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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 2.30 p.m. on Wednesday, December 2nd, 1942, at the London School of Hygiene and Tropical Medicine, London, W.C.1. Dr. E. B. Hughes, President of the Society and Vice-Chairman of the Food Group, presided.

The subject of the meeting was "Flavours in Foods," and the following papers were read and discussed:—"The Naturally Occurring Flavours of Foodstuffs," by W. A. Waygood, B.Sc., A.R.C.S., F.I.C., and "Some Aspects of the Chemistry of Flavours," by T. F. West, M.Sc., Ph.D., F.I.C., read by Miss H. M. Perry, M.Sc., F.I.C., in his absence.

NEW MEMBERS

The following have been elected members of the Society:—Edward Samuel Bodkin; Herbert Dedicoat, A.I.C.*; Andrew Nicoll Harrow, A.H-W.C., A.I.C.†; Richard Selwyn Haskew, A.C.G.I., F.I.C.; Oscar Vincent Soane, B.Sc. (Glas.), A.I.C.; Francis Derek Williams, A.M.C.T., A.I.C.*

DEATHS

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

Charles Crocker. Frederick O'Brien. Charles Henry Kosier.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held on Friday, November 27th, 1942, at the Central Station Hotel, Glasgow. Mr. J. W. Hawley presided.

The following papers were read and discussed:—"An Investigation Relative to the Milk of an Abnormal Herd of Cows," by A. Scott Dodd, B.Sc., Ph.D., F.I.C., and R. Cowan; "The Estimation of Fibre in National Wheatmeal," by James Sword, M.A., B.Sc., Ph.D., F.I.C.; "Note on the Conversion of Calcium Oxalate into Oxide," by James Sandilands, F.I.C.

Obituary

FRANCIS GEORGE HENRY TATE

1878–1942

FRANCIS GEORGE HENRY TATE was the son of Charles James and Emily Tate, and those of us who met his parents can appreciate the degree to which his upbringing contributed to the breadth and sincerity of his outlook on life. Tate was a man of deep religious convictions and took an active and fruitful part in Church activities. He was fond of music and sport, was a keen cricketer, and was throughout his career a prominent member of the Customs Sports Club. As an organiser, Tate showed outstanding ability, and it is not too much to state that had he chosen a commercial career he would, without doubt, have attained a very high position in the community. One illustration of this will be found in the Exhibition in connection with the Faraday Centenary Exhibition at the Albert Hall in 1931, when Tate was instrumental in organising a section of the exhibits and demonstrations given on that occasion.

His contributions to technical literature were extensive and his published investigations have a lasting value. He devoted a considerable amount of time to the history and development of the testing of wines and spirits and was engaged on the writing of a monograph on the subject on his retirement from the position of Superintending Chemist at the Government Laboratory. The work on "Alcoholometry," published by H.M. Stationery Office, is a standard work on the subject. It achieved a marked success, the whole edition

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

being sold within a short time of its publication. Much of his investigations on this and kindred subjects was published in the *Wine and Spirit Trades Review* from time to time. A number of his articles will be found in the later editions of *Thorpe's Dictionary of Applied Chemistry*. The subjects range from alcoholic beverages to coffee, cocoa, and so on. Several investigations by Tate and his collaborators have been published in THE ANALYST and elsewhere; Tate and Warren's modification and extension of the Dean and Stark method for determining moisture in various materials are now accepted generally as one of the best procedures for using the method.

During his official career Tate was associated with the onerous investigation of the density of spirit-water mixtures now accepted as standard in this country. His knowledge of hydrometry was profound and authoritative. The new saccharometer for the determination of the gravity of beer, now known as the "Tate Saccharometer," is probably the best instrument of its kind for service use, being designed to overcome the defects due to bubbles and to give increased accuracy of readings. In order to avoid temperature difficulties in determining the strength of spirits in hot climates, Tate prepared Spirit Tables at 80°/80° F., and these were published as an official document in 1933.

During the latter part of his official career Tate was in charge of matters concerned with the Revenue on tobacco in the Government Laboratory. In this connection he initiated many investigations into the methods for the analysis of tobacco and devised equipment for the more accurate and rapid determination of tobacco constituents. His organising ability had full scope in these matters.

Tate's position in the chemical world was recognised by his election to the Fellowship of the Institute of Chemistry and by the confidence of his fellows in this Society, where he took an active part in its affairs on the Council. An illness just before the World War of 1914–18 prevented him taking the place he would have liked, but his keenness was such that he rose from the ranks of the Territorial forces of those days to active command of training in that organisation. A serious operation in recent years restricted his activities. Nevertheless, he became C.O. of the local platoon of the Home Guard and threw himself into his duties with characteristic energy. The words used by the Senior Officer of the area in which Tate served give us a picture of Tate as we knew him:

> "In spite of prolonged ill-health, he maintained his enthusiasm to the end, and continued to give invaluable service beyond the limit of his strength. It is not too much to say that he gave his life in the service of his country. . . ."

Those of us who knew Tate and valued him as a friend are glad to acknowledge this appraisement of his devotion and loyalty.

J. J. FOX

Analytical Studies on some 12-Heteropoly-acids. I. New Method for the Determination of Silica in Presence of Molybdic Acid

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

DETERMINATION OF SILICA IN COMPLEXES.—*Fusion Methods.*—Marignac,[1] who was the first to prepare silicotungstic acid, determined its silica content by a method based on fusion with alkali pyrosulphate. Parmentier[2] used the same procedure for silicomolybdates, but found that the silica separated out in quantities varying with the time of fusion and other factors. This led him to use another method based on fusion with only sufficient alkali carbonate to neutralise the molybdic acid present; too much or too little affected the accuracy of the results. This fusion procedure, however, was inapplicable to salts, such as those of barium or thallium, which form insoluble molybdates: these he fused with alkali pyrosulphate.

Volatilisation of Molybdenum Trioxide.—Asch[3] separated molybdenum trioxide from silicon dioxide by converting it into the volatile gas $MoO_3.2HCl$, a compound discovered by Debray[4]; the formation of this was applied successfully by Péchard[5] and by Smith and Oberholzer[6] to the separation of molybdic from tungstic acid, which does not form an analogous volatile compound with hydrogen chloride. Asch analysed several silicomolybdates by this method, and from most of them effected complete removal of

MoO_3. From magnesium, calcium and silver silicomolybdates, however, the removal was not quantitative, as small amounts of molybdic acid were tenaciously held in the residues, probably by the molten chlorides. He claimed to have removed unvolatilised MoO_3 from the magnesium and calcium salts by repeated digestion of the residues with conc. hydrochloric acid, followed by strong ignition in the air. From the silver salts, to which he assigned the formulae $2Ag_2O,SiO_2,12MoO_3.12H_2O$ or $4Ag_2O,SiO_2,12MoO_3.12H_2O$, according to the method of preparation, the residual MoO_3 could be removed by repeated digestion of the solid mass with hot ammonia. It was assumed that in this treatment the silica was not affected. Another method, also used by Asch, was based on the reduction of silver chloride at a high temp. with hydrogen to metallic silver, which was then dissolved in nitric acid and separated by filtration from the silica. It is obvious that these methods are inconvenient and cannot yield results sufficiently accurate to enable one, *e.g.*, to decide from the analysis if the silver salt is a normal or a basic salt, and accordingly whether the acid is octa basic or of lower basicity.

For the analysis of an insoluble salt, such as caesium silicomolybdate, the method in our hands failed to give concordant results. The caesium salt was preferred to the acid or other salts because of its smaller water content; moreover, this salt has been studied by X-ray methods[7] and is supposed to have the definite composition $Cs_3HSiMo_{12}O_{40}.5H_2O$ (the acid is assumed to be tetrabasic). We found considerable variations (up to more than 1%) in the amounts of molybdic acid volatilised in the current of hydrogen chloride gas after heating for 5–6 hrs. at about 400° C.; in view of the very low percentage amounts of silica present the method is therefore unsuitable. When the time of heating in hydrogen chloride was prolonged to *ca.* 8 hrs., the variations were greater, owing probably to volatilisation of some caesium chloride, although all the molybdenum trioxide could not be completely volatilised. Hence, we had recourse to another method.

Organic Complexes of Molybdic Acid.—It is known that molybdic acid forms more or less stable complexes with several organic oxy-acids, such as oxalic, tartaric, citric and malic acids, and very stable complexes with salicylic and α-hydroxynaphthoic acid, as well as with pyrocatechol. Having no data on the relative instability of these complexes and the instability of silicomolybdic acid, we could not easily decide with which of them the formation of silicomolybdic acid could best be prevented, to enable silica to be determined in presence of molybdic acid. The considerations discussed in Part II, however, which involve the electrometric determination of molybdenum, led us to choose between oxalic and tartaric acids. In the course of conductivity determinations at different dilutions, of molybdic and oxalic, or molybdic and tartaric acids, Rosenheim and Bertheim[8] observed that, whilst tartaric acid caused a considerable increase, oxalic acid caused a slight decrease in the conductivity of the mixtures as compared with the additive values for the conductivities of the single acids at the corresponding dilutions. The conductivities of the mixtures, however, approached the calculated additive values at greater dilutions, indicating that these complexes break down at low concentration.

Stability of Silicomolybdic Acid.—As regards the stability of silicomolybdic acid, it is obvious that, owing to the nature of silicic acid, such conductivity measurements for the purpose of comparison are not possible. It was found, however, that the silicomolybdate colour test follows the Beer and Lambert law even at very considerable dilutions, which indicates that the heteropoly-acid does not break down at these dilutions. Conductivity measurements at different intervals and over a longer period on dil. silicomolybdate solns. also showed remarkable constancy; thus, the following typical results were obtained with a soln. made by mixing 5 ml of 50% ammonium molybdate soln., 20 ml of 0·01 *M* sodium metasilicate soln. and 10 ml of *N* hydrochloric acid at 25° ± 0·01° C.:

$K \times 10^3$ after 10 min., 163·2; 1 hr., 162·8; 2 hrs., 162·6; 24 hrs., 161·3; 3 days 160·0.

The initial fading of the colour observable[9] soon after mixing the reagents cannot therefore be attributed to the decomposition of the complex; it is due to other factors which will be discussed in a subsequent communication.

Effect of Oxalic Acid on Stability of Complexes.—Notwithstanding these obvious variations in the stability of silicomolybdic acid on the one hand and of oxalomolybdic acid on the other, quite a different picture will be obtained when the silicomolybdate is compared with the oxalomolybdate complex in presence of a large excess of oxalic acid. Under such conditions the formation of the yellow silicomolybdic acid colour is partly or

wholly inhibited. The stability of the oxalomolybdate complex in presence of excess of either constituent is also manifested in the masking of the analytical reactions of the other constituent. Thus oxalic acid is not pptd. by calcium in acetic acid medium in presence of excess of molybdate. Feigl[10] observed also that oxalic acid is oxidised only very slowly, if at all, by permanganate under the same conditions. On the other hand, it was observed by Sterba-Böhm and Vostrebal[11] that molybdenum sulphide cannot be pptd. quantitatively from a soln. of molybdate containing oxalic acid, although they did not recognise that the incompleteness of the reaction was due to complex formation. We therefore decided to ascertain if silica in silicomolybdates could be determined while oxalic acid masked the molybdenum trioxide reaction.

In the first expts., solns. containing known amounts of metasilicate and molybdate were treated with excess of oxalic acid and with hydrochloric acid, and the silica was determined in the usual way. It was found, however, that the residues left on evaporation tended to become blue, and, when dissolved in water, formed solns. which became yellow. The amounts of silica found were not concordant and in most of the expts. were too low. It was, therefore, decided to use oxalic acid alone and to omit the hydrochloric acid. As a preliminary to this, however, it was necessary to prove that silica could be determined quantitatively by means of oxalic acid alone.

DETERMINATION OF SILICA BY MEANS OF OXALIC ACID.—For this purpose several parallel determinations of silica in a sodium metasilicate soln. were made, and it was found that either with pure recrystallised oxalic acid or with hydrochloric acid satisfactory results could be obtained, as is shown by the following results:

Silica determinated with oxalic acid, g ..	0·0270	0·0801	0·623	0·621	0·615	0·619
Silica determined with hydrochloric acid, g (mean of 3)	0·030	0·080	0·622	0·622	0·612	0·622

STABILISING EFFECT OF ACIDS.—It is also noteworthy that, whilst hydrochloric acid has a great tendency to stabilise low-molecular silica at low *p*H values, oxalic acid has not to any considerable extent. This was established by means of a procedure, previously used by one of us,[9] in which mixtures of oxalic acid and metasilicate solns. of different *p*H values (as obtained from a potentiometric titration curve) were subjected to ultrafiltration, using a high-pressure apparatus and membrane filters impermeable to Congo red soln. so as to retain all colloidally dispersed silica in the mixtures. The following results, each of which represents the mean of two determinations, show the equilibrium conditions at the given *p*H in a soln. originally molar with regard to the metasilicate.

Age of mixture, hrs. ..	24	2	24
*p*H ,, ,, ,, ..	6·0	2·8	2·8
Silica retained, %	96	38	*ca.* all (soln. turbid)

DETERMINATION OF SILICA.—In expts. to determine silica in presence of molybdic acid the residues left after evaporating the oxalic acid solns. showed a tendency to decompose, although to a less extent than when hydrochloric acid was present. A slight blue colour, becoming more intense on prolonged heating, always appeared on the upper parts which dried first on the side of the platinum vessel. Apparently this indicated that oxalic acid began to decompose at *ca.* 100° C., yielding carbon monoxide, which reduced molybdic acid to molybdenum blue. To avoid this, the solns. were evaporated to the min. vol. on the water-bath, care being taken to remove any solid residue that formed on the walls above the liquid by carefully rotating the vessel. The further evaporation to dryness was carried out in a vacuum desiccator provided with an electrically heated plate maintained at 60–70° C. The dried, completely white, residue was always kept for a few hrs. at this temp. *in vacuo* before being taken up in the min. amount of water After filtration and washing with a hot soln. of 2 *N* oxalic acid, a second evaporation was made in the same manner. The amounts of silica obtained after a second filtration usually weighed 3–5% of the total quantity present. A third evaporation was usually unnecessary. It should be noted that the success of the method depends on adding a suitable excess of oxalic acid to prevent the formation of the heteropoly-complex. In presence of 100 mg of MoO_3 it is advisable to add 3–5 g of oxalic acid. The amount required also depends on the vol. of the liquid in which the residue is taken up. Moreover, care must be taken not to over-heat the residue, or a blue tinge will immediately appear. If the two conditions are fulfilled, *viz.*, addition of an appropriate excess of oxalic acid and evaporation at a relatively low temp., silica may be determined with great accuracy. If the optimum

conditions are maintained, there will be no indication of a yellow tinge in the filtrate. The following results were thus obtained in the determination of silica in an alkaline soln. containing SiO_2 and MoO_3 in the molecular ratio 1 : 12.

Silica present, g per 100 ml ..	0·1293							
Silica found ,, ,, ..	0·1285	0·1303	0·1290	0·1305	0·1301	0·1295	0·1300	0·1288

With acid solns. or insoluble silicomolybdates it is advisable to discharge the colour (or dissolve the ppt.) in alkali hydroxide soln. before adding oxalic acid, as otherwise the decomposition of the complex will be very slow and the addition of a considerable amount of oxalic acid will be required.

In presence of calcium or magnesium and molybdic acid, it is necessary to add a few drops of conc. hydrochloric acid after evaporating the soln. as described.

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March, 1942

The Preparation and Analysis for Iron of Haemin and Haemoglobin

BY G. E. DELORY, M.SC.

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

MOST methods of estimating haemoglobin are standardised by reference to a determination of the oxygen-carrying power of the blood. This procedure requires elaborate apparatus, a skilled technique, and experience in gas analysis. Another serious objection is that some of the pigment may be present as inactive haemoglobin which does not react with ferricyanide. According to Amundsen,[1] this inactive haemoglobin, which is chiefly composed of carboxy-haemoglobin, met-haemoglobin and sulph-haemoglobin, may, under the conditions of town life, amount to 2–12% of the total haemoglobin present. A further objection to the commonly used methods, such as those of Haldane[2] and Sahli,[3] is that the standards fade somewhat readily, and the standard used in one laboratory often differs markedly from that used in another.

The alkaline haematin method recently described by Clegg and King[4] avoids these difficulties by using as a standard a soln. of alkaline haematin prepared from crystalline haemin. To investigate this method fully, it was necessary to prepare, and analyse for iron, samples of haemin and haemoglobin. The methods used and the results obtained are described below.

PREPARATION OF HAEMIN.—Allow 1 litre of citrated blood to settle until there is a clear supernatant fluid. Siphon off the plasma and to the remainder add sodium chloride soln. (0·9 g per 100 ml) to give a vol. of 800–900 ml. Strain the mixture through muslin into a separating funnel. Heat 2 litres of glacial acetic acid with 100 ml of water and 0·25 g of sodium chloride and run the blood in slowly with continuous stirring. Keep the mixture at 90° C. for 15 min., and let it cool and stand overnight. Siphon off most of the supernatant fluid and re-suspend the crystals in dil. acetic acid. Leave for several hrs., remove most of the supernatant fluid again, add water, and filter off the haemin crystals on a Buchner funnel. Wash them on the filter with dil. hydrochloric acid and then with water, and dry them in a warm place. If great purity is required, the haemin may be purified by the method of Fischer[5] as follows. Dissolve 5 g of the crude haemin in 25 ml

of pyridine in a 100-ml Erlenmeyer flask, add 40 ml of chloroform and shake for 15 min. Collect on a Buchner funnel and wash with 15 ml of chloroform. Pour the filtrate in a steady stream into a mixture of 350 ml of glacial acetic acid and 4 ml of conc. hydrochloric acid, previously heated to boiling, and rinse the suction flask with 15 ml of hydrochloric acid. Leave for 12 hrs. and filter through a Buchner funnel. Wash the ppt. successively with 50 ml of 50% acetic acid, 100 ml of water, 25 ml of alcohol and 25 ml of ether and finally dry by suction. A sample of haemin thus prepared had the following ultimate composition (Weiler and Strauss): C, 62·2; H, 5·09; N, 8·84; Fe, 8·60; Cl, 5·46%. Theoretical figures for $C_{34}H_{32}O_4N_4FeCl$ are C, 62·6; H, 4·95; N, 8·60; Fe, 8·57; Cl, 5·43%.

PREPARATION OF HAEMOGLOBIN.—Centrifuge 400 ml of blood in two pots, pour off the supernatant fluid and wash the cells twice by decantation with sodium chloride soln. (0·9 g per 100 ml). Haemolise the blood-cells in each pot by adding 5 ml of water, 5 ml of ether and a vol. of aluminium hydroxide cream equal to that of the blood-cells. It is claimed (Marshall and Welker[6]) that all the proteins except haemoglobin can be thus removed. Shake the mixture and centrifuge off the cream. Decant the supernatant layer into a flask and place it in a refrigerator until the temp. is 0° C. Next add abs. alcohol, also cooled to 0° C., to make a final concn. of 25–30%. Leave the flask overnight in a cold place, filter off the pptd. haemoglobin on a Buchner funnel, wash twice with cold 25% alcohol and dry in a vacuum desiccator.

ANALYTICAL PROCEDURES.—Two methods for the determination of iron have been employed: (1) A volumetric method depending on titration with titanous chloride. (2) A colorimetric method depending on measurement of the red colour given by αα′-dipyridyl with ferrous iron. The first method is the more accurate, but requires larger initial amounts of material. The second method is as accurate as the most precise colorimetric procedures and requires only 50μg of iron, equiv. to about 0·6 mg of haemin or 17 mg of haemoglobin. The methods used and the reasons for their choice are discussed first and the full experimental details follow.

(1) VOLUMETRIC METHOD.—(*a*) *Discussion.*—The titanous chloride method for the determination of iron has long been a standard procedure described in analytical textbooks (*cf.* Treadwell and Hall[7]). In the classical form of the method the titanous chloride is kept in an atmos. of hydrogen or carbon dioxide, necessitating the use of a somewhat elaborate and cumbersome apparatus. If, however, a small amount of the commercial soln. is diluted and used without delay, special precautions are unnecessary.

