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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 2.30 p.m. on Wednesday, December 1st, 1943, at the London School of Hygiene and Tropical Medicine, London, W.C.1. The chair was occupied by Mr Theodore Rendle, F.I.C., Vice-President. The subject of the meeting was "Nutrition of the Public and Food Legislation," and the following papers were presented and discussed:—"The Essential Natural Nutrients of Fresh and Manufactured Foods," by Professor J. C. Drummond, D.Sc., F.I.C.; "The Rôle of Food Legislation in securing adequate Nutrition" by H. E. Cox, Ph.D., D.Sc., F.I.C.; and "The Part played by the Food Supplier in Safeguarding the Nutritive Values of Food," by E. B. Hughes, D.Sc., F.I.C.

NEW MEMBERS

William Bradley, M.Sc., Ph.D. (Lond.); Arthur Carroll, A.M.C.T., A.I.C., M.P.S.*; William Bernard Chapman, A.I.C.*; Edward Albert Roberts; Albert Sciver, B.Sc. (Lond.), F.I.C.; John Robert Stewart, Major, R.A.S.C., B.Sc. (Leeds); Harold Sykes, B.Sc. (Lond.)*

DEATH

WE regret to have to record the death of Leo Cooksey.

The Calibration of Fluorimeters used for Estimating Vitamins, Alkaloids, and other Substances

BY FRANK WOKES, B.Sc., PH.D., F.I.C., PH.C., JOAN G. ORGAN, PH.C., BESS M. STILL, B.Sc., AND F. C. JACOBY, PH.D.

FLUORESCENCE methods are widely employed to estimate vitamins, alkaloids and other physiologically important substances, but a study of the principles involved has hitherto been lacking. Most workers measure fluorescence intensity by photoelectric methods. The apparatus used has been given various names (*e.g.*, fluorimeter, fluorometer, photofluorometer). The term always used in our communications is fluorimeter, which has now received the approval of the panel appointed by the S.P.A. Publications Committee.[1] Our investigations have been made with the Spekker instrument,[2] but the results are in general applicable to other types.

USE OF CALIBRATION CURVES TO DETECT QUENCHING—Diminution or *quenching* of fluorescence, leading to low results, may be due to various causes, *e.g.*, *concentration quenching*,[3] which increases with increasing concn. and is due to too high a concn. of the fluorescent molecules, *dilution quenching*, which increases with decreasing concn. and may be due either to instrumental causes (*e.g.*, reflection in cells) or to the solvent (*e.g.*, fluorescence of *iso*butanol in aneurine estimations). Other causes of quenching are ultraviolet light, oxygen, salts and various impurities, also changes in *p*H.

Quenching can be detected by *recovery experiments*, in which a known amount of the specific substance is added to the material being tested, and the % recovery of this added substance is determined and used to correct the result. Such recovery expts. are employed with success in the estimation of aneurine in flour,[4] but have proved less satisfactory with other foodstuffs.[5]

In this communication quenching is demonstrated by calibration curves, the results being recorded by plotting against different concs. the optical density difference produced by doubling the concn. Since in the Spekker fluorimeter the diaphragm controlling the amount of light which reaches the fluorescing solution is calibrated logarithmically, the optical density difference produced by doubling the concn. should be log 2 or 0·301 if no quenching occurs, and the quenching which does occur can be expressed as a percentage by means of the formula: $100-(50\times\text{antilog. of the optical density difference})$.

CALIBRATION OF FLUORIMETER CELLS—Before constructing calibration curves it is necessary to calibrate the fluorimeter cells. In the Spekker fluorimeter 3 types of cells

Through the North of England Section.

have been employed: (1) with loose lids, (2) and (3) with sealed lids, containing respectively stoppered spouts or stoppered orifices for filling. Type (1) is the commonest. Types (2) and (3), which were introduced at the request of one of us,[6] possess the advantages that they can readily be filled without spilling, that the solns. they contain can be stabilised against oxygen quenching by bubbling nitrogen through the cell, and that the lids cannot be interchanged. Since a considerable amount of the dilution quenching is due to reflection of ultra-violet light from the lids of cells (see Fig. 1), interchanging of these lids may lead to

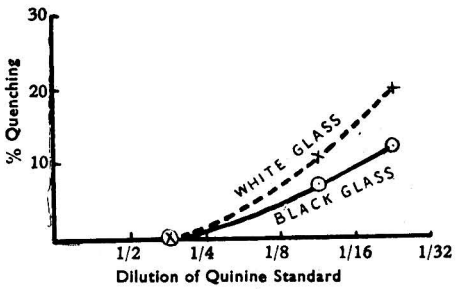

Fig. 1. Dilution quenching caused by reflection from lids of fluorimeter cells. Broken curve obtained with ordinary glass lids; continuous curve obtained with black glass lids.

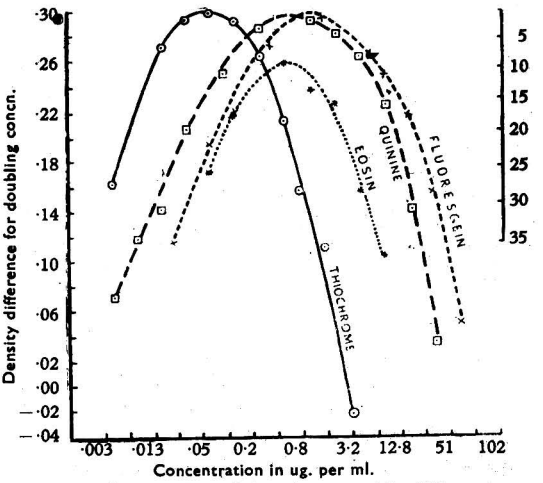

Fig. 2. Calibration of fluorimeter with different fluorescent substances. Decrease in density difference as concn. increases due to concn. quenching and as concn. decreases due to dilution quenching. In absence of quenching density difference should remain constant at 0.301 (log. 2).

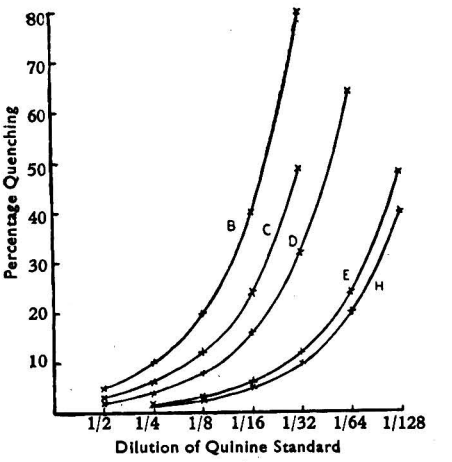

Fig. 3. Quenching caused by fluorescent impurities in different samples of *iso*butanol.

TABLE I—CALIBRATION OF FLUORIMETER CELLS

Cell	Mean value as % of B	Average error %	Coeff. of variation of mean value
Loose lid			
A	102·4	2·4	1·3
D	108·6	8·6	1·1
F	108·7	8·7	1·4
Spouts			
B	100·0	—	—
C	100·1	0·1	1·0
G	100·0	0·0	1·4
Orifices			
H	106·8	6·8	1·5
I	109·1	9·1	0·4
J	104·8	4·8	1·8

errors. Data obtained when calibrating cells of these 3 types with quinine and with thiochrome in normal concentrations show that variations as high as 9% may occur between different cells (see Table I). All new cells should therefore be calibrated with the kinds of solns. being studied. It would seem best that such calibrations should form part of the testing of all fluorimeters before they are sent out by the manufacturers.

CALIBRATION CURVES WITH DIFFERENT SUBSTANCES—Having calibrated a series of 9 fluorimeter cells, we used the most accurate of these (B, C and G) for constructing calibration curves with solns. of different quinine salts in $N/10$ sulphuric acid. Our results with quinine sulphate, plotted as density difference against concn., are given in Fig. 2.

This also includes calibration data we obtained with eosin, which has been recommended as a standard in riboflavin estimations,[7] with fluorescein (used by Cohen[8] to calibrate his fluorimeter) and with thiochrome obtained from aneurine.* The curves all show marked concentration and dilution quenching, which might exceed 10% in the comparison of 2 solns., one of which possessed a fluorescence intensity 2 or 3 times that of the other, a difference often encountered in practice. The quenching can be diminished by choosing concentrations near the top of the curves, as in fact occurs in the method of Nicholls *et al.*[9] for estimating aneurine in flour, but there may still be a considerable amount of such quenching when the fluorescence of the blanks is being measured. The occurrence of this quenching with quinine or with thiochrome has been observed by various workers,[10,11,12,13] using visual or photoelectric methods. Since these results had a bearing on the fluorimetric estimation of vitamin B_1 we studied the quenching in greater detail.

