THE ANALYST

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

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

CHANGE OF EDITORSHIP OF "THE ANALYST"

DR. C. AINSWORTH MITCHELL, who has been Editor of THE ANALYST since 1920, retired from that position on September 30th, 1945.

Dr. Mitchell has had a very long and intimate association with the Society's affairs. He became a member in 1894, served on the Council in 1899–1900 and was Secretary from 1925 to 1937. As co-author, with Dr. Bernard Dyer, of *"Fifty Years of the Society of Public Analysts,"* published in 1932, he contributed to that work a valuable detailed review of the analytical activities of the Society from its formation to 1925. He will retain a literary connection with the Society as Honorary Librarian.

Under Dr. Mitchell's editorship THE ANALYST has grown steadily, not only in size and circulation but also in prestige. Its scope, like that of the Society, has widened and now embraces all branches of analysis. It is becoming increasingly recognised as the most informative record of current analytical work, and its circulation is more than double the membership of the Society itself. On retiring, therefore, Dr. Mitchell leaves the journal in a flourishing condition.

Numerous contributors to THE ANALYST will retain grateful memories of Dr. Mitchell's helpfulness, and it will be a satisfaction to members that his retirement will not deprive the Society of his continued interest and co-operation in its work.

Dr. Mitchell is succeeded as Editor by Mr. J. H. Lane, who has acted as Assistant Editor since 1936. Communications relating to THE ANALYST should be addressed to Mr. Lane at 7–8, Idol Lane, London, E.C.3.

NORTH OF ENGLAND SECTION

AN open meeting of the North of England Section was held in Manchester on Saturday, 9th June, 1945. The Chairman (Mr. H. M. Mason), who was accompanied by Mrs. Mason, presided over an attendance of 54. The earlier portion of the meeting was of an informal nature. A resolution was unanimously passed expressing the continued loyalty of the Section to the Council and the Parent Society.

S. W. Butterworth, B.Sc., F.R.I.C., gave a most interesting paper, illustrated by lantern slides, on "Bread."

MICROCHEMISTRY GROUP

A MEETING of the Group, in conjunction with the Newcastle Sections of the Royal Institute of Chemistry and the Society of Chemical Industry, was held in Newcastle on September 14th, 1945. In the afternoon the Derwenthaugh Coke Works of the Consett Coal and Iron Co. were visited and the party then returned to the College Union for tea.

The Joint Meeting was held in the Chemistry Lecture Theatre of Kings College, under the chairmanship of Professor H. V. A. Briscoe. Thanks were accorded to the College authorities for their hospitality and to Mr. H. E. Blayden, Hon. Secretary of the Newcastle Section of the Royal Institute of Chemistry, for the excellent arrangements he had made.

The following papers were read and discussed: "Quantitative Inorganic Micro-analysis for University Students," by Dr. Christina C. Miller; "A Review of Methods for Micro-filtration," by Dr. G. H. Wyatt; "Some Aspects of the Microchemical Analysis of Ferrous Alloys," by Mr. C. Whalley.

An Apparatus for the more Accurate Determination of Hydrogen Liberated from Acids by Metals*

By B. S. EVANS AND D. G. HIGGS

THE apparatus and method of using it described in this paper were evolved in an attempt to improve the technique of the old method of evaluating the metal content of, *e.g.*, magnesium metal, by determining the vol. of hydrogen given off when it is dissolved in acid. The old method suffered from the drawbacks of the difficulty of accurate measurement of the gas produced and the fact that there was almost invariably a "dead space" of air, often considerable, for which a correction was required.   The difficulty of measuring the gas was due to the dilemma: either a necessarily small vol. of the gas was measured in a burette which could be read accurately, or, a larger measuring vessel, on which the graduations could not be read so closely, was used; in either event, there was inevitably a large percentage error.
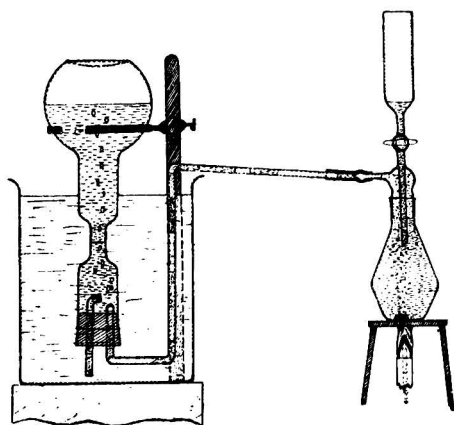
HYDROGEN EVOLUTION APPARATUS
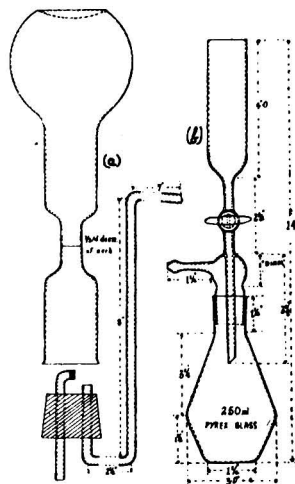
Fig. 1   Fig. 2

The difficulties adhering to "dead space" are too obvious to need comment, bearing in mind the accuracy with which it is necessary to control temperature and pressure in gas measurement.   Our aim was therefore: (*a*) to work with a fairly large vol. (say, 500 to 600 ml) of hydrogen, which could be completely water-jacketed; (*b*) to measure this vol. accurately; (*c*) to eliminate "dead space" altogether.

Our solution of the problem is: (*a*) to use as measuring vessel a flask with a single graduation placed on a constriction of the neck; (*b*) to use a sample weight calculated to produce approx. the vol. of hydrogen required; (*c*) after evolution and careful adjustment of temp. to bring the vol. of gas exactly to the mark by means of a levelling tube the difference in pressure required is read on a manometer; (*d*) to begin with the entire apparatus completely filled with boiled-out water.

The process falls naturally into two stages: (I) evolution, (II) measurement.

(I) EVOLUTION

APPARATUS—The requirements are: (*a*) A flask (of *ca.* 600 ml) with a long neck, which is drawn out into a constriction about $2\frac{1}{2}$ in. below the mouth; the constricted portion (approx. $1\frac{1}{2}$ in. × $\frac{1}{2}$ in.) carries a graduation mark in the centre.   The mouth must be wide enough for a three-holed rubber stopper (Fig. 2*a*).   (*b*) An evolution flask (250 ml) with a ground-in stopper carrying a tapped funnel, which passes down to about the centre of the flask, and a

horizontal exit tube flush with the top of the stopper.   It is important that there should be no sharp angles, which might trap gas bubbles, as it is essential that it should be possible to fill the flask completely with water (Fig. 2b).   (c) A two-holed rubber stopper fitting the mouth of the measuring flask and carrying: (i) A leading tube, bent 3 times at right angles, so that one end passes inside the measuring flask when in position, while the other fits snugly against the end of the exit tube of the evolution flask.   (ii) A tube (3/16 in. wide), bent at right angles about $\frac{1}{2}$ in. from one end, which is filled with a coarse-grained, sintered glass-filter.   This is easily made according to directions given by Stone and Weiss.[1]   When in position the end carrying the filter projects about 1 in. inside the flask, with the filter vertical; the other end, which is open, passes just outside the outer end of the stopper.   (d) A stand with a clamp ring to hold the measuring flask vertical in an inverted position.   A segment of about $\frac{3}{4}$ in. is cut out of the ring to enable the flask to be removed from it without disconnecting the apparatus.   (e) A beaker (squat form) large enough to allow of the rubber stopper being pressed into the mouth of the inverted measuring flask under water and without admission of any air; also it must accommodate the water displaced from the measuring flask. (f) A tripod or other stand on which the evolution flask may be heated during the reaction.

The complete apparatus is shown in Fig. 1 and some of the individual parts in Fig. 2.

CALIBRATION OF FLASK—The measuring flask must be calibrated as accurately as possible.   This may be done by weighing the clean, dry flask, then weighing again after filling to the mark with distilled water (taking care not to wet the neck above the mark). The difference in weights gives the number of g of water required to fill the flask to the mark at the temp. of weighing, and this must be corrected to give the capacity in ml.   The formula $v = \dfrac{a(b+1)}{d_t}$ is used, where v = required volume, a = weight of water, d_t = density of water at $t°$ (Table II), b = buoyancy constant (Table I).

TABLE I
BUOYANCY CONSTANTS "b" (Scott and Furman)[2]

Pressure mm	Temperature		
	15° C.	20° C.	25° C.
740	0·00105	0·00103	0·00101
750	0·00106	0·00104	0·00102
760	0·00107	0·00105	0·00103
770	0·00109	0·00107	0·00105
780	0·00110	0·00108	0·00106

TABLE II
DENSITY OF WATER AT VARYING TEMPERATURES "d_t" (Scott and Furman)[3]

Temp., °C.	Density	Temp., °C.	Density	Temp., °C.	Density
10	0·99973	17	0·99880	24	0·99732
11	0·99963	18	0·99862	25	0·99707
12	0·99952	19	0·99843	26	0·99681
13	0·99940	20	0·99823	27	0·99654
14	0·99927	21	0·99802	28	0·99626
15	0·99913	22	0·99780	29	0·99597
16	0·99897	23	0·99756	30	0·99567

FILLING AND EMPTYING THE MEASURING FLASK—The peculiar shape of the flask makes filling and emptying rather slow; a glass siphon tube expedites these operations considerably. If the siphon is inserted to just below the constriction, the flask fills quickly and smoothly; to empty it, pass the tube to the bottom of the flask and blow the free end.

Procedure—Pour boiled-out distilled water into the beaker to a convenient depth (say, $\frac{1}{2}$-full. Fill the measuring flask until it brims over and then insert a rubber stopper with a single hole carrying a short length of glass tube with a tap or pinchcock.   As the stopper is pressed in, the excess water overflows through the tap, which is then closed.   Immediately invert the flask and place it mouth downwards in the beaker, open the tap, remove the stopper under the water, slide the constricted part of the neck through the gap in the•clamp ring, and adjust the latter so that the flask hangs suspended with its mouth 2 or 3 inches below the surface of the water.   Take care throughout not to admit any air to the flask; if successfully done, there should now be no bubbles in it.

Place the weighed sample in the evolution flask, having calculated the weight to be taken from the formula $\dfrac{0\cdot0899\ X \times Y}{1000\ Z}$ = weight in g, where X is the hydrogen equivalent of the metal in g (where an alloy is being dealt with this of course has to be calculated from its composition). Y is the calibrated volume of the measuring flask and Z is the reading on the curve (Fig. 4) for the approximate temperature prevailing in the laboratory (*vide infra*).

If the sample consists of a readily attacked metal (*e.g.*, magnesium or zinc) in lumps or relatively coarse particles, the procedure given below is effective; aluminium and some other metals and all fine powders require special treatments which will be given later.

MAGNESIUM, ETC., IN LUMPS—Place the weighed sample in the evolution flask and fill up with boiled-out distilled water, taking care that no air bubbles are trapped in the sample; insert the stopper and continue the admission of water through the funnel (if necessary tilting the flask) until all air has been removed from both flask and exit tube. Attach the free end of the leading tube to the exit tube by a short rubber connection, seeing that the ends of the two tubes are in contact; then continue admission of the water until air is completely expelled from the leading tube also. Plunge the stopper of the measuring flask, carrying the leading tube, beneath the water in the beaker in which the measuring flask is suspended, and remove air from the small tube, containing the sintered glass filter, by sucking a rubber tube attached to the open end. Remove the rubber tube below the water and press the stopper firmly into place in the measuring flask. The system should now be completely filled with boiled-out distilled water. Support the evolution flask on a tripod stand and place a burner beneath it. Next run hydrochloric acid, a little at a time, into the evolution flask; the rate and quantity of admission must be governed entirely by the nature and state of division of the sample, but the ideal to be aimed at is the evolution of a steady stream of bubbles, which pass along the leading tube and up into the flask. Some metals require heat to promote reasonably rapid solution, and this can be supplied by the burner beneath the tripod, but, if the sample will dissolve satisfactorily in the cold, allow it to do so. Finally, when every particle of the sample has dissolved, boil the liquid in the evolution flask for a few min., to drive over any dissolved hydrogen; then remove the burner and allow to cool. It will probably be found that there is a bubble of gas remaining in the evolution flask; drive this also into the measuring flask by running in distilled water through the tapped funnel until the flask, and leading tube, are completely filled with liquid and all the gas is in the measuring flask; the meniscus should be within a reasonable distance of the graduation mark. Gently detach the rubber stopper below the surface of the water and remove it with the rest of the evolution apparatus, leaving the flask, containing the gas, suspended with its mouth below the surface of the liquid.

SPECIAL PROCEDURES—Certain metals, and certain states of division, present difficulties which necessitate special procedures. (*a*) Some metals tried dissolved too slowly in dil. hydrochloric acid alone. For these it was generally found efficacious to produce a metal couple by the addition of either a drop of mercury or a piece of platinum foil. For aluminium, mercury was the best agent, the completion of attack being sharply indicated by the sudden cessation of bubbles shortly after the drop has become spherical; only a tiny drop is required. With iron, lead and zinc, either amalgamation was difficult, or the attack of the last trace in the drop of mercury was very slow and tedious; for this reason, in these cases, platinum was a more satisfactory addition.

(*b*) *Fine powders*—These are the most difficult substances to deal with, especially if they are of a highly reactive metal. The reason is that the fine powder gets carried by the rush of gas right through the apparatus into the measuring flask. Even this might not matter, as the liquid in the flask becomes quite acid in the later stages and attack might be completed there; but usually the powder is carried up to the surface of the liquid, where it sticks to the walls and is left dry, unattacked, as the level falls. This behaviour necessitates several modifications in procedure. (i) It is impossible to fill the flask with water in the first instance without loss of sample unless the powder is first thoroughly wetted so that it ceases to cling to the surface of the water. This may generally be achieved by preliminary addition of a "wetting" agent, such as "permanol"; even so, however, addition of the first drops of acid usually caused much of the powder to rush to the top and be carried over. (ii) A very satisfactory method was found to be to fill the lower third of the flask (if necessary with prior addition of permanol) with saturated sodium chloride soln. and to run the subsequent water gently on to this so as not to cause mixing. When the acid is finally admitted, sufficient seems to diffuse into the lower layer without breaking the interface, and

the gas is then generated at this interface, leaving the metal particles trapped beneath it, at any rate, long enough to establish a gas seal in the upper part of the flask.   (iii) With certain powders even this method does not prevent carrying over, and, as a last resort, it becomes necessary to establish an artificial gas seal.   This may be readily and accurately done in the following manner.

The evolution flask, containing the weighed sample, is treated as before, the free space above the sodium chloride soln. being completely filled with distilled water; the funnel is also filled with water and then stoppered with a one-holed rubber stopper, the excess water overflowing through the hole.   Into this hole is then pushed the jet of a 25-ml burette (the jet must fit the hole tightly) containing water exactly to the lower, 25 ml, graduation; by gently sucking the upper end of the burette, and at the same time opening the tap, water is drawn up the burette from the funnel to just above the "0" graduation, its place being taken by air which enters the flask through the side tube.   If now the water in the burette is allowed to sink just to the 0 graduation, by manipulation of the tap, the flask, including the side tube, contains exactly 25 ml of air at the prevailing temp. and pressure.   All that remains
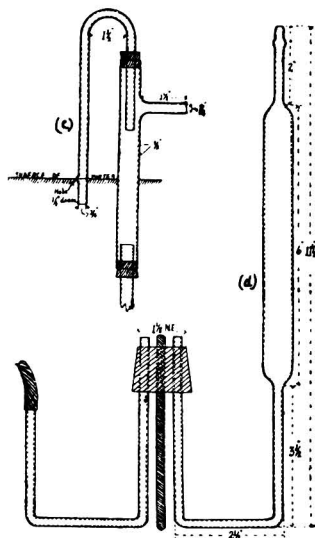
Fig. 3
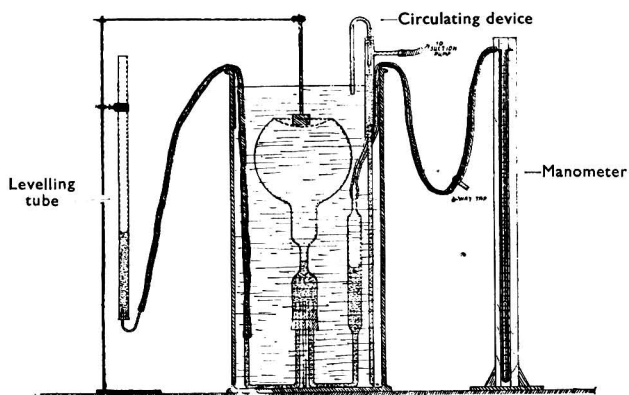
Fig. 5

is to connect the side tube still open to the air, with the leading tube completely filled with water.   The water remaining in the tapped funnel should be siphoned out before adding hydrochloric acid.   This device, which can be carried out in 2 or 3 min., seems to be completely successful in preventing the blowing-over of the powder.   It is necessary to read the temp. and pressure at the time of introducing the 25 ml, so that the vol. at N.T.P. can be calculated for deduction from the vol. of hydrogen obtained; it is desirable also to calculate the weight of sample used for a volume 25 ml less than that of the measuring flask.   It is true that this method re-introduces the "dead space," which it was our aim to exclude, but, it is an accurately measured dead space, and, in practice, does not seem to bring in any error.

(II) MEASUREMENT

APPARATUS—The complete apparatus is shown in Fig. 5.   The components required are: (a) A tall cylinder large enough to allow of the measuring flask being completely immersed in water contained therein, leaving, say, $\frac{1}{2}$ in. space of water all round.   (b) A three-holed rubber stopper carrying (Fig. 3d): (i) A leading tube with a rubber connection to the levelling tube.   (ii) A tube with a rubber connection to the manometer.   This tube carries a bulb, shaped similarly to that of a pipette, and so placed that, when the stopper is in position in the flask, the bulb is parallel to, and close to, the constricted part of the neck, the graduation mark being roughly about the centre of the bulb.   Both these tubes are bent twice at right angles.   (iii) A glass rod bent once at right angles.

These two tubes and rod are of such a length that, when their ends pass just through the

stopper, their horizontal portions lie in the same plane and, radiating outwards at 120° intervals, provide a tripod support on which the flask may rest vertically in the cylinder.

(c) A manometer filled with coloured water. (d) A three-way tap inserted in the rubber connection to the manometer. (e) A device (e.g., a bent rod held in a clamp) for keeping the flask depressed below the surface of the water. (f) A levelling tube held in a clamp. (g) An "air-lift" device for circulating the water in the cylinder. This device, which is shown in Fig. 3c, consists of a vertical wide tube, closed with a single-bored cork at each end and carrying a side tube, closer to the upper than the lower end; the upper cork carries one limb of a tube bent into a U, the end passing to about the middle of the wide tube; the other limb has a hole in the side about ⅓ in. from the end. This hole is arranged to be at the desired level of the water in the cylinder. The lower cork carries a tube which goes to the bottom of the cylinder. The side tube is connected to a filter pump. If the cylinder is filled with water nearly to the level of the side hole, the pump started and the addition of water continued cautiously until it is drawn up in a stream of bubbles through the hole, the water will continue to circulate through the cylinder so long as the pump is running. The only attention required is to replenish, from time to time, the water lost by the fairly considerable evaporation.

Procedure—Attach the levelling tube to the plain tube in the stopper by means of pressure tubing, which must be long enough to allow freedom of movement when the measuring flask is immersed in the cylinder. Plunge the stopper beneath the surface of water in a beaker and drive out all air in the tube and pressure tubing by pouring water into the levelling tube, with subsequent manipulation of the latter to ensure that all air bubbles are expelled (this can conveniently be done in the beaker in which the measuring flask is suspended if care is taken to ensure that no bubbles get into the flask); close the pressure tubing with a screw clip. Press the stopper firmly into place in the flask; below the level of the water attach the manometer lead to the top of the bulb tube, taking care that the three-way tap is open to the air, and that there are no drops of water either in the upper narrow part of the bulb tube or in the pressure tubing lead (otherwise back pressures may be set up). Remove the apparatus from the beaker and stand it on its tripod support (the glass tubes and rod) on the bench; holding the levelling tube beside the flask, with the water in each at about the same level, open the screw clip and adjust the height of the levelling tube until the water in the flask, the bulb tube and the levelling tube, is at the same level; then turn the three-way tap so that it is cut off from the air but connects the manometer with the bulb tube. Place the flask in the cylinder and clamp into place so that it rests vertically on its tripod support on the bottom as shown in Fig. 5. Set the circulating device running and suspend a thermometer in the water of the cylinder; bring the level of the water in the flask exactly to the graduation mark, by adjusting the levelling tube, which is then clamped into place. From time to time make the necessary adjustments of the levelling tube to restore the changes of volume of the gas in the flask as the temperature becomes steady. When the volume appears to be steady, exactly on the graduation mark, make the following observations: (i) temperature; (ii) height of barometer; (iii) manometer reading; (iv) difference in level between the water in the flask and the water in the bulb.

The amount of (iv) is very slight and must be subtracted from the manometer reading. Convert the manometer reading thus corrected into its mercury equivalent by dividing by 13·6 and add, or subtract, the value thus found, to, or from, the barometer reading.

If the water level in the open limb of the manometer is the higher, the value must be added. If it is lower, the value must be subtracted. Calculate the volume of dry hydrogen in the flask at 0° C. and 760 mm barometric pressure from the following formula.

$$\frac{B \times Y}{C \times 760} = \text{vol. of dry hydrogen at N.T.P.,}$$

where B = barometer reading corrected by manometer reading (mm of mercury); Y = calibrated volume of flask (ml); C = reading on the curve (Fig. 4) corresponding to the temp. of the water-jacket.

(The readings of the curve are divisors for reducing volumes of gas saturated with water vapour at t° to dry gas at 0°. The curve is plotted from the table given in H. M. Spiers's "*Technical Data on Fuel*"[4]).

Errors not corrected for—The first three are negligibly small. (i) Coefficient of cubical expansion of glass. This introduces an error (+ or −) of 0·015 ml per 1° C. of difference between the temperature of measurement and the temperature of calibration for the measuring

flask we used (575·1 ml).   (ii) Error due to reversal of position of meniscus between calibration and measurement (—).   This for our flasks (int. diam. 10·5 mm) amounts to 0·15 ml, or, 0·026%, which it would be valid to add to the readings obtained.   (iii) A negligible error (—) due to increase of pressure owing to surface tension.   (iv) Solubility of hydrogen.   This is probably the greatest source of error (—); it seems to be impossible to correct for it, but figures given in Table III appear to show that the error involved is not serious.
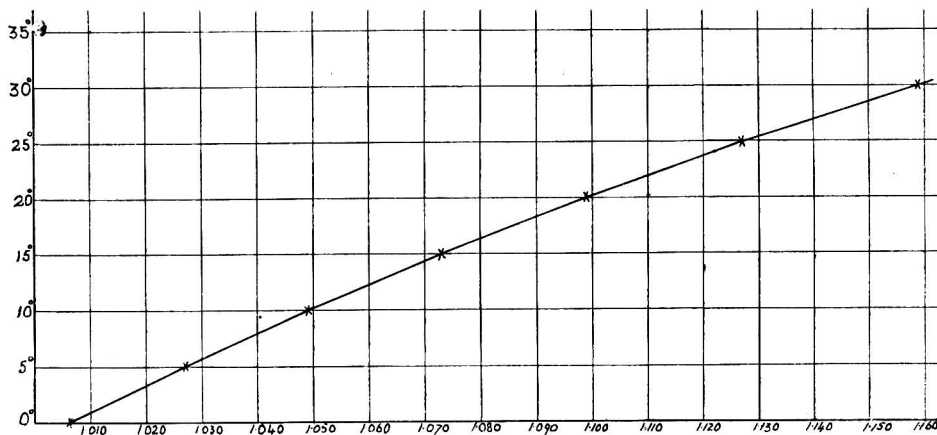
Fig. 4

(A larger scale drawing on squared paper is advised for use)

RESULTS—The following results (Table III) were obtained with the purest samples of metals available.