In preliminary expts., the weighed haemin or haemoglobin was placed directly in a muffle furnace, but this procedure proved unsatisfactory, as some of the iron was carried away in the smoke. This was avoided by preliminary addition of a mineral acid and gentle evaporation.

(*b*) *Detailed Method.*—Weigh accurately *ca.* 50 mg of haemin or 1 g of haemoglobin into a 100-ml Pyrex beaker. Moisten with 2 *N* nitric acid and heat over a mild Bunsen flame or on an electric heater until charring is complete. Place in a muffle furnace at 400° C. overnight; alternatively, heat the beaker between two evaporating basins over a strong Bunsen flame for a few hrs. After cooling, dissolve the ash by warming gently with about 10 ml of conc. hydrochloric acid, dilute with an equal vol. of water, and titrate with titanous chloride soln., as follows:

Dilute a suitable vol., usually 2 ml, of stock titanous chloride soln. to 100 ml with *N*/10 hydrochloric acid. Mix thoroughly and with this soln. fill a 50-ml burette. No more titanous chloride must be put into the burette during the determination. Pipette 5 ml of a standard iron soln. (1 mg of Fe per ml) into a flask, add 1 ml of 20% potassium thiocyanate soln., and run in the titanous chloride soln. until the red colour disappears. Titrate the test solns. in the same way, and then do a further standard titration to make sure that the titanous chloride soln. has not changed in strength during the procedure.

(*c*) *Sample Analyses.—Crystalline Horse Haemoglobin*:

Standard soln. containing 5 mg of Fe required 12·55 ml $TiCl_3$ soln.
(1) 1·0162 g of haemoglobin sample required 8·25 ,, ,, ,,
(2) 1·3650 g ,, ,, ,, ,, 10·98 ,, ,, ,,
Fe in sample: (1) $(8{\cdot}25 \times 5 \times 100)/(12{\cdot}54 \times 1{\cdot}0162 \times 1000) = 0{\cdot}324$ g/100 g
(2) $(10{\cdot}98 \times 5 \times 100)/(12{\cdot}54 \times 1{\cdot}3652 \times 1000) = 0{\cdot}321$ g/100 g

Haemin:

Standard soln. containing 5 mg of Fe required 5·92 ml $TiCl_3$ soln.
5·90 ,, ,, ,,
(1) 64·8 mg of haemin sample required .. 6·38 ,, ,, ,,
(2) 61·7 mg ,, ,, ,, ,, .. 6·10 ,, ,, ,,

Fe in sample: (1) $(6{\cdot}38 \times 5 \times 100)/(5{\cdot}91 \times 64{\cdot}8) = 8{\cdot}34$ g/100 g
(2) $(6{\cdot}10 \times 5 \times 100)/(5{\cdot}91 \times 61{\cdot}7) = 8{\cdot}33$ g/100 g

(2) COLORIMETRIC METHOD.—(*a*) *Discussion.*—For many years it has been known that the iron in the ash of blood could be estimated colorimetrically, *e.g.*, by measurement of the blue colour formed with ferrocyanides or of the red colour with thiocyanates. Many methods have been based on the latter reaction during the past 40 years, the most widely used being that of Wong.[8] Difficulty in obtaining correct results, however, have been frequently reported (Breuer and Militzer,[9] Coombs,[10] and Jenkins and Thomson[11]). The main sources of error recorded are the fading of the ferric thiocyanate, the development of turbidity in the coloured test solutions, and, in some methods, the problem of separating the iron from the rest of the haemoglobin molecule without loss. These difficulties are avoided in the method of Thorp,[12] who used the colour given by dipyridyl with ferrous iron. His published procedure, which has been supplemented by a personal communication, was chosen as the basis of the micro method to be described. It may be objected that the dipyridyl is very expensive, but 1 g is sufficient for 100 determinations and the high cost will be very considerably reduced as the demand for the substance becomes greater. Methods based on the colour with thioglycollic acid are quite accurate, but the lack of sensitivity prevents their use in a micro-method.

(*b*) *Detailed Method.*—Pipette 5 ml of the haemin soln. containing about 0·025 mg of iron into a 10-ml flask; into a second similar flask pipette 5 ml of standard iron soln. (0·025 mg Fe), and into a third pipette 5 ml of water. Place in each flask 0·15 ml of 60% perchloric acid and 0·2 ml of hydrogen peroxide (100 vols.). Heat the flasks on an electric heater or in a boiling water-bath until the solns. are clear and colourless. After cooling, add 1 ml of the dipyridyl reagent and then 3 ml of 42% sodium sulphite soln. Dilute to the mark with water, mix, and after 15 min. standing read the colour of the solns. in a colorimeter (the photo-electric colorimeter described by King[13] is particularly useful for the method outlined). The blank solution prepared from the 5 ml of water is used to set the zero in the colorimeter.

(*c*) *Sample Analysis on 5 ml of a 61·1 mg/litre Haemin Solution.*

Reading of standard, 23·5; of test (1), 24·0; of test (2), 24·0.

$$\text{Fe in sample: } \frac{24{\cdot}0 \times 0{\cdot}025 \times 1000 \times 100}{23{\cdot}5 \times 5 \times 61{\cdot}1} = 8{\cdot}35 \text{ g/100 g.}$$

SOLUTIONS REQUIRED FOR THESE METHODS.—(1) *Standard Iron Solution* (1 *mg per ml*).—Dissolve 4·318 g of ferric ammonium sulphate, $Fe_2(NH_4)_2(SO_4)_4{,}24H_2O$, in about 250 ml of water in a 500 ml flask; add 50 ml of iron-free HCl, and dilute to the mark with water.

(2) *Dilute Standard Iron Solution* (0·005 *mg per ml*).—Pipette 5 ml of the stock soln. (1) into a 1-litre flask together with 100 ml of *N* HCl, and dilute to the mark.

(3) *Stock Titanous Chloride Solution.*—A commercial preparation containing 15% of $TiCl_3$.

(4) *αα′-Dipyridyl Soln.*—1 g in 100 ml of *N*/10 HCl.

(5) *Potassium Thiocyanate Soln.*—20 g of KCNS per 100 ml in water.

(6) *Sodium Sulphite Soln.*—42 g of $Na_2SO_3{\cdot}7H_2O$ per 100 ml in water.

(7) *Hydrogen Peroxide.*—100 vols., analytical reagent quality.

(8) *Aluminium Hydroxide Cream.*—Treat 1 g of ammonium alum dissolved in 100 ml of water at room temp. with a slight excess of ammonium hydroxide (1%). Centrifuge off the ppt., wash three times by decantation with water, and suspend in 100 ml of water.

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THE BRITISH POSTGRADUATE MEDICAL SCHOOL
HAMMERSMITH HOSPITAL, L.C.C.,
DUCANE ROAD, SHEPHERD'S BUSH, W.12

September, 1942

DISCUSSION

Mr. N. L. ALLPORT expressed some surprise that Mr. Delory did not use the thioglycollic acid method for colorimetric estimation. He did not think that it was much less sensitive than αα′-dipyridyl as a reagent for iron; in his experience the sensitivity of the two reagents was approximately the same. Dipyridyl was expensive and not readily obtainable, mainly owing to difficulties attendant upon its commercial production. He wondered why the author destroyed the organic matter of the haemin and haemoglobin samples by ignition rather than wet oxidation. Was there any objection to Wong's technique in which the blood was heated with a mixture of sulphuric acid and potassium sulphate?

Mr. R. W. SUTTON asked if the coloured complex with αα′-dipyridyl could be extracted with immiscible solvents.

Mr. R. H. THORP, replying, said that, whilst at present αα′-dipyridyl was not readily obtainable, it was not difficult to prepare, and a yield of the order of 25–30% had been obtained by the method of Wibaut and Overhoff (*Rec. Trav. Chim. Pays-Bas*, 1928, **47**, 761). The thioglycollic acid method was not as sensitive as the dipyridyl method—at least 10 times less sensitive. Moreover, the absorption bands of the blue colour of the thioglycollic complex were more dispersed and not nearly as suitable for colorimetry as the precise band, centred at 525μm, of the αα′-dipyridyl iron complex. He had not yet found an organic solvent for this red complex; if one could be discovered that was immiscible with water, it would prove of immense value.

Dr. E. J. KING exhibited a photoelectric colorimeter suitable for use with the colorimetric method described.

The Spectrophotometric Determination of Vitamin A in Fish-liver Oils

BY J. I. M. JONES, M.SC., F.I.C., AND R. T. HAINES, M.A.

(*Read at the Meeting, November* 4, 1942)

SECTION II of the Medical Research Council's Special Report, No. 202, on the Standardisation and Estimation of Vitamin A (p. 28) deals with certain investigations into the spectrophotometric determination of vitamin A by measurement of the intensity of absorption at 328mμ. The observation of previous workers (Drummond and Morton,[1] Coward, Dyer *et al.*[2]) that the gross absorption of the whole oil was greater than that of the unsap. fraction is confirmed in the report in question and this is attributed to absorption by substances other than vitamin A which are removed by saponification. It is stated that since re-saponification causes no further diminution of absorption, there is no loss of vitamin A during the saponification treatment. It is also stated that the irrelevant absorption removed by saponification bears no constant relationship to the vitamin A present, *i.e.*, to the gross or residual absorption.

We have been led to investigate this matter more closely and have accumulated evidence that for oils from the same species extracted by similar methods, there is a strong correlation between the absorption of the whole oil and of its unsap. fraction. Though there is indubitable irrelevant absorption in certain cases, the evidence tends to show that the nature of the oil plays an important part.

Investigations on various fish-liver oils have been made and data are given below for cod-liver oil, and shark, halibut and blue fin tunny-liver oils, these being the only species of which a sufficient number of samples have as yet been examined. These data, presented in the tables below and in Figs. 1 to 4, cover the following ranges of vitamin A potency:

Cod-liver oil	58 samples.	700–3000 Int. Units per g
Shark-liver oil ..	24 ,,	12,000–80,000 ,, ,, ,, ,,
Halibut-liver oil ..	21 ,,	25,000–80,000 ,, ,, ,, ,,
Blue fin tunny-liver oil	12 ,,	33,000–96,000 ,, ,, ,, ,,

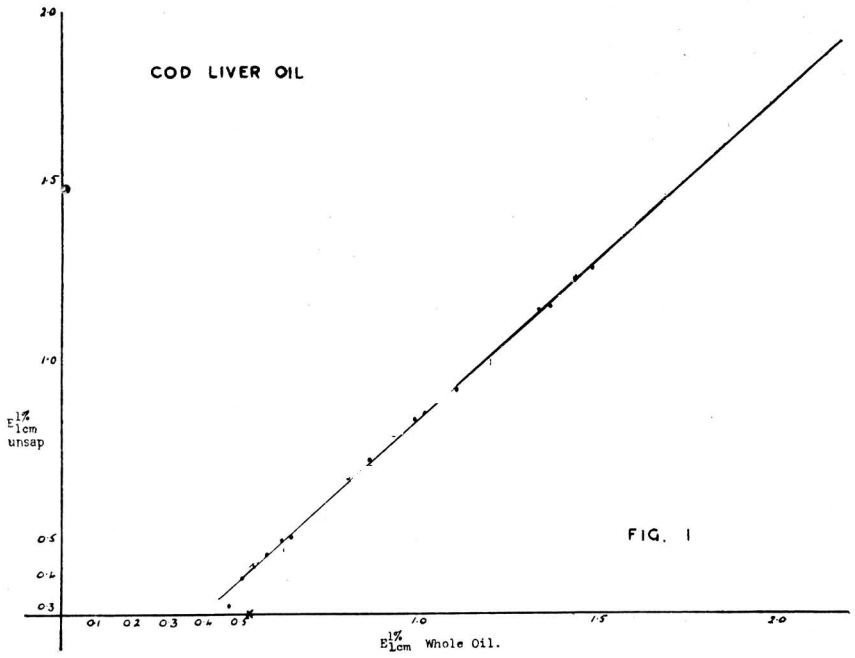
COD LIVER OIL
2·0
1·5
1·0
0·5
0·4
0·3
$E^{1\%}_{1cm}$ unsap
0·1 0·2 0·3 0·4 0·5
1·0
1·5
2·0
$E^{1\%}_{1cm}$ Whole Oil.

FIG. I

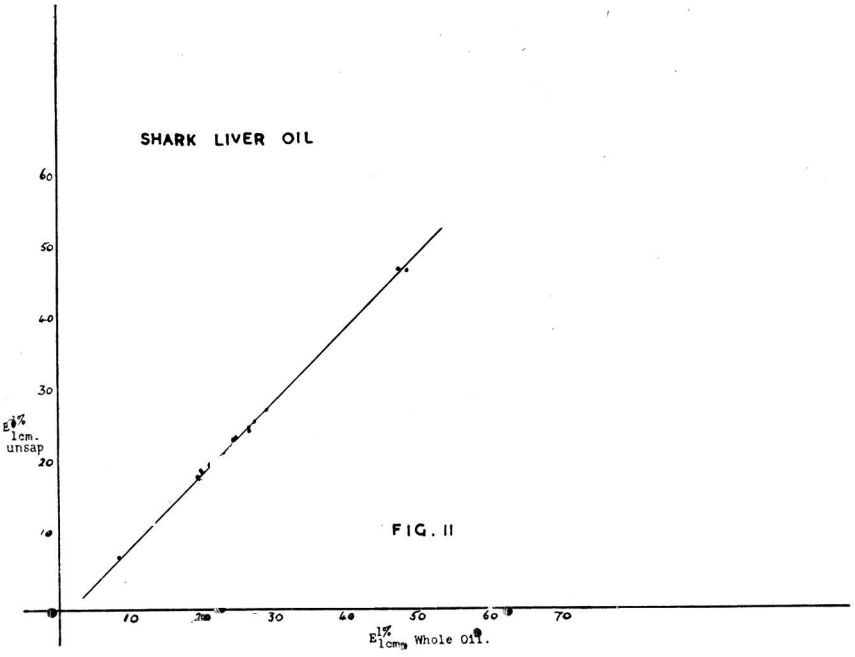
SHARK LIVER OIL
60
50
40
30
$E^{1\%}_{1cm.}$ unsap
20
10
10
30
40
50
60
70
$E^{1\%}_{1cm.}$ Whole Oil.

FIG. II

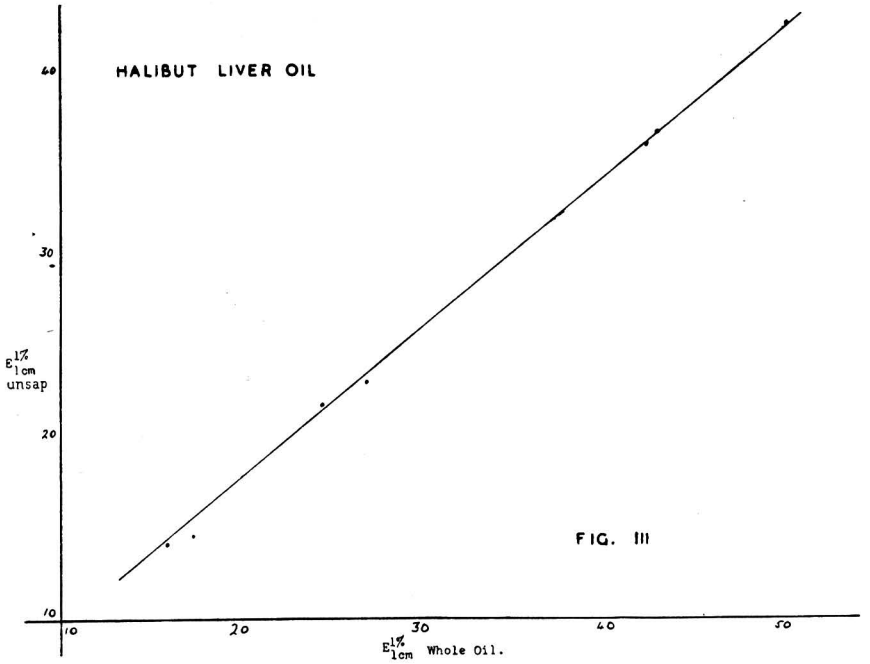

FIG. III

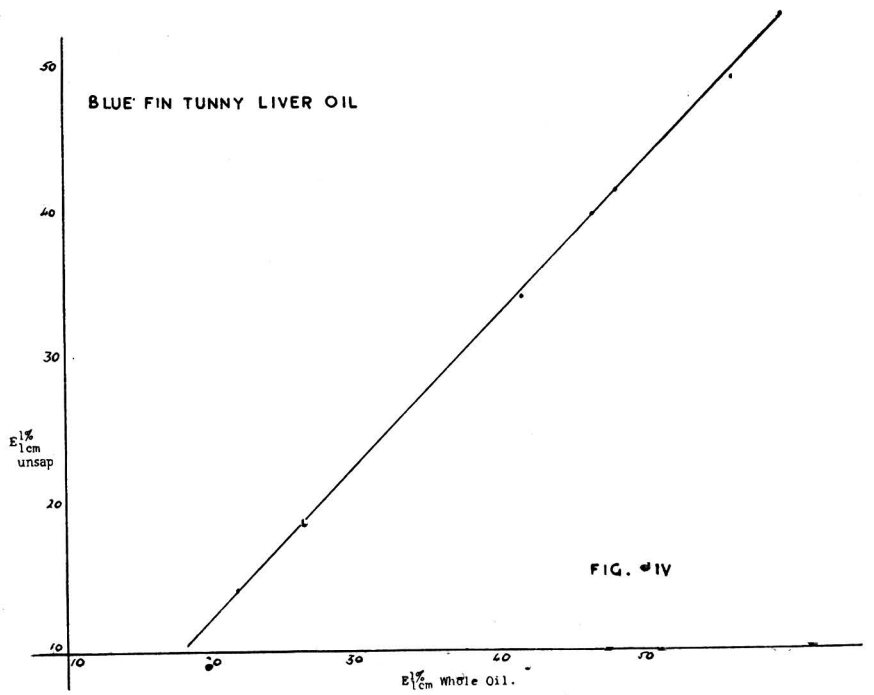

FIG. IV

Absorption was measured by means of a Hilger small quartz ultra-violet spectrograph fitted with a Spekker photometer. Match points at 328$m\mu$ were determined either from enlarged prints made from the plates or directly from the plates under the microscope. The instrument was regularly checked with an N.P.L. standardised Wood's glass.

The method of saponification was as follows:—Approx. 1 g of the oil was weighed into a 100-ml round-bottomed flask fitted with an air condenser (ground-glass joint), and 1·25 ml of 56% aqueous potassium hydroxide soln. and 10 ml of alcohol were added. The mixture was boiled on a sand-bath for 20 min. after which 40 ml of cold water were added. The cooled mixture was then extracted 3 times with freshly distilled ether (50, 30, 30 ml), the united extracts were washed once with 40 ml of distilled water, once with 40 ml of *N* sodium hydroxide soln. and finally with water until the washings were neutral to phenolphthalein. The ether was removed *in vacuo* and the residue was dried by evaporating once or twice with a few drops of abs. alcohol. The dry residue was then dissolved in cyclohexane, and the soln. was diluted as required and examined spectrographically.

Figs. 1 to 4 show at a glance that there is a marked linear correlation between the absorption of the whole oil and its unsap. matter. The correlation for halibut liver oil is not so clear from the figure as for the other oils and the spread of values is restricted. The correlation co-efficients are as follows:

Series	No. of samples	r for $E^{1\%}_{1\,cm}$	For P = ·05 r is significant if it exceeds
Cod-liver oil	55	0·985	0·25
Shark-liver oil ..	24	0·998	0·423
Halibut-liver oil ..	21	0·967	0·433
Blue fin tunny-liver oil	12	0·988	0·576

In these series there is a significant difference between the correlation in the shark-liver oil series and the other three series, which do not differ significantly in themselves. This is also shown in the average percentage recovery of vitamin A in the saponification, which was as follows:—Cod-liver oil, 84·5; halibut-liver oil, 86·4; blue fin tunny-liver oil, 86·5; shark-liver oil, 96·2%.