QUENCHING BY *Iso*BUTANOL FLUORESCENCE—*Iso*butanol, as obtained from the manufacturer, always exhibits some fluorescence in ultra-violet light. This fluorescence can be considerably diminished by fractional distillation in all-glass apparatus, but we have not yet succeeded in obtaining a sample entirely free from fluorescence. Moreover, the more often *iso*butanol is used and recovered, the more difficult it is to remove the fluorescence. These findings have been confirmed on a series of samples obtained from other laboratories, including all that had collaborated in the paper by Nicholls *et al.* The fluorescence intensity of each sample was estimated against a series of very dil. quinine solns., the weakest of which was Q/128 containing 0·008μg of quinine sulphate per ml. In order to measure the very low fluorescence intensities we had to use 3 or 4 neutral glasses before the left-hand photocell and to increase the sensitivity of our galvanometer by placing it at a distance of 3½ m from the scale. The results summarised in Table II

TABLE II—MEASUREMENT OF FLUORESCENCE IN DIFFERENT SAMPLES OF *Iso*BUTANOL

Sample	Mean optical density against the following quinine standards				Dilution of Q to which I is approx. equivalent
	Q/16	Q/32	Q/64	Q/128	
A as received (Q. at zero) ..	0·016	0·202	0·401	0·565	Q/15
	I	Q	Q	Q	
distilled by sender ..	0·087	0·144	0·299	—	Q/22
,, ,, ,, F ..	0·072	0·151	0·318	—	Q/20
,, ,, ,, F ..	0·043	0·140	0·309	0·469	Q/21
	I		Q	Q	
B ,, ,, ,, ..	0·254	—	0·113	—	Q/40
,, ,, ,, F ..	0·266	—	0·118	—	Q/40
,, ,, ,, F ..	0·202	—	0·110	—	Q/40
	I	I	Q	Q	
C ,, ,, ,, ..	0·341	0·080	0·043	—	Q/55
,, ,, ,, F ..	0·414	0·119	0·044	—	Q/58
,, ,, ,, F	0·290	0·056	0·107	0·215	Q/60
	I	I		Q	
D ,, ,, ,, ..	—	0·114	—	0·063	Q/85
,, ,, ,, F ..	—	0·199	—	0·088	Q/87
,, ,, ,, F ..	0·362	0·154	—	0·055	Q/95
	I	I	I	I	
E ,, ,, ,, F ..	0·412	0·210	0·113	—	Q/250
	I	I	I	I	
F ,, ,, ,, F ..	0·525	0·310	0·145	0·039	Q/250
G ,, ,, ,, F ..	0·578	0·442	0·210	0·061	Q/300
H ,, ,, ,, F ..	0·575	0·353	0·163	0·073	Q/320

Notes—Samples marked F examined with blue filter in front of right-hand photo-cell.
Q, obtained with quinine standard at zero.
I, obtained with *iso*butanol standard at zero.

showed wide variations in the fluorescence of *iso*butanol being used by different workers, which might lead to considerable quenching with blanks. Allowance for such quenching can be made by means of calibration curves obtained with the given batch of *iso*butanol,

* These curves summarise a very considerable number of experimental data. For example, each point in the quinine and thiochrome curves is based on the mean result of 4 separate assays, in each of which sufficient readings were taken to ensure that the differences between the results are statistically significant. The detailed data have been omitted in order to save space.

but for critical work it would seem preferable not to use frequently recovered *iso*butanol. It is of great importance to store all the solvents in glass stoppered bottles. "AnalaR" methanol and ethanol, when supplied in corked bottles, have been found to exhibit marked fluorescence, and should therefore be redistilled in all-glass apparatus.

QUENCHING BY ULTRA-VIOLET LIGHT—Thiochrome fluorescence is gradually quenched by irradiation[14] (see Fig. 4). This difficulty is overcome by matching the thiochrome

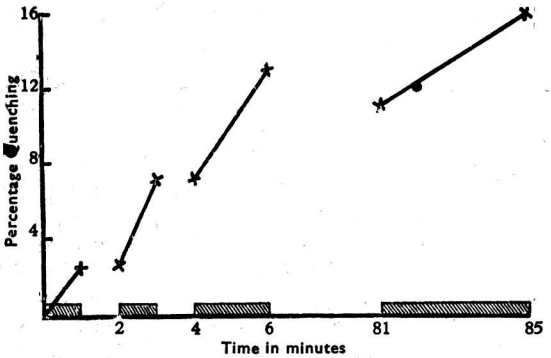

Fig. 4. Quenching by ultra-violet light. Shaded sections of time scale indicate periods during which the thiochrome soln. in fluorimeter cell was exposed to radiation from the fluorimeter lamp with diaphragm at full aperture. 75 mins. elapsed between third and fourth exposures.

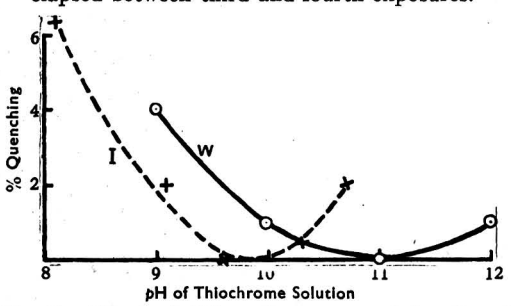

Fig. 5. Effect of *p*H on fluorescence intensity of thiochrome solns. in water (continuous line) and in *iso*butanol containing traces of water (broken line).

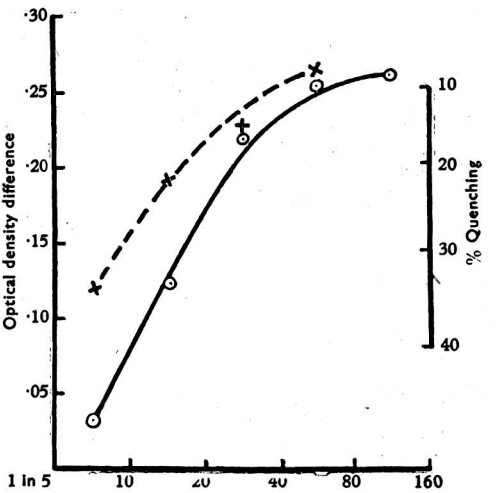

Fig. 6. Reduction of quenching by dilution and by washing with *iso*butanol. Broken curve obtained when estimations made at different dilutions by ordinary methods; continuous curve obtained when quenching substances have been partially removed by washing aqueous extract with *iso*butanol. If no quenching occurs density difference should be 0·301.

fluorescence against the more intense fluorescence of quinine. The zero is obtained on the fluorimeter with not more than 20 or 30 sec. irradiation, readings are taken with the quinine solns. at leisure, and a quick check is made on the zero. Thus the thiochrome is exposed to not more than 40 or 60 sec. total irradiation, which causes no significant quenching. Thiochrome solns. should be stored in glass-stoppered amber bottles and stabilised with nitrogen as described below. The fluorescence of quinine solns. may be gradually affected by irradiation, but under usual conditions the degree of quenching is not serious.

OXYGEN QUENCHING—Thiochrome solns. in *iso*butanol are gradually decomposed by exposure to air. This is due to atmospheric oxygen and can be prevented by their storage in nitrogen-filled containers, under which conditions no diminution in fluorescence was observed after 3 weeks. Our results on the stabilising effect of nitrogen were confirmed by Drs. S. K. Kon and S. Y. Thompson.

EFFECT OF *p*H ON THIOCHROME FLUORESCENCE—Fig. 5 summarises data obtained on this effect, using thiochrome prepared from aneurine by the usual method,[4] but varying the proportion of sodium hydroxide to alter the *p*H, as determined with glass electrode and agar bridge. The results show that, to avoid significant quenching, the *p*H should be between 9·5 and 10·5. This will normally require 0·05 to 0·3% of sodium hydroxide in the *iso*butanol, a concn. usually obtained in the above methods, but subject to alteration by presence of buffering substances or of substances affecting the distribution coefficient of sodium hydroxide between water and *iso*butanol.

QUENCHING BY IMPURITIES—This can be diminished by washing the aqueous extract with *iso*butanol or by dilution. Fig. 6 shows the effect of both these methods when applied to malt extract, which is subject to marked impurity quenching. Correction for impurity quenching may be made by means of recovery expts.; whilst these have proved quite satisfactory with materials such as flour,[4] they need modification when applied to certain other materials.

SUMMARY—Fluorescence measurements in general are complicated by various factors which diminish (quench) the fluorescence, causing too low results. *Concentration quenching* due to a high concn. of fluorescent molecules, may be avoided by dilution, but this, if carried too far, will lead to *dilution quenching*, due either to instrumental or olvent causes. This can be reduced by re-designing the instrument (*e.g.*, so as to minimise reflection of ultra-violet light from the sides or lid of the cell) or by careful purification of solvents (*e.g.*, *iso*butanol, methanol, ethanol), but in practice some dilution quenching is unavoidable. Fluorimeter cells give varying results and should be calibrated with the soln. being tested. Quenching of some substances, *e.g.*, thiochrome or riboflavin, by ultra-violet light must be prevented by a careful technique for taking readings and by using more stable standards, such as quinine or eosin. Oxygen quenching can be avoided by stabilising the solns. with nitrogen. The effect of *p*H on thiochrome fluorescence has been studied. Quenching due to the *p*H deviating from the optimal range of 9·5 to 10·5 should be avoided by ensuring that the *iso*butanol extract contains a suitable concn of sodium hydroxide (between 0·05 and 0·3% according to experimental conditions, including presence of buffering substances). Quenching by impurities can be diminished by washing with *iso*butanol and by dilution. Correction for such quenching by recovery expts. may involve difficulties with certain materials.

We wish to thank all the authors of the ANALYST paper,[9] also Drs. E. R. Dawson, A. Green, L. J. Harris, G. V. James, S. K. Kon, R. A. Morton and Y. L. Wang for advice and co-operation. We are indebted to Dr. T. A. Henry for specimens of quinine salts and to Messrs. Merck & Co. of Rahwah, N.Y., for a specimen of thiochrome.

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OVALTINE RESEARCH LABORATORIES
KING'S LANGLEY, HERTS.