TABLE III

All the determinations were made on lumps, to avoid any doubts as to the possible carrying over of fine powder; but the precautions to be adopted in dealing with powders were introduced in order to show that these in themselves did not bring in any error.

Metal	Weight taken g	Conditions	Manometer mm of water	Vol. of flask ml	Temp. °C.	Barometer mm of Hg	Corr. pressure mm of Hg	Air added	Factor	% of theoretical vol.
Zn	1·5502	Air added. Pt. wire used to promote soln.	+(468–6)	575·1	15·2	749·7	783·7	25 ml { 21·5° C. / 742·3 mm } = 22·0 ml at N.T.P.	2·917	99·85
Zn	1·5502	Same sample No air added	+(312–5)	575·1	19·0	743·7	766·3	—	2·917	99·80
Al	0·4043	Very pure sample. Air added. Hg used to promote soln.	+(62–2)	575·1	19·6	758·0	762·4	25 ml { 20·0° C. / 751·6 mm } = 22·5 ml at N.T.P.	0·8017	99·90
Al	0·4377	Less pure sample No air added	+(185–3)	575·1	16·8	762·9	766·5	—	0·8017	99·40
Fe	1·3371	No air added	+(189–3)	575·1	19·0	757·0	770·7	—	2·491	99·40
Fe	1·3347	Same sample No air added	−(104–3)	575·1	15·5	766·1	758·7	—	2·491	99·65
Mg	0·5500	Air added. Hg used to promote soln.	+(104–0)	575·1	19·7	756·4	764·1	25 ml { 20·0° C. / 755·5 mm } = 22·6 ml at N.T.P.	1·085	99·50

SUMMARY—An apparatus is described for the more accurate determination of hydrogen evolved from metals. Points of the method are: (a) relatively large volume of hydrogen; (b) single graduation mark on measuring vessel; (c) control of approximate volume of hydrogen by adjustment of weight of sample; (d) entire water-jacketing of hydrogen vessel before measurement; (e) exact adjustment of volume to the single graduation mark, measuring the pressure required on a water manometer; (f) entire elimination of "dead space" except under certain carefully controlled conditions.

A new and efficient apparatus for the circulation of water in the water jacket is described.

REFERENCES

1. Stone, H. W., and Weiss, L. C.   *Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 220.
2. Scott and Furman, "*Standard Methods of Chemical Analysis*," 5th Ed., 1939, p. 1227.
3. *Ibid.*, p. 1228.
4. Spiers, H. M., "*Technical Data on Fuel*," World Power Conference, 1928, p. 57.

September, 1944

A Composite Method for the Determination of Silicon, Manganese and Phosphorus in Cast Iron and Steel

By T. S. HARRISON

THE aim of this investigation was to determine silicon gravimetrically, phosphorus absorptiometrically by the phosphovanadomolybdate technique, and manganese by absorptiometry or titration, all on a single sample of iron or steel. The method would thus resemble the composite methods of Vaughan.[1]

Previously a Spekker method for phosphorus determination was described,[2] in which the acid concn. essential for the stability of the yellow complex was attained by evaporating the acid sample soln. to dryness and dissolving the residue in a standard amount of nitric acid. As most of the silicon was thus dehydrated to insoluble silica, the combination of a gravimetric silica assay with the absorptiometric determination of phosphorus in the filtrate seemed possible. Furthermore, aliquots of filtrate could be taken for (a) phosphorus and (b) manganese determinations, provided that the halogens could be expelled readily from (b). Complete removal of silica, however, requires the usual "bake," and this renders the solid incompletely soluble in nitric acid. Elimination of halogens by "fuming" with sulphuric acid is a lengthy operation, hardly justified by the manganese figure alone, but the fact that a method for silicon is based on the dehydration of silica by "fuming" a nitro-sulphuric acid soln. suggests a composite method, based on the observation of Kitson and Mellon[3] that phosphorus can be determined in sulphuric acid solution. Nitric acid is necessary for the complete oxidation of phosphorus to orthophosphate, any loss as phosphine being avoided, and of iron to the ferric state. Bromine, added to remove arsenic as volatile tribromide, assists oxidation; excess of each oxidant is expelled later by "fuming." The acid concn. essential for colour stability[2] is readily attained, since the sulphuric acid lost by "fuming" is negligible. Work was begun on these lines.

1. EXPERIMENTAL—(a) *Preliminary experiments*—Several modifications were applied to the existing method for phosphorus,[2] including variations in the nature and amount of acid used. Of these, the nitro-sulphuric technique alone gave accuracy and reproducibility for each element. Next many colour stability tests were made, and from the transmission/time curves the optimum conditions of acidity for development of the complex were deduced. Sufficient acid was necessary to give fluidity during "fuming," and the ammonia required to neutralise the excess was incorporated, for convenience, in the molybdate reagent. The following scheme was then evolved for cast irons of medium and high phosphorus contents.

(b) *Procedure*—Dissolve 0·5 g of the sample in 5 ml of brominated nitric acid and 50 ml of 20% sulphuric acid. Evaporate to fuming, cool, quench with distilled water, and re-dissolve in 60 ml of hot water. Filter through a Whatman No. 41 paper, wash with hot water, cool the filtrate and make up to 100 ml. Transfer the remaining silica to the paper, using a

"policeman," and wash with hot dil. hydrochloric acid, using a fresh receiver. Ignite and weigh the silica and calculate the percentage of silicon. To 40 ml of filtrate add 10 ml of 1·2% ammonium vanadate soln. and boil. Remove from the hot plate and add, dropwise and with shaking to dissolve the precipitated ferric hydroxide, 10 ml of ammonium molybdate
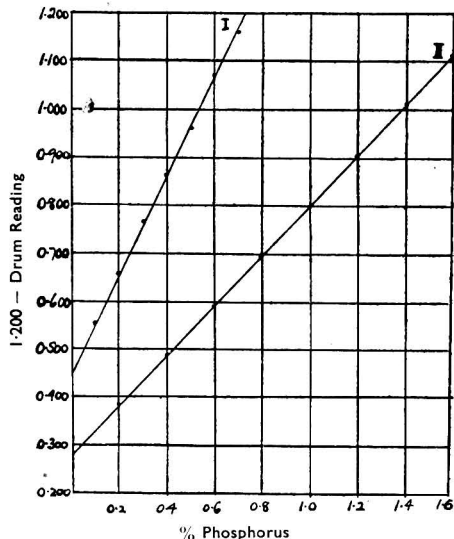
Fig. 1.   Phosphorus in cast iron.   Mercury-
vapour lamp.   Spectrum violet
filters No. 601.   Setting W:W 1·2.
Graph I, 1 cm cells.   Graph II.
0·5 cm cells.
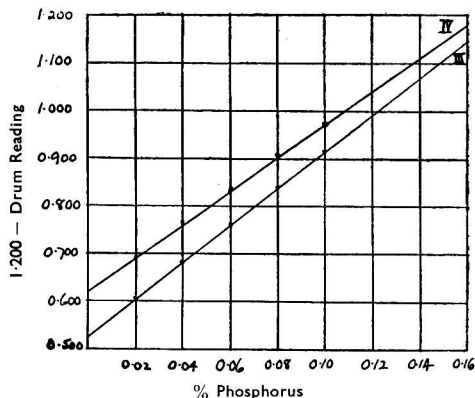
Fig. 2.   Phosphorus in haematite cast iron
and steel.   Mercury-vapour lamp.
Spectrum violet filters No. 601.   Setting
W:W 1·2.   Graph III, 2 cm cells.
Graph IV, 4 cm cells.

soln. (prepared by dissolving 80 g of the salt in water, making up to 350 ml, cooling and adding 350 ml of ammonia of sp.gr. 0·880).   Cool and make up to 100 ml.   Fill an absorptiometric cell and determine the Spekker drum reading, using Ilford violet filters No. 601 and setting "water-to-water" 1·2.   Convert the drum reading to % of phosphorus from a graph obtained with solns. of standard sodium phosphate, containing spectrographically pure iron and treated exactly as the sample (Fig. 1) Add 1·5 ml of phosphoric acid (sp.gr. 1·75) and 5 ml of 2% silver nitrate soln. to a further 50 ml of filtrate, heat to boiling, add 2·5 g of ammonium persulphate and allow to simmer for 4 min.   Cool, add 0·5 g of urea and dilute to 60 ml.   Fill a suitable cell and obtain the drum reading for Ilford yellow filters No. 606 and setting "water-to-water" 1·0.   Convert to % of manganese from a graph obtained from known solns. of standardised potassium permanganate treated as the sample (Fig. 3). Alternatively, dilute 20 ml of the filtrate, add 10 ml of 0·3% silver nitrate soln., boil and develop the colour with 0·75 g of ammonium persulphate.   Cool, add 10 ml of 0·2% sodium chloride soln., and titrate with standardised sodium arsenite solution in the usual way.
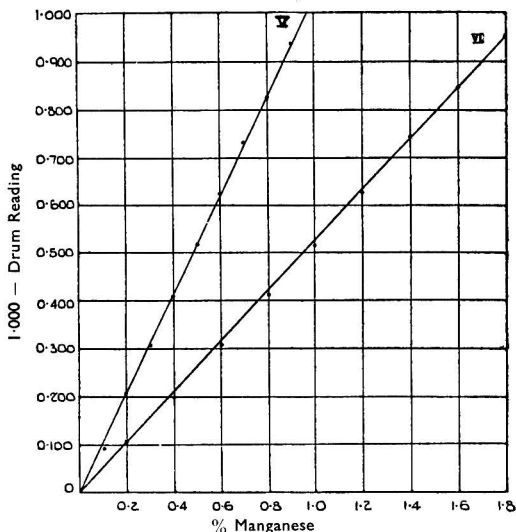
Fig. 3.   Manganese in cast iron and steel.
Mercury-vapour lamp.   Spectrum yellow
filters No. 606.   Setting W:W 1·0.   Graph V,
2 cm cells.   Graph VI, 1 cm cells.

2.   HAEMATITE IRONS AND STEELS—(a)   *Interfering elements*—When dealing with samples of low phosphorus content it is imperative to eliminate interference from arsenic and combined carbon.   The added absorptions of the yellow arsenic complex and the brownish organic compound, previously destroyed,[2] give high results.   The further addition

of hydrobromic acid completely volatilises any arsenic, and for the oxidation of carbonaceous matter ammonium persulphate[3] is preferable to the usual permanganate. The "difference" method[2] is necessary in the analysis of alloy steels containing nickel and/or chromium. The added absorptions of the green salts are cancelled by taking readings on sample "blank" solns. (molybdate omitted), subtracting from the normal "colour" readings and converting to % of phosphorus from the corresponding "difference" graphs.[2]

(b) *Conditions*—Whereas 0·5 g of the sample was adequate for the determination of silicon in haematite iron, at least 2·0 g of steel were required for accuracy. A procedure was therefore devised for steel, using 2·0 g of the sample and reserving a 0·5-g portion of filtrate for phosphorus. Tests showed this fraction to be preferable to a 1·0-g aliquot. When the optimum working conditions had been ascertained, as in 1 (a), the following method was developed.

(c) *Procedure*—Dissolve 2·0 g of the sample in 20 ml of brominated nitric acid and 100 ml of 20% sulphuric acid. Add 5 ml of 7·5% ammonium persulphate solution and boil for 5 mins. to remove excess oxygen. Evaporate until solid separates, add 1 ml of hydrobromic acid and fume. Continue as in 1 (b). To 25 ml of the filtrate add 20 ml of water (to avoid any precipitation) and 10 ml of 0·24%* vanadate soln. and develop the colour. Read the absorption in a 2-cm cell and convert to phosphorus by means of Graph III (Fig. 2). For manganese by means of the Spekker instrument, take 25 ml and make up the final coloured soln. to 120 ml.

An alternative process for phosphorus in haematite iron is as follows. Treat 0·5 g of the sample as under 1 (b), but observing the above precautions for removing arsenic and carbonaceous matter. To a 0·2-g aliquot add 10 ml of 0·24% vanadate soln., develop the colour, cool and dilute to 100 ml. Read the absorption in a 4-cm cell and convert to phosphorus by means of Graph IV (Fig. 2).

(d) *Range*—Graphs I to IV cover all requirements.

(e) *Other elements*—As the conditions simulate those of Vaughan,[1] this scheme of analysis may be extended to include nickel and molybdenum.

3. RESULTS—The following figures are selected from numerous analyses of standards and routine samples.

Procedure 1—0·5 g sample

	Silicon, %		Phosphorus, %		Taken	Manganese, % Found	
Sample	Taken	Found	Taken	Found		(a) Spekker	(b) Titration
BCS.C.1."G" ..	1·30	1·30	0·45	0·44	0·41	0·43	0·42
,, "No. 206" ..	3·32	3·32	1·51	1·49	0·325	0·33	0·32
,, Haem.C.1."A₃"	1·78	1·78	0·066	0·070	0·98	1·01	0·98
*Foundry C.1. ..	3·00	2·99	0·30	0·32	0·58	0·58	0·55
,, Haem. C.1.	0·82	0·81	0·080	0·084	0·42	0·44	0·42

* This sample contains 0·8% chromium.

Procedure 2—2·0-g sample

	Silicon, %		Phosphorus, %		Taken	Manganese, % Found	
Sample	Taken	Found	Taken	Found		(a) Spekker	(b) Titration
BCS.Haem.C.1."A₃"	1·78	1·78	0·066	0·066	0·98	1·01	1·00
,, Steel "No. 215"	0·26	0·24	0·038	0·038	0·42	0·43	0·42
,, ,, "R" ..	0·22†	0·20	0·058	0·060	0·914	0·91	0·91

These samples contain 0·02 to 0·03% of arsenic.

The heading "taken" refers to the figure on the certificate or obtained by a standard method.

† (Approx.)

4. DISCUSSION—This method gave the greater satisfaction with samples to which procedure 1 was applicable. The larger 2·0-g sample was less convenient to handle, there was greater risk of "spitting," the rate of re-solution was slow and graphs III and IV are admittedly shallow. Reproducibility of results showed that the standard acid concentration was always attained; losses during "fuming" were inappreciable. Development of the phosphovanadomolybdate in hot solution was preferred, as drum readings indicated a slow rate of formation in the cold. Evidence was produced to show that the vanadate and

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* Note the weaker concentration.

molybdate solns. can be added in one operation; further tests are under way. In every instance pure white silica ppts. and accurate manganese figures were readily obtained.

Advantages gained by using this scheme are economy of time, labour, fuel, chemicals and glassware, relief of pressure on balances and hot plates, limitation of objectionable fumes, and utilisation of "by-products"—containing required elements—formerly discarded.

I wish to thank Mr. B. Froggatt for valuable suggestions and criticism, Mr. E. Hulbert for co-operation in the analysis of works samples, and Messrs. Newton, Chambers & Co., Ltd., for permission to publish this paper.

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The Determination of Iron, Manganese and Aluminium in Bronzes and Brasses after Separation of Copper as Oxalate

BY F. H. EDWARDS AND J. W. GAILER

INTRODUCTION—The use of oxalic acid for the separation of copper in brasses and bronzes prior to the volumetric determination of tin was described by us in a recent paper.[1] Further work has shown that under the same conditions of separation iron, manganese and aluminium remain in solution, whereas nickel and zinc are partly co-precipitated with the copper. This has enabled methods to be formulated for the routine determination of the former elements in a variety of industrial alloys and provides useful alternatives to existing methods. The Spekker photoelectric absorptiometer has been employed wherever possible, using a mercury vapour lamp as the light source.

PART I. PRELIMINARY WORK ON IRON AND MANGANESE

A range of solutions was prepared corresponding to synthetic brasses containing 56% of copper, 1% of tin, 0 to 3% of iron and manganese, the remainder being zinc. The copper was separated by oxalic acid as in the tin method.[1] The oxalic acid was removed by treatment with nitro-sulphuric acid and evaporating until fumes of sulphur trioxide appeared. Iron and manganese were then determined by normal chemical methods and it was found that both elements were completely recovered.

The next step consisted in the adoption of absorptiometric methods[2] for the determination of iron and manganese after the oxalate separation. Straight line graphs were obtained for both elements except with the samples of higher manganese content. Under the conditions used at the time for the latter element the soln. consisted of minimum quantities of nitric and sulphuric acids, and the manganese was oxidised with potassium periodate. It was observed that the maximum intensity of colour was developed only after 10 to 20 min. simmering. By adding a mixture of nitric and phosphoric acids prior to the introduction of the periodate the time for development of colour was reduced to 1 min. and satisfactory results were obtained.

METHOD FOR IRON AND MANGANESE IN BRASSES—Dissolve 2 g of fine drillings in 20 ml of 50% sulphuric acid with the aid of 20 ml of hydrogen peroxide (100 vol.). The temperature should not exceed 40° C. during this operation and solution should be complete within 10 min. Remove the excess of peroxide by boiling, cool slightly, and add 10 ml of diluted hydrochloric acid (1 + 1). Warm until any ppt. dissolves. Dilute with 150 ml of 10% oxalic acid soln., boil for 15 min., cool quickly and make up to 250 ml. Decant 200 ml through a double Whatman No. 40 paper (dry). (Normally 125 ml of this liquid will be used for a tin determination.)

Take an aliquot of 50 ml, add 10 ml of sulphuric acid (sp.gr. 1·84) and 5 ml of nitric acid (sp.gr. 1·42). Evaporate rapidly to fumes with the cover glass on, then fume for 5 min. without the cover glass, and cool. Take up in 25 ml of water and warm until dissolved.

Cool and make up to 100 ml.   This forms the parent solution "S" for the determination of iron and manganese.

(a) *Iron*—Take a 10-ml aliquot from soln. "S," warm to 80° C. and "spot" in $N/10$ potassium permanganate (prepared from A.R. salt) to a faint pink.   Add one spot of sulphurous acid soln., boil briskly for a few min. and cool.   Add 10 ml of 10% hydrochloric acid, 10 ml of 5% ammonium persulphate and 20 ml of 10% sodium thiocyanate solns.   Make up to 100 ml and leave for 15 min.   Fill a 1-cm cell and determine the drum reading with the Spekker set "water to water 1·0."   Use heat absorption and Ilford 604 green filters.[2]

(b) *Manganese*—Take a 20-ml aliquot from soln. "S" and add 20 ml of an acid mixture consisting of 10 ml of nitric acid (sp.gr. 1·42), 25 ml of phosphoric acid (sp.gr. 1·75) and 65 ml of water.   Add one pellet (Analoid) of potassium periodate and allow to simmer on a hot plate until the colour is fully developed.   Cool and make up to 100 ml.   Fill a 1-cm cell and determine the drum reading on the Spekker instrument exactly as for iron.

PREPARATION OF STANDARD CURVES FOR IRON AND MANGANESE—The points on the curves shown in Figs. 1 and 2 were obtained from solns. of synthetic brasses treated as in the above methods.   The solns. used were as follows.

A. *Basic brass solution*—Dissolve 56 g of pure copper, 43 g of pure zinc and 1 g of pure tin in diluted sulphuric acid (1+1) with the aid of hydrogen peroxide (100 vol.).   Make up to 1 litre with diluted sulphuric acid (1+1).

20 ml of soln. "A" ≡ 20 ml of 50% sulphuric acid containing a 2-g sample of brass of composition 56% of copper, 43% of zinc and 1% of tin.

B. *Iron and manganese solution*—Dissolve 1 g of pure iron in hydrochloric acid (sp.gr. 1·16) with addition of hydrogen peroxide.   To this add 3·16 g of AnalaR potassium permanganate (calculated upon assay) which has been dissolved previously in water and decolorised with sulphurous acid, and the excess of sulphur dioxide boiled out.   Add hydrochloric acid (sp.gr. 1·16) to produce a total of 125 ml in the combined soln. and dilute to 500 ml.

1 ml of soln. "B" ≡ 0·10% of iron and 0·10% of manganese on a 2-g sample of synthetic brass.

It may be observed that greater accuracy is obtainable by using a 2-cm cell for iron and manganese contents of the order of 1·8 to 1·5% and a 4-cm cell for contents less than 0·8%. Graphs similar to those illustrated can be prepared for these cells.

A number of routine brass samples have been analysed by the above procedure and by normal chemical methods.   The net results are shown in Table I.

TABLE I

	Iron, %		Manganese, %	
Sample	Iodide/thiosulphate titration	Oxalate/ Spekker	Bismuthate	Oxalate/ Spekker
1	1·18	1·16	1·60	1·56
2	1·58	1·59	1·12	1·11
3	0·88	0·85	1·98	1·96
4	0·91	—	2·34	2·36
5	0·89	0·89	1·16	1·14
6	0·93	0·94	1·52	1·51
7	0·90	0·91	1·27	1·26
8	1·82	1·80	1·26	1·26
9	0·76	0·76	0·79	0·78
10	0·60	0·60	1·06	1·05

PART II.   DETERMINATION OF LOW IRON CONTENTS

Iron contents of the order of 0·05 to 0·50% can be determined satisfactorily in cartridge brass, gunmetal, phosphor bronze, copper and aluminium bronzes by an oxalate Spekker technique.   It is necessary, however, to use a "difference" method instead of plotting the direct drum readings, since there is an appreciable blank.   In the original work curves were prepared from synthetic solns. representing each of the above types of material, but it was found that the curves were strictly interchangeable.   With the phosphor bronzes it was observed that phosphorus contents up to 1% had no effect on the iron determination.

METHOD FOR LOW IRON CONTENTS—Dissolve 1 g of the sample in 15 ml of diluted sulphuric acid (1+1) and 10 to 15 ml of hydrogen peroxide (100 vol.) in a 450-ml conical beaker. If the sample is difficult to dissolve, "spot in" nitric acid (sp.gr. 1·42) to assist solution.   Boil

out excess of peroxide and remove nitric acid by evaporating until fumes of sulphur trioxide appear.   Add 10 ml of water, 10 ml of diluted hydrochloric acid (1+1) and 100 ml of 8% oxalic acid.   Boil for 15 min., cool and make up to 250 ml.   Allow the ppt. to settle and decant the liquid through a double Whatman No. 40 paper (dry).   Pipette 50 ml of the filtrate into a 250-ml conical beaker, add 10 ml of sulphuric acid (sp.gr. 1·84) and 5 ml of nitric acid (sp.gr. 1·42).   Evaporate rapidly to fumes with the cover glass on, remove the cover glass and "fume" strongly for 5 min.   Cool, add 40 ml of water and warm until the salts are in solution.   "Spot in" $N/10$ potassium permanganate (AnalaR salt) to a faint pink. Add one drop of sulphurous acid and boil out excess sulphur dioxide.   Dilute to 50 ml, add 10 ml of 10% hydrochloric acid, 10 ml of 5% ammonium persulphate soln. and 20 ml of 10% sodium thiocyanate soln.   Make up to 100 ml and leave for 15 min. before reading. A blank on the reagents is run alongside the determination.   Fill a 1-cm cell with the blank and determine the drum reading with the Spekker set "water to water 1·0," using Ilford 604 green filters and heat absorption filters.   Repeat for the sample and obtain the difference in drum readings.