In the M.R.C. Report 202 (Table V, p. 31) a series of six results is given. These have been plotted in Fig. 1 and are represented by ×. It is obvious that 5 of these samples lie close to the regression line calculated for our own series. The sixth sample is a crude cod oil of the type generally obtained by sun rotting of the livers. This process produces very dark oils with high fatty acid contents containing products of biological decomposition. A sample of crude cod-liver oil examined by us showed similar divergence in the data from those for cod-liver oil extracted by modern methods.

The equations for the regression $E^{1\%}_{1\,cm}$ for unsap. matter on $E^{1\%}_{1\,cm}$ for the whole oil are as follows:

Cod-liver oil series	$y = 0{\cdot}8933x - 0{\cdot}0474$
Shark-liver oil series	$y = 1{\cdot}0163x - 1{\cdot}3680$
Halibut-liver oil series	$y = 0{\cdot}8214x + 1{\cdot}4776$
Blue fin tunny-liver oil series ..	$y = 1{\cdot}0293x - 7{\cdot}7986$

x and y being the $E^{1\%}_{1\,cm}$ values for whole oil and unsap. fraction respectively.

These equations are of the general straight line form: $y = bx - c$, and their consideration raises two questions, *viz.*, (i) is the slope b significantly different from unity? (ii) is the intercept c significantly different from zero?

If there were no experimental or other loss, b would be unity. If b differs significantly from unity, it implies that there is a loss related to the vitamin content. Statistical examination shows that b is significantly different from unity with cod and halibut-liver oils, but not with shark and tunny-liver oils. If the intercept c is significantly different from zero, *i.e.*, if the line fails significantly to pass through the origin, it implies that there is a constant fixed loss. It is found that for shark and tunny liver oils, c is significantly different from zero, but for halibut and, possibly, cod, it is not so. It is perhaps possible for both these factors to operate together.

The interpretation of these phenomena is somewhat a matter of speculation. Among the possibilities are: (1) the presence of "irrelevant" absorbing matter in fixed relationship to the vitamin A content, or (2) errors inherent in the analytical process. Such errors might be due to destruction or other loss of vitamin A during saponification and extraction. During saponification there is the possibility of adsorption of the vitamin by the resulting soap soln. That such adsorption does occur is well known to those experienced in the

manufacture of Vitamin A concentrates. Brocklesby and Kuchel[3] have shown that the relationship between the vitamin A potency of the original oil and the amount of vitamin A removed by the soaps formed during saponification has the essentials of an adsorption isotherm.

Variations in the saponification procedure produced no variation in the final result as shown by the following data:

TABLE I

Procedure	$E^{1\%}_{1\,cm}$	
(*a*) Standard saponification	0·926, 0·898	Mean 0·912
(*b*) KOH and time of boiling doubled	0·886, 0·891	Mean 0·889
(*c*) Saponified cold for 30 min. with sodium ethylate and abs. alcohol	0·905	0·905

The fact that the unsap. matter does not show further loss of absorption at 328$m\mu$ when re-subjected to the saponification treatment is explicable by the fact that no soap is formed on re-saponifying. In the presence of soap there is a further reduction in absorption as shown by the following results, which provide confirmation that adsorption by soap occurs during the analytical process.

TABLE II

Material	$E^{1\%}_{1\,cm}$			Relative values
1. Whole oil	1·072,	1·088	Mean 1·080	100
2. Unsap. matter..	0·926,	0·898	0·912	84·5
3. Unsap. matter re-saponified	0·870,	0·898	0·884	81·8
4. Unsap. matter re-saponified in presence of fatty acid	0·751,	0·756	0·754	69·8

Proof that the extraction process is sufficient to remove the readily available vitamin A is afforded by the fact that when it is repeated on the residual soap solution, the $E^{1\%}_{1\,cm}$ is increased by only 0·01–0·02. The difference between the values for the unsap. matter before and after re-saponification is considered not to be outside the experimental error and shows that vitamin A is not destroyed by the alkali treatment. Approx. 1 g of oil was used in each determination. For the re-saponification in presence of fatty acid, 1·5 g of a 1:1 mixture of pure oleic and stearic acids was added to the unsap. matter from approx. 1 g of the oil before re-saponification. The possibility of adsorption of vitamin A by the soap was examined by pptng. the soap as the barium salt after 2 extractions with ether and re-extraction of the barium soap. The $E^{1\%}_{1\,cm}$ values so obtained were 0·013 and 0·020, showing that practically no further vitamin A is recovered by this treatment.

Further tests showed that the fatty acids from the soap after two extractions with ether did not give any blue colour in the Carr–Price test. One is therefore led to the conclusion that destruction of vitamin A had occurred during saponification in presence of fats or fatty acids. The differences already mentioned indicate that the nature of the fats is important, and this is confirmed by expts. in which unsap. matter from one class of oils was dissolved in oils of a different class and the effect of saponification examined.

Shark and halibut oils were used in these expts. These oils show after saponification an average $E^{1\%}_{1\,cm}$ value at 328$m\mu$ of 96 and 86%, respectively, of the values of the original oils. If this is true, then when halibut-liver oil unsap. matter is dissolved in shark liver oil the enhanced E value should fall only to *ca.* 96% of its value on saponification, and conversely, shark oil unsap. matter dissolved in halibut-liver oil should show a reduction to about 86% on saponification. Table III gives results which confirm this, and show that the nature of the unsap. matter is not an important factor.

TABLE III

Mixture		$E^{1\%}_{1\,cm}$		Vitamin A recovered, %	Unsap. %
Oil	Unsap. from	Whole mixture	Unsap.		
Shark liver	Shark liver	50·8	48·75	96	11·5
,, ,,	,, ,,	126·2	116·0	92	11·1
,, ,,	Halibut ,,	60·4	59·8	99·2	18·95
Halibut ,,	Shark ,,	100	69·6	69·6	18·17
,, ,,	,, ,,	180	157·8	87·7	33·3

Further evidence of the effect of the nature of the fatty material is afforded by the analysis of mixtures, as shown in Table IV. Apparently, even a small proportion of one oil produces a large effect.

TABLE IV

Mixture	$E^{1\%}_{1cm}$ Whole mixture	$E^{1\%}_{1cm}$ Unsap.	Vitamin A recovered, %
Halibut-liver oil 10% / Shark ,, ,, 90%	31·4	26·3	83·7
Halibut ,, ,, 25% / Shark ,, ,, 75%	29·8	26·01	87·3
Halibut ,, ,, 20% / Shark ,, ,, 64% / Dogfish ,, ,, 16%	21·8	18·7	86·7

Dogfish-liver oil appears to belong to the same group as shark oil. The few samples examined showed a recovery of 98 to 99% after saponification.

At present no satisfactory explanation of these phenomena is available. Further investigation of the effect of the nature of the fatty material is in progress. The observations, nevertheless, have a bearing on the work of the analyst, who may be led to erroneous conclusions, particularly with regard to oils in the lower range of potencies.

SUMMARY AND CONCLUSIONS.—A strong correlation has been observed between the $E^{1\%}_{1cm}$ values for absorption at $328m\mu$ for the whole oil and the unsap. matter for several species of fish-liver oils. Evidence is presented that this is connected with the nature of the oil and usually saponification leads to a fall in the $E^{1\%}_{1cm}$ value which, it is suggested, is due to destruction of vitamin A. The exact cause of this loss has not been ascertained and is the subject of further investigation.

Our thanks are due to Mr. E. C. Fieller, M.A., for valuable advice on the statistical treatment of the data, and to the Directors of British Colloids (The Crookes Laboratories) for permission to publish this work.

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See also

Chambers, *"Statistical Calculation for Beginners,"* London, 1941.
Fisher, R. A., *"Statistical Methods for Research Workers,"* London, 1941.

THE CROOKES LABORATORIES, GORST ROAD, PARK ROYAL, LONDON, N.W.10 *August*, 1942

DISCUSSION

Mr. NORMAN EVERS asked if the reduction in the absorption might not be due to oxidation of the fatty acids during saponification, and if the authors had observed the same results when the process was carried out in an atmosphere of nitrogen.

Dr. E. LESTER SMITH said that, as the characteristic vitamin A absorption was displayed by aqueous or alcoholic dilutions of saponification mixtures, an attempt could be made to assess destruction of vitamin A during saponification independently of loss during extraction with ether. The result might be inconclusive, however, because the intensity of absorption was known to be slightly affected by the nature of the solvent. Nevertheless, in preliminary expts. with halibut viscera oil, the same values for $E^{1\%}_{1cm}$ were given by cyclohexane solns. of the oil and aqueous solns. of the saponification mixture. As organic peroxides were known to be destructive to vitamin A on heating, was it possible that oils showing losses on saponification and extraction might perhaps have high peroxide values?

Mr. HAINES, replying, said that he did not think that oxidation of the fatty acid could be responsible for a loss in ultra-violet absorption. Previous experience had shown that working in an atmosphere of nitrogen did not affect the values obtained after saponification. Referring to the question of peroxides in the oils, he said that it had not been investigated. The oils, however, were of good quality with low free fatty acid content; there appeared to be no association of vitamin recovery with free fatty acid content, which should show some relationship to peroxide values.

Notes

A FLUORESCENCE TEST FOR β-INDOLE ACETIC ACID

IN the course of work in our laboratory on the detection of auxins in fertilisers a new test for β-indole acetic acid was desired. During a search for such a test it was found that when β-indole acetic acid was treated with conc. sulphuric acid and gently heated, a pale brownish-green soln. was obtained, which was slightly fluorescent in visible light and under ultra-violet light showed characteristic intense yellowish-green fluorescence.

The test is best made as follows: Treat 1 mg, or less, of the substance suspected to be β-indole acetic acid in a dry test tube with 3 ml of conc. sulphuric acid, and heat in boiling water for about 10 min. Cool and examine the tube and its contents under ultra-violet light, using a Wood's glass filter. A strong yellowish-green fluorescence indicates the presence of β-indole acetic acid. This test is very sensitive,

and will detect as little as 0·02 mg of β-indole acetic acid. If the sulphuric acid soln. is poured into 50 ml of cold water the resulting aqueous soln. will give the same yellowish-green fluorescence in ultra-violet light. This procedure has been found useful, if, owing to the impurity of the β-indole acetic acid, appreciable charring occurs when the sulphuric acid is heated.

Indole does not respond to the test; when it is treated as described there is charring with subsequent blackening of the sulphuric acid, but no yellowish-green fluorescence in ultra-violet light is obtained. When the sulphuric acid is poured into water the aqueous soln. shows only a slight bluish fluorescence in ultra-violet light.

β-Indole propionic acid, when treated in the same way, gives a bluish fluorescence in ultra-violet light, which may readily be distinguished from the intense yellowish-green fluorescence of β-indole acetic acid, but is not sufficiently different from the slight fluorescence given by indole to be of use as an identification test.

J. HUBERT HAMENCE

DR. BERNARD DYER'S LABORATORY, PEEK HOUSE, 20, EASTCHEAP, E.C.3 *Nov. 21st*, 1942

THE DETECTION AND DETERMINATION OF SMALL AMOUNTS OF MOLYBDENUM

THE intense blue colour formed when a molybdenum salt is evaporated to dryness with sulphuric acid is well known, but I have not found any record of a test based upon the reaction. To ascertain the sensitivity of the test, standard solns. containing from 0·001 to 0·00001 g of molybdenum were prepared by dissolving molybdenum trioxide in ammonium hydroxide, acidifying with sulphuric acid, evaporating the solns. to dryness in 1-in. porcelain basins, heating the residues strongly on a hot plate and noting the smallest amount that gave a distinct blue tint. It was found that the blue reaction may be used for the colorimetric estimation of as little as 0·0005 g of molybdenum (sulphurous acid being used as diluent) and for the detection of still smaller amounts, but is not so sensitive as the brown sulphide colour, which is quite distinct with 0·00001 g.

MOLYBDENUM IN PAINT PIGMENTS.—Many paint pigments are quite free from molybdenum, but others contain small amounts (less than 0·05%). In the usual analytical procedure molybdenum is finally concentrated by pptn. as lead molybdate. This is collected on fine-grained filter-paper and paper pulp and dissolved in hot dil. hydrochloric acid, and the soln. is treated with dil. sulphuric acid, evaporated until white fumes appear, and cooled. Cold water and a little alcohol are added as usual, and the beaker is cooled in ice-water and left overnight for pptn. of the lead sulphate. Complete removal of lead is essential for the application of the molybdenum tests. When large amounts (say, above 1%) of molybdenum are present, the usual gravimetric methods give reliable results, but when the amount of lead molybdate is very small, *e.g.*, 0·001 g, the molybdenum blue test should be applied to prove that molybdenum really is present. For this purpose, the clear filtrate from the lead sulphate ppt. is evaporated to dryness, and the residue is strongly heated on a hot plate. If the blue molybdenum oxide is formed, it is dissolved in the min. quantity of dil. ammonium hydroxide, the soln. is rendered distinctly acid with acetic acid, and hydrogen sulphide water is added. A clear yellowish brown colour is produced and is matched colorimetrically with standards containing known amounts of molybdenum (down to 0·00001 g) which have been similarly treated. After about 24 hrs. the molybdenum sulphide subsides, leaving a colourless supernatant liquid.

With quantities of molybdenum of the order of *ca.* 0·001 g the blue soln. may be diluted with sulphur dioxide soln. and the blue colour matched with standards, but I prefer to use the blue reaction for the identification and the yellowish-brown sulphide colour for the determination.

Approval has been given by the Director of Scientific Research and Experiment, Admiralty, for the publication of this note.

W. ELDER

October, 1942

ASSAY OF EUFLAVINE MUSLIN AND LINT

IN the assay of Euflavine Muslin and Euflavine Lint by the method of the B.P.C., Dressings Supplement, 1940, which uses the original method of Hall and Powell,[1] two noteworthy points arise. First, the official method stipulates that in the removal of fatty and waxy material from the hot aqueous soln. by means of chloroform, the liquid is to be cooled after the first chloroform extraction. With some samples of cotton this procedure results in troublesome slow-separating emulsions. Practically instantaneous separation can be achieved if the hot aqueous soln. be extracted with hot chloroform and the liquid cooled, if required, after the third and final extraction. Secondly, in the final titration with *N*/100 sodium thiosulphate the end-point in the B.P.C. method is by no means sharp, owing to the weakness of the volumetric soln. compared with the relatively large bulk of liquid being titrated, and to the yellowish colour at the end-point. The following modification will give results accurate to 1 drop of *N*/100 thiosulphate.—Titrate the liberated iodine with *N*/100 thiosulphate until the dark brown soln. becomes light brown. Transfer about 5 ml to a 6 in. × 1 in. boiling-tube, (tube 1), and add 1 drop of starch mucilage. Continue the titration of the bulk liquid as before, using another boiling-tube (tube 2). Transfer the liquid from tube 1 to tube 2. Finish the titration of the bulk liquid until the addition of 1 drop of starch mucilage to the liquid in tube 2 causes no change in the bright pale lemon colour. Observation of the end-point is rendered more certain by adding one drop of *N*/100 thiosulphate to the contents of tube 2, when, if the titration is really complete, no colour change occurs. This slight excess of thiosulphate in tube 2 is then made to react with the free iodine in tube 1 by mixing the contents of the two tubes. In practice, if the titration of the bulk liquid has been carried sufficiently far before using the boiling-tubes, the excess of thiosulphate in tube 2 is usually sufficient to react with the slight excess of iodine in tube 1, thus completing the titration in an exact manner.

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LABORATORIES, CROMER ROAD, NEW BARNET, HERTS.

W. P. CHAMBERS

November, 1942

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Ministry of Food

STATUTORY RULES AND ORDERS*

1942—No. 2186. The Canning of Food and Feeding Stuffs (Control) Order, 1942. Dated October 24, 1942.

The canning of food or feeding stuff in excess of 20 cwt. per annum is prohibited except by licence of the Minister. Any licence to engage in canning fruit or vegetables under Art. 2 of either the Fruit (Canning, Bottling and Freezing) (Control and Maximum Prices) Order, 1942, (S.R. & O., 1942, No. 87) or the Vegetables and Vegetable Products (Canning, Bottling and Freezing) (Control and Maximum Prices) Order, 1942 (S.R. & O., 1942, No. 88, as amended by No. 559), or to engage in making condensed milk under the Condensed Milk (Licensing and Control) Order, 1940 (S.R. & O., 1940 [No. 1788], II, p. 710), if subsisting immediately before the date of the coming into force of this Order and granted to a person who at that date is a member of the Canners' (War-time) Association shall continue to have effect so long as the holder continues to be a member of that Association.

— **No. 2189. General Licence, dated October 24, 1942, under the Food (Restriction on Dealings) Order, 1941.**

This Order authorises any person licensed to manufacture, can or bottle specified foods (viz., biscuits, etc., national butter, any canned specified food, cereal breakfast foods, cheese, sugar confectionery, etc., jam, milk powder, pickles and sauces, processed cheese, bottle vegetables), unless the licence otherwise provides, to sell or offer to sell, and any person to buy or offer to buy any of the said specified foods in accordance with such licence. Also manufacturers who do not need a licence under various named S.R. & O. may sell or offer to sell products so manufactured and any person may buy or offer to buy them from the manufacturer. Any flour miller, within the meaning of the Flour (Control and Prices) Order, 1941, may sell or offer to sell, and any person may buy or offer to buy from any flour miller (a) any semolina produced by the seller in accordance with the provisions of that Order, (b) any flour other than semolina produced by the seller.

— **No. 2258. Order, dated November 3, 1942, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1942.**

The following amendments to the Principal Order are made:—(a) In Art. 3 (7) (a) (ii) for "feeding stuffs" read "quantities of all feeding stuffs." (b) In par. 3 of Part A, Schedule I, delete the colon dash after "dried blood." (c) In par. 6 of Part A, Schedule I, for "cereal" read "cereals." (d) In sub-par. 3 of par. 10, Part A, Schedule II, for "make" read "cake." (e) In par. 3, Part A, Schedule III, for "linseed concentrates" read "licensed concentrates." (f) In par. 4 of Part A, Schedule III, insert a comma between "husk" and "bran"; for "Linseed molassed feeding stuffs" read "Licensed molassed feeding stuffs."

— **No. 2272. Order, dated November 4, 1942, Amending the Home Grown Rye (Control and Maximum Prices) (Great Britain) Order, 1942, the Home Grown Wheat (Control and Prices) (Great Britain) Order, 1942, and the Home Grown Dredge Corn (Control and Maximum Prices) (Great Britain) Order, 1942.**

The definition of "Grower" is enlarged to include the first sellers of rye, wheat and dredge corn respectively, but for the purpose of Schedules to the respective Orders does not include an approved buyer. Potentially millable wheat is here defined as: any dredge corn which contains more than 25% w/w of wheat or which was grown from seed containing more than 25% w/w of wheat, and the value of which (taking into account any cost of separation, wastage; loss of weight and the value of grain, other than wheat, extracted), is not more than 2s. per cwt. less than the value of the same weight of millable wheat.

— **No. 2329. The Canned Vegetables (Prohibition of Retail Sales) Order, 1942. Dated November 11, 1942.**

Retail sale (offering to sell, buying or offering to buy) of any canned vegetable (whether in the original container or not) is prohibited, but the Order does not apply to (a) beans baked or steamed in a sauce containing tomatoes or other fruit or vegetables, with or without pork; beans baked or steamed in a gravy containing meat extract or yeast extract, with or without pork; any beans dried in brine); (b) any sale by a ships' stores dealer for ships' stores; (c) any sale to Institutes of H.M. Forces for the purpose of providing meals for H.M. Forces or for Allied Forces. "Sale by retail" means any sale other than for re-sale, and includes sales to a caterer for his catering business, or for use in the manufacture or preparation for sale of any other article. "Vegetable" includes tomatoes and edible fungi, and any vegetable (a) whether or not mixed with any other article, (b) whether fresh or dried, wholly or partly cooked (including vegetables in vegetable rolls, etc.) or otherwise processed or prepared (otherwise than pickled), and includes vegetable juices or purées, but does not include soups or vegetable extracts, vegetable jams, or any product manufactured from vegetables except those already mentioned.

No. 2455. The Saccharin (Control and Maximum Prices) Order, 1942. Dated December 1, 1942.