April, 1943

Determination of the Volume of Gas Contained in the Particles of Dried Milk Powder

BY M. M. MUERS, PH.D., F.I.C., AND E. B. ANDERSON, M.Sc., F.I.C.

I. INTRODUCTION—It is now well known that the particles of spray-dried milk powder consist partly of hollow spheres of varying size and density. In connection with an investigation into the packing of such powders it became necessary to ascertain the volume and composition of the gas contained in the particles. The only method previously described was that employed by Lendrich,[1] later slightly modified by Hermann,[2] in which the change in volume (contraction) of a suspension of milk powder in benzene or alcohol was determined after its solution by addition of aqueous ammonia. As volume differences of 0·5–2·0 ml only were being measured, the description of the apparatus used, a 100 ml

measuring cylinder, did not suggest that the accuracy was such as we desired. Moreover, Lendrich says—"the milk particles are dissolved to such an extent that the enclosed air is liberated." In absence of experimental proof, this statement is open to question. The figures found by Lendrich varied from 0·1 to 1·4 ml/g and by Hermann from 0·2 to 0·3 ml/g.

II. EXPERIMENTAL—An obvious method for the purpose in view is, using suitable apparatus, to dissolve the particles completely and collect the gas liberated. This is essential when an analysis of the gas is also required. This led to a study of ebullition technique, as a result of which the first method described below was evolved.

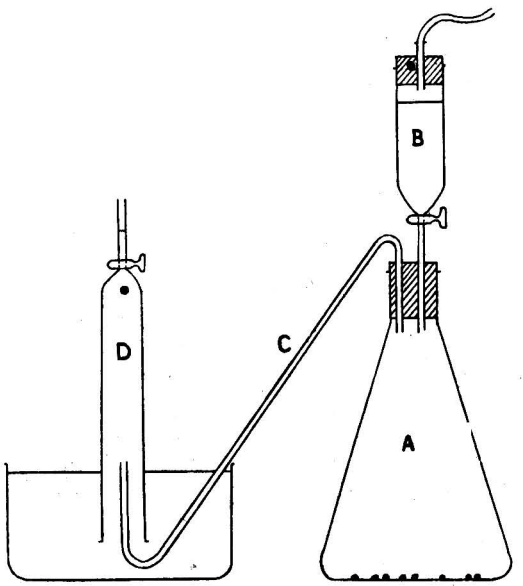

Fig. 1

(i) *Ebullition Method*—Fit a 500-ml conical flask A with a 50-ml dropping funnel B having a 5-mm bore tap and with a delivery tube C which dips into a small trough filled with water and provided with a gas collecting tube D. Carry out a blank with each test as follows.

Fill the flask completely with water, add a few glass beads to prevent bumping, and rinse the funnel into the flask with a few ml of *n*-propyl alcohol. Boil the water vigorously until 150–200 ml have been expelled or evaporated; a test tube inverted over the outlet of C prevents splashing. Place 30 ml of *n*-propyl alcohol in the funnel and the gas collecting tube full of water over the end of C. Run the alcohol continuously into the flask during ½ min., applying gentle pressure by mouth to B by means of the attachment (Fig. 1), to prevent the steam escaping into B. Continue boiling for a total time of 2 min. from the time D is placed over the outlet. Transfer the gas in D to a gas burette and, after agitating with water to remove alcohol vapour, measure and analyse in any appropriate way.

Make the actual determination in exactly the same way, using a well-stirred suspension of 20 g. of powder in 25 ml of *n*-propyl alcohol, taking care to break down lumps and eliminate entangled air bubbles. Introduce the suspension through the funnel B into A, as soon after preparation as possible, in successive small amounts with minimum intervals, otherwise the stem of B may become blocked. Finally rinse B with 5 ml of the alcohol. The powder dissolves. Collect the evolved gas and measure and analyse as before, correcting for the blank.

Duplicates agree within 5–10% and the limit of accuracy is 0·03 ml/g. *n*-Propyl alcohol was chosen because it combines the required properties of minimal solvent action on this type of powder, low density, miscibility with water and b.p. just below that of water.

The objections to the method are (*a*) the relatively large blank, (*b*) the difficulty of obtaining bubble-free suspensions with low-density powders, (*c*) the manipulation of introducing the suspension is not easy, (*d*) if the powder has been packed in CO_2 this is partially lost by solution in the water of the collecting apparatus, and (*e*) gas, especially CO_2, dissolved in the fat phase of whole milk powders is liberated together with the enclosed gas. For these reasons attention was directed to a density method.

(ii) *Density Method**—For hollow particles of density d with solid shells of density D the volume of the hollow space is $\frac{(D-d)}{Dd}$ ml/g.

For determining d the main problem is the selection of the liquid to be used. It should have (*a*) a density less than that of the powder, (*b*) minimum solvent or other action, (*c*) low viscosity and good wetting power. After several trials *n*-propyl alcohol was

* This paper had been prepared for publication before receipt of the *Journal of Dairy Research*, 1943, **13**, 162–215, in which Lea *et al.* derive another formula for calculating the gas content of spray-dried powders.

found most nearly to fulfil these conditions, as, apart from the other properties, it has little action on spray milk powder in less than 30 min. contact; beyond this time a little fat is extracted and some gas liberated.

For the actual determination, use a 25-ml sp.gr. bottle at 25° C.; adjust to this temp. as rapidly as possible, but great accuracy is not necessary. Duplicates agree within 5%.

Determination of D for full cream (26% fat) gas-free milk solids gave a range of 1·26–1·28, mean 1·27; skim milk solids gave 1·46, which is in good agreement with Stamberg and Bailey's[3] figure of 1·459. Taking the D/25° C. of butter fat as 0·920, and the figure of 1·459 for skim milk solids, the calculated value for full cream milk solids is 1·266.

Difficulties were again experienced with certain low-density powders having particles with so much enclosed gas that they were lighter than the alcohol.

III. RESULTS—Table I gives typical results for three types of full cream powder in the range within which all other samples fall. Samples 1–5 are normal powders produced by the Milkal process, 6–10 low-density powders, and 11–14 very high density ones. Comparison of results obtained by the two methods shows fair agreement, except for some low-density samples, which contained dissolved CO_2 and gave high values by the ebullition method.

TABLE I

Expt. No.	Gas, ml/g N.T.P.	
	Density method	Ebullition method
1	0·10	0·15
2	0·07	0·10
3	0·11	0·09
4	0·07	0·10
5	0·09	0·05
6	0·35	0·29
7	0·31	0·65
8	0·23	0·38
9	0·19	0·56
10	0·23	0·68
11	0·04	<0·05
12	0·03	<0·05
13	0·02	0·06
14	0·03	<0·05

These figures are, of course, average values for the enclosed gas in a sample. Reference has already been made to difficulties experienced with certain powders containing light particles, and one such was separated into a light and heavy fraction using *n*-propyl alcohol as the vehicle and centrifugal action for the separation. The results which are of interest appear in Table II.

TABLE II

	Gas, ml/g N.T.P.	
	Density method	Ebullition method
Original powder	0·39	—
Light fraction	0·88	0·88
Dense fraction	0·14	0·14

SUMMARY—The necessity having arisen for determining the gas content of spray-dried milk powder particles, a search of the literature failed to reveal a method of sufficient accuracy for the purpose in view. Two methods have been worked out, one depending on the liberation of the gas on complete solution of the particles, the other on a determination of their density. From a manipulative point of view the latter is far superior, but if an analysis of the gas is required also, the former is recommended.

The gas content of the powders examined ranged from 0·02 to 0·35 ml per g. The results obtained suggest that for normal powders the figures quoted by previous workers are high.

Our thanks are due to the Directors of United Dairies, Ltd., for permission to publish.

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The Determination of Cobalt and its Separation from Nickel by the Phosphate Method

By W. R. SCHOELLER, Ph.D., F.I.C.

The analytical text-book methods for nickel and cobalt include: electrolytic determination of nickel *plus* cobalt; separation of cobalt as the double nitrite; electrolytic determination of the separated metals; pptn. of cobalt with nitroso-β-naphthol; pptn. of nickel with glyoxime. The cyanide titration for nickel is not always given; other methods are usually ignored.

Neither the purity of the electrolytic deposit nor the completeness of the deposition[2] can be taken for granted, and appropriate tests are required. The nitrite method needs practice, and even in skilled hands has inherent defects. The double nitrite must be pptd. from conc. soln., which leads to occlusion of nickel and calls for re-treatment; the two ppts. should be left to deposit overnight, and both the ppt. and combined filtrates must be freed from nitrite. I concur with Gregory and Stevenson,[3] who discourage the use of this tedious method. Nitroso-β-naphthol is not a pure reagent; it adheres to the glassware, which must be cleaned with strong sulphuric acid. Its cobalt ppt. is too bulky for rich material, it gives off poisonous fumes when heated, and the ignition product (in which I can usually detect weighable traces of nickel) is not quite, though almost, definite in composition; with small ppts. this error is negligible.

This paper describes the methods I have gradually elaborated for the determination of cobalt and its separation from nickel. They are not those prescribed by the text-books; nevertheless I find them at least as reliable, and since they are quicker in execution and more easily acquired, their description should be of practical interest. As in my work on tantalum and niobium[4], I apply only simple classic methods; the only special reagent used is dimethylglyoxime.

A. Determination of Nickel—For substantial quantities I always use Moore's cyanide titration,[5] in my opinion one of the most elegant volumetric methods and at least as accurate as electrolysis. Smaller amounts may be weighed as the oxime complex, and if only a few mg the complex may be wrapped in the wet filter and ignited to oxide.