For iron contents of 0   to 0·3% use a 2-cm cell.
        „   „   „   „ 0·2 „ 0·6%   „   1-cm   „

The curves shown in Fig. 3 were plotted from results obtained by treating synthetic solns. of copper-tin alloys as in the above method.   These solns. were prepared upon similar lines to the synthetic brasses described in Part I.

The results upon a number of miscellaneous samples by the oxalate/Spekker method and normal chemical methods are compared in Table II.

TABLE II

				Iron, %	
Sample				Chemical	Oxalate/Spekker
Gunmetal					
Sn 7%, Pb 2%, Zn 5%	..	..	..	0·10	0·09
Gunmetal					
Sn 5%, Pb 5%, Zn 5%, Ni 0·5% ..		..		0·18	0·17
Gunmetal (iron added upon solution)					
Sn 7%, Pb 2%, Zn 5%	..	..	..	0·40	0·41
do.	..	..	..	..	0·60	0·58
Phosphor-bronze (iron added upon soln.)					
Sn 14%, P 0·7%	..	..	..	..	0·40	0·39
do.	..	..	..	..	0·60	0·62
Aluminium bronze					
Al 10%, Mn 5%, Cu 85%	..	..	..	0·11	0·12
do.	..	..	..	0·11	0·11
do.	..	..	..	0·09	0·09
do.	..	..	..	0·07	0·06

PART III.   ALUMINIUM IN BRASSES AND BRONZES

Preliminary work showed that the whole of the aluminium present in a sample remained in solution after the oxalate separation of copper.   Any convenient method may be used for the final determination following upon the oxalate separation.   In the present scheme we have employed the gravimetric method.

METHOD FOR ALUMINIUM—Using a 2 g sample, follow the method outlined for "Iron and Manganese in Brass" to the stage of precipitation of the copper and dilution to 250 ml.

For aluminium contents up to 1·5%   take a 125-ml aliquot.
        „   „   „   from   1·5 to 5·0%   „   100 „   „
        „   „   „   „   5·0 „ 12·0%   „   50 „   „

To the aliquot add 10 ml of sulphuric acid (sp.gr. 1·84), and 5 ml of nitric acid (sp.gr. 1·42).   Evaporate rapidly with the cover glass on, then "fume" for 5 min. with the cover glass removed.   Take up the salts in 20 ml of water, boil, dilute to 200 ml and remove Group II elements by means of gaseous hydrogen sulphide.   Filter on a pulp pad and wash with acidulated hydrogen sulphide water.   Boil out the hydrogen sulphide in the filtrate, oxidise with brominated hydrochloric acid, boil out excess bromine and make a basic acetate separation.   Dissolve the iron and aluminium ppt. in diluted hydrochloric acid (1+1), evaporate almost to dryness, dilute with 50 ml of water and add 30 ml of 15% potassium iodide soln.   Leave for 5 min. and titrate with $N/10$ sodium thiosulphate to determine the

iron content.   Add 5 ml of thiosulphate soln. in excess, make just alkaline with ammonia (using methyl orange as indicator) and then just acid with hydrochloric acid.   Add 10 g of sodium thiosulphate and heat to boiling.   Add 5 ml of ammonium acetate soln. and boil for 5 min.   Cool quickly, add 4 ml of phenylhydrazine soln. in alcohol (1+1), filter quickly through a large pulp pad (using suction) and wash 10 or 12 times with previously boiled cold water.   Ignite the precipitate at 1200° C. and weigh as Al_2O_3.

RESULTS ON SYNTHETIC MIXTURES AND INDUSTRIAL ALLOYS—The results by the method are shown in Table III with comparisons by normal chemical methods.

TABLE III

Sample	Aluminium, %	
	Normal chemical method	After oxalate separation
Synthetic aluminium copper		
Al 0·5% Cu 99·5%		0·51
,, 1·0 ,, 99·0		1·06
,, 1·5 ,, 98·5		1·58
,, 2·0 ,, 98·0		1·99
,, 3·0 ,, 97·0		2·98
,, 4·0 ,, 96·0		4·04
,, 8·0 ,, 92·0		8·02
,, 9·5 ,, 90·5		9·67
,, 11·0 ,, 89·0		11·07
Aluminium bronze		
Al 10%, Fe 3% (Cu remainder)	8·69	8·75
Aluminium brass		
Al 2%, As 0·03%, Cu 77% (Zn remainder)	2·04	2·07
do.	1·88	1·88
Leaded brass		
Pb 3%, Cu 58%, Zn and impurities remainder	0·50	0·53
do.	0·56	0·57

CONCLUSIONS AND DISCUSSION—The oxalate separation of copper provides a useful alternative to the electrolytic method for removing copper in copper base alloys.   The elements tin, iron, manganese and aluminium may subsequently be determined by any convenient method.   In the present work, iron and manganese have been determined by absorptiometric procedures, aluminium gravimetrically and tin volumetrically.   Where tin is present in the alloy, a single weighing of the sample suffices for the determination of all four elements.

The method outlined for iron present in gunmetals, bronzes, etc., is much quicker than those in general use.   Normally the processes of separation before the final determination of the iron are extremely laborious and slow, and with tin bronzes where the tin is removed as volatile bromide the fumes of bromine are objectionable.

Owing to the exigencies of space it is impossible to propose schemes of analysis for the many types of alloy dealt with.   It is sufficient to suggest that variations in the procedure may be introduced to meet the individual need.   For instance, with samples of the aluminium brass condenser tube type where the iron, manganese and tin are negligible in quantity and the spectrographic figure for these elements is normally accepted, the method for aluminium may be shortened appreciably.   The boiling time, after addition of oxalic acid, may be reduced from 15 min. to 2 min. and the basic acetate operation omitted.   If desired, and as an alternative to the removal of the oxalate radicle by "fuming," a quicker treatment consists in oxidation by silver persulphate.   In this case hydrochloric acid is omitted completely from the solns. employed in the analysis.   For samples of complex brasses, and where the aluminium content is known to be low, it may be advisable to use a separate amount for the aluminium determination.

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An Electrometric Titration Method for Water Determination, using the Karl Fischer Reagent

By R. J. CARTER and L. WILLIAMSON

INTRODUCTION—The attention that has been paid within recent years to the Karl Fischer method[1] for direct titration of water is a measure of the importance this method has assumed in analysis.* The Karl Fischer reagent consists of a solution of iodine and sulphur dioxide in methanol and pyridine, the essential reaction occurring between the first two constituents in the presence of water. The stoichiometric expression:

$$I_2 + SO_2.2C_5H_5N + 2H_2O + 2C_5H_5N \longrightarrow (C_5H_5N)_2.H_2SO_4 + 2C_5H_5N.HI$$
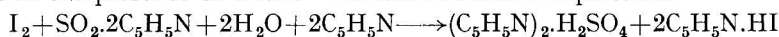
has been shown[2] to be an over-simplification, as the reaction really proceeds in two distinct steps. No stoichiometric relationship has been found between the water that reacts and the volume of Fischer reagent used, and the reagent is standardised empirically against water.

The visual method for titration, which relies on the detection of excess of iodine by its colour in the titrated solution, is limited in its application to colourless or light-coloured materials, and even with these many operators have experienced difficulty in discerning the end-point. To overcome this difficulty, alternative methods for determining the end-point have been described.[3,4,5] These are all electrical in principle, being based on potentiometric[3] or electrometric methods, the current changes in the latter methods being indicated by a galvanometer,[4] or a cathode ray oscillograph.[5] For satisfactory performance an excess of Fischer reagent is used, the excess being back-titrated with a standard solution of water. In some instances, also,[3,5] the apparatus required is not readily accessible.

THEORY OF THE METHOD—The requirements of simplicity, ease of operation and precision in the routine determination of water in solutions of resins led us to devise an electrical method employing direct titration, as opposed to the longer back-titration methods. It was also our aim to devise an apparatus of simple and inexpensive design. The method depends upon the fact that when a small potential difference is impressed upon electrodes immersed in a methanol soln. of moist material, the current which flows in the closed circuit on the addition of Fischer reagent remains fairly constant during titration, but suddenly increases at the end-point of the titration. The reverse of this phenomenon has been noted for the addition of the solution of water to the Fischer reagent.[3] An electrometric method of this nature was originally used by Foulk and Bawden[6] in the titration of aqueous solns. of iodine with thiosulphate and *vice-versa*, and termed by them the "dead-stop end-point" method. Its principle in that instance was explained on the basis of polarisation of one or both of the electrodes, and the same explanation probably holds in titrations with the Karl Fischer reagent in non-aqueous solvents.

EXPERIMENTAL

APPARATUS—The electrical circuit is shown diagrammatically in Fig. 1. The 2-volt cell B is used as the source of potential, and R_1 is a high resistance of the order of 3000 to 10,000 ohms. The shunt resistance R_2 is of the order of 1/40th to 1/10th of the galvanometer resistance, and the most suitable value of this and of R_1 are found by trial titrations, the requirement being that the change between the current reading during the titration and the surge current reading at the end-point be observable on the galvanometer scale. In the circuit used by us, the galvanometer G is of the mirror type with sensitivity of 24 mms/microamp and with a 100-mm scale for the spot movement. The galvanometer resistance is 373 ohms and the shunt 10 ohms; R_1 is 5000 ohms.

The calculated e.m.f. applied to the electrodes varies, since the resistance of the electrode-solution system decreases throughout the titration from a value greater than 15,000 ohms to a value of approx. 500 ohms at the end-point. Initially, in the methanol soln. the calculated potential being applied is between 1 and 2 volts, whereas at the end it proves to be approx. 0·15 volt. In this respect this method differs from that of previous writers, who have generally used a potential of 10 to 15 millivolts.

For titration purposes, no readings of currents or resistance are necessary. The galvanometer spot is merely observed, and when it moves with a surge up the scale the end-point of

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* *Cf.* this Vol., p. 339.—*Editor.*

the titration is noted. It is unnecessary to note the zero spot reading before the Fischer reagent is added, and in fact the galvanometer suspension setting is adjusted so that the spot is off the lower end of the scale. Hence, when the current increases to an approx. constant value during the titration, the spot appears at the beginning of the scale and is in a suitable position for observation of the sudden increase at the end-point, which may reach values of the order of 50 mm.

The titration vessel with its fittings is represented in the diagram, Fig. 2. This apparatus is designed to incorporate the features of robustness, and ease of removal of the container. The container consists of a round-bottomed tube, 95 mm × 35 mm. This is fitted with a rubber stopper, drilled to carry in permanent position the glass sleeve for the stirrer shaft, the electrodes and the tip of the burette extension. The electrodes, spaced about 20 mm apart, each consists of a glass tube, having sealed in its lower end a platinum electrode wire looped at its lower extremity. The circuit wire enters the tube at its open end, being sealed in position with sealing cement, and electrical contact with the electrode wire is effected by a column of mercury in the tube. The axle of the glass stirrer fits closely into the sleeve to avoid undue vibration. To facilitate cleaning, the burette extension is made separately and joined to the burette tip by narrow-bore thickwalled rubber tubing.

The stopper carrying the electrodes, stirrer and burette tip extension is firmly clamped, the titration vessel being changed by lowering, and the fittings are cleaned and dried by washing from an ether wash-bottle and wiping with filter-paper.

PROCEDURE—A convenient quantity of the material for test is weighed into the dry titration vessel, and 3 ml of dry methanol are added. The vessel is quickly stoppered and its contents well mixed, if necessary by gentle warming. The vessel is fixed to the electrode unit and the galvanometer and battery keys are depressed.

The Fischer reagent is then added rapidly in 0·2-ml portions with the stirrer running. The galvanometer reading becomes constant, until the end-point is approached, when small oscillations occur. Additions of Fischer reagent are made dropwise from this point onwards and when the end-point is reached the galvanometer reading makes a steady upward movement of approx. 50 mm and remains stationary at the higher value. Owing to gradual absorption of moisture the end-point is not permanent, but generally has a duration of at least 10 sec. The end-point is usually precise to 0·05 ml. A "blank" expt. is run with omission of the test sample, and allowance is thus made for moisture present in the methanol and taken up during the determination. The following figures (Table I) obtained, during routine determinations, indicate the degree of precision to be expected in standardisation of the reagent by direct titration of weighed quantities of water.

TABLE I

Weight of water g	Fischer reagent ml	Factor g of water/ml of reagent
0·0265	9·40	0·00282
0·0185	6·55	0·002825
0·0178	6·30	0·002825
0·0271	9·70	0·00280
0·0372	13·40	0·00278
0·0421	15·10	0·00279
0·0472	16·80	0·00281
0·0385	14·09	0·00273
0·0226	8·30	0·00272
0·0268	9·85	0·00272
0·0202	7·40	0·00273

Examples of the results obtained from routine duplicate determinations of water in synthetic resin solutions are given in Table II.

The average deviation from the mean of the duplicates given in Table II is 0·5%. These results compare well with those published by other workers using indirect titration by electrical methods.[2,3,4]

SUMMARY—An electrometric method for the determination of water by direct titration with Karl Fischer reagent, in which the end-point is observed by a change in current registered on a galvanometer, has been described. This method is simple and direct, and capable of wide application to coloured materials.

TABLE II

	Sample No.	Water, %				
		(a)	(b)	Mean of duplicates	Deviation from mean	Deviation, %
Synthetic resin						
Solution A ..	F.8240	0·913	0·930	0·9215	0·0085	0·90
	F.8459	0·867	0·871	0·869	0·002	0·23
	F.8241	0·837	0·843	0·840	0·003	0·36
	F.8458	0·837	0·851	0·844	0·007	0·83
	F.8460	0·981	0·981	0·981	nil	nil
	F.8462	0·974	0·972	0·973	0·001	0·10
	F.8463	1·030	1·046	1·038	0·008	0·77·
	F.8464	1·010	0·990	1·000	0·010	1·00
	F.8477	0·967	0·974	0·9705	0·0035	0·36
Solution B ..	F.8352	0·850	0·862	0·856	0·006	0·70
	F.8353	0·863	0·853	0·858	0·005	0·58
	F.8881	2·00	2·01	2·005	0·005	0·25
	F.8882	0·810	0·813	0·8115	0·0015	0·18

The authors express their thanks to Dr. N. W. Hanson for helpful advice in preparing this report.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
RESEARCH AND DEVELOPMENT DEPARTMENT
PAINTS DIVISION, SLOUGH
May, 1945

Erratum—DETERMINATION OF PHOSPHINE AND HYDROGEN SULPHIDE IN ACETYLENE, this Vol., p. 280, lines 33–34:

For ". . . dilute 1 ml of the stock soln. with 4 ml of water."

Read ". . . dilute 1 ml of the stock stannous chloride soln. with 4 ml of water."

Notes

THE VOLUMETRIC ANALYSIS OF DICHROMATE—VANADATE MIXTURES

MUCH attention has been given to the simultaneous determination by volumetric methods of vanadic and chromic acids, as may be seen from the list of references quoted in Mellor and Thompson's "*Treatise on Quantitative Inorganic Analysis,*" 2nd Ed., 1938, pp. 537–539. Practically all such methods depend ultimately upon differences in the oxidising properties of the two acids. Of the volumetric methods for determining vanadium, one of the most valuable consists in oxidising the element from the quadrivalent to the quinquevalent condition in acid solution by potassium permanganate. The reverse reaction, $V^V \rightarrow V^{IV}$ is readily effected quantitatively by numerous reducing agents, of which ferrous sulphate is in many respects the most convenient. In the course of conducting practical classes in quantitative analysis, it has been found that mixtures of ammonium metavanadate and potassium dichromate can be estimated accurately by reduction with standard ferrous sulphate, and subsequently oxidising the resulting vanadyl salt with permanganate. It should be noted that when solns. of vanadyl sulphate and chromic sulphate in concentrations of the order of 0·05 N to 0·1 N are titrated with potassium permanganate in presence of sulphuric acid, the reaction $V^{IV} \rightarrow V^V$ proceeds quantitatively without the slightest oxidation of the chromic sulphate.

Recently I have found that determinations of this kind may also be carried out by differential titration with a standard ferrous soln. as follows. The soln. of the dichromate-vanadate mixture, containing sulphuric acid, is first titrated with the ferrous soln. run from the burette, the end-point being obtained with the aid of a few drops of a 0·33% soln. of barium diphenylaminesulphonate as an internal indicator. An equal volume of the original soln. is then treated with a slight excess of hydrogen peroxide, which results in the immediate formation of perchromic and "pervanadic" acids, but the blue perchromic acid is at once decomposed, with formation of chromic salt. The soln. is then boiled for a short time. This effects the decomposition of the excess of hydrogen peroxide, while most of the vanadium is present in the quinquevalent condition. Under the conditions of my expts. a limited reduction (of the order of 10% of the vanadium present) to the quadrivalent condition occurs, but on adding dilute permanganate soln. from a dropping tube to the soln. until a pink colour is just attained, the resulting liquid contains all the vanadium in the quinquevalent and the chromium in the tervalent condition. The liquid is then titrated at the

ordinary temperature with the ferrous solution.   This second titration thus gives a measure of the vanadium, and the difference from the first titration represents the chromium.   An example may be quoted to show the value of the method.   A soln. of ammonium metavanadate and potassium dichromate in very dilute sulphuric acid when analysed by reduction with ferrous sulphate and subsequent titration with permanganate gave 4·90 g of NH_4VO_3 and 2·49 g of $K_2Cr_2O_7$ per litre.   When analysed by titration with ferrous sulphate followed by treatment with hydrogen peroxide and a second titration with ferrous sulphate, the results were 4·87 g of NH_4VO_3 and 2·52 g of $K_2Cr_2O_7$ per litre.

It may be noted that if vanadic acid is treated with hydrogen peroxide in presence of a very considerable concn. of sulphuric acid, reduction to vanadyl sulphate takes place.   A volumetric method based on this reaction has been devised by Hothersall (*J. Soc. Chem. Ind.*, 1924, **43**, 270т).   The conditions for the production of the dark red-brown peroxide derivative, which is of value for the detection of traces of the element and for its colorimetric determination, have been investigated by Meyer and Pawletta (*Z. Anal. Chem.*, 1926, **69**, 15).

CHEMICAL LABORATORY   A. J. BERRY
    UNIVERSITY OF CAMBRIDGE   *July*, 1945

THE SPECIFIC GRAVITY OF MILK WITH SPECIAL REFERENCE TO MILK WHICH HAS BEEN HOMOGENISED AND STERILISED

DURING the last year or so an increased number of sterilised milk samples have been examined in the course of the ordinary work of the laboratory, and it was noted that many of these gave, from the result of the specific gravity determination, apparent solids-not-fat by Richmond's calculation which were appreciably (up to 0·2% approx.) lower than the weighed solids-not-fat (Table I).   In some instances the figures

TABLE I

STERILISED MILK SAMPLES

Sample No.	Date	Fat	Sp.gr. 60° F.	Richmond scale		Weighed		Freezing-point (Hortvet)°C.
				T.S.	S.N.F.	T.S.	S.N.F.	
A620	18/7/44	3·3	1·0305	11·70	8·40	11·90	8·60	−0·542
A624	20/7/44	3·3	1·0305	11·70	8·40	11·82	8·52	−0·536
A661	21/8/44	3·3	1·0300	11·70	8·40	11·81	8·51	−0·532
M214	7/9/44	3·75	1·0304	12·25	8·50	12·40	8·65	−0·538

calculated from Richmond's formula were below the presumptive limit of the Sale of Milk Regulations, thereby necessitating additional work on the samples.

With a view to investigating this point a number of samples have been taken direct from two different sterilised milk plants and the figures in Tables II and III are representative of the results obtained.   The

TABLE II

DAY-TO-DAY VARIATIONS IN THE SPECIFIC GRAVITY OF STERILISED MILK

Number	Date of processing	Fat %	Specific gravity at 60° F.					Acidity 5/10/44 Lactic acid % W/v	Richmond scale S.N.F.—Calc. %		S.N.F. Weighed %
			2/10/44 10 a.m.	2/10/44 4 p.m.	3/10/44 9.30 a.m.	4/10/44 9.30 a.m.	5/10/44 9.30 a.m.		2/10/44	4/10/44	
M240	1/10/44	3·70	1·03045	1·03065	1·03070	1·03095	1·03090	0·17	8·50	8·60	8·65
M241	1/10/44	3·65	1·03045	1·03060	1·03070	1·03090	1·03095	0·17	8·47	8·59	8·68
M242	1/10/44	3·67	1·03045	1·03065	1·03070	1·03095	1·03100	0·17	8·48	8·61	8·65

TABLE III

DUPLICATE SAMPLES OF RAW AND HOMOGENISED (NOT STERILISED) MILK—VARIATION IN SPECIFIC GRAVITY

Number	Temperature Homogenised	Type	Fat %	Sp.gr. at 60° F.					Richmond scale S.N.F.—Calc. %		S.N.F. Weighed %
				10/10/44 *(a)	10/10/44 (b)	11/10/44 (c)	11/10/44 (d)	11/10/44 (e)	as recd.	heated to 40° C.	
M254	—	Unprocessed	4·07	1·0314	1·0308	1·03147	1·03135	1·03075	8·78	8·63	8·71
M255	—	,,	4·09	1·0315	1·0308	1·03147	1·03150	1·03085	8·81	8·64	8·73
M256	115°F.	Homogenised	4·08	1·0306	1·0308	1·03135	1·03132	1·03075	8·59	8·63	8·71
M257	115°F.	,,	4·07	1·0306	1·03075	1·03147	1·03140	1·03070	8·60	8·63	8·73

* (a) As received.
 (b) After heating to 40° C. read at 20° C.
 (c) Heated to 40° C. 10/10/44 re-read 11/10/44 without again heating.
 (d) Not heated to 40° C.
 (e) Portions heated to 40° C., 10/10/44 again heated to 40° C., 11/10/44 and re-read.

sp.gr. of the samples were determined at intervals (samples kept in refrigerator in the meantime), and it was found that for one plant, Table II, two or three days passed before the gravity reached a maximum figure; whereas for the other plant, Table III, reasonably constant figures were obtained in 24 hr.   These

two plants worked at homogenising temperatures of 174° F. and 115° F. respectively, and it may be that the efficiency of homogenisation has some connection with the time required for the gravity to reach a constant figure.

The behaviour mentioned above resembles closely "Recknagel's phenomenon,"[1] observed with freshly-drawn milk. A number of explanations have been offered from time to time for the increase in sp.gr. known by this name, of which the most likely would appear to be slow solidification of the fat globules, with a resulting contraction in their volume and an increase in sp.gr. The usual preliminary procedure recommended before determining the sp.gr. of milk is to keep the sample a sufficient time to allow the maximum contraction to occur. The British Standards Specification for density hydrometers,[2] on the other hand, determines the density of milk immediately after heating to 40° C. before any contraction of the fat can occur; it is claimed that by the latter method inconsistencies due to Recknagel's effect are avoided. Both these procedures were adopted before taking the sp.gr. of unprocessed and homogenised samples from the same batch of bulked milk (Table III) and all the results were corrected by Richmond's milk scale to sp.gr. at 60° F. It will be observed that the sp.gr. of the unprocessed milk was appreciably lowered by heating to 40° C., while, as was to be expected, that of the recently-homogenised milk was not seriously affected. The gravity then slowly rose, in 24 hr. in this instance, to that of the original unprocessed milk and the whole procedure could be repeated by reheating to 40° C.