This Order, which replaces S.R. & O., 1941 (No. 2129), II, p. 1125 as amended by 1942, Nos. 174, 348 and 1106, prescribes the conditions for buying or selling saccharin, etc., and fixes max. prices.

The following definitions are given:

"Dulcin" means *p*-phenetylcarbamide, and "dulcin soln." any liquid (capable of being used as a substitute for sugar) in the preparation of which dulcin has been used.

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* A summary of some Orders. Italics signify changed wording. Price 1d. each.

"Full-strength saccharin tablet" means a tablet which (i) weighs not more than 1·1 grains and, (ii) contains not more than 5% by wt. of water-insol. matter and not less than 0·27 grain and not more than 0·33 grain of saccharin mixed with sufficient sodium bicarbonate to render the saccharin soluble with or without other excipients or an equiv. amt. of soluble saccharin with the addition of sodium bicarbonate or other excipients.

"Half-strength saccharin tablet" means a tablet which (i) weighs not more than 0·9 grain and, (ii) contains not more than 5% by wt. of water insol. matter and not less than 0·135 grain and not more than 0·165 grain of saccharin mixed with sufficient sodium bicarbonate to render the saccharin soluble with or without other excipients, or an equiv. amt. of soluble saccharin with the addition of sodium bicarbonate or other excipients.

"Saccharin" means *o*-benzoicsulphinide.

"Soluble saccharin" means the sodium salt of *o*-benzoicsulphinide.

"Saccharin soln." means any liquid (capable of being used as a substitute for sugar) in the preparation of which saccharin has been used.

"Standard saccharin tablet" means a tablet which (i) weighs not more than 1·1 grains and, (ii) contains not more than 5% by wt. of water-insol. matter and not less than 0·18 grain and not more than 0·22 grain of saccharin mixed with sufficient sodium bicarbonate to render the saccharin soluble with or without other excipients, or an equiv. amt. of soluble saccharin with the addition of sodium bicarbonate or other excipients.

"Sweetening powder" means any powder (capable of being used as a substitute for sugar) in the preparation of which saccharin or dulcin has been used, but the expression does not include soluble saccharin or any powder wholly consisting of saccharin or dulcin.

"Sweetening tablet" means a tablet which (i) weighs not more than 0·9 grain and, (ii) contains not more than 0·09 grain of saccharin and not less than 0·06 grain of dulcin mixed with sufficient bicarbonate to render the tablet soluble with or without other excipients.

The Order prohibits the retail buying or selling (a) of saccharin soln., dulcin soln., saccharin or dulcin in powder form, soluble saccharin in powder form or sweetening powder; (b) tablets capable of being used as a sugar substitute other than full-strength and half-strength saccharin tablets, standard saccharin tablets and sweetening tablets; (c) after Jan. 31, 1943, any full-strength or half-strength saccharin tablets; (d) until Jan. 1, 1943, any standard saccharin tablets, (e) full-strength saccharin tablets except in packets of 50 or 100 bearing a label stating in clearly legible letters the number of tablets and the saccharin content of each package; (f) any half-strength saccharin tablets or standard saccharin tablets except in packets of 100 bearing a label stating in clearly legible letters the number of tablets and the saccharin content of the package; (g) any sweetening tablets except in packets of 100 bearing a label stating in clearly legible letters the number of tablets and the saccharin and dulcin content of the package.

Records of all sales other than by retail must be kept. The order came into force on Dec. 1, 1942.

SAMPLING OF FOOD BY INSPECTORS OF WEIGHTS AND MEASURES

THE Minister of Food has made an Order conferring on Inspectors of Weights and Measures powers already exercisable by Sampling Officers under the Food and Drugs Act, to take samples of food free from the restrictions of any other Orders, such as the Rationing Orders, made by the Minister.

The Order takes effect on Dec. 3, 1942. (*Cf.* ANALYST, 1942, **67**, 163, 195.)

Dec. 1, 1942

NATIONAL FLOUR AND BREAD: 2ND REPORT FROM THE SCIENTIFIC ADVISER'S DIVISION*

SUMMARIES of routine tests on 322 samples of flour and 459 samples of bread produced since March, 1942, are presented. Of the samples of flour examined, 85·1% had fibre contents of 0·9 % or less, some being as low as 0·4%. The determined vals. were increased by 0·05 % to compensate for the permitted addition of Canadian G.R. Flour. Such addition does not affect the vitamin B_1 val., which was 0·90–1·10 I.U. or more per g (93·5% of the samples had at least 0·90 I.U. per g), because G.R. flour is fortified. The riboflavin contents of 6 random flour samples (fibre contents, 0·7%) were 1·3–1·9 (aver. 1·7) μg per g, and the germ contents (determined microscopically by comparison with standards) were approx. 1·0–2·0%. There is no official specification for granularity, but the Medical Research Council's recommendations mean in practice that all the flour should pass through a No. 5 silk (aperture 0·27 mm). The most recent average vals. for flours from 100 mills were: tails 32 GG, 1·2%; throughs 32 GG and tails 5 silk, 6·4%. Milling conditions are being adjusted so as to scrape the endosperm from commercial bran without chopping the bran, and in this way an 85% meal containing 0·35–0·40% of fibre has been produced. Loaf qualities were classed as:—good, 60·6; fair-good, 22·2; fair, 12·6; poor, 4·6%. The chief defect was poor fermentation and/or inadequate baking, giving a weak crumb (*cf.* G. N. Jenkins, E. I. McDougall and P. Herbert, ANALYST, 1942, **67**, 364). G.

Legal Notes

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

ARTIFICIAL VINEGAR: INVOICE NOT A WARRANTY

BRECONSHIRE COUNTY COUNCIL *v.* THE MEADOW DAIRY CO., LTD.

ON Oct. 29, 1942, a Divisional Court, consisting of the Lord Chief Justice Mr. Justice Tucker and Mr. Justice Cassells, gave judgment, with costs, in favour of the Breconshire County Council in an appeal against a decision of the Brecknockshire Quarter Sessions on July 30, 1941, confirming the conviction

* *Nature*, 1942, **150**, 538–539.

by the Brynmawr Justices of the Meadow Dairy Co., Ltd., who were fined £5 for selling to the chief inspector of Food and Drugs a bottle of vinegar which was not of the nature, substance or quality demanded by him.

The main contention of the appellants was that the invoice received by them from the manufacturers, which read "12 dozen vinegar at 4s. 9d., £2 17s. 0d.," constituted a warranty within the meaning of Sec. 84(5) of the Food and Drugs Act, 1938, and was a good defence to the charge under Sec. 84(1) of that Act.

The Public Analyst (Mr. H. J. Evans) had certified that the vinegar which was the subject of the appeal was artificial vinegar deficient in acetic acid to the extent of 43%, calculated on the basis that vinegar should contain not less than 4% of acetic acid.

The Divisional Court were unanimously of the opinion that in these circumstances the invoice was not a warranty entitling the appellants to sell the vinegar in question to the chief inspector of Food and Drugs as the pure vinegar asked for by him.

Notes from the Reports of Public Analysts

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

BIRMINGHAM: REPORT OF THE CITY ANALYST FOR THE THIRD QUARTER, 1942

FRUIT CORDIALS.—A sample, labelled "Grape Fruit Flavour Cordial," consisted of an artificially flavoured 1·8% soln. of citric acid. The makers have omitted the word "cordial" and are now selling the product under a trade name. Another sample, labelled "Lime Flavour Cordial," consisted of an artifically flavoured 20% soln. of citric acid. A picture of a lime fruit on the label was "calculated to deceive" the purchaser. The article was withdrawn from sale, and the stock was returned to the makers.

MILK PUDDING POWDER.—The label of the sample had the words "contains no milk" in very small type on the side panel. The powder consisted of a mixture of wheat and maize flours. After an interview the makers agreed to have the words "contains no milk" printed on the front of the carton in type equal in size to "Milk Pudding Powder."

"LIQUID SACCHARIN."—This was a 1% soln. of saccharin. The sale of such solns. is forbidden by Sec. 3(*a*) of the Saccharin (Control and Maximum Prices) Order, 1941, and the stock was destroyed.

H. H. BAGNALL

Department of Scientific and Industrial Research

INVESTIGATION OF ATMOSPHERIC POLLUTION: 1941–42

IN continuation of the policy outlined in last year's Summary Report (ANALYST, 1942, **67**, 229), the following brief account of recent observations for the year ended March 31, 1942, has been prepared for the information of the Co-operating Bodies.

The numbers of instruments maintained by the Co-operating Bodies during the last three years were:—Deposit gauges, 127, 106 and 101; automatic filters, 11, 9, 6; volumetric sulphur apparatus, 12, 7 and 6; lead peroxide apparatus, 60, 41 and 40, respectively.

The greater uniformity of occupation throughout the country has the effect of simplifying the problems of atmospheric pollution, while Government control of labour and industry makes possible an unusually accurate knowledge of industrial activity. For the first time comprehensive records are being kept of the consumption of coal in different districts, by different industries and by private consumers. A special effort is being made to use coal as efficiently and economically as possible, and a decrease in soot deposit and suspended impurity is likely to occur. These are reasons for making every effort to maintain and increase observations of atmospheric pollution, despite the scarcity of labour and other difficulties which beset the Co-operating Bodies.

DEPOSIT GAUGES.—There is strong evidence that deposited matter has increased in Great Britain as a whole since the war, continuing the upward trend which was noted a year ago. Comparing results for summer 1941 with summer 1939, from the 87 deposit gauges which were in operation for both periods, there was an average increase of 13% in "soot," *i.e.*, insoluble carbonaceous matter other than tar, and 8% in insoluble ash. Although 11% less rain fell, the average increase in dissolved sulphates was 10%. Smaller increases in total solids, tar and total dissolved matter were found, but it is now possible to calculate whether such changes are likely to be accidental, and it is customary to view with suspicion any result which appears to have more than one chance in twenty of being an accident. The increases in total solids, tar and total dissolved matter, fall into this class, and they are technically described as "not significant." There was no change in average deposits of chloride ion. It is to be noted that there were significant increases in ash and sulphate ion, which are believed to correlate most closely with industrial activity. The greatest increase, 13%, was in soot, which is more characteristic of the open domestic grate than the industrial boiler; but in the special circumstances of war much of the extra smoke may have been from industrial chimneys.

The most remarkable example of increased deposited matter is at Southampton, half a mile north-east of the power station. Here the soot deposit was more than trebled in 1941–2, and the insoluble ash nearly trebled, while total solids were doubled. This is ascribed in part, locally, to the use of inferior and unsuitable fuel owing to the exigencies of war.

There is evidence that air raids have affected deposits in some localities. At one station, fires increased the month's deposit of insoluble ash more than four-fold; and building operations in a later month were blamed for a nine-fold increase in dissolved matter, including a seven-fold increase in chloride ion. It would be helpful if analysts could add notes to their returns of atmospheric pollution, when they suspect such abnormalities.

SULPHUR MEASUREMENTS.—(*a*) *Lead Peroxide*.—The 32 stations where the lead peroxide method was used during the past two years show an average increase of 7% in the sulphur for the summer of 1941 compared with the summer of 1939. This agrees well with the increase of 10% in deposited sulphur, and,

making the reasonable assumption that sulphur emission is proportional to the quantity of coal burned, it seems probable that the country as a whole burned about 10% more coal during summer 1941 than summer 1939. During winter 1941–2 about 10% more sulphur was collected by the lead peroxide method than during winter 1939–40.

It should be noted that the summer increase of 7% is not by itself significant, because its standard error is 4·1% and the corresponding accident-probability is 0·09. It is usual to treat as significant any deviation which is more than twice the standard error, the accident-probability then being less than 0·05, or 1 chance in 20. However, since the winter increase is significant, having an accident-probability of 0·01, it is most probable that there was a small increase also in summer.

(*b*) *Volumetric.*—The 5 stations having complete records for the years 1941–2 and 1940–1 are not sufficiently representative of the whole country for a similar comparison to be made. An average increase of 40% on the previous year was recorded at Newcastle in 1941–2, and the other stations show smaller increases which are not statistically significant.

(*c*) *Sulphur Measurements by Three Methods.*—A table presents a summary of sulphur measurements (*a*) by analysis of soluble matter in deposit gauges, (*b*) by the lead peroxide method, and (*c*) by the volumetric method.

The mean results (mg of SO_3 per sq.m per day) recorded at 24 stations in 15 large towns are classified under summer and winter headings. The highest figures were:—*Method* (*a*).—London, 31–34 (S), 28–31 (W); Glasgow, 21 (S), 42 (W); Manchester, 23–25 (S), 20–42 (W); Halifax, 15–26 (S), 22–40 (W). The lowest figures were: Godalming, 9 (S), 8 (W), and Southport, 10 (S), 11 (W). Mean of 24 stations, 21 (S), 25 (W).

Method (*b*).—Highest: London, 98–190 (S), 207–436 (W); Manchester, 94–227 (S), 244–477 (W); Halifax, 136–214 (S), 302–411 (W); Sheffield, 58–184 (S), 112–375 (W). Lowest: Godalming, 16 (S), 51 (W). Mean of 24 stations, 139 (S), 284 (W).

The means of the 4 stations using the volumetric method (*c*) were: 212 (S), 386 (W).

The figures for method (*c*) are about 10 times those for method (*a*). This means that if sulphur dioxide is uniformly distributed with height, there is as much of it in a layer of air 1000 m thick as is deposited on the ground in 10 days. It is known of course that sulphur dioxide is removed from the air by some physical or chemical process, or else its concentration would increase indefinitely, and the process is likely to be connected with the washing effect of clouds and falling rain. The average height of nimbus clouds is about 1500 m, and rain occurs at Kew on an average of about 1 day in 2, so that if rain were a perfectly efficient agent for washing sulphur dioxide from the air, it would deposit the contents of a 750-m layer each day, and the figures for method (*a*) would be about 0·75 times those for method (*c*). Since this is not so, the purifying effect of rain cannot be particularly rapid. There will therefore be no very obvious reduction in sulphur dioxide concn. after a downpour of rain.

There is additional evidence in the recorded results that the sol. deposit of sulphur is not identical with sulphur dioxide in the air, because of differences in the range of annual variation. For suspended sulphur dioxide the ratio of summer to winter by the lead peroxide method is 0·49, and the ratio for the four stations using the volumetric method is 0·56. For deposit, on the other hand, the ratio is 0·84. If suspended sulphur dioxide were deposited within a few score miles of its point of emission, the annual range by all three methods would be nearly the same, provided that most of the sulphur in coal leaves the chimney in gaseous form and not, *e.g.*, combined with ash. It is known that most of the sulphur in coal is converted into sulphur dioxide on combustion, so that the argument is valid, and sulphur dioxide cannot usually be deposited within a few score miles of its point of emission.

The area enclosing Liverpool, Preston, Leeds, Nottingham and Birmingham might burn ½ million tons of coal for all purposes on a winter day. If the weight of sulphur dioxide emitted is 3% of the weight of coal burned, this would correspond to an emission of about 300 mg of SO_3 per sq.m per day. The rate of deposition of sulphur dioxide is only about one-tenth of this, even in the centres of Sheffield and Salford. The conclusion must be that the sulphur dioxide which is emitted in the given area is deposited over a very much larger area. This confirms qualitatively the conclusion already drawn. The processes by which sulphur dioxide and smoke escape from the atmosphere are not fully understood, but they are important, because if they fail, abnormally high concentrations of pollution will be set up, endangering health and causing general inconvenience to the community.

Automatic Filter.—Complete sets of readings are available for two Glasgow stations, three London stations and Stoke-on-Trent. The aver. suspended impurity at Stoke was about 20% less in summer 1941 than in summer 1940, and the improvement occurred chiefly between 4 p.m. and 1 a.m. No other significant changes have been noted.

Light Measurements.—Measurements of light have been made at 6 stations. There were no noteworthy differences from the figures for the previous year.

Summary.—Atmospheric pollution has increased appreciably since the war began. The increase is due principally to industrial activity, but there is evidence that both air-raids and air-defence measures are also to blame. The effect of rain and clouds in removing sulphur dioxide from the air is shown to be a slow process, a matter of days and weeks rather than hours.

National Research Council of Canada

POLARIMETRIC DETERMINATION OF STARCH IN CEREAL PRODUCTS

I. Rapid Determination of Starch in Crude Gluten.*—Neither the original Mannich–Lenz procedure (*Z. Nahr. Genussm.*, 1920, **40**, 1) nor its recent modifications (*e.g.*, Hopkins, *J.A.O.A.C.*, 1939, **22**, 523; 1940, **23**, 489; Munsey, *id.*, 1937, **20**, 350) have proved suitable for the polarimetric determination of

* By K. A. Clendenning, Paper No. 195, of the Associate Committee on Grain Research and N.R.C. No. 1065. Div. of Applied Biology, National Research Laboratories, Ottawa, Canada. (*Canadian J. Res.*, 1942, C, **20**, 403.) Price 10 cents.

starch in gluten. The principal defect lay in the means of removing or correcting for proteins dissolved with the starch, and there was also need of improvement in the technique for starch dispersion. In the present method the starch is dispersed by 15 min. boiling in calcium chloride soln. of sp.gr. 1·30 and *p*H *ca.* 2·5, the vol. being kept constant by addition of water and frothing controlled by adding a few drops of *n*-octyl alcohol. The dissolved proteins are pptd. with 20% stannic chloride soln. prior to the filtration and polarisation (Mannich and Lenz, *loc. cit.*). This procedure underestimates the starch, but application of a factor enables the actual starch content to be determined with an accuracy of ±0·5%.

Reagents.—(1) *Calcium chloride soln.*—Dissolve 2 parts of calcium chloride ($CaCl_2.6H_2O$) in 1 part of water and adjust the sp.gr. to 1·30, and the *p*H to 5·50 with dil. hydrochloric acid and sodium hydroxide soln.

(2) *Stannic chloride soln.*—Dissolve 20 g of stannic chloride ($SnCl_4.5H_2O$) in *ca.* 80 ml of (1) and make up to 100 ml with the same soln.

Procedure.—Weigh 2 g of the gluten sample (140 mesh) into a 300- to 400-ml beaker, add 50 ml of the calcium chloride soln. and 2 ml of 0·8% acetic acid, and immediately whip the mixture into a smooth suspension. Add 1 or 2 drops of *n*-octyl alcohol, heat to b.p. in *ca.* 5 min., with constant stirring, and maintain steady boiling for 15 min., rubbing all gluten particles from the side of the beaker by means of a rubber-tipped rod. Add water from time to time to keep the contents at the original level. After 15 min. cool to room temp. in running water. Pour the mixture into a 100-ml graduated flask containing 3.5 ml of the stannic chloride soln., rinsing the beaker out with the calcium chloride soln. (1), add 1 or 2 drops of 95% ethyl alcohol to destroy froth, and make up to 100 ml with (1). Shake vigorously for 3 min. and run 10 ml through a fluted Whatman 15-cm. No. 12 filter-paper, taking care to wet the whole filtering surface thoroughly. Discard the first filtrate, filter the remainder of the soln. without suction, and polarise in a 1-dm tube. Calculate the starch content of the crude gluten by means of the formula

$$\frac{(50\alpha)(1{\cdot}21)}{\text{(wt. of sample in g)}} = \%\ \text{starch},$$

where α represents the observed optical rotation in degrees.

The relationship of the apparent starch content (Mannich and Lenz's equation) to the actual content was found to be linear over the range 0 to 18% of starch and is represented by: % present = 1·21 (% found). By the use of this correction factor analyses of two glutens prepared to contain 8 and 16% of starch respectively showed agreement within ±0·3% between the known and determined starch contents. With practice, it is thus possible to determine the starch contents of 12 or more samples of gluten in 6 hrs.

Ministry of Labour and National Service

ANNUAL REPORT OF THE CHIEF INSPECTOR OF FACTORIES FOR 1941*

THE number of reportable accidents in 1941 again shows an increase on 1940. There were 1646 fatal and 269,652 non-fatal accidents as compared with 1372 and 230,607 in the previous year, and 944 and 179,159 in 1938. The percentage increase of 1941 over 1938 was: adult males, 42; adult females, 192; boys, 21; girls, 20. The gross increase in accidents to women is to be attributed to the larger number employed, especially in dangerous processes. Industries such as metal extracting, engineering, machinery making and aircraft production account for about 19,000 of the additional 28,000 accidents to women and for more than the additional 1500 accidents to girls under 18. The most distressing accidents to women are due to the hair being caught in moving machinery; this caused 179 accidents in 1941.