B. Determination of Cobalt—The experience of many years has led me to modify my earlier views on the advantages of volumetric over gravimetric methods,[6] and I now use the simple gravimetric methods given below, to the exclusion of volumetric ones.

Substantial amounts of cobalt I always weigh as pyrophosphate obtained by ignition of cobalt ammonium phosphate. The latter is an almost ideal ppt. for gravimetric work and its ignition product is perfectly definite in composition and hence superior to the oxide, sulphate and metal (electrolytic or reduced from the oxide pptd. by alkali). The double phosphate is soluble in acids and insoluble in neutral or feebly ammoniacal soln.; but its solubility increases with the ammonia concn., complete pptn. requiring a rather delicate adjustment. To secure quantitative results without adjustment, Schoeller and Powell[7] simply add a slight excess of ammonia and recover the remaining trace of cobalt from the filtrate as sulphide. The introduction of this simple step has induced Evans[8] to include the phosphate method among those "not quite good enough when one requires great accuracy." This criticism can hardly be based on actual experience. I need only say that our combination procedure has been constantly used for over 20 years, and that considerable transactions are based on its results. I find it simpler than, and as accurate as, electrolysis —which also involves an ammonium sulphide treatment, *i.e.*, of the spent electrolyte.[9]

Phosphate-sulphide procedure—Treat the neutral soln. (0·1–0·3 g Co; 150 ml) with 10 ml of hydrochloric acid and 10–20 ml of 10% diammonium phosphate soln. Heat to *ca.* 80° C., add ammonia (1 : 1) until the blue amorphous ppt. partly re-dissolves and the liquid becomes slightly purple. Stir the boiling-hot soln. until the ppt. is converted into lilac crystalline cobalt ammonium phosphate. Set aside on a water-bath for *ca.* 10 min., with occasional stirring, collect the ppt. on a No. 41 Whatman filter, wash with cold water and transfer the wet paper and ppt. to a tared porcelain crucible. Dry on an asbestos mat, heat to char the paper and complete the ignition on a triangle. Cool, cautiously crush any lumps with a smooth glass rod, re-ignite with the full flame of a Bunsen burner, and weigh as $Co_2P_2O_7$ (major cobalt fraction).

Minor cobalt fraction: treat the hot filtrate in the pptn. beaker with 2–3 ml of yellow ammonium sulphide and a little creamed filter fibre, and allow the ppt. to flocculate on a water-bath, leaving the liquid perfectly clear. Collect and wash with warm water containing ammonium sulphide and nitrate, and ignite wet in porcelain, finally over a strong burner. Weigh as Co_3O_4. This fraction normally weighs less than 0·002 g.[10]

C. Separation of Nickel and Cobalt—John Clark's phosphate method,[11] strongly recommended by Hope[12] after several years' daily use, is given in Grossmann's monograph[13] with the comment that it yields satisfactory results after some practice. Double pptn. can be effected in under 30 min., the products being a cobalt ppt., which is ignited and weighed, and a nickel filtrate suitable for direct cyanide titration.[14] The only valid objection against the adoption of this simple and rapid method for accurate work is that total cobalt pptn. is difficult without slight co-pptn. of nickel, whilst pptn. of the cobalt free from nickel normally entails a slight solubility loss of cobalt. This is due to the peculiar course of the reaction, which hinges on the ammonia concn.: (1) neutralisation of the acid phosphate soln. produces at first an amorphous ppt. of cobalt and nickel phosphates. Should the addition of ammonia now be interrupted and the hot soln. well stirred, the ppt. gradually becomes crystalline and total pptn. of (Co, Ni) NH_4PO_4 ensues; the ppt. may be ignited to, and weighed as $(Co, Ni)_2P_2O_7$ (see Expt. 4). (2) The amorphous nickel phosphate readily dissolves in more ammonia, the liquid becoming blue; the ammoniacal nickel soln. is stable. (3) Additional ammonia causes partial soln. of the cobalt ppt., the soln. becoming purple. (4) A large excess of ammonia completely dissolves the cobalt ppt.; this soln. is unstable, the bulk of the cobalt crystallising out as the double phosphate while the balance is oxidised to a cobaltammine salt. (5) At stage 3 the amorphous cobalt ppt., still slightly contaminated with nickel, readily changes to the crystalline double phosphate, insol. in ammonia. To remove the co-pptd. nickel, Clark dissolves the ppt. in acid and repeats the operation. (6) The ammoniacal nickel filtrate, when neutralised or evaporated, yields pale green crystalline nickel ammonium phosphate.

I succeeded in working out rapid separation processes based on the above reactions, yielding accurate results without delicate adjustments or re-pptn., by using glyoxime to complete the separation. It may be argued that this reagent by itself brings about a perfect separation, but actually it has a limited range of applicability, the nickel ppt. being too bulky for any but small quantities, while substantial amounts of cobalt interfere by forming a stable glyoxime complex[15]; moreover, the method takes no account of the cobalt determination, which is the object of my procedure.

D. Small Amounts of Nickel from much Cobalt: Crystallisation Method—This surprisingly simple method excels where direct glyoxime pptn. is unreliable,[16] as in the determination of traces of nickel in the weighed cobalt pyrophosphate, or in metallic cobalt or its salts. Clark's phosphate method usually gives a poor nickel recovery here, the amorphous cobalt ppt. still retaining nickel during its conversion to the crystalline double phosphate. I overcame the difficulty simply by pouring the cobalt soln. into a cold ammoniacal phosphate soln. This gives a clear wine-red liquid which, when heated, becomes lilac, while cobalt ammonium phosphate gradually crystallises out. This treatment is so efficient that no re-treatment of the cobalt ppt. is required; a little cobalt is oxidised to cobaltammine salt, which colours the filtrate pink. Pptn. of the nickel glyoxime is not retarded.

Procedure—Pour the cold, feebly acid soln. (1 g of Co) into a cold soln. (200 ml) of ammonium phosphate (5 g) and chloride (5 g) and strong ammonia (25–30 ml); heat to boiling. Should an amorphous ppt. appear on heating, add more ammonia to dissolve it. Stir to prevent bumping as crystallisation proceeds. Keep boiling for 1–2 min., set aside on a water-bath for 15 min., filter on loose-textured paper and wash with cold water. Slightly acidify the filtrate with acetic acid, ppt. with glyoxime and proceed as usual.

Expt. 1. *Rapid Determination of Nickel in a Cobalt Salt*—A soln. of 1·0006 g of cobalt ammonium sulphate was poured into 100 ml of soln. containing 1 g each of ammonium phosphate and chloride and 10 ml of strong ammonia. Further procedure as above gave 0·0060 g NiO ≡ 0·47% Ni. Time taken: 1 hr. Re-treatment of the double phosphate ppt. by the same procedure: the filtrate from the second cobalt ppt. gave no glyoxime ppt. after 24 hrs. standing.

Expt. 2. *Test Separation*—4·035 g of cobalt pyrophosphate, obtained from doubly re-crystallised cobalt ammonium phosphate, dissolved in 10 ml of hydrochloric acid (1 : 1):

0·0104 g of standard nickel ammonium sulphate (≡ 0·00154 g Ni; Co-Ni ratio, 1058). This mixture was poured into 250 ml of soln. containing ammonium phosphate (2 g) and chloride (3 g) and 30 ml of strong ammonia. Found, 0·0019 g NiO ≡ 0·00149 g of Ni; error, −0·05 mg. Time taken: 2½ hrs.

E. SUBSTANTIAL AMOUNTS OF BOTH METALS: PHOSPHATE-GLYOXIME METHOD—This combines the speed of the phosphate separation with the accuracy of the glyoxime method. It consists in a single pptn. of cobalt ammonium phosphate, giving a filtrate free from cobalt (major nickel fraction) and a slightly nickeliferous ppt., which is ignited to $(Co,Ni)_2P_2O_7$. The weighed ppt. is treated by the crystallisation method (*D, supra*), and the nickel in the small glyoxime ppt. is titrated with cyanide and calculated to $Ni_2P_2O_7$; cobalt is found by difference. The titrated liquid is added to the major nickel fraction, and the titration is continued for total nickel. The whole operation takes 4–5 hrs.

The *procedure* for the pptn. of the double phosphate is given under *B*, but greater care is required in the addition of ammonia, since total cobalt pptn. must be achieved at this stage, whilst the amount of co-pptd. nickel does not affect the final result. Any cobalt passing into the nickel filtrate would count as nickel and be detected with certainty, as it imparts a slight brown tint to the (otherwise straw-yellow) titrated cyanide soln. The weighed pyrophosphate is digested with hydrochloric acid for the conversion of the phosphoric acid into the ortho form; the other steps do not require further description.

Expt. 3. *Test Separation*—Taken: 0·1237 g Co, 0·1193 g Ni. Found: 0·3246 g $(Co, Ni)_2P_2O_7$ containing 0·0070 g Ni ≡ 0·0174 g $Ni_2P_2O_7$. $Co_2P_2O_7$ found, 0·3072 g ≡ 0·1241 g Co: error + 0·0004 g. Total Ni found, 0·1196 g: error + 0·0003 g. Time taken, 4¾ hrs.

