the development and application of chemistry to the many ramifications of our daily life. T. J. WARD.

SELECT METHODS OF METALLURGICAL ANALYSIS. By W. A. NAISH, Ph.D., A.R.S.M., F.I.C., and J. E. CLENNELL, B.Sc. With an Introduction by Sir H. C. HAROLD CARPENTER, F.R.S. Pp. xii+495. London: Chapman & Hall. Price 30s. net.

The 500 pages of this book are allotted in the following proportion:—Qualitative analysis, sampling, general methods of separation, etc., 69 pp.; selected methods for the determination of the elements, 248 pp.; analysis of commercial metals, 34 pp.; analysis of ores, slags, refractory materials, etc., with the analysis of coal, 22 pp.; electrometric titrations (by A. Hebdon), 4 pp.; analysis of minerals (by C. Stansfield Hitchen), 31 pp.; and spectrum analysis (by A. A. Fitch), 21 pp. There is also an appendix of tables and an index.

The preface and introduction make certain claims:—(*a*) The methods have

been chosen for their accuracy and general suitability; (*b*) most of them have been tried by the authors themselves; (*c*) the authors' aim has been to describe the methods in minute detail; (*d*) "References indicated in an extensive bibliography . . . which should adequately cover the demands of the metallurgical chemist."

If these claims were substantiated, they would go far towards the making of an ideal text-book of analytical chemistry, but, unfortunately, this is not the case. No indication is given as to which of the processes have been tried (except in perhaps two or three cases, *e.g.* p. 298), and even then, no figures are given to show the measure of success. The merit of the book, then, rests on an *ex cathedrâ* statement of various processes, and an examination of the accounts of these processes reveals the fact that they bristle with inaccuracies; a few of the latter are given below:—

On p. 12, mercuric sulphide is stated to be insoluble in sodium hydroxide, and sodium sulphide; and on p. 67 chromates, *after hydrogen sulphide treatment*, are stated not to be precipitated by ammonia. The statement (p. 171), that ferric iron can be quantitatively reduced by sulphur dioxide, is worse than useless, unless directions are given *re* acid condition. As most text-books recommend heating precipitated silica over the blow pipe for a considerable time, it is a little startling to find (on p. 252 [115*b*]) directions to "dry to constant weight" and "weigh as SiO_2." To give a factor 1 c.c. of $N/10$ iodine=·005935 grm. tin in a process which counsels reduction of stannic chloride with iron or nickel and "the addition of a small piece of marble before titration" (p. 285) does not look as if this were amongst the processes which "have been tried by the authors themselves." That this is not due merely to an oversight is proved by the fact that they recommend metallic tin and arsenious oxide as alternative standards for the setting of the iodine solution.

A very serious mistake (due presumably to careless proof-reading) in an otherwise good process, occurs on p. 344, where the words "cathode" and "anode" have been transposed; and on p. 345, directions are given for washing lead sulphate precipitate with hot water!

For the determination of oxygen in brass (p. 348), the authors recommend heating drillings at a dull red heat in a current of hydrogen; the loss in weight is supposed to represent the sum of the oxygen and sulphur. This is really a most remarkable process, for the following reasons:—

(*a*) The reaction $ZnO+H_2=Zn+H_2O$ is reversible, and the current of hydrogen does not remove more than a fraction of the water formed, since the reverse reaction is much the faster in the cooler parts of the tube.

(*b*) The vapour pressure of the zinc in 70: 30 brass at the temperature used (say 700° C.) is quite appreciable (600° 1·3—800° 29 mm. of mercury), and the loss in weight due to volatilisation of zinc alone might easily be several hundred times that due to reduction of ZnO (the oxygen content of brass is usually of the order of 0·004 per cent.). (See West, *J. Inst. Metals*, 1913, **10**, 375; and B. S. Evans and H. F. Richards, *J. Inst. Metals*, 1926, **35**, 173.)

Another serious error occurs on p. 369, where it is stated that 0·00135 grm. of phosphorus corresponds to 1·0 c.c. of *N*, not *N*/100, sulphuric acid.

In dealing with the determination of alkalis in slag, the authors advise evaporating the filtrate from the magnesia determination to dryness, "heating strongly," and "weighing as KCl+NaCl." What they consider happens to:

(*a*) The P_2O_5 from the excess ammonium phosphate in the solution;
(*b*) The alkali phosphates formed from the above;
(*c*) The alkali chlorides evaporated by "heating strongly"—does not transpire.

In view of the above examples, and others like them, one is inclined to endorse the caution on p. 73. "It is not necessary to determine the weight of the sample closer than to within 1/10 milligram."

A distinctive feature of the book is the bibliography at the end of the description of each element; an analysis of the contents of these bibliographies is, however, not altogether satisfactory. The number of references to quite a small number of text-books is out of all proportion to those to original papers. (*Treadwell* and *Hall* receives no less than 231 references in the bibliographies, quite apart from those in the text.) Of the references to original papers, those to old sources (say 1846–1866) are relatively common, those to anything so late as 1927, very rare; for instance, it seems ridiculous to give a reference to Herapath, 1853 (*re* thiocyanate colour with ferric salts, p. 178), and to miss out the Bureau of Standards work on the same subject in 1907.

Many sections might, with advantage, have been considerably expanded at the expense of others which are comparatively useless; for example, the complicated analytical chemistry of tantalum is dismissed in half a page. The one method given dates back to 1909, and no reference is made, in the text, to the work of Schoeller and his collaborators; on the other hand, the reader is told exactly how to titrate the alkali in pure borax, together with much similar information.

Mr. Hebdon's chapter on electrometric titration is clear and well written, but in the space of four pages it is obviously impossible to give more than an outline of a subject of growing importance.

The same criticism attaches to Mr. Fitch's chapter on spectrum analysis; exigencies of space have prevented the author from giving more than a very clear and interesting introduction to the subject. Mr. Stansfield Hitchen's excellent chapter on the analysis of minerals makes no claim to treat the subject fully "in the short space available"; in his aim of "giving helpful advice of a thoroughly practical nature" he has succeeded in a masterly manner.

The English of some of the earlier sections of the book has an irritating way of passing from imperative to passive in the middle of the description of a process. The repeated invitation (in the analysis of alloys) to add "a spot" of this or that reagent, has much to be said against it on one or two counts.

The general presentation of the book, printing, binding, illustrations, etc., are very good indeed, and there are very few obvious misprints; there are three or four cases of wrong words or figures being used (one of these is noted above), which may be either printing errors or slips in the manuscript. B. S. EVANS.