We, therefore, find that two different yet constant gravities can be obtained for the same sample of milk depending on the procedure adopted, *i.e.* whether it is allowed to stand or whether it has recently been heated. Richmond, knowing about the Recknagel effect, chose the first procedure, and his method of determining the sp.gr. of milk, therefore, normally records its maximum figure. It is doubtful whether it has been fully realised that these two determinations are carried out under totally different conditions of the milk; the former when the milk is completely contracted and the latter under conditions of no contraction. Unless appropriate corrections are made solids-not-fat calculated on the one hand from sp.gr. determined by Richmond's technique and on the other hand by sp.gr. determined after the milk has been heated to 40° C., will not agree. Similar results and conclusions with regard to raw and pasteurised milk have been reported by Boden and Campbell.[3]

An additional point of some importance brought out by the present note is that homogenising with or without sterilisation may under certain conditions result in contraction being so delayed that the milk will require several days to reach it maximum sp.gr., and that the Richmond formula cannot be applied to milk so treated. The results in Table II indicate that the specific gravity was rising over a period of three days and may not then have reached its maximum.

While consistent results can undoubtedly be obtained by heating all milks to 40° C., there are two inherent objections to this procedure: (1) the Public Analyst is usually dealing with milk which is one day or more old and there is the possibility, particularly in hot weather, that the milk will form butter or curdle if heated, thereby rendering further analysis difficult, (2) the results obtained are lower than those obtained by Richmond and other authors who adopted the procedure of allowing the milk to stand, and there is a danger, unless solids are determined gravimetrically on all milks, of assuming that the average quality of milk has deteriorated in recent years.

There is evidence, therefore, that sterilised milks and milks generally which have been recently heated to 40° C. or higher give figures for solids-not-fat by hydrometry which are not in agreement with those obtained by allowing the milk to stand under cool conditions until maximum contraction has occurred, and that homogenised milks may take a longer time than usual to reach this condition.

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City Analyst's Laboratory   G. H. Walker
    Salford   *August,* 1945

SPECTROPHOTOMETRIC ESTIMATION OF DIPHENYL AND OF o-PHENYL-PHENOL

As both diphenyl and o-phenyl-phenol have a well marked absorption in the ultra-violet, measurement of it affords a simple and accurate method for the estimation of these compounds. Their extraction from orange wrappers or orange peel has been described by Tomkins and Isherwood (this vol., p. 330), whose method requires only slight modification to enable the final estimation of the separated substances to be made spectrophotometrically. In hexane solution diphenyl shows an absorption maximum at wave-length approximately 251 $m\mu$ and o-phenyl-phenol one at 245 $m\mu$. At these points the molecular extinction coefficients are 18,500 and 9800 respectively. These figures correspond to $E_{1\,cm}^{1\%}$ values of 1200 and 580 respectively; so a solution containing about 0·001% of the phenyl compound gives a convenient reading in a 1 cm cell.

As pure chloroform shows considerable absorption at and below 250 $m\mu$, it cannot be used as a solvent. Spectrographically pure cyclohexane is satisfactory, and the procedure of extraction and purification described by Tomkins and Isherwood is applicable.

For o-phenyl-phenol there is a further alternative method, which does not require a spectrophotometer. This compound, in slightly alkaline solution in alcohol, shows a strong blue-violet fluorescence under ultra-violet light, and the intensity of this can be matched visually, just as is done in the estimation of aneurine, the colour or fluorescence of which it closely resembles.

As the proposed limit for diphenyl in orange wrappers is 40 mgms per 100 square inches and the above methods are easily applicable to 0·1 mgm of the diphenyl, it is only necessary to extract about a square inch of the paper.

10, Billiter Square   H. E. Cox
    London, E.C.3

PREPARATION OF SCHIFF'S REAGENT

THE preparation of a satisfactory Schiff's reagent, both colourless and sensitive, is very much dependent upon the quality of the magenta used. It has been found practically impossible at the present time to obtain magenta of sufficiently high purity to give a colourless solution after sulphite treatment. As the residual brown colour remaining after sulphite treatment masks the reaction of small quantities of methyl alcohol, the possibility of removing this residual colour by various adsorptive agents without affecting the sensitivity of the reagent was examined.

Tests were carried out with a number of different qualities of magenta at present commercially available in this country and using a variety of adsorptive materials. The most satisfactory from the aspects of decolorisation and effect on sensitivity proved to be the B.D.H. "Fuller's Earth for Adsorption Purposes."

The method of preparation finally adopted was as follows:—Dissolve 0·2 g of magenta (basic) in 120 ml of hot water and cool; add 2 g of anhydrous sodium sulphite dissolved in 20 ml of water, followed by 2 ml of conc. hydrochloric acid and dilute to 200 ml. Then add 0·1 g of B.D.H. Fuller's Earth per 100 ml of reagent, shake for a few minutes and filter. If decolorisation is not complete add a further 0·1 g of the Fuller's Earth (but usually 0·1 g was found sufficient). The reagent should be tested for sensitivity before use.

The sensitivity of the reagent prepared as above varied with the type of magenta used. Five samples of magenta were compared for sensitivity by the colours given in the two lower standards described in the A.O.A.C. Handbook,[1] i.e., 0·25 ml of 0·02% and 0·04% solutions of methyl alcohol in 22% ethyl alcohol. The test was carried out according to the procedure of the A.O.A.C.[1] A reagent which gave a colour in the lowest standard was considered to be of satisfactory sensitivity. The results obtained are given in Table I.

TABLE I

Magenta sample used	Reaction obtained with 0·02% MeOH soln.	Reaction obtained with 0·04% MeOH soln.
A	No colour	Colour
B	Slight colour	,,
C	No colour	,,
D	Good colour	Good colour
E	,,　　　,,	,,　　　,,

The reagents were then tested by the B.P.[2] procedure, except that they were allowed to stand for one hour. A 0·1% v/v solution of methyl alcohol in 10% ethyl alcohol was used for this purpose; 0·5 ml of this solution was used in each test and 0·45 ml of absolute alcohol was added to give the required 10% alcohol concn. The results obtained are given in Table II.

TABLE II

Magenta sample used	0·5 ml of 0·1% MeOH in 10% EtOH
A	Slight colour
B	,,　　　　,,
C	,,　　　　,,
D	Good colour
E	,,　　　　,,

D and E were marked special for Schiff's reagent and were obtained from Baird & Tatlock and from Hopkin & Williams, respectively. They gave an immediate reaction in absence of methyl alcohol, but this faded during the hour. It is therefore essential to allow one hour for the test in order to prevent this initial reaction being taken to indicate the presence of methyl alcohol. This initial reaction has also been found to occur with Schiff's reagent prepared with Kahlbaum's magenta.

I wish to thank the Directors of Boots Pure Drug Co., Ltd., for permission to publish this note.

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1. A.O.A.C., "Methods of Analysis," 1940, p. 176.
2. British Pharmacopoeia, 1932, p. 45.

ANALYTICAL DEPARTMENT　　　　　　　　　　　　　　　　　　　　F. CLERMONT SCOTT
　　BOOTS PURE DRUG CO., LTD., NOTTINGHAM

THE SPECIFIC ROTATION OF ERGOTAMINE ISOLATED FROM ERGOTAMINE TARTRATE

THE United States Pharmacopoeia XII includes a monograph on Ergotamine Tartrate and under "Optical Rotation" it is stated "The specific rotation $[\alpha]_D^{25°}$ of a solution of Ergotamine Tartrate in chloroform containing 0·4 g. in each 100 c.c., and using a 100 mm tube, is not less than $-125°$ and not more than $-155°$." We have recently had occasion to examine some of this salt and, when attempting to follow the directions of the U.S.P., found it impossible to take the specific rotation in chloroform, in which solvent the salt is nearly insoluble.

"New and Non-Official Remedies" (1939 Edition, p. 242) states that the ergotamine base extracted from the tartrate and re-crystallised from dilute acetone, possesses $[\alpha]_D^{20°}$ of $-125°$ to $-155°$ (c, 0·6 in chloroform). No difficulty was experienced in carrying out the N.N.R. directions, but we found that, owing to the ease of transformation of ergotamine into ergotaminine, the specific rotation of the extracted alkaloid showed marked variation. For example, one batch of salt gave alkaloid having specific rotation

of $-90°$ to $-130°$ in different expts., carried out apparently under identical conditions. It was discovered that evaporation of the chloroform extract of the alkaloid to dryness and its subsequent re-crystallisation was the cause of the discrepancies, and we found that the following process for determining the specific rotation gave more reliable results.

Place about 0·34 g of ergotamine tartrate, accurately weighed, in a separating funnel, and add 25 ml of water, followed by 0·5 g of solid sodium bicarbonate. Gently mix and, after adding 10 ml of chloroform, shake vigorously. Allow to stand and run off the chloroform extract through a 7-cm No. 41 Whatman filter-paper, previously moistened with chloroform, into a 50-ml standard flask. Repeat the extraction with further successive portions (10 ml) of chloroform, passing the extracts through the same filter into the flask, until nearly 50 ml of extract have been obtained. Place the flask in a bath at 20° C. for 10 min. and finally adjust the volume of the extract to 50 ml with chloroform at 20° C. Thoroughly mix and measure the optical rotation of the soln. at 20° C. Calculate the specific rotation of the ergotamine base, assuming the tartrate to contain 88·5% of ergotamine in the anhydrous salt or, alternatively, evaporate a known volume of the chloroform extract to dryness, dry the residue to constant weight at 100° C. *in vacuo* and use the figure thus obtained for calculating the specific rotation of the base.

Using this technique, we have obtained figures of $-150°$ or over for the specific rotation of the alkaloid extracted from commercial ergotamine tartrate.

We wish to thank the Directors of the Wellcome Foundation for permission to publish this note.

WELLCOME CHEMICAL WORKS   A. E. BEESLEY
DARTFORD   G. E. FOSTER
                                                           September, 1945

Ministry of Food

INTER-DEPARTMENTAL COMMITTEE ON FOOD STANDARDS
SELF-RAISING FLOUR: SUPPLEMENTARY REPORT

THE Food Standards (Self-raising Flour) Order, made in January, 1944, created the following standard for self-raising flour:

1. Self-raising flour shall yield not less than 0·45 % of available carbon dioxide and not more than 0·65% of total carbon dioxide, the available carbon dioxide and the total carbon dioxide being determined in the manner specified in paragraph 2 of this Schedule.

2. (1) The total carbon dioxide shall be determined by ascertaining the weight thereof evolved when the self-raising flour is treated with excess of dilute sulphuric acid at room temperature, the evolution being completed either by boiling for five minutes or by means of reduced pressure.

(2) The available carbon dioxide shall be determined by ascertaining the difference between the total carbon dioxide and the residual carbon dioxide; and the residual carbon dioxide shall be determined in the following manner:

The self-raising flour shall be treated with water first for twenty minutes at room temperature, then for twenty minutes on a boiling water bath and subsequently by boiling for one minute. The residual carbon dioxide is the weight thereof evolved when the self-raising flour so treated is further treated with excess of dilute sulphuric acid at room temperature, the evolution being completed either by boiling for five minutes or by means of reduced pressure.

This standard was recommended by the Committee for the reasons set out in the précis of its report issued in March, 1944. The Committee felt it undesirable that an Order should describe analytical procedures in greater detail than was necessary to ensure uniform results being obtained by different analysts, but shortly after the issue of the Order complaints were made that the prescribed analytical details were not sufficient. While individual analysts could obtain uniform results by applying their own procedures within the general framework of the method set out in the Order, appreciably different results were being obtained in different laboratories, even by trade chemists who were familiar with the difficulties arising in the analytical examination of self-raising flour. In laboratories where the examination of self-raising flour was comparatively infrequent, the difficulties were more serious, and as a result proceedings were instituted in a number of areas for alleged infringement of the standard in respect of samples made by well known manufacturers and which, when examined by the manufacturers' own chemists, were found to be fully up to standard. One manufacturer arranged for assays to be carried out by ten trade and other chemists on different portions of a carefully prepared sample of self-raising flour. Although all the participating chemists had considerable experience of the determination, the range in the results obtained was as follows:

For total carbon dioxide	..	..	0·52 to 0·60	(average	0·571)
For residual carbon dioxide		..	0·033 to 0·110	(,,	0·063)
For available ,, ,,		..	0·46 to 0·535	(,,	0·508)

Comments made by the analysts who took part indicated some divergence of opinion as to the reason for the differences in the results and hence it was impossible to say, without further investigation, in what respects the details set out in the Order required amplification. Moreover, at the time the recommendation of the Standards Committee was first submitted to the Ministry, the acid-aerating agent used almost exclusively was calcium acid phosphate, but subsequently sodium acid pyrophosphate became available in larger quantity, and it was necessary to consider whether the method set out in the Order would apply to pyrophosphatic flours. Accordingly a meeting was held between the analyst members of the Standards Committee and a number of trade chemists, at which the standard was fully discussed. The supply position with regard to ingredients had eased considerably since the Committee's recommendation had first been made, and it was agreed that the whole question of the standard should be considered afresh.

TOTAL CARBON DIOXIDE—As was stated in the Committee's original report, the reason for the inclusion of a maximum limit for total carbon dioxide was the necessity at that time of exercising strict economy in the use of phosphorus compounds for food purposes. The imposition of this limit, however, had the effect of preventing some manufacturers from supplying a product containing what they considered to be a sufficiently high excess over the minimum standard to allow for deterioration during storage. In the circumstances prevailing to-day, acid calcium phosphate is in sufficient supply and there is no longer any necessity to retain the maximum figure for total carbon dioxide. Its deletion would make it possible to resume the supply for special purposes of self-raising flours containing a high proportion of sodium bi-carbonate, and would allow manufacturers to make whatever provision for deterioration they consider necessary.

AVAILABLE CARBON DIOXIDE—At the meeting with the trade chemists suggestions were made that the standard of 0·45% of available carbon dioxide was unnecessarily high. On the other hand, when the matter was originally considered by the Committee, trade representatives had suggested that a flour containing less than 0·40% available carbon dioxide would probably be unsatisfactory for household use. The figure of 0·45% was, therefore, adopted by the Committee to make provision for some slight deterioration taking place after purchase by the consumer. The trade representatives now suggested that 0·35% was a reason-able minimum figure and pointed out that this would be advantageous because a sample containing between 0·5 and 0·6% of available carbon dioxide when stored for a long time tended to reach a condition of stability when the available carbon dioxide content had dropped to 0·35%.

In the Committee's view, the sole criterion of the minimum figure should be whether or not the product would give satisfactory results to the domestic user. Baking experiments conducted at an Army bakehouse in the preparation of scones suggested that self-raising flour containing below 0·32% of available carbon dioxide would be unsatisfactory, and even when it contained 0·42% the scone was distinctly less satisfactory then when a higher proportion was present.

Further tests were conducted for the Committee by the Ministry's Cereals Research Station. Samples of self-raising flour containing bicarbonate in amounts corresponding to various total carbon dioxide values within the range 0·60% to 0·35% with appropriate quantities of calcium acid phosphate, were used to make steamed puddings, experience having shown that self-raising flour which gave satisfactory steamed puddings could be relied upon to give satisfactory results with other products. The results showed that the lowest total carbon dioxide which gave satisfaction was 0·45%, the residual carbon dioxide being 0·03 to 0·04%. It followed that the lowest figure for available carbon dioxide would be just over 0·4%, thus confirming the view expressed by the trade representatives when the matter was originally considered by the Committee.

In view of these results, the Committee feel unable to recommend that any change should be made in the existing figure of 0·45% for the available carbon dioxide in self-raising flour.

RESIDUAL CARBON DIOXIDE—The present standard does not prescribe a limiting figure for residual carbon dioxide but, since available carbon dioxide is determined by ascertaining the difference between total carbon dioxide and residual carbon dioxide, the residual carbon dioxide is, in fact, limited to 0·20%. With the suggested removal of the standard for total carbon dioxide, this consequential limit would disappear and the Committee have, therefore, considered whether it is desirable to include a limit for residual carbon dioxide so as to prevent the use of excess of sodium bicarbonate to produce the standard amount of available carbon dioxide. The imposition of a limit for residual carbon dioxide would be difficult in present circum-stances. The smaller manufacturers of self-raising flour may use flour fortified with chalk, and since chalk does not react with calcium acid phosphate to any significant extent, the carbon dioxide from the chalk increases the residual carbon dioxide found on analysis. If, however, allowance were made for the residual carbon dioxide contributed by the chalk, a self-raising flour not containing chalk could contain an unduly high excess of sodium bicarbonate while still conforming to the limit for residual carbon dioxide. Moreover, although the standard amount of chalk incorporated in flour is 7 ozs. per sack, difficulties occur in securing uniform admixture and a standard for residual carbon dioxide would have to make allowance for some variation on each side of the theoretical figure. In these circumstances, the Committee have reached the conclusion that it is not at present practicable to include in the standard a figure for residual carbon dioxide. When the present standard was recommended, acid ingredients were in short supply and although excess alkalinity produces discoloration in baked goods, it was felt that at a time when the food supply position was difficult, unsatisfactory articles might find a ready market with the public. The supply position is now easier and it is reasonable to hope that competition will discourage the sale of unsatisfactory products containing an excess of sodium bicarbonate.

METHOD OF DETERMINATION—Discussion with trade chemists indicated that a major source of difficulty was probably the method of mixing the sample with water at the commencement of the determination. If the mixing were carried out in such a way that lumps were formed, the full amount of carbon dioxide was not liberated. It was also the general opinion that vigorous boiling at the end of the determination was an important factor. It was further suggested that in order to obtain trustworthy figures in the determination of residual carbon dioxide, the amount of the sample taken for the assay should not be less than 5 g. A method based on these recommendations was drawn up and tried out on a sample of self-raising flour pre-pared by one of the manufacturers and circulated to all the chemists who had participated in the collabora-tive tests previously mentioned. The results showed a fair measure of agreement, but having regard to the fact that those participating were thoroughly familiar with the assay of self-raising flour, it was felt that there was still a danger that those less familiar with the determination would fail to secure satisfactory results. Some further details were therefore added and the revised method was tested by the same chemists as before on samples fortified with chalk, two being prepared with calcium acid phosphate and two with sodium acid pyrophosphate. The results showed reasonably satisfactory agreement and the Committee feel confident that, with the exercise of the necessary care on the part of the analyst, the method specified below will be found satisfactory in practice with all varieties of self-raising flour at present on the market. It is recognised that other methods may be available which would give equally satisfactory results, but the Committee's object has been to give detailed particulars of one reliable method suitable for inclusion in an Order.

The Committee accordingly recommend that:
(1) the standard for self-raising flour should be amended by:
    (*a*) deleting the figure for total carbon dioxide.
    (*b*) replacing the method for the determination of residual carbon dioxide at present set out in the Standards Order by the following:

        "Not less than 5 g of the self-raising flour shall be mixed to a smooth paste with distilled water and a further quantity of distilled water amounting in all to not less than twenty times the weight of the flour shall then be incorporated. The liquid shall be heated in a boiling water-bath for thirty minutes being vigorously stirred for the first five minutes and thereafter for periods of half a minute at intervals of five minutes. The liquid shall immediately be brought to the boil and shall be maintained boiling for three minutes being vigorously stirred to ensure escape of available carbon dioxide and to avoid charring. The liquid shall be transferred to an apparatus for determining carbon dioxide and that apparatus and liquid shall be freed from available carbon dioxide by passing carbon dioxide-free air through them for ten minutes. The residual carbon dioxide is the weight thereof evolved when the self-raising flour so treated is further treated with excess of sulphuric acid, the evolution being completed either by boiling for five minutes or by means of reduced pressure."

(2) the standard should not include a maximum limit for the proportion of residual carbon dioxide.
(3) on the basis of the information available at present, no change should be made in the existing figure of 0·45% for the minimum content of available carbon dioxide.

PROPOSED REVISION OF STANDARD FOR SELF-RAISING FLOUR*

THE Ministry of Food has under consideration a revision of the existing standard for self-raising flour in the light of a report from the Inter-Departmental Committee on Food Standards.

Any manufacturer or other party desiring to make any comments on the recommendations contained in the Report were asked to send them in writing to the Ministry of Food, Cereal Products Division, Bryn Euryn, Colwyn Bay, Denbighshire, not later than August 31st, 1945.

British Standards Institution

THE Hon. Secretary of the Society has a few copies of the following Draft Specifications, issued for technical comment only, drawn up by the B.S.I. Microchemical Apparatus Sub-Committee C/8/9. He will be pleased to send them to members who apply for them.

    Third Draft for Part 3 of B.S. Specification for Burettes: Burettes with Pressure Filling Device and Automatic Zero.

    Third Draft of B.S. Specification for Wash-out Pipettes.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Experimental Studies on Decomposition of Oysters used for Canning [Determination of Indole]. W. H. King, F. F. Flynn and J. N. Gowanloch (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 385–398)—A study of biological and chemical aspects of decomposition in oysters used for canning, with special reference to the organoleptic judgment of edibility. Tryptophan, which has been detected among the products of hydrolysis of crustacean and mollusc proteins, gives rise on putrefaction to indole and skatole. Neither of these is sufficiently odorous to account for the bad odour of putrid oysters, and it is believed that one or more of the accompanying products of decomposition accounts for most of the organoleptic evidence of decomposition. Preliminary expts. showed that no indole is formed during the processing of good oysters, and that oysters allowed to spoil without being washed free from their shell liquor contain indole. Expts. with washed and unwashed oysters allowed to spoil showed that washing with clean water removes putrefying reagents, whether autolytic or bacterial, leaving those that prompte fermentation and souring rather than putrefactive decomposition. A sample of cultivated oysters was set aside for 6 days. At the end of that time 60% were dead with partly separated valves ("gapers") and in an advanced state of putrefaction. Those with closed valves were dead and decomposed, but the putrid odour was not so pronounced as that of the gapers. Both sets were canned by the usual commercial process, and two days later a can from each set was opened and the indole content was determined. The closed oysters contained 16·1 μg of indole and the gapers 89·8 μg per 100 g. To determine the indole content and organoleptic characters of oyster reef mud and water, samples were steam-distilled and the apparent indole content of the distillate was determined. No sample had any odour of decomposition and the indole content ranged from 0·6 to 1·4μg/100 g. Six samples of oyster reef bottom water and one of mud yielded no hydrogen sulphide; the remaining five samples of mud showed presence of hydrogen sulphide in the distillate. In order to determine the apparent indole content of sound oysters, samples selected carefully from consignments taken directly from the dredging boats were canned by the usual commercial process. The apparent indole content ranged from 1·4 to 2·2 μg/100 g. Expts. with mixtures of good and bad oysters in varying proportions gave 1·9μg/100 g as the indole content of good oysters, 6·6 to 13·2 μg for those with a slight odour of decomposition and 34 μg for a sample consisting

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of 76·6% by wt. of passable oysters, 13·8% of decomposed oysters and 9·6% of putrid oysters. In the samples containing from 6·6 to 13·2 μg of indole/100 g a slight odour of decomposition was apparent to the experienced nose.