Expt. 4. *Total pptn. of Nickel and Cobalt*—Taken: 0·0880 g Co, 0·1042 g Ni. Ammonia (1 : 1) added drop by drop to the hot, slightly acid phosphate soln. until the smell became evident; heated and stirred until the bluish amorphous ppt. became a dirty pink and crystalline. Filtered after 10 min. digestion on the water-bath. Found 0·4767 g $(Ni, Co)_2P_2O_7$ (pale grey). Calculated 0·2179 g $Co_2P_2O_7$ + 0·2587 g $Ni_2P_2O_7$ = 0·4766 g.

F. SMALL AMOUNTS OF COBALT FROM MUCH NICKEL: PERSULPHATE-PHOSPHATE METHOD—For the determination of small amounts of cobalt in presence of much nickel (*e.g.*, in nickel salts), the gravimetric method given below is as sensitive as pptn. by nitroso-β-naphthol; the rapid colorimetric method is suitable for serial work, but its sensitivity does not exceed 0·5 mg of Co per 100 ml. The assay is set aside overnight to promote the complete pptn. of the nickel; otherwise the actual manipulations for the gravimetric determination require only 3–4 hrs.

The process is based upon conversion of the cobalt into cobaltammine salt and pptn. of nickel as the double phosphate. Clark, who devised the method, treated the soln. with excess of bromine, ammonia, and hydrogen peroxide to complete the oxidation.[17] In my experience this mode of working gave low cobalt recoveries, which I ascribe to incomplete conversion into the cobaltammine complex; by conducting the oxidation with persulphate, however, I secured satisfactory results.

Procedure—Treat the neutral or feebly acid soln. (100–150 ml; 1 g Ni) with ammonium phosphate (5 g) and chloride (5 g), add a soln. of 2 g of potassium persulphate in 40 ml of ammonia (1 : 1), heat to 80° C. and stir in 2 g of persulphate. Boil for 1 min., cool in running water, partly immerse a strip of litmus paper in the soln. and cautiously add hydrochloric acid (1 : 1) to neutral reaction. Set aside overnight; filter on loose paper and wash with cold water.

(*a*) *Colorimetric*—Transfer an aliquot part of the purple filtrate to a Nessler tube and match the colour against that of a soln. containing a known amount of cobalt which has been treated like the assay, or that of a standard soln. of chloropentamminecobaltic chloride (see Expt. 8). The quantity of nickel left in the filtrate is much too small to affect the colour (Expts. 5–7).

(*b*) *Gravimetric*—Treat the warm filtrate with a little ammonium acetate and about 10 ml of glyoxime reagent, set aside for ½ hr. and filter off the small ppt. Briskly boil down the filtrate in a 1000-ml beaker, adding 10 ml of strong nitric acid after expelling the alcohol. To the conc. soln. add potassium chlorate in two 0·5 g portions. Boil down until a blue colour (cobaltous chloride) appears, cool, transfer to a 250-ml. beaker, dilute, treat with ammonia, yellow ammonium sulphide and creamed filter fibre, etc. (as under *B*, minor cobalt fraction); ignite to, and weigh as, Co_3O_4.

Expts. 5–7. *Test Separations*—The nickel was added as nickel sulphate AnalaR with a declared cobalt content of 0·0005%.

Exp.	Ni, g added	Co, g taken	Co, g found	Error, g	Ni in Co-filtrate, g
5	2·090	none	trace	—	0·0171
6	1·045	0·0101	0·0101	0·0000	0·0031
7	1·045	0·0010	0·0010	0·0000	0·0106

The last column gives the nickel found by glyoxime pptn. in the filtrate from the double phosphate.

Expt. 8. *Colorimetric Standard*—Pure chloropentamminecobaltic (purpureocobaltic) chloride is readily prepared from cobalt chloride,[18] which need not be pure. The reddish-violet crystalline ppt. is washed with dilute hydrochloric acid, and dried. Analysis: 0·5007 g, gently ignited in a porcelain crucible, then dissolved in dilute hydrochloric acid, gave 0·2869 g $Co_2P_2O_7$ and 0·0015 g Co_3O_4 by Method *B*. Found: 23·37% Co. Calculated for $(CoCl(NH_3)_5)Cl_2$: 23·53%. Use an aqueous soln. of 0·0425 g in 100 ml.

G. SEPARATION OF COBALT AND NICKEL FROM IRON—A further useful application of phosphate in cobalt and nickel analysis is the pptn. of ferric phosphate from slightly acid acetate soln.; the ppt. does not adsorb cobalt or nickel, hence a single pptn. effects a separation. The process was recently described by North and Wells.[19] I can recommend it, having evolved it 10 years ago as a very convenient step in the routine analysis of commercial cobalt salts.

Procedure—Heat an aliquot portion of soln. (≡ 1 g of salt), add ammonium chloride and phosphate (2 g) and enough ammonia to produce a blue amorphous ppt., stir and cautiously add acetic acid until the ppt. dissolves, leaving a pink turbid liquid. Set aside on a water-bath until the white ppt. has settled, stir in filter pulp, filter, and wash with hot water containing a little ammonium acetate and acetic acid. Ppt. the cobalt in the hot filtrate with ammonia as under *B*, using a moderate excess. Filter, dissolve the ppt. in dil. acid and repeat the pptn. Ignite and weigh as $Co_2P_2O_7$. Treat the combined filtrates with ammonium sulphide (see *B*), and ignite the ppt. to Co_3O_4 + NiO. Fuse with bisulphate, leach, ppt. with glyoxime, and ignite the ppt. to NiO. Determine iron colorimetrically in the phosphate ppt. If copper is present, it should be pptd. with hydrogen sulphide in the original acidified soln. of the salt.

SUMMARY—Rapid and reliable procedures are described for the separation and determination of cobalt and nickel in any relative proportions, even with extreme ratios. The procedures are combination methods utilising ammonium phosphate and dimethylglyoxime. Substantial amounts of cobalt are determined gravimetrically as pyrophosphate, minute quantities as oxide after pptn. as sulphide. Iron can be conveniently separated from nickel and cobalt by pptn. as ferric phosphate from acetate soln.

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A Combined Chemical and Spectrographic Method for the Determination of Traces of Tungsten in Rocks

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If spectrographic analysis is to be used most effectively so as to justify preference over chemical methods, there are two principles to which due regard should be paid. First, a spectrographic method is most efficient in comparison with chemical analysis, if, as is usual, a large number of elements can be determined together with little more effort than is required for one. Secondly, one of the advantages of spectrographic analysis is that little time is expended in the chemical preparation of the samples. For the estimation of very low concentrations of an element, the minimum of chemical treatment is desirable so as to avoid contamination from reagents and vessels. However, there are occasions when one or other of these principles must be disregarded. There are great differences in the sensitivity of detection of the various elements, and it may not be possible to detect low concns. of certain ones without chemical concn. For the determination of a single element, colorimetric analysis will generally be chosen in preference to spectrographic analysis, but suitable colorimetric methods have not yet been evolved for all elements. For example, we have found[1] that, in rock analysis, strontium may be conveniently determined by a spectrographic method.

In the present investigation, it was found that spectrographic analysis could be used for the determination of tungsten in samples of schist, although the choice of a spectrographic method is not in accordance with the principles mentioned above. As there seems to be no colorimetric method suitable for the determination of small amounts of tungsten in rocks, the use of a spectrographic method was indicated, but preliminary trials showed that considerable concn. would be required. Even when silica had been removed by means of hydrofluoric acid, tungsten could not be detected when pellets of the residue were arced on graphite electrodes. It was then decided to ppt. tungsten together with titanium by means of organic reagents, and to determine tungsten spectrographically in the ignited titania. This method proved sufficiently sensitive.

Chemical Preparation—The chemical treatment used for the concn. of tungsten required the removal of silica, and solution of the rock sample by evaporation with hydrofluoric and sulphuric acids. The method for the pptn. of titanium was that devised by Moser, Neumayer, and Winter[2] (*cf.* Mitchell and Ward[3]). Titanium is pptd. by means of tannin and antipyrine. According to Moser and Blaustein,[5] in a method also made available by Mitchell and Ward,[4] tungsten is also pptd. by these reagents. However, it was thought better to be certain of the pptn. of tungsten, by using cinchonine as well (Schoeller and Jahn[6]).

The silica-free residue from 1 g of schist was taken up with water and a little sulphuric acid and rinsed into a beaker. Ten ml of conc. sulphuric acid were added and then 4 g of ammonium chloride, and the beaker was heated until solution was complete. The warm soln. (50° C.) was treated with 40 ml of a 10% soln. of tannin, and diluted to 400 ml. A soln. was prepared containing 10 g of antipyrine (phenazone), 5 g of cinchonine and 10 ml of hydrochloric acid. Five ml of this reagent were added with stirring. The orange-red ppt. was left over-night, and the clear liquid was decanted through a fluted filter-paper. Macerated filter-paper was added to the ppt., which was transferred to the filter and washed once. The wash soln. contained 5 g of ammonium sulphate, 5 g of antipyrine, 0·1 g of cinchonine and 0·5 ml of sulphuric acid in 100 ml.

The filter-paper was dried in a small silica crucible and ashed in a muffle furnace. Ignition was completed by addition of 1 : 1 nitric and sulphuric acids, followed by re-heating. It was hoped by careful heating to drive off excess of sulphuric acid, leaving sulphates, but the residue was very hygroscopic, probably owing to the presence of aluminium sulphate, and it was necessary to heat it to redness over a burner.