Other parts of this section of the Report deal with personnel, management and welfare supervision, ventilation and general conditions, agreements with various industries, civil defence and fire prevention.

The Report on Health by Dr. J. C. Bridge, Senior Medical Inspector, forms a special section of the main Report. It discusses *inter alia* the influence of monotony on work, the effect of longer hours of work on the health, and diseases due to the materials handled in various industries.

LEAD POISONING.—The number of cases reported (59) is the lowest since notification has been enforced. This is partly due to the discontinuance of certain industries in which lead poisoning was prevalent (*e.g.*, ship breaking), but the fall does not appear to be entirely incidental to the extent of employment in processes using lead.

ANILISM.—This term is now used to include cases of T.N.T. poisoning, in which cyanosis with accompanying dyspnoea and digestive disturbances occur. Recovery is usually rapid, but cases of jaundice due to damage to the liver, which may follow the cyanosis, are more serious. There have been 41 cases (12 males and 29 females), of which 12 (3 males and 9 females) ended fatally. The greater number of cases among women is attributed to the larger number exposed to the risk rather than to greater susceptibility. Chlorinated naphthalene caused the death of a woman engaged in scraping the wax from metal parts. This chemical produces the same damage to the liver as T.N.T.

ANTHRAX.—There were 22 cases (3 fatal), compared with 37 (5 fatal) in 1940. The fall is attributed to a reduction in the importation of infected material.

PHOSPHORUS POISONING.—One case (necrosis of jaw) is referred to as being the only case since 1919.

GAS POISONING.—There was an increase of nearly 200 cases and 10 more deaths over the figures for 1940, the increase being shown in practically all the main classes of gassing. Carbon monoxide—258 with 24 deaths—showed the greatest increase over 1940 (162 with 20 deaths). There was a slight decrease in the number of cases caused by blast furnaces, but 56 more cases with 9 deaths from miscellaneous sources, *e.g.*, coke braziers and exhaust gases from internal combustion engines.

The number of cases due to nitrous fumes was somewhat lower (217 as against 236 in 1940) and only 1 was fatal. Other gases were responsible for 115 cases, making up a total of 782 cases (41 deaths) as compared with 585 (31 deaths) in 1940.

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* H.M. Stationery Office. Cmd. 6397. 1942. Price 6d. net.

DERMATITIS.—There are few things used in industry which will not cause dermatitis in some people, but, with proper care and effective cleanliness, few should be affected. It is hoped that soon it may be possible to standardise methods of protection, but their success must depend upon the co-operation of the individual worker.

The Institute of Petroleum

STANDARD METHODS FOR TESTING PETROLEUM AND ITS PRODUCTS*

THE new edition of this work contains most of the methods described in the 3rd Edition and all of those published in the journal of the Institute. All the methods have been re-written and incorporate recent improvements. Special attention has been given to the International Standard definitions of accuracy, precision and tolerance. An attempt has been made to include all test methods required in official specifications and especially those of the Services, *e.g.*, the oxidation test for aircraft lubricating oil required by the Ministry of Aircraft Production specification.

New methods described in detail include aniline points of products developing high pressures at the aniline point, Abel flash-point of viscous petroleum products, sp.gr. by displacement, by flotation and by the Westphal balance, and a rapid method of determining sulphur by combustion in a quartz tube.

Among other useful new features are methods for calculating Diesel Index and Viscosity Index, tables for converting kinematic viscosity into Redwood viscosity, and various methods standardised by the American Society for Testing Materials, such as, *e.g.*, the Cleveland Flash Point, Gum Stability, Aviation Full Knock Rating, the Centrifuge Method for Sediment in Fuel Oil, and Measurement of Colour by the Saybolt Chromometer.

Certain tests, published in the 3rd Edition, but no longer in general use, have been omitted.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Measuring the Concentration of Dissolved Oxygen in Dairy Products. A Voltammetric Method. G. H. Hartman and O. F. Garrett. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 641–644.—The method is based on Vitek's application of Heyrovsky's dropping mercury electrode to the determination of oxygen in water (ANALYST, 1925, **50**, 94; *Collect. Czechoslov. Chem. Commun.*, 1935, **7**, 535). Ingol's apparatus is used (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 256), the light reflected from a sensitive mirror galvanometer being focussed on a photoelectric cell, and the current thus produced is amplified and recorded on a Type-B, Cambridge thread recorder. The dropping electrode consists of a 50-ml pipette containing a sealed-in platinum contact, which serves as reservoir, and to the end of which is sealed a micro-capillary tip (length, 2–3 cm; bore, 60–70μ). The dropping-rate (optimum, 1 drop per 1·5–2·5 sec.) is controlled by a levelling-bulb and manometer, and special cleanliness of mercury and glass surfaces is essential. The flow of drops should begin before the electrode is inserted in the milk (immersion depth of tip, 3–4 cm), and allowed to continue after withdrawal and while the tip is being swabbed and washed with ether; the flow is stopped with the capillary immersed in distilled water. The sample is collected in a brown glass, wide-mouthed bottle, which is allowed to overflow and then closed with a rubber stopper fitted with a glass tube terminating in a piece of rubber tubing and a pinchcock; trapped air is thereby avoided. The sample is stored in ice. A saturated calomel electrode is used as the reference electrode, but it should previously be maintained for several days at the temp. at which the determination is made (*vide infra*) to prevent disturbances due to temp. variations. The error due to the residual current is relatively small for milk, and the same for different milks, but it must be deducted from the total deflection; it is determined from measurements made after removing all oxygen from the milk (*e.g.*, physically, chemically or by yeast or bacterial cultures). Allowance should also be made for the presence of abnormal quantities of copper or iron. Since the method is empirical, it must be standardised with milks of known oxygen content, *e.g.*, produced by mixing definite proportions of oxygen-free and oxygen-saturated milks prepared as follows:—Deoxygenate 1 quart of milk with yeast, and cool to 1° C. to retard further growth; pass air under pressure into another 1 quart at 1° C., through a gas-diffuser, for 15 min. The graphs for mixtures of the two show that the % saturation is linearly proportional to the corrected galvanometer deflections, and if they are extrapolated, the line passes through zero. Readings may be made over a potential range of 0·8–1·2 volt, but the use of 1·2 volt enables the full scale of the drum recorder to be used. The method of Sharp, Hand and Guthrie (*id.*, 1941, **13**, 593) is used to check the oxygen concn. during the initial calibrating process. Then the concn. of oxygen (in p.p.m.) of a given sample is $C(D_1 - D_0)/(D_2 - D_0)$, where D_0, D_1 and D_2 are the galvanometer deflections corresponding with the residual current, the sample being tested, and the milk having the known oxygen concn. C. Tests on a large number of air-saturated samples of whole milk, skim milk, cream and sour milk gave the same result, indicating that the method determines the oxygen concn. in the aqueous phase only, and that this is not influenced by the amount of fat or serum solids. Consequently allowance must be made for the concn. of total solids in calculating the dissolved oxygen content in any given dairy product, although with milk, the approx. constancy of these solids for a mixed dairy herd enables an aver. value to be taken for all milks without introducing a significant error. For the air-saturated samples the mean galvanometer deflection was 19·46 ± 0·05, and the mean concn. of dissolved oxygen 11·17 ± 0·03 p.p.m.; the corresponding

* 4th Edition. Pp. 400 with 97 diagrams. Published by the Institute of Petroleum, c/o Imperial College of Science and Technology, London, S.W.7. 1942. Price 15s.

standard deviations being 0·30 and 0·18. Oxygen concns. before saturation were, 5·77 (Guernsey, Grade A, pasteurised) to 9·07 (Guernsey, Grade B, pasteurised, in paper bottle) p.p.m. J. G.

Identification of Sugars by the Microscopic Appearance of Crystalline Osazones. W. Z. Hassid and R. M. McCready. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 683–686.)—To 2 ml of a clear, neutral soln. containing 5–20 mg of the sugar in a 15 × 150–mm. test-tube, add 0·3 g of powdered sodium acetate and 0·2 g of phenylhydrazine hydrochloride, and shake until the contents have dissolved. Place the loosely-stoppered tube in boiling water for 30 min., cool slowly (important) to *ca.* 20° C., place 1 drop of the suspension of crystals (removed in a pipette with a wide jet) on a microscope slide, and note their crystal form. Since the osazones of disaccharides are sol. and those of monosaccharides are insol. in the hot soln., mixtures of these are dealt with by identifying the latter while the soln. is still warm, filtering, and cooling the filtrate slowly so as to obtain the former. A limitation of the method is the fact that certain sugars having a common enolic form give the same osazone (*e.g.*, *d*-glucose, *d*-mannose, and *d*-fructose; *d*-galactose, *d*-talose and *d*-tagatose; *d*-idose, *d*-gulose and *d*-sorbose). Also, corresponding groups of sugars of the *l*-series having the same configuration beyond the second carbon atom give the same osazone, and this differs from the corresponding *d*-osazone only in the direction of optical rotation. Glucose, mannose and fructose are therefore distinguished as follows:—Oxidise the glucose with nitric acid by the method of Morrow and Sandstrom (*"Biochemical Laboratory Methods,"* 2nd Ed., 1935, p. 157) and neutralise the soln. with potassium carbonate soln.; this produces crystals of potassium acid saccharate. Mannose forms an osazone which is insol. in the cold, and fructose is identified by the Seliwanoff reaction, which is also given by sorbose. Galactose is distinguished from its other enolic forms by means of its *l*-tolylhydrazone derivative, and *d*-gulose is distinguished from *d*-sorbose and *d*-idose by the formation of its potassium acid saccharate (*loc. cit.*). Sorbose osazone is amorphous, but changes to crystalline needles after 24 hr. Fructose diphosphate (Harden-Young ester) forms diphenylhydrazine fructose diphosphate phenylhydrazone after 15 min. heating, but after 1 hr. it is hydrolysed to the same phenyl hydrazine hexose monophosphate osazone as is obtained from Robison ester (glucose-6-phosphate). Photomicrographs of the osazones of 20 hexoses, pentoses, methylpentoses, disaccharides, uronic acids and hexosephosphates (×45) are reproduced, to supplement those published by Morrow and Sandstrom (*loc. cit.*); (*cf.* Niemann, Schoeffel and Link, *J. Biol. Chem.*, 1933, **103**, 337). J. G.

Food Value of the Potato. Anon. (*Nature*, 1942, **150**, 518.)—The following average figures are given for potatoes:—moisture, 78; proteins, 1·2; carbohydrates, 16·2%; calcium, 8; iron, 0·7; vitamin C, 9 (freshly lifted, 30; before Christmas, 12; after Christmas, 6); riboflavin, 0·07; nicotinic acid, 1·4; vitamin B_6, 0·15; pantothenic acid, 0·3; folic acid, 0·12 mg per 100 g; vitamin B_1, 40 I.U. per 100 g; calories, 70 per 100 g. The food val. of the potato as compared with other foods is indicated on the basis of the above data. J. G.

Preparation of Certain Derivatives of Starch. E. Pacsu and J. W. Mullen. (*J. Amer. Chem. Soc.*, 1941, **63**, 1487–1488.)—To prepare starch solns. or pastes suitable for esterification or etherification reactions, boil the starch in water, add pyridine, and continue boiling with distillation so as to remove the water as a pyridine-water azeotrope, b.p. 92–93° C. The products range from clear solns. when the pyridine contains *ca.* 4% of water, to thick jellies in absence of water. Alternatively, the α- and β-amyloses of starch may be separated (Pacsu and Mullen, *J. Amer. Chem. Soc.*, 1941, **63**, 1168) and gelatinised separately in pyridine. The starch so gelatinised is highly reactive towards esterification and etherification reagents, *e.g.*, the anhydrides and acyl halides of aliphatic and aromatic acids, benzyl chloride, etc., and is trifunctional, giving rise to triesters and ethers in practically quantitative yield. The products are thermoplastic, yielding clear, glass-like substances. E. M. P.

Determination of Moisture in Living Tissue. E. G. Hallsworth and R. L. Reid. (*Nature*, 1942, **150**, 524.)—The results obtained with dehydrated vegetables by the following methods were compared:—(1) Dean and Stark, toluene distillation (ANALYST, 1920, **55**, 270); (2) vacuum oven at 70° C. and less than 50 mm pressure; (3) hot-air oven at 115° C.; (4) steam-oven at 96° C. The respective results were: Carrots, 11·2, 5·45, 15·08, 8·52; cabbage, 11·1, 5·44, 16·05, 12·04; potato (var. Factor), 11·5, 10·50, 11·98, 10·37%. These and other results obtained by the 4 methods show so little concordance that it is doubtful if a moisture figure has any meaning unless the method is specified. Method (1) gave no indication of reaching a constant val. with carrots, onions or cabbages after 13 hr. distillation, but with potatoes or parsnips it gave a constant val. fairly rapidly. Method (2) required 5–8 hr. with all the vegetables. Methods (3) and (4) required 8–16 hr. for onions, parsnips or potatoes, but showed no approach to a constant val. with carrots or cabbage. This is probably due to the structural changes that occur on heating, for marked colour changes occurred with methods (3) and (4), but not with (2). The standard error of the mean was lowest for method (2), higher for (4), and much higher for (3). J. G.

Determination of Caramel in Wines. C. Milos. (*Amer. J. Pharm.*, 1942, **114**, 138–140.)—To 2 separate portions of 25 ml of wine add 50 ml of ethyl alcohol and 50 ml of ether and shake well. If, on standing, 2 immiscible layers form, add more alcohol (25 ml are usually sufficient), shake again and leave overnight if no ppt. forms in a few hrs. Filter the soln. through filter-paper in a Buchner funnel. Wash the residue in the flask and on the paper with 10–15-ml portions of hot 85% alcohol containing 0·5% of hydrochloric acid until the filtrate is no longer red or pinkish. Wash several times with hot 85% alcohol. Place the filter-paper in the flask, add 10 ml of water and heat gently until the residue dissolves. Cool, filter and test for caramel. If the ppt. does not dissolve readily, make the aqueous soln. alkaline with 1–2 drops of 2% potassium hydroxide soln. and heat gently until solution is complete. The method does not give exact quantitative results, but, in general, quantities of caramel sufficient to give an appreciable colour to wine can readily be detected. The following tests for caramel are given. (1) To 2 ml of the soln add 0·5 ml of 10% sodium hydroxide soln. and boil. Caramel darkens slightly. Cool, add 10 ml of Marsh reagent (*"Methods of Analysis," A.O.A.C.*, 1940, **34**, 180). Caramel does not

dissolve in the Marsh reagent (Horn, *Amer. J. Pharm.*, 1910, 151). (2) Add a few drops of hydrochloric acid to 2 ml of the solution. Caramel becomes paler. Add 10 ml of Marsh reagent and shake well. Caramel does not dissolve. A change of colour to pink or red on addition of the hydrochloric acid indicates the presence of wine colours. (3) To 2 ml of the soln. add 3–5 times the vol. of paraldehyde and just enough alcohol to form a homogeneous soln., and stopper the tube. A brownish ppt. indicates caramel. Samples containing small amounts of caramel may need to stand overnight. (4) To 2 ml add 2 ml of a reagent consisting of phenylhydrazine hydrochloride 2 parts, sodium acetate 3 parts, water 20 parts. A dark brown ppt. indicates caramel (Amthor, *Z. anal. Chem.*, 1885, **24**, 30). (5) Shake 10-ml portions separately with chloroform, ether, light petroleum, ethyl acetate, carbon tetrachloride, and amyl alcohol. Caramel is insol. in all these solvents.

E. M. P.

Colorimetric Data for the Assay of certain Alkaloidal Hypodermic Tablets and Injections. N. L. Allport and N. R. Jones. (*Quart. J. Pharm.*, 1942, **15**, 238–250.)—To provide rapid methods of assay, previously published colour tests have been modified and made quantitative. *Morphine.*—The colour reaction of Radulescu (*Bull. Soc. Pharm. Bucuresci*, 1905, **14**, 602; ANALYST, 1906, **31**, 234) is less reliable for use with the Lovibond tintometer than with Nessler glasses, but, within a limited range of colour, determinations within ±10% of the true value can be made. Dissolve 1 tablet (or 1 ml of injection) in approx. *N* hydrochloric acid and dilute to 100 ml with the same solvent. Treat an aliquot portion (≡0·8–1·3 mg of morphine) with 5 ml of freshly prepared 0·5% aq. sodium nitrite soln. and, after mixing, with 2 ml of dil. ammonia (10% w/w of NH_3) and dilute to 25 ml with water. Measure the colour in a 1-cm cell and correlate the yellow component with the values in a table constructed from results thus obtained with known amounts of morphine. *Diamorphine.*—Dilute an aq. soln. of the tablet or injection, ≡ *ca.* 1 mg of diamorphine, to 5 ml with water, add 5 ml of dil. hydrochloric acid (10% HCl), boil under reflux for 10 min. and proceed as for morphine. *Atropine, hyoscyamine and hyoscine.*—Dilute the sample with water so that 1 ml ≡ *ca.* 0·8–1·2 mg of atropine. Treat 2 ml with 0·5 ml of dil. ammonia and extract the mixture with three 3-ml portions of chloroform. Wash the combined extracts with 2 ml of water and shake with 20 ml of 6% acetic acid (made with 5% alcohol). After complete separation, filter 5 ml of the aqueous layer, evaporate 1 ml of the filtrate just to dryness on the water-bath and immediately add 0·2 ml of fuming nitric acid (sp.gr. 1·5), moistening the whole of the residue. Evaporate to dryness on the water-bath and leave the dish containing the residue on the bath for 3 min. in all. Add *ca.* 3 ml of acetone, stir and rinse the soln. into a cylinder with acetone until the vol. is 10 ml. Cool, add 0·1 ml of 3% potassium hydroxide soln. in methyl alcohol (not more than 14 days old) and, after inverting the cylinder once, let it stand for exactly 5 min. Measure the colour in a 1-cm cell and correlate the red component with the values found for standard amounts of atropine. The relation between the quantity of alkaloid and the colour is identical for atropine and hyoscyamine, and the method may be applied to hyoscine, the intensities of the colours produced by equal wts. of hyoscyamine and hyoscine being inversely proportional to their molecular wts. Morphine disturbs this colour reaction, but the datura alkaloids do not affect the colorimetric assay of morphine. In presence of morphine treat 5 ml of a soln. of the drug containing *ca.* 1 mg of atropine with 0·5 ml of 5% w/v ferric chloride soln., set aside for 2 min., add 2 g of sodium citrate, render the mixture alkaline with ammonia and proceed as already described. *Procaine.*—The method of Willstaedt (*Biochem. Z.*, 1934, **269**, 182), in which the base is coupled with 1-amino-8-naphthol-3:6-disulphonic acid (H acid), is modified by increasing the concn. of alkali in the coupling reagent and by the use of ammonium sulphamate instead of urea to remove excess nitrous acid. Dilute an aq. preparation (≡ 0·02–0·05 mg) of procaine base to 5 ml, add 1 ml of hydrochloric acid and 0·5 ml of fresh 1% sodium nitrite soln. and set aside for 30 sec. Add 1 ml of 15% aq. ammonium sulphamate soln., shake vigorously and, after 30 sec., add 1 ml of fresh 1% soln. of 1-amino-8-naphthol-3 : 6-disulphonic acid in 20% w/v sodium hydroxide soln. After mixing, dilute the soln. to 10 ml with water. Correlate the red component of the colour with the values in a table of standards. *Emetine.*—The method will detect only gross errors in dispensing. Treat 1 ml of the diluted preparation (0·4–0·7 mg of emetine base) with 2 ml of conc. hydrochloric acid and 0·5 ml of hydrogen peroxide (10 vol.) and set the mixture aside, away from direct sunlight, for 5 min. Correlate the yellow component of the colour with values in a standard table. *Strychnine.*—Deniges' modification of Malaquin's colour test is applicable. Treat 5 ml of a dilution of the preparation in 10% hydrochloric acid (≡ 0·03–0·05 mg of strychnine) in a dry test-tube with 0·2 g of zinc amalgam containing 40% of mercury and recently treated with mercuric chloride and washed with water. Immerse the tube in boiling water for 7 min., cool rapidly, add 0·05 ml of fresh 0·1% aq. sodium nitrite soln. and measure the pink colour. Correlate the red component with values in a standard table. *Ergometrine and Ergotoxine.*—The preparation should contain *ca.* 0·05 mg of alkaloidal base per ml in 1% tartaric acid. Treat 1 ml with 2 ml of 0·125% w/v soln. of *p*-dimethylaminobenzaldehyde in sulphuric acid (65% v/v) containing 0·005% w/v of ferric chloride. After 5 min. measure the blue-violet colour in a 1-cm cell. Calculate the amount of alkaloidal base on the basis that 0·05 mg of solvent-free ergometrine produces a colour ≡ 7·6 blue units, or that the corresponding value for the same amount of ergotoxine is 4·1 blue units. *Pilocarpine.*—The procedure is essentially that of Shupe (*J. Assoc. Off. Agr. Chem.*, 1941, **24**, 757; ANALYST, 1941, **66**, 467). Treat 10 ml of an aq. soln. containing 0·8–1·5 mg of pilocarpine base with exactly 1 ml of 10% acetic acid, exactly 5 ml of chloroform, 1 ml of 5% aq. potassium chromate soln. and 1 ml of approx. 3% w/v hydrogen peroxide soln. and shake the mixture for 30 sec. Filter the chloroform layer and correlate the blue component of the colour with the values in a standard table. *Ephedrine.*—The colour reaction is that of Chen and Kao (*J. Amer. Pharm. Assoc.*, 1928, **15**, 625). Treat 5 ml of an aq. preparation containing 5–7·5 mg of ephedrine with 1 ml of dil. ammonia and 2 ml of methylene chloride. Wash the methylene chloride layer with 2 ml of sat. sodium chloride soln. and repeat the extraction of the aq. layer twice, washing the extracts with the same sodium chloride soln. Evaporate the extracts in a weighing bottle with 1 ml of 1% benzoic acid soln. in methylene chloride,

and to the residue add exactly 1 ml of water and then, after warming gently and cooling, 0·1 ml of 10% aq. copper sulphate soln. and 2 ml of 20% sodium hydroxide soln. Add 3 ml of cyclohexane, shake vigorously and correlate the red component of the colour of the upper layer with the values in a table of standards. A. O. J.