The method used for the detm. of indole was a modification of that of Clarke et al. (J. Assoc. Off. Agr. Chem., 1937, 20, 475; ANALYST, 1937, 62, 806). Drain the entire contents of the can for 2 min. on an 8-mesh sieve. Weigh the meat and disintegrate it thoroughly in a Waring Blendor, adding a weighed amount of the drained liquor if necessary and calculating the final result to the drained sample. Steam-distil 100 g of the blended sample, collect 325 ml of distillate in 40 to 50 min. and steam out the condenser. Acidify the distillate with 5 ml of diluted hydrochloric acid (1+19), add 25 ml of chloroform, shake the mixture ca. 150 times during 1 min., and after several min. draw off the chloroform layer and shake it vigorously 10 times with 25 ml of water. Finally pass the chloroform layer through a small plug of cottonwool, avoiding any inclusion of water in the filtrate. Repeat the extraction with 20-ml and 15-ml portions of chloroform successively. To the combined extracts add exactly 10 ml of a reagent prepared by dissolving 0·4 g of p-dimethylamino-benzaldehyde (m.p. 72°–73° C.) in 5 ml of glacial acetic acid and mixing the soln. with 92 ml of phosphoric acid and 3 ml of conc. hydrochloric acid and shaking vigorously ca. 400 times during 2 min. After 10 min. draw off exactly 9 ml of the aq. layer into a glass-stoppered graduated 50-ml cylinder, avoiding inclusion of the chloroform layer. Dilute the liquid to ca. 40 ml with a mixture of glacial acetic acid and peroxide-free ether (1+1), mix, cool and finally adjust to 50 ml with the acetic acid and ether mixture. If the soln. is not brilliantly clear, filter it through a small paper in a covered funnel. Transfer the liquid to a suitable photometer cell and record the scale reading within 15 min., making further dilutions if necessary. Correct the result by means of a blank determination, including the reagent blank and the distillation blank and convert the reading into μg of indole by means of a standard curve. Prepare the standard curve with a soln. of indole in chloroform (1 ml≡1 μg) freshly diluted from a stock soln. (1 ml≡100 μg). To the amount of chloroform required to make a final vol. of 50 ml add enough dil. indole soln. to make a series of standards of 1, 2, 5, 7, 10, 15 and 25 μg. Add the p-dimethylaminobenzaldehyde reagent as previously described and read the colour with the photometer at 560 mμ. Plot a separate curve for each cell after deducting the reagent blank. A series of spectrophotometric curves prepared with good oysters, bad oysters, pure indole and skatole showed that the principal substance measured in bad oysters is indole and that the yellow or brown to light pink colour obtained with good oysters is not due to indole.   A. O. J.

Preservative, Chemical and Bacteriological Effect of Hypochlorite Solution added to Milk [Rupp's Test]. G. H. Hauser and W. H. King (J. Assoc. Off. Agr. Chem., 1945, 28, 417–424)—To obtain direct evidence of the germicidal and preservative effects of the addition of hypochlorite preparations to milk the following expt. was made. From a gallon of milk twenty-four 100-ml portions were placed in sterilised glass jars. A 10,000 p.p.m. chlorine soln. was prepared from a commercial product, and also a ten-fold dilution of this soln. One or other of these solns. was added to 18 of the milk

samples to give three groups of samples containing respectively 10, 50 and 100 p.p.m. of free chlorine. The six control samples were divided equally among the three groups, and each group thus contained two series of 4 samples containing respectively 0, 10, 50 and 100 p.p.m. of free chlorine. One series was carefully pasteurised at 143° F. for 30 min., cooled and subjected to a standard plate count within 1 hr. The companion series of unpasteurised milks was counted in the same manner at the same time. The other two groups were held at 50° F., and at the end of 1 day the second group was examined similarly, and at the end of two days the third group. In the first group the original standard plate count of the raw milk was of the order of 4 million, decreasing with increasing free chlorine content to 132,000. The corresponding count for pasteurised milk in the same group decreased from 800 to 200. In the second group at the end of 1 day the raw milk count of ca. 7 million (free chlorine, nil) decreased to ca. 79,000 (free chlorine, 100 p.p.m.). In pasteurised milk of this group the count decreased from 3000 (free chlorine, nil) to 500 (free chlorine, 100 p.p.m.). In the third group at the end of 2 days the raw milk count of over 13 million decreased to 920,000 (free chlorine, 100 p.p.m.) and the pasteurised milk count from 6,700 to 1,000. In the raw milk of the second group and in the raw and pasteurised milk of the third group samples containing only 10 p.p.m. of free chlorine tended to give higher counts than the samples containing no free chlorine. None of the pasteurised milks had soured after 8 days. After 4 days raw milk samples containing no free chlorine were sour; after 7 days, those containing 10 p.p.m. were sour; and after 8 days those containing 50 p.p.m. and one out of three of those containing 100 p.p.m. were sour. The flavour of free chlorine was not perceptible in samples containing less than 100 p.p.m.

The Rupp test is suitable for detecting free chlorine in milk, there being little necessity for a more sensitive method, since the purpose of the test is to distinguish those traces of chlorine introduced by the proper use of sterilising solns. for cleansing containers and equipment from the larger amounts of chlorine introduced by the illegal use of such solns. as preservatives. To 5 ml of milk in a test tube add 1·5 ml of freshly prepared 7% potassium iodide soln., mix thoroughly and note the colour of the mixture. If the colour is unaltered, add 4 ml of diluted hydrochloric acid (1+2), mix with a flattened rod and note the colour of the curd. Place the tube in a water-bath at 85° C. for 10 min., cool rapidly and note any colour change that may have occurred either in the curd or in the liquid below. Finally add 0·5 ml of 1% starch soln. to the liquid beneath the curd and note the result. If a yellow colour occurs before adding the starch soln., or if a light purple to dark blue colour forms after adding the starch soln., more than 10 p.p.m. of free chlorine is present. If the free chlorine content of the sample amounts to 200 p.p.m. or more, the sample will be coloured yellow after addition of potassium iodide soln. If the milk has been processed in contact with chromel metal or other metal containing copper and a positive Rupp test is obtained, the copper content of the milk should be determined ("Methods of Analysis of the A.O.A.C.," 1940, 414). If the copper content exceeds 2·5 p.p.m., the Rupp test is not a reliable indication of free chlorine. Milk treated with compounds containing chloramine-T also answers to the test. In the experimental series of milk

samples examined only those containing 50 and 100 p.p.m. of free chlorine answered to the test.

A. O. J.

Simplified Method for the Estimation of Iron in Milk. W. R. Ruegamer, L. Michaud and C. A. Elvehjem (*J. Biol. Chem.*, 1945, 158, 573–576)

Most methods of estimating iron in milk involve ashing. This is avoided in the method of Kitzes, Elvehjem and Schuette (*J. Biol. Chem.*, 1944, 15%, 653), in which the proteins are pptd. with heat and trichloroacetic acid, the buffered supernatant liquid is reduced with thioglycolic acid and the colour is developed with αα'-dipyridyl. As originally described, however, this is not applicable to milk, as firmly bound iron is not liberated, and very low values (0·13 to 0·18 mg per ml) are obtained. The recovery of added iron is also poor, averaging only 45%. These disadvantages were overcome by adding hydrochloric acid to the digestion mixture and varying the amount of trichloroacetic acid.

To 5 ml of whole milk in a 15-ml centrifuge tube, add 5 drops of thioglycollic acid, 2 ml of 25% trichloroacetic acid and 1 ml of conc. hydrochloric acid. Prepare a blank with all the reagents. Stir well and heat in a water-bath at 90–95° C. for 5 min. Stir very thoroughly to break up the ppt., and centrifuge at 3500 r.p.m. for 15 min. Decant the supernatant into a 25-ml volumetric flask, wash the ppt. with a mixture of 2 ml of redistilled water, 1 ml of trichloroacetic acid and 1 ml of conc. hydrochloric acid and repeat the heating and centrifuging as above. To the combined supernatant liquids add 1 drop of 0·1% p-nitrophenol soln. and 6 N ammonium hydroxide slowly until the soln. becomes yellow. Make acid with 1 or 2 drops of 6 N hydrochloric acid, add 1 ml of buffer soln. (27·2 ml of glacial acetic acid and 33·4 g of sodium acetate in water, diluted to 250 ml) and dilute with water to 25 ml. Pipette a 5- or 10-ml aliquot containing 1 to 3 μg of iron into an Evelyn colorimeter tube and make up to 10 ml. Reduce any ferric iron present with 2 drops of thioglycollic acid and determine the centre point reading. Add 1 ml of αα'dipyridyl reagent (0·2 g in 5 ml of glacial acetic acid, diluted to 100 ml with water) and evaluate the colour in each tube, using the respective centre point readings previously obtained. Calculate the amount of iron present by reference to a standard curve. The values obtained for raw milk ranged from 0·49 to 0·52 mg per kilo. Recoveries of added iron were 95 to 106% of the theoretical.

F. A. R.

Colour Test for certain Resins in Vanilla Extract. J. Fitelson and T. Riggs (*J. Assoc. Off. Agr. Chem.*, 1945, 28, 427)

Since the last report by Sale (*J. Assoc. Off. Agr. Chem.*, 1926, 9, 446) on the application of the tests now used as tentative qualitative tests for vanilla resins ("*Methods of Analysis of the A.O.A.C.*," 1940, 322) to certain resins, two plant extracts not previously encountered have been used as adulterants of vanilla extract. These plant materials, *viz.*, wild cherry bark and St. John's wort, contain alcohol-soluble resins that resemble vanilla resins superficially. However, adulterated extracts containing these resins behave abnormally in the tests for vanilla resins. Removal of alcohol produces only a slight turbidity, but subsequent addition of acid gives a considerable ppt. The foreign resins when dried are brittle, are easily removed from the vessel and have a distinct red colour, whereas the true vanilla resins are oily, adherent and have a yellow or brown colour. The foreign dried resins are much less soluble in cold alcohol than the vanilla resins, and the alcoholic solns. give transient green colours with ferric chloride soln. The terpene-like odour evolved by these resins during ignition suggested that a modification of the Komarowsky test could be used for their detection in adulterated samples. Mix 5 to 10 mg of the dried resins with 10 ml of a 0·1% soln. of p-dimethylaminobenzaldehyde in alcohol, cautiously add 25 ml of sulphuric acid, mix and heat on the steam-bath for 5 min. Cool and add 150 ml of water. A light brown colour is given by vanilla resins. The dried resins of wild cherry bark and St. John's wort give intense colours, and mixtures of these resins with vanilla resins also give distinct red colours. Authentic vanilla extracts give only a light brown colour.

A. O. J.

Separation of Amylose and Amylopectin by certain Nitroparaffins. R. L. Whistler and G. E. Hilbert (*J. Amer. Chem. Soc.*, 1945, 67, 1161–1165)

In addition to alcohols, as found by Schoch (*Cereal Chem.*, 1941, 18, 121; *J. Amer. Chem. Soc.*, 1942, 64, 2957; Wilson, Schoch and Hudson, *J. Amer. Chem. Soc.*, 1943, 65, 1380; see also Wiegel, *Koll. Zeit.*, 1943, 102, 145) certain other organic compounds, such as esters, ketones, mercaptans, carboxylic acids, nitroparaffins and pyridine, which can form hydrogen bonds with amylose, form complexes with this carbohydrate and can serve as agents for fractionating starch. "Crystalline" amylose-nitroparaffin complexes are formed when starches are fractionated with nitroethane, 1-nitropropane and 2-nitro-propane, average yields from corn (maize) starch being 22–23%, 26–28% and 29–31%, respectively. With 1-nitropropane, potato starch, wheat starch and tapioca yield 26–27%, 31–32% and 20–21% respectively of crude amylose. The yields are therefore approximately the same with these nitro-paraffins as with butanol, but satisfactory fractionation is effected with much less of the nitroparaffin. The properties of the amylose-nitroparaffin solvates are similar to those of the amylose-butanol solvate.

Prepn. of complexes—The procedure is similar to that used by Schoch. Stir 240 g of starch (moisture content 10%) with water to form a thick slurry, which is poured slowly into 6 litres of water 85–90° C.) with rapid stirring. Dilute the uniform paste to 8 litres and heat in an autoclave at 120° C. under steam pressure for 3 hr. Clarify the hot soln. by passing at 50,000 r.p.m. through a Sharples supercentrifuge fitted with a clarifier bowl. Heat the centrifugate at 85° C., add nitroparaffin (200 ml of 1- or 2–nitropropane, or 500 ml of nitroethane) and stir vigorously. If necessary, adjust the pH to 5·5 to 6·0 by adding sodium bicarbonate; variation of pH from 5·5 to 7 does not affect the yield of complex. Fit a rubber gasket over the mouth of the container and round the stirrer shaft to prevent evaporation of the nitroparaffin, and decrease the rate of cooling by insulating the container with cloth. After 24 hr. standing the soln. has cooled to room temp. and pptn. is practically complete; when stirring is stopped the ppt. settles as a flocculent white mass. Centrifuge the soln. in cups in a laboratory centrifuge. For more accurate yield measurements, collect the ppt. in a Sharples' supercentrifuge; stir the pasty ppt. into ethanol (about 1 vol. of ppt. to 5 vols. of ethanol), filter, and repeat twice the ethanol treatment; dry over calcium chloride *in vacuo* to a colourless fine powder. Ordinarily, purify the crude complex from the

centrifuge bowl by dissolving in water and nitro-paraffin to make a 1–2% amylose soln., the water containing more than enough nitroparaffin to saturate it at room temp. Cool slowly to room temp., separate the ppt., wash and dry as above. The crude ppt. can also be purified by suspending in water saturated with nitroparaffin, stirring vigorously for several min., and removing the ppt. by centrifuging; the yields are the same within 1 to 3%. The quantity of nitroparaffin used to ppt. the amylose was about 1/3 more than saturates 8 litres of water, but addition of just sufficient 2-nitropropane to saturate the soln. caused no decrease in yield of ppt.; half the amount of solvent caused a noticeable decrease.

Prepare amylose fractions from aqueous extracts of swollen starch granules by gently stirring a 3% corn starch paste at 85° C. for 16 hr. Separate the swollen granules by centrifuging the paste in cups. The water extract contains 12% of the starch. Heat this soln. to 85° C. and ppt. by 1- or 2-nitro-propane or nitroethane. Fine needle-like crystals form on slow cooling. Collect the product by centrifuging.

Potentiometric iodine titration—Use the method of Bates, French and Rundle (*J. Amer. Chem. Soc.*, 1943, **65**, 142), as modified by Schoch, Wilson and Hudson (*J. Amer. Chem. Soc.*, 1943, **65**, 1380), but dissolve the starches in N potassium hydroxide at 0° C., when soln. is usually complete after 1 or 2 hr., whereas at room temp. many hr. or several days are required. Quantitative data are shown in Table I.

TABLE I

AMOUNT OF IODINE BOUND BY REPPTD. AMYLOSE PREPNS.

Source of amylose	Pptng. agent	Iodine bound in complex, mg/g amylose ($\pm 1.5\%$)
Corn (maize) starch	1-nitropropane	149
,,	2-nitropropane	142
,,	nitroethane	161
,,	butanol	178
Wheat starch	1-nitropropane	167
Potato starch	,,	182
Tapioca starch	,,	173
Corn starch (a)	,,	199
,, (a)	2-nitropropane	195
,, (a)	nitroethane	182

(a) From aqueous extract of swollen granules.

X-ray diffraction patterns—Obtain by exposing for 1 hr. a 1-mm thick portion of fresh moist complex in a thin-walled glass cell to unfiltered copper radiations at 37 K.V.P., 15 ma and a specimen-to-film distance of 5 cm. Identical patterns are obtained by the use of filtered radiation. On drying to a transparent horny film the specimens show a "B" X-ray diagram. X-ray diffraction patterns of the nitroethane- and 1- and 2-nitropropane complexes appear to be identical; the spacings and relative order of intensity of the rings are shown in a table.

Extent of retrogradation—Dissolve 5 g of amylose in 100 ml of 3% sodium hydroxide by allowing the mixture to stand at 0° C. under nitrogen for 18 hr. Dilute the soln. to 400 ml, neutralise with sulphuric acid and bring to 500 ml. Keep the soln. at 3° C. for 24 days, centrifuge aliquots in cups at 2800 times gravity, and analyse the supernatant liquids for starch content by a modification of the di-chromic acid oxidation method (Launer, *Bur. Standards J. Res.*, 1937, **18**, 333). Obtain by

difference the amount of amylose degraded. Amyl-ose prepd. from whole corn starch with nitroethane, 1-nitropropane or 2-nitropropane retrogrades rapidly from 1% aqueous solns. at 3° C. and to the extent of 84, 93 or 71%, respectively, in 24 days.

Separation of amylopectin—After removing the ppt. formed by treating dispersions of corn or potato starch with 1- or 2-nitropropane, concentrate the resulting amylopectin soln. under reduced pressure to a thick paste and ppt. by pouring slowly into ethanol (1 part of paste to 5 parts of ethanol). Filter off the ppt., stir vigorously three times with fresh portions of ethanol and dry in a vacuum desiccator over calcium chloride. The fraction retrogrades extremely slowly from water at 3° C. Films of the triacetate are self-supporting, but so brittle that the tensile strength could not be determined. Corn amylopectin is coloured violet by iodine and absorbs 11·6 mg of iodine/g, whilst potato amylopectin is coloured magenta and absorbs 0·1 mg of iodine/g.

β-Amylase hydrolysis—Various modifications of procedures described in the literature were in-vestigated but none was found to give trustworthy values.   E. M. P.

Biochemical

Estimation of Iodine in Rat Thyroids. **V. L. Koenig and R. G. Gustavson** (*Arch. Biochem.*, 1945, **7**, 41–45)—The method is sub-stantially that of Hunter (*J. Biol. Chem.*, 1910, **7**, 321), modified by distillation of the iodine as hydrogen iodide from the acidified fusion mixture before oxidising the iodide to iodate, followed by colorimetric estimation of the iodine by the starch-iodine reaction. Store the thyroids until required in individual tubes containing small amounts of abs. alcohol. Put the thyroid and alcohol into a 60–70 ml nickel crucible, add 1 ml of saturated potassium hydroxide soln. and heat the crucible in an oven at 105° C. overnight. Transfer the crucible containing the dried material to a cold electric furnace and raise the temperature to 400° C. over a period of about 30 min. Cool and transfer the fused material to a 50-ml distilling flask with not more than 25 ml of re-distilled water. Put a glass bead into the flask and connect the apparatus with a condenser, the tip of which dips under the surface of water in the receiver. Add 4 drops of 3% sulphuric acid to the receiver and maintain an atmosphere of bromine vapour in it throughout the distillation. Add 5 ml of 50% sulphuric acid to the distilling flask through a separating funnel and distil rapidly over a free flame until fumes of sulphur trioxide appear at the top of the condenser. Remove the distilling flask and rinse the condenser into the receiver. Transfer the contents of the latter to a 125-ml conical flask containing a bead and evaporate on a hot plate to *ca.* 5 ml. Concen-trate further to 2 or 3 ml over a free flame, and transfer to a Klett colorimeter tube, rinsing out the flask two or three times and adding the washings to the contents of the tube. Cool in running water (10–15° C.), add 6 drops of starch-iodide soln. (put 0·5 g of soluble starch and 0·5 g of potassium iodide in 10 ml of re-distilled water and slowly pour into 40 ml of boiling re-distilled water; prepare the soln. fresh every other day). Dilute to the 5-ml mark and evaluate the colour in a Klett colorimeter. Calculate the result from a standard curve. The values obtained are accurate within 10% with amounts of the order of 3 μg and 5% with more than 2 μg.   F. A. R.

Estimation of Basic Organic Compounds. Application to the Cinchona Alkaloids. B. B. Brodie and S. Udenfriend (*J. Biol. Chem.*, 1945, **158**, 705–714)—Many organic bases combine with certain sulphonic acids to form molecular complexes which are very soluble in organic solvents. In some instances the concn. of base in the organic solvents may be estimated indirectly by measuring the concn. of the sulphonic acid with which it is combined. The specificity of the method can be tested by comparing the solubility of the pure compound with those of substances that accompany it in the biological material and measured in the analytical procedure. The general method is illustrated by reference to cinchonidine, using as the sulphonic acid methyl orange. This is a very suitable sulphonic acid, as it is very soluble in water, has a high colour index and forms with cinchonidine a complex which is very soluble in ethylene dichloride or chloroform. It only enters the organic phase in amounts equivalent to that of the base. The cinchonidine is extracted from the sample with ethylene dichloride at an alkaline *p*H, the extract is washed with alkali to remove degradation products of cinchonidine, and an aliquot of the washed ethylene dichloride soln. is then shaken with a saturated methyl orange soln. at *p*H 5. The excess of methyl orange is removed, the ethylene dichloride is acidified, and the conc. of the highly coloured acid salt of methyl orange is measured photometrically. The procedure is specific in that substances normally occurring in tissues and body fluids and degradation products of cinchonidine do not react. Recoveries of as little as 5 μg of added cinchonidine averaged 95% of the theoretical.

Estimation in plasma—Add 1 to 5 ml of plasma and 1 ml of N sodium hydroxide to 20 ml of ethylene dichloride in a 60-ml glass-stoppered bottle and shake for 5 min. Decant the contents of the bottle into a 40-ml tube and centrifuge for 10 min. at 2500 r.p.m. Discard the supernatant layer and return the ethylene dichloride soln. to the bottle. Shake with an equal vol. of 0·1 N potassium hydroxide in 20% ethyl alcohol for 10 min. and centrifuge for 1 min. Discard the supernatant liquid and transfer the ethylene dichloride soln. to a 60-ml glass-stoppered bottle. Add 0·5 ml of a saturated soln. of methyl orange in 0·5 M boric acid (add an excess of the sodium salt of methyl orange to the boric acid soln., heat gently, cool to room temp. and filter; wash the soln. several times by shaking with an equal vol. of ethylene dichloride) and shake for 5 min. Decant into a 25-ml test-tube and centrifuge for 5 min. at 3000 r.p.m. Discard the supernatant layer and re-centrifuge the ethylene dichloride phase for 5 min. Pipette 10 ml into a colorimeter tube containing 1 ml of alcoholic sulphuric acid soln. (2 ml of conc. sulphuric acid in 100 ml of abs. alcohol) and evaluate the colour with a filter transmitting maximally at 540 $m\mu$. To set the instrument at 100% transmission, use a reagent blank in which water is substituted for the plasma. This should not give a transmission of less than 97 when ethylene dichloride and the alcoholic sulphuric acid are used to set the instrument at 100.

Procedure for urine—Add 1 ml of diluted urine, containing 10 to 40 μg of cinchonidine, and 1 ml of N sodium hydroxide to 20 ml of ethylene dichloride in a 60-ml glass-stoppered bottle and shake for 5 min. Discard the supernatant layer, add an equal vol. of 10% sodium hydroxide soln. and again shake for 5 min. After it has separated again discard the aqueous layer, add a few ml of water, shake and transfer to a 40-ml tube and centrifuge for 2 min.

Discard the supernatant layer, add 0·5 ml of methyl orange reagent to the ethylene dichloride soln. and proceed as described above.

Method for faeces—Add 20 ml of conc. hydrochloric acid to the sample and dilute to a known vol. with water. Shake until a homogeneous mixture is obtained and analyse a suitable aliquot as described for plasma. Washing with alcoholic potassium hydroxide is not necessary. Prepare a standard soln. containing 100 mg of cinchonidine per litre of 0·1 N sulphuric acid and to 1 ml add 1 ml of N sodium hydroxide and 20 ml of ethylene dichloride. Proceed as described for the estimation of cinchonidine in plasma, but omit the alcoholic potassium hydroxide wash, and construct a calibration curve; as the relation between concn. and colour is linear, standards need not be run with each set of estimations, but the results can be calculated from this curve.

The above procedure is applicable to a large number of basic drugs, *e.g.*, codeine, pamaquine, amphetamine, ephedrine and demerol, but the modifications for each of these substances have not yet been worked out. A reaction similar in principle has been used for the estimation of certain organic acids which form with basic dyes, such as rosaniline, complexes that are soluble in ethylene dichloride.   F. A. R.