The weight of residue obtained ranged from 12 to 30 mg. As the samples contained 0·55–0·85% of titania, the amount of impure titania was sometimes more than 3 times as much as should have been obtained. For a spectrographic examination, pellets of the

residue were mixed with ammonium sulphate and arced on copper electrodes. It was concluded from a comparison of the spectrograms with those of standard mixtures that the heaviest residues contained titania, alumina, calcium sulphate and magnesium sulphate in the proportions 30, 67·5, 2·5 and 0·5% respectively.

A soln. was made up containing the nitrates of aluminium, calcium and magnesium, which on ignition with sulphuric acid would give alumina together with calcium and magnesium sulphates in the above proportions. Sufficient of this soln. was added to each of the residues to bring the weight up to 30 mg after ignition. An equal weight of ammonium sulphate was then added, and the mixture was made up into 10-mg pellets.

Standards were required with different contents of tungsten for comparison with the samples. For this purpose solns. were made of about the average composition for the main constituents of the soln. obtained by digesting 1 g of schist with hydrofluoric and sulphuric acids. In particular, each contained 10 mg of titanium dioxide, added as a soln. of sodium titanate. Measured amounts of a standard tungsten soln., containing 0·01 mg of tungsten per ml, were added to each. The same procedure as described for the samples was followed, and the residues were made up to 30 mg in the same way.

Spectrographic Method—The electrodes were graphite rods, 6·5 mm in diameter. The lower, negative electrode was sharpened to a diam. of 4 mm and a hole was made in the end into which the pellet (3 mm in diam.) was fitted, so as to be level with the surface. The upper electrode was sharpened to leave an end surface 3 mm in diam. The pellet was burned for 60 sec. in a short D.C. arc, the electrode separation being 1·5 mm. The initial current was 3 amp., and this was raised to 5·5 amp. after 10 sec., and to 7 amp. after the next 30 sec.

The Hilger Automatic Large Quartz Spectrograph was used. Uniform illumination of the spectrograph was obtained by projecting an image of the arc on the prism with a short-focus quartz lens (F_v 7 cm). In order to reduce random variations in the intensity, each spectrogram was the result of the super-imposed exposures of three separate arcings. A single spectrogram for each sample was first obtained, and this required half the concentrate available. After determination of the tungsten by comparison with standards on the same plate, spectrograms for the remainder of the material were again made by super-imposing either 2 or 3 arcings, according as the tungsten content was relatively high or low. The plates used were Ilford Zenith, developed for 5 min. in ID2.

A suitable wavelength range for the instrument was 2400–3200A. The line 2947·0 is suitable for the determination of tungsten. The tungsten line 2944·4 is liable to interference if there is any iron to give the iron line 2944·4A. Another line that is suitable if the content of tungsten is not very low is 2551·2A. This line is not listed in the table of the lines of tungsten given by Brode,[7] but it has been found to be a useful one.

Estimates of the content of tungsten were made by comparing the spectrograms with those of standard samples. Ten standards were made, and in these the content of tungsten in the equivalent of 1 g of schist ranged from 0·0008 to 0·013 mg. Spectrograms of both samples and standards were made on the same plate, but, to facilitate comparison, the spectrograms of the samples were together towards the bottom of the plate, and the standards together towards the top. After development, the plate was cut in half lengthwise. When the two halves were placed on a Judd Lewis Comparator, it was possible to compare the spectrogram of a particular sample with that of any one of the standards. The difference between two standards was *ca.* 50% of the higher standard. As it was possible to say whether the intensity of the line in the spectrogram of a sample was the same as that of the line in the standard, or was intermediate between the intensities of lines of two standards, the contents could be estimated within 25%. Estimates from different plates, or by different observers on the same plate, did not always agree to this extent, but the content reported was the average obtained by two observers on several plates.

Analytical Results—The method described was used to determine tungsten in 33 samples of schist collected on lines cutting across well-known scheelite-bearing lodes of the Glenorchy District, New Zealand.

The limit of detection was about 0·00007%, and in only 2 of the samples could no tungsten be detected. Most of them contained from 0·0001 to 0·0002%, and this seemed to be the normal content of the schist. There were a few samples with a content up to 0·0015%, and a single sample with over 0·0025%. No relation could be found between the tungsten content and the distance from the lode at which the sample was taken. The

full results will be discussed by R. W. Willett[8] in the Geological Survey Bulletin on the Glenorchy Subdivision.

The analyses were carried out at the request of the Director of the Geological Survey, Dr. J. Henderson, who has kindly allowed us to use the data. Thanks are due to Mr. R. L. Andrew Director, Dominion Laboratory, for permission to publish the paper.

ADDENDUM—Since this paper was prepared a spectrochemical method for the determination of traces of tungsten in ores has been published by Scobie.[9] In his method tungsten is collected by pptn. of aluminium hydroxide. By using a 10-g sample, 0·00002% of tungsten can be estimated. However, the preliminary chemical treatment is much longer and more complicated than that described here, and the method would not give sufficient concentration with rocks that contain a large proportion of aluminium oxide.

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Notes

ESTIMATION OF ADDED CALCIUM CARBONATE (CRETA PRAEPARATA) IN NATIONAL FLOUR

IN an earlier paper[1] three methods of estimating Creta Praeparata in National flour were described, and of these, the third method of direct determination as carbonate was preferred inasmuch as it avoided the necessity for a control flour sample containing no added Creta; in fact, generally speaking, it is impossible to obtain a sample of the control flour. During the past year more than 11,000 flour samples have been tested in these laboratories by the carbonate estimation method and, in the light of experience gained in working the method as a routine, certain modifications designed to improve reliability and convenience have been adopted.

The original method, as published, provided for the aeration of an acidified flour batter with CO_2-free air at the rate of 50 ml of air per min. for 1 hr. The air stream was subsequently dried over calcium chloride, and carbon dioxide from the reaction of calcium carbonate and hydrochloric acid was absorbed in a weighed soda-lime tube.

The following modifications in procedure are now employed. (1) The time of aeration has been reduced from 60 to 30 min. (2) The useful life of the absorption tubes is markedly prolonged by substituting soda asbestos for soda lime as an absorbing agent. (3) The drying agent in the absorption tubes ($CaCl_2$) was found to require frequent renewal if moisture losses were to be avoided. Phosphorus pentoxide has proved more useful for this purpose. In consequence, P_2O_5 tubes are also fitted on both sides of the absorption tube in the drying train. (4) To prevent any possibility of acid spray being carried from the reaction vessel to the drying train, small splash traps have been interposed in the system. (5) It has been found more satisfactory to aerate the flour batter by blowing air through it and through the drying and absorption train rather than by sucking air through the system. To this end, a small diaphragm pump has been mounted on the baseboard of the apparatus and is operated by an arm attached to the reciprocating board.

The apparatus at present in use carries 12 reaction vessels, one of which is used for the estimation of the reagent blank in each batch of samples. The apparatus is found to be extremely convenient and rapid in use, as may be judged by the fact that two operators can carry out estimations at the rate of about 100 per day.

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A MODIFIED CERIC PROCESS FOR THE DETERMINATION OF SERUM CALCIUM

CALCIUM in serum is usually determined by the Clark and Collip[1] procedure of centrifugal separation as calcium oxalate. This is washed with ammonium hydroxide, and dissolved in dil. sulphuric acid, and the soln. is titrated with potassium permanganate. A micro-filtration technique described by Holt and Callow[2] also includes a final permanganate titration. In laboratories where serum calcium is determined only at

irregular intervals this entails the preparation each time of fresh $N/100$ potassium permanganate and standardisation against freshly prepared $N/100$ sodium oxalate.

A sulphuric acid soln. of ceric sulphate is much more stable than permanganate soln., so that its use is advantageous. Moreover, in ceric titrations there is only one valency change, Ce^{IV} to Ce^{III}, during oxidation. Willard and Young[3] mention that the yellow colour of dilute ceric solns. can be made the end-point "indicator," but no reference has been found to the adoption of this finding on the micro-scale.

More complicated cerate processes have been described for determining the oxalate radical. Thus, Larson and Greenburg[4] add excess of $M/100$ ceric sulphate soln., leave for 30 min., and titrate back with $M/200$ ferrous ammonium sulphate soln., with phenanthroline ferrous sulphate as indicator. Reitemeier[5] has recently described the Smith and Getz[6] procedure of titrating calcium oxalate at room temp. in perchloric acid soln. with ammonium hexanitrato cerate and nitroferroin indicator. In view of the simplicity of the Willard and Young procedure, an investigation was made of the accuracy of the visual yellow end-point on the micro-scale.

PREPARATION OF $N/100$ CERIC SULPHATE—Prepare an $N/10$ soln. from anhydrous ceric sulphate by solution in N sulphuric acid, as described by Scott.[7] Dilute with 2% sulphuric acid, when required, to $N/100$ soln.—bright yellow in colour. This may be checked occasionally against $N/100$ sodium oxalate by the method given below.

METHOD—Calcium is separated as oxalate in accordance with the Clark and Collip centrifugal procedure. In this laboratory duplicate 2-ml samples are taken and the calcium oxalate is pptd. by leaving overnight. Precipitation and titration are carried out in 5-ml conical sedimentation tubes graduated in 0·1 ml.