Observations on Indian *Artemisia*. N. A. Qazilbash. (*Quart. J. Pharm.*, 1942, **15**, 323–331.)—*Artemisia* growing in the Kurram has been referred to different species, *viz.*, *A. Maritima* L., *A. brevifolia* Willd. and *A. cina* Berg, no botanical distinction being made between the santonin-bearing and santonin-free species. The santonin-bearing form resembles *A. cina* (Berg) Willkomm, but has apical marginal hairs upon the involucral bracts of the flower-heads. The foliage leaves of the Kurram *Artemisia* are covered with white hairs, and this, with other morphological details, suggests that the Kurram santonin-bearing plant is a distinct sub-species of the *A. cina* group and apparently peculiar to the Kurram valley. Wild and cultivated samples were collected from different places in the Kurram and in Waziristan, the fresh top branches being dried in the sun before removal of the leaves and flower-heads for chemical examination. To detect santonin, shake 1 g of the powdered material with 10 ml of benzene or carbon tetrachloride for 10 min., filter and evaporate the filtrate to dryness on the steam-bath. Treat the margins of the residue with 2 or 3 drops of potassium methoxide. An orange-red or carmine colour indicates santonin. For its determination, heat 15 g of the powdered drug with 65 ml of hot water on the steam-bath for 15 min. and, after addition of 25 ml of *ca.* 4 *N* hydrochloric acid, for a further 5 min. with thorough stirring. Transfer the liquid while still lukewarm to a separator, and, when it is cold, add 15 g of karaya gum (Indian gum tragacanth) and 150 ml of chloroform and shake thoroughly for 30 min. and at frequent intervals for a further 30 min. Filter the chloroform layer through cotton wool and distil 102 ml (≡ 10 g of the herb) until the vol. is reduced to *ca.* 15 ml. Rinse the conc. extract into a beaker with two or three 5-ml portions of chloroform and heat the extract with 100 ml of freshly prepared 5% barium hydroxide soln. (in hot water) on the steam-bath. The subsequent procedure has been described elsewhere (*Bull. Sci. Pharm.*, 1935, **42**, 129; *Current Science*, 1935, **4**, 51). The santonin content of 40 samples ranged from 1·04 to 2·78%. It was found that the root and woody stem of the Kurram plants are devoid of santonin, whereas the leaves, flower-heads and seeds contain a good percentage. The santonin-content of the herbaceous portions of the stems and stalks of the flowering tops was low, but in the leaves and immature flower-heads of samples from Burki, Nastikote, Chamkani and elsewhere in southern Afghanistan it was high. The flower-heads of *A. fragrans* Willd. from Kandahar showed 1·56% of santonin during September. To give a high manufacturing yield of santonin, the material should be completely freed from pieces of stem and branches and from thin pieces of the stalk. *M.* O. J.

Synthetic Menthols. W. E. Huggett. (*Quart. J. Pharm.*, 1942, **15**, 218–237.)—Each of the 4 isomeric forms of *p*-menthan-3-ol, *viz.*, menthol, *neo*-menthol, *iso*menthol and *neo-iso*menthol, occurs in 2 optically active and one racemic modification. *d-neo*-Menthol occurs naturally in small amounts with *l*-menthol and remains in the "dementholised" peppermint oil left after crystallisation of *l*-menthol. There is no authentic evidence that any of the menthols derived from the *iso*menthones occur naturally. *dl*-Menthol can exist in 2 crystalline modifications with m.p. 28° and 38° C. respectively, and the form of higher m.p. may pass over to the other form, so that melting may occur at any temp. from 28° to 38° C. If *ca.* 10 g of *dl*-menthol are melted and stirred with a thermometer during cooling, the mass begins to solidify at 27°–28° C., but suddenly becomes more opaque and the temp. rises to 38° C. and remains constant until crystallisation is complete. This double setting-point is very marked with pure samples and is itself a criterion of purity. *l*-Menthol also occurs in several forms, but the lower-melting forms are unstable and the m.p. of 43° C. is usually well-defined, although a setting-point curve shows 2 and sometimes 3 slight inflections under favourable conditions. The optically active and inactive forms of *neo*-menthol exhibit a great tendency to remain in the liquid state when once molten, and *d-neo*-menthol (m.p. −15° C.) is extraordinarily difficult to solidify in absence of a seeding crystal. The $[\alpha]_D$ of menthol isomers is not a reliable index of purity unless the conditions of determination are strictly defined, especially with *neo-iso*-menthol and *iso*menthol, on which the solvent has an abnormal influence. When any menthol is mixed with phosphoric acid a solid addition compound is formed (Blagden and Huggett, *J. Chem. Soc.*, 1934, 317). This reaction is the basis of a reliable test for isomeric impurities in menthol. Mix 1 g of phosphoric acid (sp.gr. 1·75) with 4·25 g of the menthol previously dried by boiling. The mixture will have a well-defined setting-point and m.p. A setting-point of 60° C. (or m.p. 61° C.) indicates absence of isomers, and lower values indicate their presence. The test is independent of the optical activity, and when the impurity is *iso*menthol an estimate of the amount to within 1% for any mixture containing up to 40% can readily be obtained. The first steps in the manufacture of synthetic "menthol" lead to the formation of a mixture of isomers which is very difficult to resolve into its components, and, consequently, partly separated mixtures, of more or less constant composition, appear on the market and are used to replace natural menthol, especially in preparations for external use. A group of observers described, with moderate agreement, the tastes of solns. of the isomers in tap water (1:4000), as follows:—*neo*menthol, dank, earthy, fusty, mouldy, slightly bitter and, to some, slightly salt with a slight delayed cooling effect; *iso*menthol, musty, stale, mildew-like, hay- or vegetable-like, distinctly bitter and persistent without cooling effect; *neo-iso*menthol, between the other two with relatively non-persistent taste. *iso*-Menthol has an over-powering effect in spoiling the taste of menthol, as little as 1% leaving an unpleasant, stale after-taste. The recent supplement to the German Pharmacopoeia prescribes, as a test for absence of isomeric impurities in synthetic menthol, absence of a stale or musty taste. The m.p. (°C.) of isomerides not given above are:—*dl-neo*-menthol, 52·0°; *d-iso*menthol, 82·5°; *dl-iso*menthol, 53·5°; *neo-iso*menthol, −8·0°; *dl-neo-iso*menthol, 13·5°. A. O. J.

Determination of Calcium as Oxalate. G. J. W. Ferrey. (*Quart. J. Pharm.*, 1942, **15**, 264–267.)—Calcium is most completely pptd. as oxalate from acid soln. at *p*H 4·4–4·6. Treat the

sample (*e.g.*, 10–20 ml of a galenical, accurately weighed) with an equal vol. of water and 4 ml of nitric acid and, after boiling to oxidise iron, with 20 ml of ammonium citrate soln. (the B.P. lead test soln. adjusted to *p*H 7) and dilute to 150 ml. Heat the mixture to b.p., test its *p*H with bromocresol green, add 50 ml of ammonium oxalate soln., boil gently for 15 min., collect the ppt. in a tared sintered glass crucible (Jena 1GK.3/<7 or Pyrex F.2C.4), and wash it thoroughly with cold water. Dry at 100° C. for 1 hr. and weigh as $Ca(COO)_2H_2O$. If desired, adjust the *p*H of the filtrate for pptn. of iron by oxine by adding 32 ml of *N* sodium hydroxide soln. to neutralise 2 of the 4 ml of nitric acid originally added. The method was tested on a standard soln. of pure Iceland Spar in dil. hydrochloric acid. The presence of iron, manganese and magnesium had no significant effect on the accuracy of the method. For determination of calcium in Compound Syrup of Ferrous Phosphate, B.P., Compound Syrup of Hypophosphites, B.P.C., and Compound Syrup of Glycerophosphates, B.P.C., the method gave results agreeing satisfactorily with those obtained by the official method of assay of the first preparation. The method serves also for the removal of calcium before the use of oxine, especially in the determination of magnesium. A. O. J.

Biochemical

Colour Test for *p*-Aminobenzoic Acid, the Chromotrichia Factor. H. Tauber and S. Laufer. (*J. Amer. Chem. Soc.*, 1941, **63**, 1488–1489.)—Add to 5μg of *p*-aminobenzoic acid in 1 ml of glacial acetic acid 1 ml of a 1% glacial acetic acid soln. of *p*-dimethylaminobenzaldehyde, and use as control a mixture of 1 ml of glacial acetic and 1 ml of the *p*-dimethylaminobenzaldehyde soln. After 5 min. at room temperature a deep yellow colour develops in presence of the amino acid. To study the solns. colorimetrically, add 5 ml of glacial acetic acid. The colour, which is probably a Schiff base, is given also by *o*- and *m*-aminobenzoic acids and their alkyl esters, and by aniline and its derivatives, but the latter are not normally found in biological material. The reaction is not given by aliphatic amino acids or their aromatic derivatives, *e.g.*, tyrosine and phenylalanine, or by glutathione, urea, pantothenic acid, nicotinic acid, nicotinic acid amide, or thiamine. Thiamine and some other compounds with primary or secondary amino groups react only on prolonged boiling or complete evaporation of the acetic acid. E. M. P.

Vitamin C in Rose Hip Syrup. F. Wokes, E. H. Johnson, J. Duncan, J. Organ and F. C. Jacoby. (*Quart. J. Pharm.*, 1942, **15**, 314–322.)—An examination of rose hips from a 5-ton crop, gathered in Hertfordshire during Oct. and Nov., 1941, gave the following data. The vitamin C content was highest in the middle of November (400 mg per 100 g). For several weeks before this it was lower and fell to 2/3rd of the max. val. by early December. A small sample collected in Jan., 1942, gave only 104 mg per 100 g. The aver. content for the whole of November lay between 300 and 400 mg per 100 g. According to Melville (private communication) the predominant species in Hertfordshire are *Rosa canina* and *R. dumetorum*. Loss of vitamin C began as soon as the hips were collected. When dried whole *in vacuo* (8 hr. at 50° C.) they lost 10% immediately, and when dried at 105° C. for 12 hr. they lost 86%. Mincing before drying caused still greater instability, and solid extracts made *in vacuo* lost vitamin at the rate of 5% per week at 5° C., and at higher temp. still more rapidly, especially during the first week (46%). Of 6 commercial syrups, 1 contained only 10% of the potency stipulated by the Ministry of Health; of the other 5, only 1 was more than 10% below the Ministry's requirement. Syrups stored at 37° C. lost 5–10% per week, and a black-currant purée showed a similar loss. At 5° C. the rate of loss was *ca.* 1% per week. The rate of destruction of the vitamin at the *p*H of rose hip syrup (*ca.* 3·8) is about 10 times the rate at *p*H 1. Sugars (sucrose, maltose and mixtures of maltose and glucose) have a marked stabilising effect which is not specific for any one sugar. The concn. of sugar should be high, and the rate of loss is reduced but not entirely prevented by storing the syrup in nitrogen-filled containers. In the determination of vitamin C, the chloroform method gave erratic results, but the potentiometric method of Harris, Mapson and Wang (*Biochem. J.*, 1942, **36**, 183; ANALYST, 1942, **67**, 304) was satisfactory. The titration mixture should contain enough potassium chloride soln. to give a final concn. of 0·046 *N*, and addition of 20% of acetone eliminates certain disturbing influences. Use oxygen-free nitrogen for stirring, record the initial e.m.f., and add the dye soln. (≡ 0·5 mg of ascorbic acid per ml) from a micro-burette. Precisely 30 sec. after beginning to add the dye soln. (addition should take not more than 10 sec.), read the e.m.f., which at the end-point will rise rapidly from an initial value of 100–120 mv to over 300 mv. Take at least 2 readings between 200 and 300 mv, plot them against vols. of dye soln. added, and extrapolate downwards those between 200 and 300 mv to intersect a horizontal line drawn through the initial readings, thus locating the end-point. The results can be made more accurate by the use of more conc. solns. The following details are important:—Daily plating of the electrode with mercuric chloride; rinsing the electrode and agar bridge with water after each titration; the presence in the titration mixture of 20% of a freshly prepared 25% soln. of metaphosphoric acid and of 20% of acetone; precise timing of the additions of dye soln.; daily cleaning of the electrode by immersion in 6 *N* nitric acid for 5 min.; standardisation of the potentiometer. With these precautions the method gives good results with a wide variety of food and medicinal preparations. Losses of vitamin C during manufacture can be avoided by subjecting the hips to heat treatment as soon as possible after collection and then preparing *in vacuo* an extract which is stabilised by addition of a high concn. of sugar at *p*H 4 and storage at a low temp. A. O. J.

Vitamin C from Green Tomatoes. F. Wokes and J. G. Organ. (*Nature*, 1942, **150**, 523–524.)—When green and red tomatoes are pulped, 92 and 27% of the vitamin C may disappear, respectively, in 7 min. Pyke's conclusions regarding the importance of avoiding too fine a state of subdivision of green vegetables (*cf.* Lampitt, Baker and Parkinson, ANALYST, 1942, **67**, 305) are confirmed; *e.g.*, chopped and coarsely-sliced unripe tomatoes lost 18 and 11%, respectively, of their original vitamin C content overnight; pulped green tomatoes in 10% vinegar (*p*H of mixture, 3·7) lost 80% overnight. The oxidising enzymes responsible for these losses are probably concentrated in the skin and flesh,

and almost absent from the juice. The vitamin contents of ripe or unripe or of large or small (*e.g.*, 1 g) tomatoes showed no significant differences. J. G.

Use of Formaldehyde in the Determination of Ascorbic Acid and Dehydro-Ascorbic Acid. J. W. H. Lugg. (*Nature*, 1942, **150**, 577.)—The determination of ascorbic acid by reduction of 2 : 6-dichlorophenolindophenol is rendered more specific by pre-treatment under controlled conditions (not stated) with formaldehyde at *p*H 1·5 and 3·5. Cysteine, sulphites, thiosulphates, hydrogen sulphide and pyruvic acid treated with hydrogen sulphide readily react ("condense") with the formaldehyde at both *p*H vals.; quinol, 2 methyl-1 : 4-naphthaquinone treated with hydrogen sulphide, thiourea, reductone and ferrous salts do not react at either *p*H val.; ascorbic acid is the only substance yet found which reacts readily at *p*H 3·5, but only very slowly at *p*H 1·5. Dehydro-ascorbic acid is reduced by hydrogen sulphide and determined as ascorbic acid. The actual titration should then be made, under standard conditions at *p*H 1·5, with a standard soln. of reagent, in drops of definite size, to a "persistence" of limited time. A definite fraction of the colour (*e.g.*, 95, 90 or 80%) should be discharged, and a correction made for the small amount of untitrated ascorbic acid remaining. J. G.

Vitamin P in Blackcurrants. A. Pollard. (*Nature*, 1942, **150**, 490–491.)—The following method is used to isolate a fraction containing vitamin P in high concns.:—Depectinise and concentrate the juice, extract it with amyl alcohol, remove the anthocyanins from the extract by extraction with dil. hydrochloric acid, and ppt. and separate the lead salt of the active fraction in alcoholic soln. by the method of Szent-Györgyi (*Z. physiol. Chem.*, 1938, **255**, 126). On decomposition of the lead salt, a water-sol. red-brown amorphous material is obtained, which appears to consist mainly of anthoxanthin glycosides. The yield from black currants is 0·03% of the original juice, and the activity is 100 and 10 times greater than that of recryst. hesperidin and water-sol. citrin (from oranges), respectively, as determined by the method of Bacharach, Coates and Middleton (*Biochem. J.*, 1942, **36**, 407). A similar product was obtained from wild rose hips by the same method. J. G.

Bacteriological

Staining Solution for Micro-organisms. P. H. H. Grey. (*Canad. J. Res.*, 1941, C**19**, 95–98; *J. Text. Inst.*, 1942, , A427.)—The spores and vegetable cytoplasm of bacteria, yeasts and certain fungi may be stained differentially with an aqueous soln. of 0·5% of malachite green and 9·05% of basic fuchsin. For a saline soln. 2 parts of this dye soln. may be diluted with 8 parts of a 0·8% soln. of sodium chloride. Heating is not necessary with these solns., although it is recommended for staining "ripe" spores of bacteria. Decolorisation and counter-staining are not required. The spores of bacteria and yeasts are stained blue or greenish-blue, whilst cytoplasm is stained light violet or pink. Young bacterial cells are stained deep violet, older cells light violet; the soln. can thus be used as a general stain. The acid-fast organism *Mycobacterium berolinensis* is stained greenish-blue with the granules violet. The saline soln. is recommended as a differential stain for young colonies of *Aspergillus* and *Penicillium*; the terminal growing tips and young branches of hyphae are stained blue, older hyphae light violet, spores and conidiaphores blue.