Estimation of Uric Acid in Human Blood. H. Brown (*J. Biol. Chem.*, 1945, **158**, 601–608)—Uric acid estimations in blood by direct colorimetric procedures have hitherto suffered from three disadvantages: (*a*) colour intensity is not proportional to uric acid concn.; (*b*) recoveries of uric acid added to tungstic acid filtrates are low; (*c*) the reaction is not sufficiently specific. A method has now been developed which gives recoveries of 95% and is more specific than the older methods. This has been achieved by altering the composition of the phosphotungstic acid reagent and the concn. of the cyanide soln. The new phosphotungstic acid reagent gives maximum colour with uric acid without a marked increase in blank values; it contains just enough sulphuric acid to change all the tungstate and phosphate into free acids without giving a ppt. of inert white solid when the reaction mixture is cooled. The cyanide concn. has been increased to 12%, as it was found that the amount of colour produced when the phosphotungstic acid reacts with uric acid increases with increasing cyanide concns. The concn. cannot profitably be increased beyond 12%, however, owing to the destructive effect of the increased alkali on the coloured complex.

Method—To 2 ml of a 1 : 10 tungstic acid filtrate in a test-tube graduated at 10 ml add 2 ml of 12% sodium cyanide soln. and 2 ml of 50% urea soln. Add 1 ml of phosphotungstic acid reagent (dissolve with the aid of heat 100 g of sodium tungstate and 20 g of Na_2HPO_4 in *ca.* 150 ml of water, mix 25 ml of conc. sulphuric acid with 75 ml of water and pour the warm soln. slowly and with continual shaking into the flask; boil gently under reflux for 1 hr., cool and dilute to 1 litre with water), leave for 50 min. at room temp., dilute to the mark and compare the colour in a colorimeter with that of 2 ml of a standard uric acid soln. (2·5 mg per 100 ml) treated in the same way. Alternatively, the colour can be evaluated in a photoelectric colorimeter, using filter 54 and a blank prepared from 2 ml of water treated in the same way as the unknown or standard.   F. A. R.

Field Method for the Direct Estimation of Mepacrine in Plasma and Blood. E. J. King and M. Gilchrist (*Lancet*, 1945, i, 814)—A modification of the procedure of Masen (*J. Biol. Chem.*, 1943, **148**, 529) is described in which a portable visual fluorimeter is used. *Method*—Place 10 ml of 0·3 N sodium hydroxide, 25 ml of light petroleum and 25 ml of a mixture of equal parts of redistilled *iso*propyl and redistilled *iso*butyl alcohol in a separator, add 5 ml of plasma (potassium oxalate or citrate, or heparin) or 5 ml of whole blood, shake vigorously for 1 min. and allow to separate. Discard the blood layer, wash the mixed solvents with 10 ml of 0·3 N sodium hydroxide by shaking vigorously for 1 min. and reject the alkali layer. Repeat this washing twice and separate as completely as possible. Extract the mepacrine into 5 ml of a 30% soln. of *iso*propyl alcohol in 0·1 N hydrochloric acid by shaking vigorously for 1 min., draw off the separated acid layer and centrifuge until clear. To 4 ml of this clear soln. add 1 ml of a buffer consisting of a filtered soln. of 4 g of sodium borate in 100 ml of 1·35 N sodium hydroxide and compare the fluorescence with that of standards.

Standards—Prepare freshly every month a soln. containing 100 mg of mepacrine per litre of 0·1 N hydrochloric acid and store in the dark. Make the fluorescent standards by diluting 0·1, 0·2, 0·4, 0·8, and 1·2 ml of a freshly-prepared dilute standard containing 1 mg per litre of 0·1 N hydrochloric acid to 5 ml with the acid—*iso*propyl alcohol mixture and treating 4 ml of these solns. with 1 ml of the buffer as above; these are equivalent to 20, 40, 80, 160 and 240 μg of mepacrine per litre respectively. For very high mepacrine concns. 2 ml of whole blood may be used, while for very low plasma concns. 10 ml should be taken. J. A.

Interference of Cystine with the Quantitative Nitroprusside Test for Methionine. W. White and F. C. Koch (*J. Biol. Chem.*, 1945, **158**, 535–536)—When the colorimetric method of McCarthy and Sullivan (*J. Biol. Chem.*, 1941, **141**, 871) for the estimation of methionine was applied to hydrolysates of bovine serum albumin it was found that the red colour developed on acidification faded considerably during the 5–10 min. standing recommended by the authors. This was found to be due to the presence of cystine and, when the proportion of cystine to methionine is high, the colour fades markedly, so that readings made after 10 min. standing are useless. If the readings are made immediately, satisfactory results are obtained. F. A. R.

Colorimetric Estimation of Creatinine by the Jaffe Reaction. R. W. Bonsnes and H. H. Taussky (*J. Biol. Chem.*, 1945, **158**, 581–591)—Although the Jaffe reaction has been used for estimating creatinine for many years, there are no published data to indicate how the particular conditions were arrived at; these have now been investigated in solns. containing 1 to 50 μg per 5 ml. It was found that the colour produced by addition of picric acid was independent of the concn. of picric acid above a certain limit, and that the colour was greatest with low concns. of alkali and decreased progressively with increasing concns. The rate at which the colour developed was inversely proportional to the concn. of both the picric acid and the sodium hydroxide. The colour formed was not directly proportional to the concn. of creatinine except at concns. below 10 μg per 5 ml. By diluting more concentrated solns. to this value, the linearity is improved.

The following method was devised for the estimation of creatinine in blood filtrates and in diluted urine. To 3 ml of unknown soln., containing up to 50 μg of creatinine, add 1 ml of 0·04 M picric acid followed by 1 ml of 0·75 N sodium hydroxide, leave for 15 min. and evaluate the colour. Calculate the creatinine content of the soln. from a standard curve obtained by averaging the results in six different expts. with 1 to 10 and 20, 30, 40 and 50 μg of creatinine. In a series of 30 urines, the average deviation from the value obtained by the Folin method was −0·4%, with a maximum range of ±10%. Blood filtrates were found to contain 1·4–1·7 mg % by the Folin method and 1·2–1·5 mg % by the above procedure.

Creatine is converted to the extent of 80% into creatinine by heating with picric acid in a boiling water-bath for 45 min.; this avoids the use of an autoclave as required by Folin's method. Heat 3 ml of the creatine soln. with 1 ml of 0·04 M picric acid for 45 min. in an unstoppered tube graduated at 5 ml, in a vigorously boiling water-bath. Cool, add 1 ml of 0·75 N sodium hydroxide, dilute to 5 ml and evaluate the colour as before. To calculate the amount of creatine as creatinine, multiply the result by 1·25. F. A. R.

Use of diazotised p-Aminoacetophenone in the Estimation of Pyridoxine. E. B. Brown, A. F. Bina and J. M. Thomas (*J. Biol. Chem.*, 1945, **158**, 455–461)—To 10 ml of an alcoholic soln. of pH 7·0 to 7·3, containing 5 to 25 μg of pyridoxine, add 2·0 ml of water, 2·0 ml of 50% hydrated sodium acetate soln. and 1 ml of diazo reagent. (Dissolve 3·18 g of p-aminoacetophenone in 45 ml of conc. hydrochloric acid and dilute to 500 ml. Put 2·0 ml of this soln. into a 25-ml brown glass-stoppered graduated cylinder, immerse in an ice-bath and add 2·0 ml of 4·5% sodium nitrite soln. Leave for 10 min. with occasional shaking and add a further 8·0 ml of nitrite soln. Leave for a further 20 min. and use within 1 hr. of preparation.) It is advisable, after addition of the acetate soln., to let the cylinder stand for a few sec. to allow air bubbles to escape from the soln. and then to run the diazo reagent slowly down the inside of the cylinder to avoid formation of air bubbles. The pipette used for adding the diazo reagent should be carefully rinsed with water after use. Evaluate the colour of the soln. at intervals in a fluorophotometer with a combination blue and yellow filter transmitting maximally at 420 mμ; maximum colour develops 3 to 5 min. after adding the diazo reagent. To obtain the zero setting use a blank consisting of all the reagents. The results agreed closely with those obtained with sulphanilic acid. The method has the advantage over the original method in which sulphanilic acid is used, that removal of interfering substances can readily be accomplished by means of Amberlite IR-4 instead of the complicated sodium tungstate pptn.

To a sample containing 100 to 200 μg of pyridoxine add 50 ml of 0·1 N sulphuric acid and autoclave at 15 lb. for 30 min. Cool and incubate at 40° C. for 2 hr. in an acetate buffer soln. of pH 4·5 (54·4 ml of glacial acetic acid diluted to 100 ml with water) containing 0·2 g each of takadiastase and papain. Dilute to 100 ml, filter, add 0·5 g of Amberlite IR-4 to the filtrate and pipette 40 ml of the soln. into a glass-stoppered centrifuge tube containing 0·6 g of Super Filtrol. Leave for 30 min. with occasional shaking, centrifuge and decant and discard the supernatant liquid. Wash the Super Filtrol with 40 ml of water,

centrifuge and discard the washings. Add 20 ml of alkaline ethyl alcohol (dilute 2 ml of 25% sodium hydroxide soln. to 100 ml with 95% ethyl alcohol), heat at 60–65° C. for 30 min. with occasional shaking, cool ,and centrifuge. Decant the pyridoxine soln. into a 50-ml beaker, wash the residue with 10 ml of alkaline alcohol, centrifuge and add the washings to the beaker. Adjust the combined extract to pH 7·3 with 8% v/v acetic acid and dilute to 40 ml with alcohol. Use 10-ml portions for colour development as described above. Results ranged from 95·8 to 98·7% of the theoretical.

F. A. R.

Estimation of Aromatic Amidines. A. T. Fuller (*Biochem. J.*, 1945, **39**, 99–102)—When aromatic amidines are heated with glyoxal at pH 9 with sodium borate as catalyst, a brilliant stable magenta colour is produced in dilutions down to 1 : 100,000 of amidine. A method of estimating amidines based on this reaction offers certain advantages over a boric acid method of Devine (*Ann. trop. Med. Parasit.*, 1944, **38**, 35). Heat 10 ml of a soln. containing 0·1 to 0·4 mg of amidine in a boiling water-bath for 10 min. with 1 ml of glyoxal reagent (0·1% aqueous soln. of glyoxal sodium bisulphite) and 1 ml of borate buffer soln. (4 g of boric acid neutralised to pH 9, made up to 100 ml and heated to dissolve). Cool, acidify with 0·2 ml of 2 N hydrochloric acid, and evaluate the colour either in an absorptiometer, using an Ilford spectrum green filter, or in a visual colorimeter against standard solns. of the amidine. The addition of acid, although it somewhat reduces the intensity of the colour, is advisable, as many biological fluids are cloudy at alkaline reactions. The colour is maximal with 1 to 2 moles of glyoxal per mole of amidine, and excess of glyoxal inhibits colour production. The reaction is specific for unsubstituted amidines directly joined to an aromatic nucleus. Guanidines, bi-guanides, aliphatic amidines and N-substituted aromatic amidines do not react. Amidines in blood in concns. exceeding 1 mg per 100 ml could not be estimated, as they were pptd. when tungstic acid, trichloroacetic acid, toluene-sulphonic acid, metaphosphoric acid or zinc hydroxide were used as protein precipitants. Precipitation with methanol allowed some amidine to pass into the filtrate, ethanol gave better results, whilst *iso*propanol gave satisfactory recoveries.

Method—Mix 1 ml of blood with 4 ml of *iso*propanol, filter and boil 2 ml of the filtrate with 0·2 ml of borate buffer and 0·2 ml of glyoxal reagent for 10 min. Cool, acidify with a drop of 2 N hydrochloric acid, make up to 2·5 ml with *iso*propanol and evaluate the colour as described above. *Iso*propanol cannot be used with concns. of amidine less than 1 mg per 100 ml if the pigment formed from the amidine is fluorescent, because *iso*propanol extracts of blood give a fluorescent blank. Dialysed iron should be used in such instances, the correct strength necessary to ppt. the blood proteins without leaving excess being determined by preliminary tests. Mix 1 ml of blood, 3 ml of water and 1 ml of dialysed iron and heat in a boiling water-bath with stirring until the mixture coagulates to a brownish mass (*ca.* 1 min.). Filter and treat 2 ml of the filtrate as described above. To estimate amidine in urine, neutralise to thymol blue or phenolphthalein and, if the content of amidine is unknown, develop the colour on the undiluted sample and on the sample diluted 10- and 100-fold.

F. A. R.

Replacement of Pyridoxine for some Micro-Organisms by *d*(-)Alanine and an Unidentified Factor from Casein. E. E. Snell (*J. Biol. Chem.*, 1945, **158**, 497–503)—It has been suggested that alanine is a precursor of pyridoxine for *Streptococcus faecalis* R (*S. lactis* R) and that organisms thus able to utilise alanine synthesise the remainder of the pyridoxine molecule and couple it with alanine. *Lactobacillus casei*, however, is unable to dispense with pyridoxine even in presence of excess of alanine, although in absence of pyridoxine growth is better with than without *dl*-alanine. This observation can be explained by assuming that *L. casei* is unable to synthesise the remainder of the pyridoxine molecule. It has now been shown that an enzymic digest of vitamin-free casein when added to the basal medium in amounts too small to produce a growth effect alone, together with *dl*-alanine, stimulates the growth of *L. casei* to the same extent as does pyridoxine. In presence of the casein hydrolysate, therefore, *dl*-alanine can replace pyridoxine for *L. casei*, just as it normally replaces pyridoxine for *S. faecalis* R in absence of casein hydrolysate. Under these conditions, *dl*-alanine is more effective than *l*(+)alanine for both organisms, whereas in presence of pyridoxine *l*(+)alanine is more effective than *d*(—)alanine. It therefore appears that *d*(—)alanine, together with one or more substances present in an enzymic digest of casein, is required for the synthesis of pyridoxine, whilst *l*(+)alanine is required for protein synthesis.

F. A. R.

Microbiological Assay of Riboflavin. S. A. Price (*Nature*, 1945, **156**, 171–172)—The need for a more critical examination of microbiological assays (see Wood, *Nature*, 1945, **155**, 632; ANALYST, 1945, **70**, 312) is stressed and it is stated that, although stimulation of *Lactobacillus helveticus*, occurring with certain types of sample, may be diminished by ether extraction of the sample (Bauernfeind, Sotier and Boruff, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 666) or pptn. of the acid extract at pH 4·5 (Strong and Carpenter, *Id.*, 1942, **14**, 909), it is frequently not eliminated. The fact that $(a-A)$ (see Wood, *loc. cit.*) is usually positive is considered cause for concern, and it is suggested that a medium giving enhanced responses with sub-optimum doses of riboflavin might yield more valid assays. The superiority in this respect of the modified medium of Greene and Black (*J. Amer. Pharm. Assoc.*, 1943, **32**, 217) has been confirmed and higher responses have been obtained by increasing the concn. of the yeast factors. In these enriched media the curve relating dose and response conforms closely to the normal semi-logarithmic form and a linear relationship between response and log dose has been repeatedly observed over the dosage range 0·04 to 0·125 μg. Many of the discrepancies between figures published by different workers for the riboflavin content of the same or similar cereal samples are considered incompatible with the precision usually claimed for microbiological assays, viz., ±10%. It is considered that pre-treatment of extracts by a method such as that of Strong and Carpenter (*loc. cit.*) is essential when the present media are used, and it is suggested that since extracts giving a positive value for $(a-A)$, i.e., giving invalid assays, yield considerably lower results on the enriched media referred to above, the lower result rather than the higher is likely to be a closer estimate of the true riboflavin content. It is considered that the real solution of the difficulties lies in improving the medium, since

attempts to remove stimulatory factors from the sample extracts are in themselves an admission of its incompleteness.   J. A.

Microbiological Assay Methods for Nicotinic Acid. H. P. Sarett, R. L. Pederson and V. H. Cheldelin (*Arch. Biochem.*, 1945, **7**, 77–85)— Most improvements in microbiological assay methods have been made by adding pure growth factors; in the present method natural extracts treated to remove the vitamin being assayed are used. This is claimed to increase the response of the organism to the added vitamin and to overcome the extra growth stimulation by the materials under test. The basal medium is as follows:

Glucose	40 g
Sodium acetate, anhydrous ..	36 ,,
Hydrolysed casein, vitamin-free ..	10 ,,
Lloyd's reagent-treated { Peptone	5 ,,
Liver extract	2 ,,
Yeast extract	2 ,,
Norit-treated yeast extract ..	2 ,,
Cystine	400 mg
Calcium pantothenate	200 μg
Inorganic salts A, B* ..	10 ml each

Distilled water to 1000 ml, *p*H 6·6–6·8

The constituents are prepared as follows:
Hydrolysed casein—Autoclave 100 g of "vitamin-free" casein for 16 hr. at 15 lb. pressure with 500 ml of 25% sulphuric acid. Adjust the *p*H to 3·0 with·baryta, dilute to *ca.* 2 litres, filter, shake for 20 min. with 10 g of Norit and again filter. Re-adjust the *p*H to 3·0 and repeat the charcoal adsorption and filtration. Centrifuge under reduced pressure to 100 ml and adjust the *p*H to 6·6. Sterilise by steaming and store under toluene.
Lloyd's reagent-treated peptone, liver and yeast extract—Autoclave 25 g of bacto-peptone, 10 g of liver extract (Lederle or Wilson) and 10 g of yeast extract (Difco) for 15 min. with 400 ml of *N* sodium hydroxide. Cool, acidify to *p*H 0·8–1·0 with conc. hydrochloric acid, dilute to 500 ml and add 45 g of Lloyd's reagent. Shake for 20 min., centrifuge, decant the supernatant liquid, re-adjust the *p*H to 0·8–1·0 and repeat the adsorption twice. Add 18 g of K_2HPO_4 and adjust the *p*H to 6·6. Sterilise by steaming and store under toluene.
Norit-treated yeast extract—Steam 10 g of yeast extract (Difco) for 10 min. in 200 ml of water, adjust the *p*H to 1·5 with conc. hydrochloric acid and add 10 g of Norit. Shake for 20 min., filter, re-adjust to *p*H 1·5 and repeat the adsorption. Neutralise to *p*H 6·6 and sterilise by steaming.
Method—Hydrolyse the samples to be assayed with *N* sulphuric acid according to the method of Krehl *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1945, **15**, 471), but filter the extracts after neutralisation before using them for assay. Dilute the samples (*p*H 6·6 to 6·8) to contain *ca.* 0·06 μg of nicotinic acid per ml and use 4 tubes for each sample, the vols. ranging from 1 to 4 ml. With each batch of samples include a series of standards containing 0·05, 0·1, 0·15, 0·2, 0·3, 0·4 and 0·5 μg of nicotinic acid. Dilute the contents of each tube with water, add 5 ml of basal medium, plug the tubes and autoclave for 10 to 15 min. at 15 lb. Prepare a dilute inoculum from a stock culture of *Lactobacillus arabinosus* 17–5 by direct transfer to a tube containing 5 ml of basal medium diluted to 10 ml containing 1 μg of nicotinic acid, 5 mg of liver extract (Lederle or Wilson) and 5 mg of yeast extract (Difco). After incubation at 37° C. for 16 to 24 hr.

* *Cf.* ANALYST, 1945, **70**, 139, footnote.

dilute 1 ml of the culture with 15 ml of 0·9% saline. Inoculate each of the tubes with one drop of this diluted inoculum, incubate in a water-bath at 37° C. for 40–72 hr. and titrate the acid produced. Recoveries of nicotinic acid added to various materials were 102 ± 10% of the theoretical.
F. A. R.

Colorimetric Estimation of Diethylstilboestrol, Hexoestrol and Dienoestrol. F. H. Malpress (*Biochem. J.*, 1945, **39**, 95–98)—Put an acetic acid soln. of diethylstilboestrol containing 0·5 to 2 mg into a 50-ml flask and dilute to 6 ml with glacial acetic acid. Add 0·1 ml of conc. sulphuric acid, followed by 0·04 ml of conc. nitric acid, and heat on the steam-bath for 1¼ min., with gentle shaking every 15 sec. Add 10 ml of water, make alkaline with 15 ml of conc. ammonia (sp.gr. 0·88), allow to cool and dilute to the mark with water. Compare the orange colour with that given by 1 ml of a 0·1% *w/v* soln. of diethylstilboestrol in glacial acetic acid treated in the same way, using a spectrum green colour filter (Ilford 604, 520 *m*μ). To estimate hexoestrol, use the same procedure up to the addition of conc. nitric acid and then without heating make alkaline by addition of 25 ml of 25% sodium hydroxide soln. Dilute to 50 ml as before and compare the colour with that given by 1 ml of a 0·1% soln. of hexoestrol in glacial acetic acid. To estimate dienoestrol, follow the same procedure up to the addition of conc. nitric acid, but instead of heating the soln., leave it for 1 hr. at room temp., then dilute with 10 ml of water, make alkaline with 15 ml of conc. ammonia and dilute to 50 ml. Compare the colour with that given by 1 ml of a 0·1% dienoestrol soln. in glacial acetic acid. The procedure used in preparing solns. for assay depends on the nature of the preparation under investigation. In some instances ether extraction of an aqueous suspension, evaporation of the ethereal extract to dryness and re-solution in glacial acetic acid gave satisfactory results. For non-oily preparations having a high solids content, drying in a steam-oven, followed by direct extraction of the residue with hot acetic acid, was used. Dienoestrol should be extracted at room temp., as it is destroyed when acetic acid solns. are heated.
The method is non-specific, since a variety of substances which might be present in biological materials give similar colours. Satisfactory results were obtained in the estimation of oestrogens in tablets, the recoveries ranging from 99·7 to 101·4, 95·4 to 98·8 and 97·7 to 102·1% of the theoretical with different concns. of diethylstilboestrol, hexoestrol and dienoestrol, respectively.   F. A. R.

Bacteriological

Aniline Oil-Methylene Blue Stain for the Direct Microscopical Count of Bacteria in Dried Milk and Dried Eggs. W. R. North (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 424–426)—An aniline oil and methylene blue stain has been recommended for use in the microscopical examination of dried and liquid egg (North, *J. Assoc. Off. Agr. Chem.*, 1945, **28**, 61), and although little work has been done with these products to afford a direct comparison of this stain with the plain methylene blue stain, examinations of authentic samples by different workers have shown exceptionally close agreement. Such agreement is apparently not possible with the plain methylene blue stain (Schneiter, *J. Assoc. Off. Agr. Chem.*, 1942, **25**, 740). A limited series of comparative examinations of the

two stains has been made with dried milk and the results warrant attention. Thirty-four such comparative counts were made with dried milk smears prepared as follows: A smear was stained with the standard Breed stain (0·3 g of certified methylene blue powder to 30 ml of 95% alcohol), and a duplicate smear was prepared with the aniline oil stain (aniline oil 3 ml, 95% alcohol 10 ml, conc. hydrochloric acid added slowly 1·5 ml, sat. alc. methylene blue soln. 30 ml and water to 100 ml) and the film prepared with the standard stain was counted, decolorised and re-stained with the aniline oil stain. In all expts. higher counts were obtained on duplicate smears with the aniline oil stain than with the standard stain. In 30 out of the 34 samples the counts were at least twice as high, in 12 at least five times as high and in 3 at least ten times as high as those obtained with the standard stain. Similar results were obtained when the plain methylene blue stains were decolorised and re-stained with the aniline oil preparation. Comparative examination of a limited number of samples of fluid raw milk has not revealed significant differences between the counts obtained with the two stains. The aniline oil and methylene blue stain has the advantage of producing a very pale blue background against which the organisms are deeply stained and there is little probability of over-staining even in 24 hr., so that the necessity for reducing the stain with alcohol is avoided. Also, the smears require no special handling, but may be transferred from xylol to alcohol and then to the stain without any intermediate treatment. The special fixation necessary with smears of milk for staining with plain methylene blue is unnecessary with the aniline oil and methylene blue stain.