To the pad of calcium oxalate ppt. add 2 ml of N sulphuric acid. Dissolve by heating at 70° C. in a water-bath, agitating with a narrow ball-end glass stirring rod. Add the $N/100$ ceric sulphate drop-wise from a 2-ml micro-burette graduated in 0·01 ml, returning the tube periodically to the water-bath to maintain the contents at 70° C. At the end-point there is a definite yellow colour which persists when the tube is returned to the water-bath. The judging of the end-point is greatly facilitated by titrating over a white tile and having for comparison two sedimentation tubes, one containing 3 ml of distilled water and the other 3 ml of N sulphuric acid + 1 drop ($\equiv$ 0·04 ml) of $N/100$ ceric sulphate; the latter has a distinct yellow colour when viewed vertically from above. The recorded titration value, less 0·04 ml, gives the amount due to the calcium oxalate present. One ml of $N/100$ ceric sulphate is equiv. to 10 mg of calcium per 100 ml of serum when 2 ml of serum are taken for analysis. The normal range of calcium in human sera is 9 to 11 mg per 100 ml, *i.e.*, the titration value usually obtained lies within the limits 1 ± 0·1 ml of $N/100$ ceric sulphate.

REPRODUCIBILITY OF RESULTS—Six 2-ml portions of a bulk sample of horse serum were examined by this method, with the following results:

Calcium, mg per 100 ml of serum: 10·5, 10·5, 10·2, 10·3, 10·5, 10·5.

Permanganate titration gave 10·4 mg of calcium per 100 ml.

EXACTNESS OF END-POINT—To determine how far the visual end-point had exceeded the actual end-point the six tubes referred to above were quickly cooled, and their contents were titrated back with $N/100$ ferrous ammonium sulphate, phenanthroline ferrous sulphate being used as indicator. $N/100$ Ferrous ammonium sulphate required: 0·02, 0·02, 0·02, 0·02, 0·03, 0·03 ml, *i.e.*, within the 0·04 ml correction applied.

COMPARISON WITH PERMANGANATE TITRATION RESULTS—Samples of human sera have been examined by both titration procedures, using freshly standardised $N/100$ permanganate each time, and the same stock solution of $N/10$ ceric sulphate. Good agreement (± 0·2 mg of calcium per 100 ml) has been obtained during a period of 3 months.

SUMMARY—A ceric sulphate process for titrating calcium oxalate obtained in the Clark and Collip procedure for serum calcium determination is described and compared with the permanganate titration. It is shown that the visual yellow end-point is accurate and that the standard stock $N/10$ soln. of ceric sulphate is stable over a long period.

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W. R. SMITH

R.N. MEDICAL SCHOOL

September, 1943

SOME NEW FLUORESCENCE REACTIONS

A 0·001% soln. of acenaphthene-5-carboxylic acid in conc. sulphuric acid gives a strong greenish-yellow fluorescence in ultra-violet light when gently heated with formaldehyde. To carry out the test, put 0·1 ml of test soln. in a small test-tube, add 1 ml of reagent, mix and heat until the liquid begins to darken. Leave for 2 min., cool, examine under the ultra-violet lamp, then dilute with 4 ml of water, cool and re-examine. In presence of 4μg of formaldehyde in 0·1 ml of test soln. a dull greenish-yellow and a greenish yellow fluorescence are observed in the conc. and dil. liquids, respectively. Other aldehydes do not react, nor do the various natural gums and carbohydrates, dextrins, monohydric alcohols or formic, citric or oxalic acid. Tartaric acid, carbitol, glycerol and ethylene glycol give a similar reaction when 0·1 ml of a 0·1% soln. is taken for the test, and with these compounds the examination of the conc. reaction mixture is of some assistance in distinguishing between them, as they show yellowish-blue, bright, slightly greenish-yellow and a golden-yellow fluorescence, respectively The fluorescence colours shown on dilution are all similar to that given by formaldehyde.

The splitting off of formaldehyde from polyhydroxylic compounds in Malaprade's reaction, by the use of periodic acid in the cold, cannot be employed in conjunction with the above test to detect this class of compound, as the iodine compounds in the soln. interfere with the fluorescence reaction. By treating 0·4 ml of a soln. of certain polyhydroxylic compounds with a crystal of ammonium persulphate and 2 drops of 1 : 4 sulphuric acid on a boiling water-bath for 5 min., then adding 1 ml of the above reagent, boiling for 5 sec., cooling and examining under the lamp, the following results were obtained.

Substance	*Concentrated reaction mixture*	*Diluted reaction mixture*
Formaldehyde	Bright greenish-yellow	Strong, deep greenish-yellow
Citric acid	Bright strong yellow-green	Bright greenish-yellow
Gum Senegal or arabic	Dull reddish-brown	Strong, dull, bluish-green (like lubricating oil)
Gum tragacanth	Bright greenish-yellow	Weak greenish-yellow
Gum ghatti	Strong greenish-brown	Powerful bluish olive-green
Manucol V	Bright yellow	Very faint milky blue
Carbitol	Powerful yellowish-green	Weak milky blue

Under these conditions ethylene glycol, glycerol, tartaric acid, glucose, sucrose, starch, dextrin (yellow potato) and oxalic acid give dull shades of greenish-blue in the conc. reaction mixtures, whilst in the diluted state only faint milky-blue fluorescence colours are shown. Formic acid shows a pronounced bluish-green and a strong lavender-blue fluorescence in conc. and dil. reaction mixtures, respectively.

These results are obtained by using 0·4 ml of the solns. containing 1 g of the various compounds per litre and, although a positive result cannot be regarded as specific, it has been found a useful confirmatory test for the various compounds in absence of others in the group.

My thanks are due to I.C.I. (Dyestuffs) Ltd. for permission to publish this note.

11, Wilton Road
Crumpsall, Manchester, 8

J. A. Radley

September, 1943

Official Appointments

The following amendments to the list of Public Analysts appointed by Local Authorities with the approval of the Ministry of Health were notified by the Ministry on December 21st, 1943.

Authority	*Public Analyst*
St. Helens County Borough	John Graham Sherratt (Deputy)
Colne Borough	Malcolm McFarlane Love
	John Linley Wilson (Deputy)
Leigh Borough	John Robert Stubbs
Oxford County Borough	Alan West Stewart
Reigate Borough	Daniel Donald Moir (Additional)

Ministry of Food

STATUTORY RULES AND ORDERS*

1943—No. 1497. The Feeding Stuffs (Maximum Prices) Order, 1943. Dated October 22, 1943. Price 10d. net.

This Order consolidates the Feeding Stuffs (Maximum Prices) Order, 1942, and its amending Orders, and makes minor amendments *relating mainly to prices and conditions of sale.* Feeding stuffs sold for industrial purposes, raticides and other vermin destroyers are exempted from the Order (Art. 9).

Schedule II, giving data on which basic prices are fixed, is important to agricultural analysts. Thus the basic prices for bone flour or meal, decorticated cottonseed cake or meal, decorticated groundnut cake or meal, hempseed cake or meal and sunflower cake or meal depend on the albuminoid and oil contents, whereas the price of linseed cake is based upon the oil content only (i.e., up to 8% or over 8%). For fish meals (other than herring meal) the albuminoid content is related to the oil content, whereas for herring meal the amount of oil is immaterial.

— **No. 1593. The Fish Cakes (Maximum Prices) Order, 1943. Dated November 9, 1943.** Price 1d.

This Order prohibits the manufacture or sale of fish cakes containing less than 25% by weight of fish or weighing less than 2½ ozs. In any proceedings for infringement of the Order relating to the composition of fish cakes a certificate signed by or on behalf of the Government Chemist or the Government Chemist for Northern Ireland or a Public Analyst shall be sufficient evidence of the facts stated therein, unless the other party requires the person who made the analysis to be called as a witness. Inconsiderable variations in the weight or fish content of any single fish cake are to be disregarded and the average weight or fish content of a reasonable number (not less than 5) is to be taken. If the fish cakes are sold otherwise than by the piece the average fish content of a sufficient quantity of similar fish cakes to bring the total quantity up to 1 lb. net shall be taken.

* A summary of some Orders. Italics signify changed wording. Obtainable from H.M. Stationery Office.

Notes from the Reports of Public Analysts

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

CITY OF BIRMINGHAM: REPORT FOR THE THIRD QUARTER, 1943

Of the 1124 samples submitted under the Food and Drugs Act, 4 were taken formally.

Curry Powder—A sample contained about 11% of common salt. The packers, in reply to a letter, stated that in their experience and that of the importers salt was a normal constituent of such powders. To this a reply was sent to the effect that of 16 samples examined since January, 1940, only this sample and one other had contained salt, and the makers of the latter admitted that it was incorrectly compounded. Moreover, grocers' publications, pharmaceutical works and cookery books agreed that curry powders should be a mixture of spices. In a further letter the importers cited 14 recipes from *The Grocer's Manual*, 3 of which contained salt. Reference to this publication showed that even in these formulæ the amounts of salt were only 1·5–3·7%. The reference in the *Manual* also deprecated the fact that "much of the curry powder sold here simply consists of ground coriander seed, turmeric, cayenne and fenugreek, mixed with a large proportion of farinaceous matter and salt."

"Lemon-flavoured Beverage".—Samples bought from 3 different shops were roughly powdered and not homogeneous. Analyses showed them to contain 76–77% of citric acid, 5–7% of salt, and 21–22% of magnesium sulphate (calculated as Epsom Salt, B.P.). Information given by the Soft Drinks Section of the Ministry of Food showed that the formula supplied by the makers specified 1·3% of salt but made no reference to magnesium sulphate. The makers explained that they had introduced Epsom Salts with a view to improving the flavour. They were asked to include on the label the information that magnesium sulphate was an ingredient of the powder.