Agricultural

Ergot in Cereal Crops. [Separation of Ergot from Contaminated Grain.] W. A. R. D. Weston and R. E. Taylor. (*J. Agric. Sci.*, 1942, **32**, 457–464.)—Several cases of ergot in barley were met with in 1941. Records of the occurrence of ergot in cereal crops in Britain during the past 24 years show that ergot is found more frequently on rye than on wheat, barley or oat, in descending order. The disease is more prevalent in northern districts than in the south. It is rare on wheat and barley, and little is known as to the susceptibility of their varieties, although it has been found on Rivet wheat (*Triticum turgidum*) on several occasions and its occurrence on crosses of Rivet and *Triticum vulgare* has been recorded. With the exception of one specimen found in a field of mixed corn in Cambridgeshire, there is no record of its occurrence on oat in this country. The disease may be contracted from an infected seed supply, from soil into which ergots have fallen from a previous crop, and from ergotised grasses in the immediate neighbourhood. The threshed grain of infected 1941 barley crop investigated contained 0·03–0·88% w/w of ergot, and the alkaloidal content of one sample, assayed by the B.P. Addendum, 1936, method, was 0·216% as ergotoxine. A flotation method of separating ergot from contaminated barley, similar to that used for rye by various workers (*e.g.*, Eriksson, "*Fungous Diseases of Plants*," London, 1930, pp. 314–5; Craigie, *Economic Survey Board, Manitoba*, 1939, pp. 12–13) was studied. The best separation was effected by soaking the barley for 3 hr. in water and removing the floating fraction by skimming. The water was then drained from the grain and replaced by a soln. of 14·5–17% of sodium chloride or 16–19% of potassium chloride. All floating matter was skimmed off twice after successive stirrings. In each expt. the floating fractions and cleansed grain were washed, dried and weighed. From 98·1 to 99·6% of the total ergot was thus removed. The material removed with the ergot consisted largely of chaff, "tail corn" and weed seeds. The preliminary soaking in water greatly reduced the loss of grain in the skimmings without lessening the effective removal of ergot. Grain thus treated with sodium chloride showed better germination than before, but the potassium chloride treatment slightly reduced the germinating capacity. The percentage of total grain removed was 5·7 as compared with 22 when pre-soaking was omitted. In one series of expts. the seeds of two weeds scheduled as "injurious" in the Seeds Regulations, 1922 (Seeds Act, 1920) were removed in the skimmings together with the bulk of the other weed seeds and much of the inert matter. Control measures recommended are: (1) to use only seed free from ergot; (2) to plough the stubble from a contaminated crop deeply so as to bury fallen ergots; (3) so far as possible, to cut wild grasses on field margins before they flower (*cf.* Barger, ANALYST, 1937, **62**, 340).

Organic

Separation of Linolic and Oleic Acids. J. Frankel and J. B. Brown. (*J. Amer. Chem. Soc.*, 1941, **63**, 1483–4.)—Dissolve the mixed fatty

acids, *e.g.*, of maize oil, in acetone (75 g per litre), chill, separate the fractions crystallising at −20° C. and −50° C., and cool the filtrate to −70° C. Dissolve the crystalline product (*ca.* 90% linolic acid) in light petroleum (65 g per litre) and chill to −48° C.; this gives a fraction with iodine val. *ca.* 176 (purity *ca.* 95%). Dissolve this mixture in sufficient light petroleum to keep the oleic acid in solution at −60° C. and cool to that temp.; practically pure linolic acid crystallises out, with very little mixed crystal formation. The physical and chemical constants of the acid thus directly obtained agreed closely with those of linolic acid prepared by bromination and repeated re-crystallisation, but its slightly lower tetrabromide value indicated that it probably contained 1–2% of an isomeric linolic acid.

New Colour Reactions of Phenols. A. Steigmann. (*J. Soc. Chem. Ind.*, 1942, **61**, 180.)—The following reactions are described. (A) To 5 ml of 1 : 7500 aqueous sodium β-naphthaquinonesulphonate soln. add the test soln. and then 0·3 ml of dil. ammonia (50 ml of 0·880 soln. + 150 ml of water). Prepare a blank test. A blue or bluish-green colour in 3–5 min. indicates a monohydric phenol or resorcinol; chlorinated monohydric phenols mostly give a bluish-green colour. The colour changes to red-brown or brown on addition of 0·5 ml of 5 *N* acetic or sulphuric acid. Pentachlorophenol, some *p*-phenols, *e.g.*, tyrosine and 2.4.6-trichlorophenol, concentrated and easily oxidised phenols, *e.g.*, pyrocatechol, quinol and metol, and polyhydric phenols, *e.g.*, phloroglucinol, do not give the test. Substances with reactive NH_2 and CH_2 groups may cause discolorations in the test, but can be distinguished from phenols, as they react in absence of ammonia. (B) Add 0·1–1 ml of the neutral test soln. to 5 ml of 1 : 8000 aqueous *p*-aminodiphenylamine hydrochloride soln. (from a 1 : 100 stock soln.) and then 0·5 ml of 3% chloramine-T soln. A blue, red-violet or violet colour indicates a monohydric phenol if the soln. does not give a similar colour with chloramine-T alone at the same dilution as with easily oxidised phenols. Strong reactions are obtained with phenol, cresol, and 8-hydroxyquinoline (violet); *o*- and *m*-chlorophenols (blue); chlorothymol and *iso*-chlorothymol (red-violet). The reaction is inhibited by proteins such as glue and gelatin. The dyes are sparingly sol. in water, but sol. in sodium hydroxide soln., the blue dyes becoming violet. The reaction may be used for spot tests, the sensitivity limit being 1–20μg, according to the phenol. The following data are given for 11 phenols:

Phenol	Dilution limit A	Dilution limit B	Colour A	Colour B
Phenol	1:250,000	1:125,000	Blue	Violet
Cresol	1:250,000	1:125,000	Blue	Violet
β-Naphthol	1:50,000	1:75,000	Yellow-green	Greenish
Resorcinol	1:3,000,000	1:250,000	Dark blue	Brown
8-Hydroxyquinoline	1:500,000	1:250,000	Blue	Red-violet
Pyrocatechol	1:100,000	Not characteristic	Yellow-green	Brown
Phloroglucinol	1:100,000	Not characteristic	Violet	Brown
m- and *o*-Chlorophenols	1:40,000	1:125,000	Blue to blue-green	Blue-violet
p-Chloro-*m*-cresol	1:40,000	1:25,000	Blue to blue-green	Violet
Chloro- and *iso*chlorothymols	1:100,000	1:50,000	Blue to blue-green	Red-violet
2.4.6-Trichlorophenol	No reaction	1:25,000	No reaction	Blue

The sensitivity limits (in μg) of the *p*-aminodiphenylamine spot tests are: phenol and cresol 5; resorcinol 1–2; β-naphthol 5–10; 8-hydroxyquinoline 3–4; *m*- and *o*-chlorophenols 10; *p*-chlorophenol 50; *p*-chloro-*m*-cresol 25; chloro- and *iso*chlorothymols 10; 2.4.6-trichlorophenol 20. ***Pentachlorophenol.***—The silver salt of pentachlorophenol is obtained at *p*H 8–9 as an intense yellow, voluminous ppt., insol. in dil. acetic acid, but somewhat sol. in ammonia. The dark red-brown copper salt is obtained at *p*H 5–6; it is changed to white by acetic acid (formation of free pentachlorophenol) and dissolves in ammonia, forming a colourless soln. These salts are obtained in colloidal soln. in presence of protective colloids. The salts of other chlorophenols are sol. in dil. acetic acid. ***Procedure.***—Place 1 drop of 0·1 *N* silver nitrate on filter-paper and when it is almost dry add the test soln. (*p*H 8–9); when dry, the yellow spot, if formed, is touched with a drop of 5% acetic acid. A yellow spot or ring indicates pentachlorophenol. The sensitivity limit is 10μg. E. M. P.

Colorimetric Determination of Phenothiazine with Palladous Chloride. L. G. Overholser and J. H. Yoe. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 646–647.)—Dilute a mixture of 50 ml each of 1·0 *M* sodium acetate and 1·0 *N* hydrochloric acid to 250 ml; this produces a buffer soln. of *p*H 2·6. To 5·0 ml add 1·0 ml of a standard soln. of palladous chloride in 1·0 *N* hydrochloric acid, which has been diluted so as to contain 0·03 mg of palladium per ml. Dilute to *ca.* 15 ml, add a suitable vol. of a soln. of the sample in acetone (*vide infra*), dilute to exactly 25 ml, and mix well; the resulting soln. thus becomes buffered at *p*H 2·9. At the same time prepare standards in the same way from a fresh soln. of phenothiazine (purified by recrystallisation from ethyl alcohol; m.p., 182° C.) in acetone (0·05 mg per ml), taking care that the acetone contents of the sample and standards are equal. Match the colours immediately. The sensitivity is 2·5μg for phenothiazine concns. of 0—40μg; 5μg for 45–100μg. Optimum acetone content of the test soln. 8–10% v/v; sensitivity much less with concns. over 20%. The dark blue complex formed, $Pd(C_{12}H_9NS)_2Cl_2$, is relatively unstable if more than 100μg of phenothiazine are taken. It is sol. in ethyl acetate giving a red soln., but this colour is too unstable for colorimetric determination, although it may be used as a qualitative test when interfering colours are present (*e.g.*, in some phenothiazine insecticides). Under the conditions described, up to 0·05 ml of 1·0 *N* hydrochloric acid and 0·2 ml of 1·0 *N* sodium chloride do not affect the results. The complex may be isolated by mixing 5 ml of the palladous chloride soln., 95 ml of water, 80 ml of acetone and 10 ml of a filtered soln. containing 40 mg of phenothiazine, collecting on a sintered glass crucible after *ca.* 3 hr., and drying at 120° C. for 2 hr. Relative absorption curves for solns. of the complex in the buffer soln. and in ethyl acetate are plotted for

wavelengths of 400–750$m\mu$; they are similar in general character, but have a min. at *ca.* 425$m\mu$, and max. at 540 and 480$m\mu$, respectively. J. G.

Determination of the Cellulose Content of Wood by Extraction with Monoethanolamine. P. Bloom, E. C. Jahn and L. E. Wise. (*Paper Trade J.*, 1942, **115**, *T.A.P.P.I. Sect.*, 33–40.)—Grind the sample (60–80 mesh), extract it in a Soxhlet apparatus for 6 hr. with a mixture of alcohol and benzene (2 : 1, vol.), dry the residue in air, and add *ca.* 2 g to *ca.* 100 g of anhydrous, clear monoethanolamine (b.p., 168–170° C.) in a conical flask. Cover the flask with a watch-glass, place it in a oil-bath at 168–170° C. for 2 hr., remove and leave for 3 min. Add water until the vol. is doubled, filter through a tared, sintered glass crucible (medium porosity), and wash the residue with 500 ml of cold water. Transfer the wet residue to a 250-ml beaker containing fresh, cold, *ca.* 1·0% chlorine water (≡ 0·4 g of available chlorine per g of original wood). After 5 min. at *ca.* 20° C., filter the mixture in the same crucible, wash successively with 50 ml of 0·67% sulphur dioxide water, 50 ml of cold water, 50 ml of 2% sodium sulphite soln. and with water. Add sufficient hot 2% sodium sulphite soln. to cover the residue in the crucible, surround the crucible with the hot soln. in a beaker, boil gently for 3 min., stirring the residue with a pointed glass rod. Remove the excess of sulphite by suction, wash the residue with cold water, and repeat the chlorine water and sulphite treatments (once for hardwoods, twice for softwoods). Wash the residue with 500 ml of water and 50 ml of acetone in succession and dry to constant weight. The effects of variations in technique are recorded in detail for largetooth aspen (*Populus grandidentata* Michaux) and sitka spruce (*Picea sitchensis* Carr). The results agree with those obtained by the Cross and Bevan method, and the celluloses obtained by the two methods are broadly similar. The residual cellulosans may, however, differ, as the two types of reagent have different selective extraction effects. Although delignification is complete by both methods, the monoethanolamine cellulose has less colour, and the particles are more discrete and resemble the original wood particles in structure. Since they do not tend to gelatinise, filtration and washing proceed easily, so that there is a considerable saving in time as compared with the Cross and Bevan method, particularly with softwoods. A further advantage is the smaller personal equation involved. As with other analytical methods for cellulose, the procedure is an arbitrary one, and must consequently be carefully standardised in detail (*cf.* Wise, Peterson and Harlow, ANALYST, 1939, **64**, 227). J. G.

Determination of Alkoxy-Groups in Cellulose Ethers. E. P. Samsel and J. A. McHard. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 750–754.)—An improved Vieböck modification of the Zeisel method is used (Vieböck, *et al.*, *Ber.*, 1930, **63B**, 2818, 3207; Clark, ANALYST, 1932, **57**, 402). The all-glass apparatus is described in detail, and illustrated with full dimensions. Important features of it are pressure-equalising and other devices to ensure a steady flow of carbon dioxide, which enters the bulb of the boiling flask at a point near the neck through a 1-mm capillary tube. The flask is fitted with a vertical condenser tube (1·6 × 28 cm), at the top of which is a water trap containing an aqueous suspension of red phosphorus (to scrub out any entrained iodine or hydriodic acid), and a connecting tube to two absorbing vessels in series. A suitable hydriodic acid reagent is prepared as follows:—Place 70 g of red phosphorus in a 3-necked, 2-litre round-bottomed flask fitted with a reflux condenser, dropping funnel and thermometer, add *ca.* 1 litre of water, warm gently, and then add slowly through the funnel a soln. of 800 g of iodine in 800 ml of hydriodic acid. Boil for 30 min., allow the excess of phosphorus to settle, and decant the soln. into a suitable all-glass distillation flask with an efficient fractionating column. Pass a slow stream of carbon dioxide through the flask during distillation by means of an inlet-tube which dips below the liquid level. Collect the portion of the distillate of sp.gr. 1·690–1·700 and store it in small, brown, glass-stoppered bottles, which have previously been swept out with carbon dioxide and are sealed with paraffin wax and stored in the dark. This is a constant boiling mixture (b.p. 126°–127° C.) containing 57% of hydriodic acid.—Grind the sample (*e.g.*, in a Wiley micro-laboratory mill), and dry it at 105° C. for 30 min. Pour a little 0·06% aqueous suspension of red phosphorus into the trap of the apparatus (*vide supra*), rinse it in with water until the trap is half full, and put into the first and second receivers, respectively, 6–7 and 10–12 ml of a fresh soln. of 5 ml of bromine in 145 ml of a 10% w/v soln. of anhyd. potassium acetate in a mixture of glacial acetic acid and acetic anhydride (9 : 1 pts. by vol.). Weigh exactly 50–60 mg of the sample into a gelatin capsule (Parke, Davis, Size 0), drop it into the boiling-flask, and add some small glass beads, chips of porcelain, and 6 ml of the hydriodic acid reagent. Immediately attach the flask to the condenser, bubble a stream of carbon dioxide (2 bubbles per sec.) through the side-arm of the boiling-flask, which is heated at 150° C. in a thermostatically-controlled oil-bath for 40 min. Wash the contents of the receivers into a 500-ml flask containing 10 ml of 25% sodium acetate soln., dilute to 125 ml with water, add 90% formic acid (sp.gr., 1·20) a drop at a time, with swirling, until the brown colour of the bromine is discharged (*e.g.*, 9–12 drops), and then add 3 drops more. After 3 min. add 3 g of potassium iodide and 15 ml of 10% sulphuric acid, and titrate the liberated iodine with 0·1 *N* sodium thiosulphate. If allowance is made for any blank, then, 100 × net titration/wt. of sample (corrected for ash and moisture contents) = % alkoxyl; factor for methoxyl groups, 0·000517; for ethoxyl, 0·00075. Successful results were obtained with the lower alkyl ethers, but a solvent (*e.g.*, phenol or propionic anhydride) is necessary with compounds particularly resistant to attack. The method is unsuitable for benzyl cellulose, as the b.p. of the benzyl iodide is higher than that of the hydriodic acid. The effects of variations in experimental technique on the accuracy of the method are described. J. G.

Determination of Methylpentoses in Presence of Pentoses. B. H. Nicolet and L. A. Shinn. (*J. Amer. Chem. Soc.*, 1941, **63**, 1456–1458.)—The method depends on the fact that methyl pentoses are the only sugars that form acetaldehyde when treated with periodic acid. The apparatus consists of 3 Pyrex test-tubes (2·5 × 20 cm.) arranged as a gas-absorption train, except that the first tube is fitted with a dropping-funnel, the stem of which reaches almost to its base. Into this tube put (in the following order) the sample (equiv. to 5–15 mg of methyl pentose in a vol. of *ca.* 5 ml); *ca.* 0·2 g of alanine; 1 drop of Nujol (to prevent foaming);

5 ml of *N* sodium bicarbonate (10 ml if large amounts of other carbohydrates are present); 10 ml of 0·1 *N* sodium arsenite containing 20 g of sodium bicarbonate per litre (*i.e.*, sufficient to reduce all the periodate not reduced by reaction with the sugars). Into the other 2 tubes put, respectively, 5 and 3 ml of a 1·9% soln. of sodium metabisulphite, and dilute each soln. to 25 ml. Pass carbon dioxide through the system for a few sec. to mix the contents of the tubes, stop the flow, and place 1–2 ml (*vide infra*) of periodic acid in the dropping funnel (outlet closed). Deliver this reagent into the tube under carbon dioxide pressure (to prevent losses of acetaldehyde, back-pressure effects and contamination from the atmosphere). Pass the gas through the tubes for 1 hr. at 1 litre per min., and then mix the contents of the 2 absorbing tubes and titrate the acetaldehyde present in the usual way, *e.g.*, by the method used by Clausen (ANALYST, 1922, **47**, 363) in the determination of lactic acid, except that 0·02 *N* iodine (1 ml ≡ 1·64 mg of methyl pentose) is desirable. If "other carbohydrates" are to be determined, the residual soln. in the first tube should be aerated and treated as in the authors' method for the determination of serine (*id.*, 1941, **61**, 343, 426). With samples of completely unknown composition first estimate the vol. of hydriodic acid required by neutralising the wt. of sample to be used in the determination with the sodium bicarbonate soln., adding 10 ml in excess, and then 2 ml of 0·5 *M* periodic acid. After 2 min. add 2 ml of 20% potassium iodide soln., and titrate the liberated iodine with a standard arsenite soln.; this soln. is equiv., mole for mole, to the excess of periodic acid; if no excess is found, repeat the expt. with more periodate. At least twice the quantity of periodic acid that can be consumed by the sample rapidly is required. Recoveries of methyl pentose recorded for experiments with mixtures containing rhamnose, *l*-fucitol, glucose, xylose and galacturonic acid (heated under reflux for 6 hr. with 1·5% sulphuric acid before analysis) range from 97·7–100·0%; 6 expts. with a seaweed (heated under reflux with 4% sulphuric acid for 6 hr.) gave 10·02–10·40% of fucose. The specificity of the method may be limited by the fact that, theoretically, reaction should occur with substances which contain the groupings –CHOH.CHOH.Me, –CHOH.CHNH*R*.Me or CHNH*R*.CHOHMe (where *R* is H or a substituent other than acyl), but this point has not yet been fully investigated. J. G.

Reactions of Gelatin Impurities which are Important Photographically. A. Steigmann. (*J. Soc. Chem. Ind.*, 1942, **61**, 162–164.)—The following test is useful for studying natural and artificial restrainers and inhibitors in photographic gelatins. Mix 5 ml of a 10% soln. of the gelatin at 40° C. with 0·5 ml of dil. ammonia (1 vol. of 0·880 with 3 vols. of water), 1 ml of *N*/10 silver nitrate and 1 ml of a 1 : 5000 methylene blue soln.; make a parallel test with the same solns. after addition of 0·5 ml of 0·3% sulphurous acid soln. (to make sure that the reaction is not due to sulphurous acid). Expose the tubes to bright diffuse daylight; in direct sunlight the reaction may be too rapid. With good natural photographic gelatins which have not been made inert or filtered through active carbon, the solns. become greenish owing to the formation of yellow colloidal silver, this change being followed, especially in bright sunlight, by bleaching of the methylene blue; the soln. then darkens, owing to the silver ammine salt being reduced to brown colloidal silver. Inert or filtered gelatins do not bleach the methylene blue. The compounds or natural impurities responsible for the test appear to be thiol compounds, some of which (*i.e.*, very good sensitisers) have no inhibiting or restraining power. The author's new restrainer test (*J. Soc. Chem. Ind.*, 1942, **61**, 67) indicates powerful restrainers by a negative reaction with gelatins which are not completely inert; completely inert gelatins also do not react. Since powerful restrainers or inhibitors prevent a positive reaction even in presence of "sulphur bodies," it is advisable to make the test with gelatin that has not been made inert and also after addition of 0·2–0·5 ml of 1 : 10,000 sodium thiosulphate soln. Inert gelatins which are not strongly restrained will then give a positive reaction, whereas much-restrained or inhibited gelatins will show a negative reaction. E. M. P.