A. O. J.

Agricultural

Application of a Modified Red Colour Test for Rotenone and Related Compounds to *Derris* and *Lonchocarpus*. M. A. Jones (*J. Assoc. Off. Agr. Chem.*, 1945, 28, 352–359)—It has been shown by Jones et al. (*J. Econ. Entomol.*, 1935, 28, 285) that, of several criteria examined, the red colour test of Gross and Smith (*J. Assoc. Off. Agr. Chem.*, 1934, 17, 336; ANALYST, 1934, 59, 567) for rotenone and related compounds (rotenoids) is most closely correlated with toxicity to house flies, some of the non-rotenone constituents of the total extractives contributing to the insecticidal value. The test is not specific for rotenone and should be applied with caution to plants not of the genera *Derris*, *Tephrosia* and *Lonchocarpus*. The method here described is a modification of that of Goodhue (*J. Assoc. Off. Agr. Chem.*, 1936, 19, 118; ANALYST, 1936, 61, 355). To 2 ml of an acetone extract containing the rotenone and related compounds in a 50-ml conical flask add 2 ml of a reagent made by mixing 40% aq. potassium hydroxide soln. with 7 times its vol. of a 0·1% soln. of sodium nitrite in 95% alcohol purified by refluxing for 1 hr. with 5 g of zinc and 8 g of potassium hydroxide and distilling. Place the flask in a thermostat for 5 min., quickly invert a test tube containing 5 ml of diluted sulphuric acid (1+4) into the flask and swirl the liquid for a few sec., keeping the flask partly immersed in the bath. After 5 min. determine the transmittance of the red soln. by comparing it with water in a Coleman double monochromator spectrophotometer with a 30 $m\mu$ slit set at wavelength 540 $m\mu$. Other methods of colour comparison may

be used. Purification of the alcohol used is essential to prevent inclusion of impurities that become brown in presence of potassium hydroxide. Contact between the reagent and rubber must be avoided. The test is more sensitive at lower temp. but a thermostat set above room temp. is convenient, and the gain in sensitivity at lower temp. is counterbalanced by increased experimental error. Slow addition of the sulphuric acid causes an erratic increase in the transmittance and the formation of a white ppt. The max. extinction per unit vol. was found when 4 ml of acid was used, but 5 ml remove more effectively the evanescent ppt. first formed. If the interval between addition of the reagent and acidification exceeds 6 min., the transmittance begins to increase and the time was therefore fixed at 5 ± 0·5 min. The red soln. has max. absorption at 540 $m\mu$ with minima at 700–1000 $m\mu$ and at 430 $m\mu$. Beer's law is valid, but the best results are obtained with concns. giving transmittances of over 20%. The red colour value of some compounds related to rotenone, viz., *l*-elliptone (m.p. 157° C.), deguelin (m.p. 169° C.), toxicarol (m.p. 209° C.) and tephrosin (m.p. 195° C.) were determined at a concn. of 0·06 mg per ml, the reference soln. being a blank soln. prepared from pure acetone and the reagents. This soln. compensated for a slight turbidity in the red soln. affecting the transmittance at low wavelengths and, compared with water, gave ca. 56% transmittance at 380 $m\mu$. These compounds gave different spectrograms, but the curves were similar in form, and it was apparent that rotenone-bearing material containing these compounds will have a colour value greater than that due to rotenone alone. Attempts to estimate the proportions of these compounds by measuring transmittances at several wavelengths and solving simultaneous equations failed, owing to the similarity of the curves and lack of knowledge of the other components of the plant extractives. The relation between the red colour value and the rotenone content of plant extracts was investigated, rotenone being determined gravimetrically ("Methods of Analysis of the A.O.A.C.," 1940, 64) and the red colour value being expressed as the amount of rotenone required to give the same colour density. It was found that 500-mg samples of root, ground through a ½-mm sieve in a No. 1 Wiley mill, could be satisfactorily extracted by soaking for 16 hr. in 50 ml of acetone. With 77 samples of *Derris elliptica* and 49 of *Lonchocarpus* sp. the ratio of rotenone to red colour value was ca. 0·4, but 4 specimens of *Lonchocarpus chrysophyllus* gave ratios averaging 0·779.

The colorimetric method is particularly useful in the analysis of small samples and was applied to the examination of various parts of the root system. No consistent relation between red colour and diameter or position was found, although fine roots (ca. 1 mm diam.) tended to be inferior. In field work rigorous analysis seems futile when it is considered that cuttings may not produce roots of the quality of the parent plant. The red colour test may then be used and the colour value multiplied by 0·4 to obtain an estimate of the rotenone content (unless the ratio is known to be different), or the selection can be based upon the red colour alone, promising material being subsequently examined by the gravimetric method. A. O. J.

Determination of Thiocyanate Nitrogen in Organic Thiocyanates and Mixtures. J. W. Elmore (*J. Assoc. Off. Agr. Chem.*, 1945, 28, 363–371)—Methods for determination of organic

thiocyanate in insecticides depending upon determination of the total nitrogen or sulphur content are satisfactory provided that no other compounds containing these elements are present, but they are of little value for analysis of mixtures. The following method is recommended. To the sample ($\equiv ca.$ 0·03 g of thiocyanate nitrogen) in a 200-ml flask fitted with a cork stopper carrying a reflux condenser add 35 ml of the mixed sulphide soln. (*infra*) and 1 g of 10 to 20-mesh carborundum crystals, and place in the flask a glass tube enlarged at the top to fit loosely over the orifice of the condenser to act as a funnel leading the condensate through the mixed sulphide soln. during the reflux distillation. Connect the flask with the condenser and boil over a small flame for 30 min. Transfer the liquid into a separating funnel with *ca.* 200 ml of water, add 50 ml of light petroleum, shake and separate the aq. layer. Wash the petroleum layer with two 10-ml portions of water and add the washings to the main soln. Discard the petroleum layer. Dilute the aq. soln. to *ca.* 300 ml, neutralise to litmus paper with diluted sulphuric acid ($1+4$) and add 2 ml in excess. Boil the liquid for 8 min. to remove hydrogen sulphide, cool and, if fatty acids are present, remove them by extraction with light petroleum. Filter the liquid through a Buchner filter, neutralise the filtrate to litmus paper with 10% potassium hydroxide soln., and add 1 ml of dil. sulphuric acid. Add *ca.* 1 g of sodium bisulphite ($Na_2S_2O_5$ or $NaHSO_3$), and when this has dissolved add excess (*ca.* 15 ml) of 20% copper sulphate pentahydrate soln. and pass in sulphur dioxide for 10 min. Allow the pptd. cuprous thiocyanate to settle for 15 min., filter by suction through a small Buchner filter, transferring all the ppt. to the filter with the aid of a washing soln. made by adding 1 ml of dil. sulphuric acid, 1 g of sodium bisulphite, 10 ml of the copper sulphate soln. and 12 g of sodium sulphate to 300 ml of water and passing in sulphur dioxide for 10 min. Wash the ppt. once or twice with the washing soln., suck the filter pad (*infra*) dry and transfer it to an 800-ml Kjeldahl flask, wiping out the funnel if necessary with small pieces of filter-paper and including these with the pad. Add a few glass beads, 35 ml of conc. sulphuric acid, 10 g of potassium sulphate and *ca.* 0·7 g of mercuric oxide (or its equiv. of mercury) and digest the mixture until it is colourless and for 15 min. afterwards. Determine the nitrogen by the official method ("*Methods of Analysis of the A.O.A.C.*," 1940, 26), beginning with the words "After cooling dilute . . ." Make a blank determination with the filter-pad. Filtration of the cuprous thiocyanate is difficult. A 2-in. Buchner funnel coated with a fine suspension of "rock wool," upon which is placed a filter-paper and then a suitably adjusted layer of fine asbestos and a little diatomaceous earth, is recommended. As an alternative procedure, treat the sample in a 50-ml glass-stoppered Erlenmeyer flask with 35 ml of the mixed sulphide soln., shake the flask vigorously at room temp. for 10 min., heat to 70° C. on a steam-bath, carefully releasing the pressure, and shake at this temp. for 15 min. Cool and extract with light petroleum as already described. To prepare the mixed sulphide soln., dissolve 180 g of potassium hydroxide in 120 ml of water, thereby producing *ca.* 200 ml of soln. Saturate 100 ml with hydrogen sulphide while cooling, add the other 100 ml and 80 g of sulphur, and shake until all has dissolved. To 100 ml of this stock soln. add 50 g of sodium sulphide ($Na_2S.9H_2O$), 30 g of potassium hydroxide and 200 ml of water. The procedure described is

applicable to spray liquids. Analysis of insecticidal dusts is made by the alternative shaking procedure described, but a larger vol. of the mixed sulphide soln. (*ca.* 50 ml) may be needed to keep the contents of the flask fluid. When reaction is complete, dilute the cooled soln. with 200 ml of water, filter through a Buchner filter and treat the filtrate as previously described. If the organic thiocyanate in the insecticide is of definite and known composition, the amount present can be calculated. If the preparation is manufactured from mixed thiocyanates, the thiocyanate found is expressed as thiocyanate nitrogen unless the thiocyanate nitrogen content of the mixed thiocyanate component is known, when the proportion in the actual sample can be calculated. Cyanides, when present, are converted into thiocyanates during the analysis, but no interference from other organic compounds containing nitrogen was encountered. A common ingredient accompanying organic thiocyanates in fly sprays is *iso*butyl-undecylenamide added as an intensifier. The amount of this substance present may be ascertained by determining the total nitrogen by the Kjeldahl method and deducting from it the thiocyanate nitrogen.   A. O. J.

Gravimetric Determination of Phenothiazine R. Payfer and C. V. Marshall (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 429–430)—Phenothiazine is frequently used as an anthelmintic in the treatment of sheep and swine for round worms, being administered either in capsule or tablet form or introduced into the food. One vermicide on the Canadian market contains phenothiazine (10%) with oilcake or bone meal and iron and other mineral salts and laxative material. The green ppt. given by phenothiazine with platinum chloride soln. (Bernthsen, *Ann. Chem.*, 1885, **230**, 73) may be used for gravimetric determination. Grind the anthelmintic preparation finely and extract with 95% alcohol in a Soxhlet extractor until the extract becomes colourless. Dilute the extract with alcohol to 200 ml and allow colloidal matter to settle. Treat a 2-ml aliquot in a 10-ml beaker with 1 ml of chloroplatinic acid soln. (40 g per litre), allow the ppt. to settle for 1 hr., transfer it into a tared sintered glass crucible and wash with alcohol until the washings are colourless. Dry the ppt. at 100° C. to constant wt. The wt. of ppt. multiplied by 0·5416 gives the wt. of phenothiazine in the aliquot. The green compound is assumed to be an addition product of the composition

$$Pt(C_{12}H_9NS)_2Cl_4$$
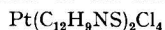
and its elementary analysis confirms this assumption. Analyses of material of known composition indicated a recovery of *ca.* 96%.   A. O. J.

Inorganic

Detection of Beryllium in Copper-Base Alloys. F. Culcsar (*Chemist-Analyst*, 1945, **34**, 28–29; 39)—The spot test developed by Komarovski and Poluektov (*Mikrochem.*, 1934, **14**, 315) provides a fairly rapid and reliable means of detecting beryllium in copper-base alloys. *Method*—Dissolve *ca.* 1 g of drillings in *ca.* 40 ml of conc. nitric acid, heating on the hot plate to complete solution and to expel oxides of nitrogen. Dilute to *ca.* 300 ml, heat just to boiling and add ammonia until the soln. has a strong odour. Filter through a No. 41H Whatman paper, wash twice with slightly ammoniacal 2% ammonium nitrate soln. and discard the filtrate. Treat the residue on the

paper with 10 ml of 10% sodium hydroxide soln. (if the residue is gelatinous, break up immediately after adding the sodium hydroxide). Allow to drain completely and discard the paper. Place 2 drops of *p*-nitrobenzene-azo-orcinol reagent (dissolve 25 mg in 100 ml of *N* sodium hydroxide. Store in a rubber-stoppered bottle. Renew every 2–3 months) on a double thickness of filter-paper and add 1 drop of 25% potassium cyanide soln. Place a small drop of the alkaline extract in the centre of the spot. A pink colour indicates the presence of beryllium. With very small amounts of beryllium, the colour may not develop immediately; allow the paper to dry, watching carefully. If no colour has appeared when drying is complete, the sample contains less than 0·05% of beryllium. (No conclusions should be drawn after the paper has dried, as the colour tends to fade; direct sunlight should be avoided.) *Ca.* 0·2 mg of beryllium can be detected in 1-g samples of various alloys. (An exception was an alloy containing 1·54% of silicon; in this 0·5 mg of beryllium could be detected.) Besides copper, the following elements, present in amounts indicated, do not interfere; Ni (0·0771 g), Mn (0·0321 g), Al (0·0400 g), Cd (0·0116 g), As (0·0027 g), Fe (0·0167 g), Si (0·0154 g), Sn (0·0991 g), Pb (0·0974 g), Sb (0·0055 g), P (0·0062 g) and Zn (0·2707 g). Further, Komarovski and Poluektov (*loc. cit.*) state that Co, Ag, Ca, Sr, Ba, La, Pr, Nd, Ce, Zr and Th do not interfere. J. T. S.

Colorimetric Estimation of Traces of Metal in [Beer] Wort. R. E. Essery (*J. Inst. Brewing*, 1945, **51**, 185–188)—The methods described have been used to study the suitability for brewery equipment of alloys of the stainless steel type. A bulk of wort was prepared (sp.gr. 1,040°, filtered bright) and used in 2-litre portions; these were either treated in a small vessel made of the alloy under test and then boiled down to 1,500 ml in Pyrex glass, or were used as blanks and boiled down to 1,500 ml in Pyrex without any other treatment. For the determination of the metals the carbohydrates are destroyed by fermentation and the residue is dry-ashed and dissolved. Standard colorimetric procedures are then employed with modifications which are made necessary by the large amount of phosphate present.

Method—(*a*) *Preparation of soln.*—Treat each 1500-ml portion of conc. wort with 200 ml of the same 10% cold water extract of the same malt, add 4 g of the same bottom-fermentation yeast and ferment in Institute of Brewing vacuum fermentation vessels. Rouse at intervals until the sp.gr. ceases to fall (10 to 14 days, final sp.gr. 1003° approx.). Rouse back any outcrop yeast. Transfer the whole mixture to a Pyrex vessel, stir the yeast in thoroughly to avoid burning, and boil down to a thick syrup. Evaporate in a silica dish, on a water-bath until the sample has the consistence of soft toffee (*ca.* 2 days). Ignite until all evolution of vapours ceases and the mass is completely charred. Grind the residue in a glass mortar, restore to the dish, and complete the ignition, stirring occasionally with a platinum wire. Sweep the ash into a Pyrex beaker, warm the residue in the dish with conc. hydrochloric acid and rinse into the beaker. Extract the whole with boiling dil. hydrochloric acid and decant the liquid through an ashless filter-paper. Repeat the extraction and decantation several times, then transfer the insoluble material to the filter and wash thoroughly with boiling water. Ignite the paper and insoluble matter in the silica dish and extract the residue again by the same method. Ignite the final

insoluble residue in platinum and fuse with sodium carbonate and a little potassium chlorate. Dissolve the melt in water, acidify with hydrochloric acid and add the soln. to the combined filtrates. Evaporate to 150 ml, cool and make up to 200 ml, so that 10 ml of the final soln. are equivalent to 100 ml of the original wort.

(*b*) *Analysis*—Prepare a soln. containing 4 g in 200 ml of a mixture of 70% of potassium dihydrogen phosphate, 10% of calcium sulphate dihydrate, 10% of magnesium sulphate heptahydrate and 10% of sodium chloride. Add suitable quantities of this soln. to the colour standards before developing the colour, to reproduce approx. the salt content of the wort soln.

Nickel—To a suitable aliquot add 10 ml of 5 *N* citric acid (35% w/v) and 5 ml of saturated bromine water, mix and leave for 5 min. Decolorise with diluted ammonia (1+1) soln., cooling under a tap. Transfer the soln. to a 50-ml Nessler cylinder, add 2 ml of 1% alcoholic soln. of dimethylglyoxime, make up to 50 ml and mix. After a few moments compare with similarly treated standard solns. made up at the same time from a nickel sulphate soln. containing *e.g.*, 0·1 mg of nickel in 50 ml. If matching is delayed a ppt. (probably largely magnesium ammonium phosphate) forms, which may be removed by centrifuging. Iron (3 to 4 p.p.m. in some worts) does not interfere.

Iron (i) *Ferrocyanide method*—Treat an aliquot of the soln. with ammonia soln. until the odour persists, dilute to 40 ml, add 3 ml of conc. nitric acid and cool. Transfer to a Nessler cylinder, add 1 ml of fresh potassium ferrocyanide soln., dilute to 50 ml and compare with standards after 15 min. (ii) *Thioglycollic acid method*—Mix an aliquot with 10 ml of 5 *N* citric acid and add 1 ml of reagent made by mixing 4 ml of thioglycolic acid with 50 ml of water containing 8 ml of conc. ammonia soln. Add ammonia soln. to develop the colour (*p*H *ca.* 9), cool, dilute to 50 ml, and compare with standards. Any ppt. may be dealt with as in the nickel test.

Copper—Neutralise an aliquot with ammonia soln. and add 5 ml of ammonium acetate soln. (100 g of ammonium acetate and 100 ml of glacial acetic acid in 500 ml). Mix in 1 ml of 0·1% alcoholic soln. of dithio-oxamide and dilute to 50 ml. The amount of copper should not exceed 0·06 mg for satisfactory matching.

Chromium—Considerable difficulty (discussed in some detail) was experienced in working out a method and the following is the best of the procedures tried, although it is not very satisfactory. Treat an aliquot with a few ml of bromine water and mix with 10 ml of 10% sodium hydroxide soln. After a short time add conc. hydrochloric acid carefully until the yellow bromine colour reappears and the soln. becomes clear. Avoid excess of acid. Stir in one or two crystals of phenol to remove the excess of bromine and cool. Add 0·2 ml of reagent (0·5 g of diphenylcarbazide dissolved in 10 ml of glacial acetic acid and diluted to 100 ml with 95% alcohol), stir, dilute to 50 ml and leave for 20 min. before matching against a standard made from 0·02 mg of chromium as chrome alum. In tests with known amounts of chromium recovery of up to 80% was found. Attention is drawn to the possibility of forming and losing chromyl chloride during the treatment of the sample. L. A. D.

Volumetric Determination of Phosphorus in Iron and Steel. T. S. Harrison and T. Parratt (*J. Soc. Chem. Ind.*, 1945, **64**, 218–219)—A rapid

and accurate procedure for the determination of phosphorus is described, in which arsenic is expelled as tribromide before the phosphorus is pptd. as phosphomolybdate. *Nitro-molybdate reagent*—Dissolve 50 g of molybdic acid in 100 ml of water and 50 ml of ammonia soln. (sp.gr. 0·880) and pour the soln. into 625 ml of nitric acid (sp.gr. 1·20), cooling and stirring.

Procedure—Dissolve 2·0 g of the sample in 20 ml of conc. nitric acid, adding a little water if necessary. Add 10 ml of brominated hydrochloric acid, evaporate the soln. gently to dryness, and continue heating, without baking, to expel excess of acid vapour. Cool, dissolve in 45 ml of nitric acid (sp.gr. 1·2), filter to remove silica and graphite, and wash the paper with cold 2% nitric acid. Add 12 ml of ammonia soln. (sp.gr. 0·880) to the filtrate, with shaking, heat to boiling, cool to 70° C. and add drop by drop 20 ml of nitro-molybdate reagent, shaking the soln. all the time. Leave for 20 min., filter, wash the ppt. with ammonium nitrate soln. (2%) and dissolve it in an excess of standard sodium hydroxide soln. containing a few drops of phenolphthalein soln. (1%). Boil and titrate with standard sulphuric acid as usual. Alternatively, redissolve, after evaporation of the solvent acids, in 20 ml of nitric acid, filter, neutralise with ammonia soln. and add 7 ml in excess. Neutralise with nitric acid (sp.gr. 1·43), add 3 ml in excess and proceed as above. With cast irons of medium or high phosphorus content use 0·2 g of sample and half the above amounts of solvent acids. L. A. D.

Some Reactions of Thionyl Chloride and their Application to the Determination of Sulphur Chlorides in Technical Thionyl Chloride. T. Harrington and T. H. Boyd (*J. Soc. Chem. Ind.*, 1945, **64**, 209–211)—The chief impurities in technical thionyl chloride are sulphuryl chloride, sulphur monochloride and sulphur dichloride, with traces of chlorine, sulphur dioxide, phosgene and iron. On hydrolysis with excess of water pure thionyl chloride undergoes the reaction

$$SOCl_2 + H_2O \longrightarrow SO_2 + 2HCl$$
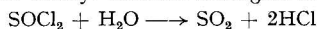
Sulphur monochloride in presence of a large excess of thionyl chloride is found to be hydrolysed by water at 0° C. to form tetrathionic acid almost quantitatively:

$$S_2Cl_2 + 2SO_2 + 2H_2O \longrightarrow H_2S_4O_6 + 2HCl.$$
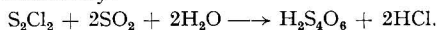
Sulphur dichloride under the same conditions produces trithionic acid with *ca.* 10% of tetrathionic acid:

$$SCl_2 + 2SO_2 + 2H_2O \longrightarrow H_2S_3O_6 + 2HCl.$$
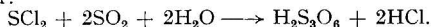
Irrespective of the exact reactions during hydrolysis it is found that the hydrolysed soln. will require 14 atoms of bromine for each mol. of sulphur monochloride and 8 atoms of bromine for each mol. of sulphur dichloride originally present. The method of analysis is based on determining the bromine titre of the mixed sulphur chlorides, and also of a second sample in which all the sulphur dichloride has been converted into monochloride by boiling with sulphur. With technical thionyl chloride erratic results may be caused by presence of chlorine which reacts with the sulphur to give sulphur monochloride, or of iron which catalyses reaction between thionyl chloride and sulphur and also leads to the formation of sulphur monochloride. Iron and chlorine are therefore removed by mixing the sample with tetrachloroethane and distilling.

Procedure—(a) *Preliminary distillation*—Dilute *ca.* 80 ml (weight *w* g) of the thionyl chloride sample to 100 ml with tetrachloroethane and distil at a pressure of 120 mm of mercury in an all-glass apparatus. To avoid loss, a vertical condenser, cooled with solid carbon dioxide and ether, is fitted above the receiver and the vacuum is applied to the top of this condenser. A dry-air leak tube is fitted to the distilling flask. The air sweeps away the chlorine, but the flow must be kept to a minimum to avoid loss of sample. Continue distillation from a water-bath to dryness, and allow the condenser to drain for 10 min. Dilute the distillate to 100 ml with tetrachloroethane and divide into two 50-ml portions, one for determination (b) and one for (c). *Hydrolysis and titration*—Dilute the 50 ml portion to 100 ml with tetrachloroethane, take 2 ml and hydrolyse by shaking for 2 min. with 50 ml of ice-cold water. Warm to 50° C. and pass a rapid stream of carbon dioxide or nitrogen for 10 min. Cool to room temp. and add approx. 0·1 N iodine until the first yellow colour forms. Ignore subsequent slow fading. These operations remove sulphur dioxide. Add 30 ml of conc. hydrochloric acid and 2 drops of methyl orange soln. (0·5%) and titrate with 0·1 N bromate-bromide soln. until the pink colour fades (t_1 ml). Add immediately 10 ml of potassium iodide soln. (10%) and dilute to 200 ml. Titrate the liberated iodine with 0·1 N thiosulphate (t'_1 ml). The vol. of bromate-bromide soln. used = $t_1 - t'_1 = T_1$ ml.

(c) *Sulphur reflux treatment*—Boil the 50-ml portion of diluted distillate gently under reflux with 2 g of powdered sulphur until the red sulphur dichloride colour is removed (*ca.* 30 min.). Cool and dilute to 100 ml with tetrachloroethane. Take 2 ml and treat as in (b) above. The vol of 0·1 N bromate-bromide used = $t_2 - t'_2 = T_2$ ml.

(d) *Calculation*—One ml of 0·1 N bromate-bromide $\equiv 135/(14 \times 10^4)$ g of S_2Cl_2 or $103/(8 \times 10^4)$ g of SCl_2. If $x = \%$ S_2Cl_2 and $y = \%$ SCl_2, then as $w/100$ g of sample is used in each titration—

$$T_1 = 14\ xw/135 + 8\ yw/103$$
$$T_2 = 14\ xw/135 + 14\ yw/103.$$

From these equations

$$x = \%\ S_2Cl_2 = (3·21/w)(7T_1 - 4T_2)$$
$$y = \%\ SCl_2 = (17·2/w)(T_2 - T_1).$$

Details of the checking of this method, of the identification of the main products of hydrolysis and of the experimental evidence in support of the equations used are also given in the paper.

L. A. D.

Microchemical

Identification of Monochloroacetic Acid as Barium Monochloroacetate. W. V. Eisenberg (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 427–428)—In the method described by Wilson and Keenan (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 446; ANALYST, 1944, **69**, 381) for the identification of monochloroacetic acid by means of the optical-crystallographic properties of barium monochloroacetate monohydrate, the barium monochloroacetate occasionally crystallises out as the anhydrous salt. This serves as an additional check, since the optical-crystallographic properties of the anhydrous salt can be determined before converting it into the monohydrate by dissolving it in a drop of water on a microscope slide and allowing the soln. to evaporate at room temp. The following are the optical-crystallographic properties of the anhydrous salt, those of the monohydrate being given in brackets for comparison. Crystal habit, six-sided plates (six-sided plates); extinction, parallel (parallel),

elongation, positive (negative); n_α, 1·512 (1·582); n_γ, 1·638 (1·611). The anhydrous salt when heated at 100° to 104° C. for 4 hr. showed no change in optical properties. A. O. J.

Micro-estimation of Dicarbonyl Compounds.

C. Neuberg and E. Strauss (*Arch. Biochem.*, 1945, **7**, 211–230)—Simple dicarbonyl compounds, such as glyoxal, methyl glyoxal and dimethyl glyoxal, form *bis*-dinitrophenylhydrazones (dinitrophenyl osazones) quantitatively; these dissolve in alcoholic alkali soln. to give blue to violet colours. This reaction has been made the basis of a method of estimating such compounds. Put 1 ml of the test soln. into a small test-tube and add 1 or 2 ml of a soln. of dinitrophenylhydrazine reagent (dissolve 0·1 g in 5 ml of 2 N hydrochloric acid by heating and dilute the hot soln. to 0·02% with 2 N hydrochloric acid; dilute the soln. ten-fold with N hydrochloric acid immediately before use). Heat on a boiling water-bath for 1 hr., cool, add *ca.* 0·5 g of kaolin, shake and centrifuge for 2 or 3 min. Remove the supernatant soln. and mix the ppt. with 0·1 N hydrochloric acid, centrifuge and wash similarly with water. Remove the water and mix the ppt. with 3 ml of a 0·3% soln. of solid sodium hydroxide in 96% alcohol or of a 0·3% sodium ethylate soln. prepared by dissolving 3 g of metallic sodium in 1 litre of abs. alcohol. Stir, leave for 5 min. and centrifuge. Remove the coloured soln. and again stir the ppt. with 3 ml of sodium ethylate soln. and centrifuge. Dilute the combined extracts to a convenient vol. with sodium ethylate soln. and compare the colour with that of a suitable standard soln. Carry out a blank with the reagents only and subtract the colorimetric value from that of the test soln. The colours of the solns. obtained with different dicarbonyl compounds are similar but not identical, and the standard soln. should therefore be prepared from the carbonyl compound under examination. Where this is not readily available, the monoximes can be used as initial materials, as the hydroxylamine radical is replaced quantitatively by the hydrazine radical on boiling with excess of dinitrophenylhydrazine for 2 hr. The reaction is also applicable to the acyloins. F. A. R.

Qualitative Micro-analysis of the Alkaline Earth Group.

R. Belcher and F. Burton (*Metallurgia*, 1944, **31**, 42)—Extraction of the nitrates with ethanol to remove calcium, conversion of the residue into the chlorides and the subsequent removal of strontium by a further ethanol extraction has the advantage that almost complete separation is obtained. Evaporations are rapidly carried out in a glass micro-spoon (Belcher, *Metallurgia*, 1944, **30**, 280) and the cost of ethanol is negligible.

Method—Ppt. as carbonates, separate and wash the ppt. by the usual centrifuge technique. Dissolve in dil. nitric acid and evaporate to dryness without decomposing the nitrates. Extract twice with ethanol.

Problems concerning the Microchemical Balance. I. Installations. II. Cleaning.

C. L. and D. W. Wilson (*Metallurgia*, 1944, **31**, 101–102; 1945, **32**, 85–87)—I—Five major factors, *viz.*, vibration, temp., humidity, dust and fumes, have to be considered. If the laboratory is being designed *ab initio* a pillar standing on the ground and independent of the building is preferable. Where vibration is not abnormal, a slate or concrete slab bolted to a solid wall is often adequate, and a site on an upper floor may be advantageous in reducing street vibration. Several thicknesses of cork, linoleum or leather, or a sheet of lead between glass plates, placed beneath the balance deaden vibration. An appreciably flexible mounting should be avoided owing to the risk of serious errors caused by displacement of the balance during operation. Temperature variations may cause much greater error than the most serious vibration. A room on the north side of the building is advantageous for equable temp. and for avoiding direct sunlight on the balance. Owing to direct draught the balance should not be placed between two doors or windows. If there is a radiator, the balance should be placed *exactly* opposite to it, and lights should be arranged symmetrically with respect to the balance. Humidity changes can have an appreciable effect, but this is not generally serious if the temp. is well controlled. Air-conditioning, often suggested in connection with dust and fumes, as well as with humidity, is not essential. In industrial districts a fan maintaining a slight positive pressure of filtered air in the room may have some advantage, but is rarely necessary.

II—The period between cleanings is a matter of experience, depending upon the exclusion of dust as well as on the frequency of use. It varies from a few weeks to *ca.* 6 months. Erratic swings or non-repeatable weighings indicate that attention is required. Observe through a lens the pointer tip and central knife-edge. A single hair, which can be removed from the pointer or pointer scale by gentle brushing, can upset the swinging. General cleaning is advisable when dust or fibres are observed on the knife-edge. Thoroughly dust the bench and the ledges of the outer case, if any (a small glass attachment to a vacuum line will remove dust without stirring it up). Remove the complete balance case for cleaning, if possible; otherwise, clean the outside of the case, remove the front window and clean its inside surface. To avoid corrosion, use chamois finger-stalls when handling metal parts. Remove the rider and detach the rider- and pointer-lenses. After noting the markings, remove the pans and then the stirrups, and place each on its own side of the balance on plates of clean glass. Hold the beam by the upper part of the pointer, lift clear of the supporting cups, tilt the pointer clear of the scale and lift off. If the beam cannot be removed without lowering the arresting mechanism, first take the weight of the beam by the

Solution—Evaporate. A white residue shows **Ca**. To confirm, dissolve in a drop of water, saturate with solid NH₄Cl, and add 2 drops of fresh K₄Fe(CN)₆ soln.; a white ppt. results.	Residue—Add 1 drop of conc. HCl and evaporate to dryness. Repeat. Extract twice with ethanol.	
	Solution—Evaporate to dryness. A white residue shows **Sr**. To confirm, dissolve in 1 drop of water and spot on paper treated with fresh sodium rhodizonate soln. A red colour, removed by 1 drop of 0·1 N HCl, results.	Residue—Shows **Ba**. To confirm, either (a) Dissolve in 1 drop of water and treat as for Sr; a scarlet colour, *not* removed by 0·1 N HCl, results. Or (b) Dissolve in dil. CH₃COOH and add 1 drop of K₂CrO₄; a yellow ppt. results.

fingers. Take care that the central knife edge does not touch its agate plate during manipulations. Lay the beam with the pointer tip clear of the glass plate. Clean the interior of the case, rider hook and mechanism, pillar and beam support first with camel-hair brushes, then with a chamois square. If possible, remove the pan arrests and wipe. Clean the supporting cups with chamois wrapped round a pointed matchstick. Lightly rub the central agate plate with the fold of a spill of special lens tissue and brush off any particles of dust. If necessary, clean and lightly oil the mechanism under the case and approximately readjust the level. Brush the beam, giving the pointer tip special attention, wipe the supporting points with chamois and the knife edges with folded squares of special tissue. Inspect for freedom from dust and replace the beam. This requires even more care than its removal. Brush the stirrups, wiping the supporting points with chamois and the agate plates with special tissue. Replace correctly on their supporting cups. Polish the pans with chamois; a little metal polish, subsequently thoroughly removed, should be used only if they are stained or corroded. Clean the tips of the pan arches and tube-holders and replace the pans on their respective hooks. Brush the rider and replace it on its hook. Clean, replace and adjust the lenses, replace the case or front window, carry out the final levelling, and test the mechanism. Up to 1 hr. may be required for the balance to "settle down"; during this time leave open the window and side doors. Check and, if necessary, adjust the zero. Clean the weights with chamois (or, occasionally, with 1 : 1 pure alcohol and distilled water, drying thoroughly). Clean the box, using the vacuum attachment for the velvet lining. Wipe the tares and replace in the drawer after cleaning the latter. Finally, after closing the balance case, wipe the surroundings, polish the windows of the outer case, and clean the lamp bulb.

J. T. S.

Physical Methods, Apparatus, etc.

Polarographic Determination of Antimony in Hard Lead. R. Kraus and J. V. A. Novak (*Die Chemie*, 1943, **56**, 302–303)—In soln. strongly acidified with hydrochloric acid, hydrolysis is suppressed and adsorption of antimony salt by lead chloride, which is pptd. in less acid soln., is prevented. Further, Sb^V is polarographically reducible under these conditions, giving two waves. The first represents the reduction of Sb^V to Sb^{III}, and the second, of Sb^{III} to Sb. The height of the second wave, which is proportional to the total concn. of antimony in the soln., is measured. *Procedure*—Heat 0·2 g of the sample with 15 ml of conc. hydrochloric acid until solution of lead is complete. Add a few drops of bromine to dissolve antimony, boil off the excess, cool, and dilute to 50 ml with diluted hydrochloric acid (2+1). (The final acidity should be *ca.* 8 *N*.) To a 5-ml aliquot, add 0·1 ml of 0·5% gelatin soln. and polarograph between 0 and 0·4 v. after passing hydrogen or nitrogen for 1 min. Compare the height of the second step with that given by a standard soln. of antimony (dissolve 0·1 g of pure antimony in 20 ml of conc. hydrochloric acid and a few drops of bromine; boil off excess of the latter, and dilute to 500 ml with diluted hydrochloric acid (2+1). Polarograph 5 ml after adding gelatin and passing the gas stream as before). The determination takes *ca.* 30 min. Silver is pptd. as chloride and does not interfere; neither does arsenic, which is

oxidised by bromine to As^V (not reducible polarographically). Copper interferes only when its concn. exceeds that of antimony. Since the amount of bismuth present in hard lead is usually less than 0·1%, its interference can be neglected for control purposes.   J. T. S.

Spectrographic Limit of Detection of Phosphorus, Titanium and Zirconium in the Direct Current Arc. D. P. Norman and W. A. Johnson (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 233–235)—The work of determining the spectrographic limits of detection of various elements in a variety of matrices has been continued. The technique used was generally similar to that already described for potassium (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 152; ANALYST, 1943, **68**, 293). The limits of detection* of phosphorus, in nine Bureau of Standards standard samples, ranged from 0·05 to 0·80 µg, using the most satisfactory phosphorus line, 2553·28A. The samples used included a variety of base compositions with percentage phosphorus contents ranging from 0·0013 to 0·59%. There was, however, no correlation between the limit of detection and the percentage of phosphorus in the sample. For titanium, using the line 3371·45A, the limit of detection ranged from 0·04 to 4 µg in 12 samples whose titanium contents lay within the range 0·0018 to 0·23%. For zirconium, the line 3391·98A of the singly ionised atom was found to be most satisfactory. The limit of detection varied from 0·5 to 4 µg in 6 samples containing zirconium ranging from 0·0037% to 0·19%.   B. S. C.

Spectrographic Detection of Selenium in the D.C. Arc Flame. C. Feldman (*J. Opt. Soc. Amer.*, 1945, **35**, 180–184)—Selenium can be detected spectrographically in the d.c. arc flame by means of the line 2413·517A. Using a Littrow model large quartz spectrograph, the spectra are most conveniently excited in the graphite arc, the sample being placed in a drilled cavity in the anode. The recommended currents are 9 to 11 amps. with ¼ in. diam. anode and ⅛ in. diam. cathode, and 4 to 6 amps. when both rods are ⅛ in. diam. The former combination is suitable for 25 to 60 mg portions, the latter for 8 to 10 mg portions. The electrode separation should be 6 mm, and the centre 1·5 mm of the flame photographed. No line is obtained with as much as 4% of selenium in 60 mg of quartz powder. In suitable combination, however, the sensitivity is very markedly increased. The following table shows the limits of sensitivity using the 2413·517A line under the above mentioned conditions.

Form of selenium	Matrix	Weight of sample mg	Weight of selenium mg	Per cent. of selenium
Na_2SeO_3	Quartz	60	0·6	1
CuSe or PbSe	CuS or PbS resp.	60	0·15	0·25
CuSe or PbSe	CuS or PbS resp.	10	0·1	1
Se	Te	60	0·006	0·01
Se	Te	10	0·025	0·25

The addition of 10% or more of tellurium to the copper selenide/copper sulphide mixture enhances the selenium line. The only interference is from the cobalt line 2413·580A. The iron line 2413·309A

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* For the purpose of the investigation the limit of detection of an element in a sample is defined as the smallest amount that can always be detected with certainty.—ABSTRACTOR.

and the "SiO" band head at 2413·8A do not interfere when the large Littrow instrument is used.

B. S. C.

Extinction Coefficients of Spectrophotometric Standards. J. M. Vanderbelt, J. Forsyth and A. Garrett (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 235–237)—The use of absorption standards for spectrophotometric calibration is becoming of increasing importance owing to the frequent use of the instrument for analytical determinations. For the calibration of instruments used for vitamin A determinations, potassium chromate in 0·05 N potassium hydroxide has long been used. Both it and potassium nitrate have the desirable purity and stability, but their absorption maxima are not at the wavelength of the absorption maximum of vitamin A. Both anthraquinone and salicylaldehyde have been suggested as more suitable standards, but their precise extinction coefficients have not hitherto been determined. The molecular extinction coefficients of all the above materials have now been determined, using a Beckman spectrophotometer with a hydrogen discharge tube source. The $E_{1\,cm.}^{1\%}$ value for a distilled vitamin A ester has also been determined, using the same instrument. Series of data on these various standards indicate that optimum extinction coefficients are obtained when the instrument density is within the range 0·5 to 1·9. The following is a summary of the absorption data obtained.

Compound	Wavelength of maximum ($m\mu$)	Mean extinction coefficient (ϵ)
Potassium nitrate (in water)	301	7064
Potassium chromate (in 0·05 N potassium hydroxide)	{ 373	4800
	{ 273	3688
Anthraquinone (in ethyl alcohol)	323	4954
Salicylaldehyde (in ethyl alcohol)	326	3662
Vitamin A ester (in ethyl alcohol)	327	$E_{1\,cm}^{1\%} = 106·0$

The values for potassium chromate and potassium nitrate are in good agreement with previous values for these standards obtained, using other forms of instrument. The values for anthraquinone and salicylaldehyde are the first careful evaluations. The value for the vitamin A ester is in almost exact agreement with the mean values of data from 17 laboratories, using Beckman spectrophotometers and from 11 laboratories using quartz spectrographs with photometers.

B. S. C.

Spectral Analyses of Solid Substances. J. Eeckhout (*Nature*, 1945, **156**, 175)—A method has been developed whereby it is possible to deduce from a single spectral photograph not only the qualitative but also the approx. quantitative composition of a totally unknown mineral powder. For this purpose, mixtures have been made of all the spectrographically detectable elements at the author's disposal in groups of 10, such that the sensitive lines of the elements in any one group do not coincide, by mixing quantities of the pure compounds equiv. to 100 mg of the oxide in an agate mortar under alcohol. Various dilutions of this 10% mixture down to 0·0001% were prepared with pure washed quartz-sand. Before the photographs were taken each mixture was further diluted with half its weight of pure sodium carbonate. The photographs were taken by means of a Hilger E.315 quartz spectrograph with an arc burning continuously for 60 sec. at 4 amp. and then for 30 sec. at 7 amp. between very pure plane carbon electrodes of 5-mm diam. A diffusely and uniformly illuminated slit was obtained by focussing the arc sharply on a screen containing a rectangular diaphragm which was then focussed enlarged on the collimator lens of the instrument. A rotating step sector (factor r = 3·5) was placed in front of the slit. The intensities of the lines chosen for the analysis were compared with those of certain silicon lines from the spectrum (the concn. of silica being approx. constant), a Zeiss spectral projector being employed for the visual comparison under a 20-fold enlargement. The relative intensity (rather the relative exposure) is denoted by the number of "steps" to be applied to the two lines under comparison in order that they may show equal blackening (see Breckpot, *Spectrochim. Acta*, 1939, **1**, 2). For the lowest concns., a spectrum was taken with illumination forming an image of the arc on the slit, without a step sector, the cathode layer effect being used. Tables have been drawn up giving the relative intensities as a function of concn. for the most prominent lines of 56 different elements. A table is appended giving results of the analysis of two synthetic mixtures and a natural mineral. Complete details of this work are to be published elsewhere.

J. A.

Rigidity Testing of Paper Board. T. H. Farebrother (*Paper Maker*, 1945, **110**, July, TS2–4)—A strip of the sample is clamped rigidly in a horizontal plane in a metal bridge supported over the pan of a box-scale type of balance. A metal strip is mounted vertically on the pan below, and by placing a succession of weights on the other pan, the sample is flexed to varying extents. The resulting deflection is measured by means of a microscope having an eyepiece-scale and a fixed magnification. The rigidity is expressed by the ratio, load (in g) : corresponding deflection (in cm). Data are presented illustrating the effects on it of varying thicknesses and substances of the sample.

J. G.

Definition and Measurement of Gloss and Smoothness of Paper. V. G. W. Harrison (*Paper Maker*, 1945, **109**, Midsummer No., 4–5, 7–8, 10,13)—The sensation of the "finish" of paper is the resultant of 3 sight sensations, *viz.*, surface texture, sharpness of the mirror image, and gloss or glare (*i.e.*, lustre), and 2 touch sensations, *viz.*, frictional resistance and smoothness. Suggested methods for measuring each of the above properties are described briefly and discussed.

J. G.

Reviews

A TEXTBOOK OF DAIRY CHEMISTRY. By E. R. LING, M.Sc., F.R.I.C., A.R.C.S. 2nd Ed. Vol. II: Practical. Pp. viii + 124. London: Chapman & Hall, Ltd. 1945. Price 10s. 6d.

This volume presents a series of experiments in dairy chemistry to supplement the theoretical considerations which were the subject of Volume I (reviewed, ANALYST, 1945, **70**, 277).

The treatment is simple, being intended primarily to meet the educational needs of students taking our shorter courses of dairy science. With this object the author found it necessary "to preserve a balance between the manifold ramifications of dairy chemistry and the limited chemical knowledge and experimental skill possessed by many of those who are called upon to perform the various tests."

A description of the usual laboratory operations of weighing, filtration, titrations, and so on, and of methods of sampling milk and dairy products, is followed by a description of sixty-six selected qualitative and quantitative experiments which cover the majority of the more frequently required analyses of milk, cream, condensed and dried milks, butter, butterfat, cheese, dairy detergents, and hypochlorite sterilising solutions. References to the literature and standard works are given, where possible, for the methods described, and references for some determinations not included are appended.

Students and dairy laboratory testers will find this convenient collection of methods very useful; although they may rightly think that it is rather highly priced at 10s. 6d. Others of us will regret that the standard and scope of Volume II has been deliberately limited, for it is, as a result, hardly a worthy companion of the excellent Volume I—which is of wide appeal.   S. J. Rowland

THE THEORY OF RESONANCE AND ITS APPLICATIONS TO ORGANIC CHEMISTRY. By GEORGE WILLARD WHELAND. Pp. iv + 316. New York: John Wiley & Sons; London: Chapman & Hall. 1944. Price $4.50.

This survey of resonance from the standpoint of an organic chemist is comprehensive and interesting and written in an attractive and lucid style; it should make a strong appeal to chemists and senior students.

That the subject with which this volume deals is of fundamental importance and wide significance is now generally realised and no less an authority than Professor Sidgwick has stated that "The conception of resonance is the most important development which structural chemistry has had since it was extended to three dimensions by van't Hoff in 1874" (*Organic Chemistry of Nitrogen*, p. xv).

On the other hand, as the author of the book under review does not fail to point out, the basis of the theory "lies in the mathematical depths of quantum mechanics . . . it can be presented precisely and completely only in highly mathematical language. Some sort of working compromise must therefore be reached."

This book represents the author's method of compromise—the theory of resonance being approached mainly in a qualitative and descriptive manner—and the subject matter is drawn largely from the field of organic chemistry wherein the most fruitful applications of the theory are to be found.

In the opening chapter the conditions for resonance are dealt with, its mechanical analogy and mathematical basis being briefly outlined. The second chapter treats skilfully and concisely of the nature of valency, including resonance between ionic and covalent bonds, and devotes some 8 pages to a detailed consideration of the hydrogen bond. The third chapter deals with the determination of resonance energies from thermo-chemical data of hydrogenation and of heats of combustion, while the fourth discusses the steric effect of resonance, including inter-atomic distances.

The remaining chapters are concerned successively with (*a*) the bearing of resonance on dipole moments and on molecular spectra, (*b*) chemical equilibrium, including sections on acid- and base- strengths, free radicals and tautomerism, (*c*) chemical reaction, which includes amongst other topics—rates of reaction, the activated complex, normal and abnormal addition reactions, polymerisation, the orientation of substituents in cyclic compounds, concluding with an interesting discussion of molecular rearrangements.

This summary will serve to indicate the numerous and widespread sections of organic chemistry in which the applications of the theory of resonance has brought, and is continuing to bring, further aid to the study of the intricate and fascinating behaviour of organic compounds.

There is an extensive list, 9 pages in length, and believed to be complete in June, 1943, of the measurements of inter-atomic distances in a wide variety of organic compounds.

The matter of the book is carefully arranged in numbered sections so that cross references are easily and quickly made. The type is clear and the book is well produced and provided with adequate indexes of both authors and subjects.   J. Kenyon

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