Inorganic

Kjeldahl Distillation without Absorbing Acid. J. A. Bradley. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 705–706.)—The usual Kjeldahl distillation apparatus is modified by attaching a filter-flask with a side-arm to the lower end of the condenser by means of a rubber stopper in its neck, and attaching a small rubber balloon to the side-arm so as to relieve the pressure set up in the flask as distillation proceeds. The flask should be arranged so that it can be lowered during the last few mins. of distillation to permit drainage. Distil *ca.* 400 ml of the sample with 25 ml of 10% potassium hydroxide soln., in presence of fine capillary tubes, sealed at one end with their open ends down (to ensure smooth boiling); the end of the condenser in the receiving flask should be just covered with water. When the distillate amounts to 250 ml, disconnect and lower the flask, continue distillation for a few min. longer (to allow for drainage), add 2 drops of methyl orange soln., and titrate with standard (*e.g.*, 0·2 *N* sulphuric) acid to the first pink shade. The method is quicker and simpler than the usual procedure and requires less materials. Comparative distillations were made of solns. produced by neutralising 0·2 *N* ammonia with the acid to methyl orange, by both the above and Kjeldahl methods, with a closed and an open receiver in each instance; 70–200 mg of ammonia were actually distilled. With the proposed method the ammonia recovered (12 expts.) was 99·4–100 (mean, 99·8)%. The recoveries by the ordinary Kjeldahl method (21 expts.) were 99·4–100·0 (mean 99·7)%, or 99·9–100·0 (mean, 99·9)% when the closed receiver was used (4 expts.). By the proposed method with an open flask the mean recovery (7 expts.) was only 98·7%. Tests by the ordinary Kjeldahl method with up to 100% excess of acid in the open receiver indicated that the amount of acid in excess is without effect on the results. J. G.

Use of Calcium Hydride in the Determination of Water. A. Elitsur. (*J. Appl. Chem. Russ.*, 1941, **14**, 682; *Chem. Age*, 1942, Oct. 17, 337.)—The following procedure is recommended for determining water in compounds and mixtures. To 3–4 ml of abs. alcohol add 0·1–0·5 g of calcium hydride powder. When evolution of hydrogen (due to reaction with the trace of water in the alcohol) ceases, connect the flask to a gas burette and add 0·15–0·25 g of the substance to be analysed. Measure at frequent intervals the vol. of hydrogen

evolved; each 22·4 ml (N.T.P.) ≡ 18 mg of water. Hydration curves reveal differences between water of crystn. and hygroscopic water. E. M. P.

5-Brom-2-aminobenzoic Acid as an Analytical Reagent. R. J. Shennan. (*J. Soc. Chem. Ind.*, 1942, **61**, 164.)—5-Bromo-2-aminobenzoic acid is preferable to anthranilic acid for the determination of certain bivalent metals, as the salts are insol. in acetate and tartrate solns. in presence of excess of the reagent. Calcium, strontium, barium or magnesium cannot be determined in this way. *Copper.*—Dilute a copper soln. of known conc. to 100 ml, add a few drops of 5 *N* acetic acid and heat. Add 50 ml of reagent (prepared by neutralising 5 g of 5-bromoanthranilic acid with sodium hydroxide and diluting to 500 ml) per 0·05 g of metal and heat gently for 30 min. Remove the copper 5-bromoanthranilate on a No. 4 sintered glass crucible, wash with hot water, and dry at 105–110° C. for 1 hr. The factor is 0·1288. Sodium acetate does not interfere. *Cobalt.*—Proceed as for copper (factor 0·1206). *Nickel.*—Proceed as for copper, but wash the ppt. with hot water containing 10% of the reagent; wash finally with a little cold water (factor 0·1200). *Zinc.*—Proceed as for nickel (factor 0·1319). By using *ca.* a 3-fold excess of reagent the results with each of the metals are satisfactory in presence of sodium and ammonium acetates and of Rochelle salt. E. M. P.

Separation of Calcium from Strontium. M. M. Tillu and M. S. Telang. (*J. Indian Chem. Soc.*, 1942, **19**, 231–232.)—The separation is carried out by extraction of the nitrates dried at 170° C., not with the usual alcohol-ether mixture, but with pure acetone. The solubility of strontium nitrate in acetone was found to be 1 : 15,000 at 35° C.; the calcium salt is readily soluble. Collect and weigh the strontium nitrate in a sintered glass crucible, recover the calcium nitrate by evaporating the filtrate and dry the residue at 170° C. W. R. S.

Determination of Sodium Sulphide, Sulphite and Thiosulphate, in Presence of One Another. Anon. (*Paper Trade J.*, 1942, **115**, *T.A.P.P.I. Sect.*, 93–96.)—These methods form part of the TAPPI Tentative Standard T 625 m-42, and have special application to sulphate black liquors. *Sodium sulphide.*—Pipette (*e.g.*) 50 ml of the soln. of the sample into a 150-ml distillation flask fitted with a dropping funnel and a glass inlet-tube with a constricted orifice (both extending below the surface of the liquid), and with an outlet tube leading, through a condenser, to two wash-bottles in series, and containing a known vol. of 0·1 *N* iodine (corresponding with an excess of at least 10 ml). A third wash-bottle contains exactly 2 ml of 0·1 *N* sodium thiosulphate to trap any iodine carried over, and sufficient water is added to each bottle to ensure efficient absorption. Place in the dropping funnel sufficient standard *N* hydrochloric acid to neutralise the sample to *p*H 4 (as determined on a separate sample), and sweep out the apparatus with carbon dioxide or nitrogen (which is first passed through a wash-bottle containing water). Stop the gas, run in the acid with *ca.* 20 ml of water, close the funnel, and resume passage of the gas at *ca.* 2 bubbles per sec. Place the distillation flask in boiling water for 30 min., rinse the condenser into the first wash-bottle with 5 ml of water, combine the contents of the wash-bottles, and titrate the excess of iodine with 0·1 *N* sodium thiosulphate, with starch as indicator; include the vol. of sodium thiosulphate in the third flask in the titration figure. *Sodium sulphite and thiosulphate.*—Ppt. the sulphides and most of the organic matter in the sample by adding to a mixture of 25 ml of glycerin and 50 ml of soln., excess of a freshly-prepared suspension of zinc carbonate (made by stirring 150 ml of 10% sodium carbonate soln. with a soln. containing 300 g. of zinc sulphate ($7H_2O$) at *ca.* 20° C.). Dilute in a flask to *ca.* 300 ml, leave to settle, and test 1 drop with lead acetate soln. for absence of sulphides; if these are present, more zinc carbonate suspension is added. Dilute to exactly 500 ml, filter at least 300 ml through a dry paper on a Buchner funnel into a dry flask, and pipette 100 ml of filtrate into a 500-ml conical flask containing 100 g of ice. Add 3 ml of starch indicator soln. and, quickly, sufficient acetic acid to acidify the soln. (as determined by a rough test on another portion of the filtrate). Immediately cover the flask with a rubber diaphragm (secured with a rubber band), and insert the tip of a burette containing 0·1 *N* iodine through a hole made in the diaphragm with a needle. Titrate until the blue colour produced on addition of 1 drop of iodine soln. persists for 10–15 sec. (*A* ml). To a second 100-ml portion of filtrate, add in succession ice, 10 ml of formalin, starch soln. and the same amount of acetic acid as used in the first titration, and titrate to a similar end-point (*B* ml). Then 0·63 $(A-B)$ = g of sodium sulphite per litre; 1·58 *B* = g of sodium thiosulphate per litre. The latter figure may be somewhat high if much organic reducing material is present. J. G.

Microchemical

Colorimetric Micro-determination of (Ferrous) Iron [in Plant Tissue]. C. P. Sideris. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 756–758.)—Dissolve the ash from *ca.* 5 g of dry plant-tissue in 10 ml of 5 *N* hydrochloric acid in a platinum dish on a hot-plate, add *ca.* 20 ml of water, heat to *ca.* 90° C., transfer to a 100-ml flask, cool, and dilute to the mark. Neutralise a 10-ml aliquot portion with 5 *N* sodium hydroxide (to litmus paper), and add in succession 1 ml of a 10% soln. of hydroxylamine sulphate (prepared in the cold), 1 ml of soln. of nitroso-R-salt (*vide infra*) and 2 ml of a 54·43% soln. of sodium acetate (prepared hot, cooled and filtered). If a ppt. (due to calcium phosphate or sulphate) appears at this stage, remove it by centrifuging. Dilute to 15 ml, and after at least 20 min. transfer the soln. to a cell (thickness, 2·5 mm), and determine the intensity of the green colour with a photoelectric colorimeter (filter No. 47; transmission limits, 445–505$m\mu$), which has been calibrated with standard solns. of iron (0·2–20·0μg per ml). The nitroso-R-salt soln. (*vide supra*) is prepared by dissolving 0·5 g of the solid reagent (1-nitroso-2-hydroxy-3,6-naphthalene disodium sulphonate) in 70 ml of water, and diluting to 100 ml with redistilled (iron-free) acetone; it remains active for some months. The sensitivity is 0·2–50μg of ferrous iron, and the optimum conditions are obtained with 0·25–20μg of iron at *p*H 8–10 (produced by the acetate buffer). The relationship between ferrous iron and the nitroso-R-salt is stoichiometric, and the compound formed has the formula $Fe(C_{10}H_5NO_8S_2Na_2)_3$ and retains its colour unchanged for *ca.* 48 hr. at room temp. Cobalt interferes by producing a wine-red colour. Copper

and nickel (1μg per ml, or more) produce a brown-yellow shade, but only at *p*H vals. below 7·0, and their effect is very small at *p*H 8–10; the max. deviation recorded is 11·4% (for 5μg of nickel in presence of 1μg of iron per ml). The yellow colour of the nitroso-R-salt soln. also interferes, unless the light filter is used. The method is equal in sensitivity to those using the *o*-phenanthroline and αα′-dipyridyl reagents, but the green colour is better suited colorimetric measurement (*cf.* Sideris, *id.*, 1937, **9**, 145). J. G.

Physical Methods, Apparatus, etc.

Testing Writing Inks with Rubber Fountain Pen Sacs. R. S. Casey and R. Macdonald. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 720–721.)—To test a new fountain pen diaphragm or sac, cut the sample in half longitudinally. Immerse one-half in distilled water (as a control), and the other in 25 ml of the ink to be tested in a test-tube (25 × 150 mm) loosely-stoppered with non-absorbent cotton; to eliminate possible variations in the sacs, place 3 half-sacs from the same batch in the same ink. The ink may then be subjected to the stability test of the U.S. Federal Specification for Writing Inks (TT–I–563, 1931). Remove the half-sacs, blot them dry, and place them (*e.g.*, in a Bierer–Davis oxygen bomb) under 300 lb. per sq. in. pressure at 70° C. Gentle flexing with the fingers at intervals reveals the progress of deterioration, *i.e.*, first a gradual softening until tearing readily occurs, followed by hardening, eventually to a brittle mass. The time necessary to attain the same (*i.e.*, soft or hard) stage may be taken as a numerical measure of the relative service life of sacs in different inks. The test may be varied by alternate immersions in ink and bomb ageing, and it may also be applied to other fountain-pen parts. The effect of the sac on the stability of the ink may also be noted, and measured in terms of the ppts. produced. Shake the residual ink, centrifuge 10 ml, wash the residue free from soluble matter, centrifuge again, and measure the vol. of the resulting sediment (*cf.* Waters, *Nat. Bur. Standards*, 1940, *Circ.* C426, p. 14); if 10-ml Constable proteir tubes are used, this may be read to 0·005 ml. Some inks were found to retard the ageing of the sacs. J. G.

Quantitative Evaluation of Metal-cleaning Compounds. O. M. Morgan and J. G. Lankler. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 725–726.)—The principle of the method is the photography of the fluorescence of the oil remaining on a metal strip after it has been treated with the cleansing agent. Scrub the test-strips (5 × 10 cm) with a 0·5% soln. of Nacconol NR at 110° F., rinse them successively in warm water and alcohol, and allow them to dry in air. Saturate strips of wool flannel (4·7 × 9·7 cm.) with a fluorescent mineral oil composition (*e.g.*, as used in brass-rolling); animal and vegetable oils can be made fluorescent by adding a suitable dyestuff (*e.g.*, Fluorescent Oil Green, H.W.). Make a stack of alternate metal and oil-saturated wool strips with protecting metal plates at the top and bottom, place the stack in a hydraulic press, and apply a pressure of 35 kg per sq. cm until no more oil oozes out at the edges; wipe the edges of the stack continuously with a clean cotton cloth. Release the pressure, but retain the strips in a horizontal position until they are ready for the cleansing test. A soaking method of cleaning is preferred, as it is widely used and simple, and shows most clearly the intrinsic cleaning ability of a preparation as distinct from the effect of mechanical action. Hang a test-strip in 800 ml of cleansing soln. in a 1000-ml beaker immersed in a thermostat at the desired temp. for the desired time. Then rinse the strip in running water at 100° F. for 1 min., dry in air, and photograph it in filtered ultra-violet light on Tri-X film through a Wratten K-2 filter (f. 4·5; exposure, 10 min.; distance of camera to strip, *ca.* 30 in.). A film developer giving max. contrast (*e.g.*, DK–60A) is desirable. Photographs showing the cleansing effects of 2, 4, 6, 8 and 10% sodium metasilicate solns. at 140° F. for 5 min. on strips carrying 0·113 mg of mineral oil per sq. cm. are reproduced; they correspond with residual oil contents of 0·038, 0·021, 0·014, 0·004 and 0·004 mg per sq. cm, respectively. The test indicates not only the amount of oil on the metal surface, but also the distribution of the oil; this distinction may be of great importance in investigating localised damage to surface coatings on the metal. J. G.

Evaluation of the Scaliness of Animal Fibres. N. H. Chamberlain and J. B. Speakman. (*Nature*, 1942, **150**, 546.)—In the apparatus described a single (*e.g.*, wool) fibre is held vertically with the tip uppermost in a clamp attached to a calibrated spring, and rubbed at a standard rate between two opposed, reciprocating plane surfaces (clothed with silk bolting-gauze) under controlled pressure. The rubbing is such that the fibre creeps downwards against the spring-tension, the rate of increase of which and the max. tension developed measure the scaliness. Typical curves relating rubbing-time and tension for human hair, New Zealand Romney, Lincoln and Cotswold wools, mohair and Kemp (Blackface fleece) are reproduced. They show that with human hair, the max. tension is developed relatively slowly and maintained over long rubbing-periods, whilst with the other fibres it is developed very rapidly and is followed by a rapid fall, which subsequently continues more gradually. J. G.

Reviews

HYDROGEN IONS. THEIR DETERMINATION AND IMPORTANCE IN PURE AND INDUSTRIAL CHEMISTRY. By HUBERT T. S. BRITTON, D.Sc., D.I.C., F.I.C. Third Ed. Vol. I, pp. xix + 420; Vol. II, pp. xix + 443. London: Chapman & Hall, Ltd. 1942. Price 36s. each vol.

Few are the chemical operations uninfluenced by *p*H, and a proper understanding of the factors affecting it adds considerably to the success of such operations, whether performed in beakers or in industrial plant. Such an understanding can be obtained by study of the present work. The earlier editions have filled an important and readily accessible

niche in the library of the modern laboratory and have established a well-deserved reputation.

The first volume deals with electrometric methods and electrodes and with the theory of indicators; the second volume with precipitation, electro-deposition and other analytical processes and with the application of *p*H in a variety of industries, including those connected with leather, sugar, paper, brewing, milk, agriculture, ceramics and textiles. All the matter of previous editions has been expanded and new chapters are included covering abnormal acids, the theory of the ionisation of dibasic and polybasic acids the activity theory of solutions, indicator papers, the reaction between weak organic acids and inorganic bases, solutions containing complex ions, and the importance of *p*H control in the detection of metals with organic reagents. The increase is approximately 40%, accounting for the re-arrangement in two volumes.

The importance that the author properly attaches to *p*H leads to occasional loose statements, implying that factors which cause changes in *p*H are results of such changes. For example, on page 303 of Volume II it is stated that "the composition of milk varies with its *p*H value when fresh, as may be seen from the data given in Table 167." Surely in this instance *p*H is a dependent variable, and fat and lactose are independent variables as regards *p*H.

This new edition can rightly be regarded as indispensable to all analysts.

J. R. NICHOLLS

CHROMATOGRAPHIC ADSORPTION ANALYSIS. By HAROLD H. STRAIN, Ph.D. Pp. x + 222. New York: Interscience Publishers, Inc.; London: Imperia Book Co., Ltd. 1942. Price 22s. 6d.

The publication of this monograph so soon after the appearance of an English translation of Zechmeister and Cholnoky's "*Chromatographische Adsorptionsmethode*" is evidence of the increasing interest in chromatography. A few years ago used only by a select group of workers engaged in the investigation of natural pigments, it is now an indispensable weapon in the chemists' armamentarium for attacking a variety of analytical, biochemical and industrial problems. Furthermore, chromatography appears to have been successfully used in some technical operations, although the inclusion in Dr. Strain's book of dehydration and decolorisation processes and water purification by the base-exchange process as examples of chromatographic processes is surely not legitimate.

It is inevitable that much of the material used by the author had previously been used by Prof. Zechmeister and Dr. Cholnoky, for, not unnaturally, both have picked out from the literature the most striking experiments and those which most effectively illustrate the principles of chromatography. In spite of this considerable overlapping, which could not have been avoided if both monographs were to be in any sense complete, the book now under review contains much that is new and useful. Most valuable, perhaps, are the practical hints given on such matters as the packing of the column, the amount and concentration of the solution used, the best solvents for adsorption and for subsequent development of the chromatogram and the separation of the resulting zones from one another. Dr. Strain has himself made important contributions to the technique of chromatography, and his words are therefore authoritative and not merely *obiter dicta*. The chapter on adsorbents I would single out for especial mention. The choice of a suitable adsorbent is, for a beginner, perhaps the most difficult step in chromatography, and even with the skilled worker this stage is more a matter of instinct or intuition than of positive knowledge—distinctly an art and not a science! Dr. Strain gives a clear account of the properties of the adsorbents used for different substances, and he has collected together a good deal of data on the subject from which he has made generalisations which, though perhaps not strictly true in ever instance, are invaluable because they enable the novice to determine what type of material will best suit his purpose. He will realise, perhaps for the first time, the great difference that exists between various types of alumina and what is the significance of that mysterious phrase "standardisiert nach Brockmann," for Dr. Strain gives a description of Brockmann and Schodder's metnod for measuring the adsorptive activity of alumina, published in the *Berichte* in 1941. Few people in this country will have had the opportunity of seeing this paper, and it is therefore useful to have a description of the method.

The chapter on the "Location of Colourless Adsorbed Substances" also contains some interesting new material. Zechmeister's technique for detecting the presence of a colourless zone by streaking the column with a brush dipped in a suitable reagent has been extended

to a variety of substances, as might have been expected, and the coupling of colourless hydroxyl compounds, such as sterols and sugars, with azobenzene-*p*-carboxylic acid chloride is mentioned, together with other illustrations of the same principle.

Omissions are often as significant as inclusions, and one is struck by the almost complete absence of a theoretical discussion of the subject. Barely a page is devoted to theory, apparently because only one paper, that by J. N. Wilson, has so far appeared in which anything like a serious attempt to work out the theory of chromatography has been made. It is surely surprising that, with the wealth of data now in the literature and the widespread use of this technique, more people have not essayed to enter this field. It may confidently be predicted that Wilson's paper will stimulate others to turn their attention to this matter.

Dr. Strain's book is well printed and well bound, but the price is rather high, for the actual text occupies only 162 pages. It contains a number of errors, most of them trivial. Incorrect formulae are given for cholesterol and its ester, and it is erroneously stated (p. 104) that "Both 7-dehydro-cholesterol and ergosterol may be regarded as provitamins D_3"; ergosterol is of course provitamin D_2. Vitamin K_2 is probably 2-methyl-3-difarnesyl-1:4-naphthaquinone and not 2:3-difarnesyl-1:4-naphthaquinone as stated.

F. A. ROBINSON

Society of Public Analysts and Other Analytical Chemists

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Volume 68 **January, 1943**
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Contents

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