Soft Drinks—Of 6 samples (lemonade and ginger beer) taken 5–6 weeks after the appointed date under the Soft Drinks Order, 1943, not one had the correct composition. Another sample was sold under a name ("Jaffa Squash") not recognised by the Order. Further samples are being taken from the same manufacturers.

H. H. Bagnall

Legal Notes

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

MILK WITH ADDED CREAM

Analysts will be interested in details of cases brought before New Zealand Courts, where suppliers to dairy factories have been prosecuted for falsifying their milk by adding cream to it. This raises the fat content so that they thereby obtain a higher pay-out than they are entitled to when the milk is used for cheese-making. One such case was successfully brought before the Magistrate's Court in December, 1935, at Otaua, Auckland, added water being associated with the extraction of skim milk.

In another case, at Palmerston North, in June, 1941, the milk sent by a supplier to a cheese factory was found to have excessive fat in his can of evening's milk, amounting on successive days to, *e.g.*, 12·3, 12·1, 12·9, 10·2%; the corresponding cans of the respective following morning's milk showed 5·2, 5·4, 4·8, 4·6% of fat.

On two separate occasions a Dairy Division Inspector visited the farm and, after watching the cows being milked, procured a sample from the can containing all the evening's milk. The same can was again sampled on delivery to the factory next morning. Babcock tests on these samples were done in the factory by the Inspector, and on each occasion the fat content of the sample from the can as delivered to the factory was much higher (each was 7·0%) than that of the sample taken from the can the previous evening (5·8% and 5·3%).

These tests appeared to provide a basis for prosecution, but the case was prepared without the assistance of a competent analyst. The defendant pleaded ignorance about addition of any cream or butter-fat, and this, together with other unforeseen lines of argument brought forward by the defence, led to the failure of the prosecution. In summing up, the Magistrate questioned the reliability of the Babcock test, and emphasised the lack of an analyst's certificate dealing with the samples in question. Even without this, but with fuller evidence about the reliability of the Babcock test and about other aspects of the case, the prosecution might have succeeded, especially as the essential evidence was linked up with the well-established principle of English law known as the "Appeal to the Cow."

In another case, dealt with by the writer, samples were procured in bottles containing 0·1 g of mercuric chloride preservative per pint of milk. After a first sample had shown small amounts of both added cream and added water, a second sample yielded the following results.

Sp.gr. at 15·5° C., 1·022; f.pt., −0·46° C.; acidity (as lactic acid), 0·153%; fat (Gerber method), 11·1%, (Werner–Schmidt method), 11·08%; total solids (gravimetrically), 19·40%; solids-not-fat, 8·30%; casein (by formol method), 2·42%, (by pptn. and Kjeldahl), 2·44%; casein/fat ratio, 0·22.

Added cream is indicated by the low sp.gr., by the very high fat content in contrast to the low solids-not-fat, and by the very low casein/fat ratio (the normal range is 0·50–0·70). Besides addition of cream, addition of water is indicated also by the very low sp.gr., low solids-not-fat, and by the f.pt. being −0·46 instead of −0·55, representing about 16% of added water. It would appear, therefore that in order to cover up the addition of a large amount of cream the supplier had also added a considerable amount of water. Put in another way, this supplier removed a substantial amount of skim milk and delivered the residue to the factory and thereby deprived the factory of the essential cheese-making constituents of the skim milk. Apart from the amount of water added to the milk, calculations indicated that for every 100 lbs. of whole milk which the supplier had available to bring to the factory, he brought only about 33 lbs., having removed about 67 lbs. of skim milk. (The skim-milk was fed to calves.) Examination of the fat for refractive index and Reichert–Polenske values showed it to be pure butter-fat.

In presenting the case, the practice of paying for milk for cheese-making on its butter-fat content was considered in some detail, along with the cheese yield obtained from milk of varying fat content. Milk of low fat content regularly makes more cheese per lb. of fat than milk of high fat content, owing to the higher proportion of casein present in low-fat milk. Milk of higher fat content usually has a higher casein content, but not in same proportion. The increase in casein content lags behind increase in fat content, so that as the fat content is higher, the casein/fat ratio becomes lower, and the yield of cheese per lb. of fat becomes lower. If the important amount of casein contained in skim milk is removed from what is supplied to the factory, the expected yield of cheese cannot be obtained and the equity of the pay-out is thereby upset. Owing to the high *normal* fat content of the defendant's milk (from Jersey cows), he would in the ordinary way get a shade more for his milk than he was entitled to on its cheese-yielding capacity. If he adds cream to his milk, *which is the same thing as removing skim milk*, this position of over-payment would be accentuated.

It has been held that addition of water, which is a normal constituent of milk, renders the milk "not pure"; so, too, it would seem that addition of other milk constituents, *e.g.*, butter-fat, casein or milk sugar, to "pure milk" should render the resultant product "not pure." Addition of butter-fat in the form of cream or abstraction of skim milk amounts to one and the same thing, and this addition of cream to the milk would render the milk "not pure" as defined in Sec. 2 of the Dairy Industry Act, 1908, which reads as follows: " 'Pure milk' means the whole of the milk (including what is commonly known as the 'strippings') drawn at time of milking; but does not include milk containing less than three per centum of butter-fat, or mixed with any preservative or chemical matter of any kind."

When the case was heard in the Court at Greytown in July, 1942, before the same Magistrate who had heard the preceding similar case, the defendant's counsel admitted the facts, but submitted argument on the meaning of the term "pure milk" as defined in the Act.

In a reserved decision, after quoting authorities to emphasise that "pure milk," as defined in the Act, means the whole of the milk, the Magistrate stated: "Nevertheless his act in abstracting the skim-milk and adding cream did constitute an infringement of the statute, for there was an abstraction by the process adopted, of the casein, and other solids-not-fat. . . . When these essentials in cheese-making are extracted or reduced, the amount of cheese which can be produced from a given quantity of milk is also reduced. The evidence tendered is to the effect that when milk is to be used for cheese-making the casein content is nearly twice as important as the fat content in assessing the amount of cheese which can be made from it. . . . The milk in one sense was 'pure'—that is free from impurities; and also it was richer in quality as regards fat; but what the defendant supplied was not 'pure milk' within the meaning of the Act, as some of the constituents of natural milk had been extracted. Accordingly he must be convicted."

DAIRY DIVISION'S LABORATORY
WALLACEVILLE, WELLINGTON, N.Z.

GEORGE M. MOIR

The British Pharmacopoeia, 1932

NOTICE has been given of the following alterations and amendments in THE SCHEDULE.

CONFECTIO SULPHURIS—Tincture of Orange may be omitted.

EXTRACTUM COLCHICI CORMI LIQUIDUM—Liquid Extract of Colchicum Corm contains 0·3% w/v of colchicine (limits 0·27–0·33%) [Preparation from powdered Colchicum Corm and 60% alcohol is described].

Assay—Evaporate 20 ml to dryness on a water-bath and complete the assay as directed under "Colchici Semen" beginning at "wash the residue into a separator with 20 ml of a 20% w/v aqueous soln. of *sodium sulphate*" and including the modification described under "Colchici Cormus."

Preparation—Tinctura Colchici.

Doses—0·12 to 0·3 ml. 2 to 5 minims.

Liquid Extract of Colchicum Corm contains in 0·3 ml 0·0009 g, and in 5 minims about 1/70 grain of colchicine.

EXTRACTUM COLCHICI LIQUIDUM—When Liquid Extract of Colchicum is prescribed, or demanded, Liquid Extract of Colchicum Corm may be dispensed, or supplied.

MISTURA SENNAE COMPOSITA—Aromatic Solution of Ammonia may be used, in place of Aromatic Spirit of Ammonia and a mixture of 1 part of Conc. Compound Tincture of Cardamom and 3 vols. of water may be used, in place of Compound Tincture of Cardamom, in making this Mixture.

PARAFFINUM LIQUIDUM—The requirement "remains clear when dried, cooled to 0° and kept at that temperature for 4 hours (limit of solid paraffins)" is deleted.

TINCTURA COLCHICI—Liquid Extract of Colchicum Corm may be used, in place of Liquid Extract of Colchicum, in making this Tincture.

TROCHISCI—A mixture of 1 vol. of Conc. Tincture of Tolu and 3 vols. of water may be used, in place of Tincture of Tolu, in making lozenges.

British Standards Institution

BRITISH STANDARDS SPECIFICATION OF TEST SIEVES (B.S. No. 410)*

A REVISION of B.S. No. 410 for Test Sieves has been issued. Whilst making no fundamental changes in the B.S. series of meshes, it deals with the question of the tolerances on the apertures. As a result, modified tolerances on "average" and maximum apertures, more evenly graded throughout the series, have been adopted. An additional tolerance, termed the "intermediate tolerance," has been introduced and the max. % of apertures that may exceed this tolerance has been specified. To meet special requirements,

* Obtainable from Publications Dept., 28, Victoria Street, London, S.W.1. Price 2s. post free.

e.g., in research and control work, a second series of "Special Test Sieves" with more stringent tolerances has been designed for manufacture after the war.

For the present revision, wire cloth has been retained for the medium series, but the manner of expressing tolerances on the apertures has been brought into line with that now adopted for the fine mesh sieves. The coarse mesh series has been extended to include a selected number of larger apertures.

An appendix, prepared in consultation with the National Physical Laboratory, indicates the type of projection apparatus required for examining the aperture widths and wire diameters of fine and medium mesh sieves, describes in detail the method of measurement, and specifies the number of apertures that must be measured to ascertain if a sieve conforms to the requirements of the specification. General information on other sieves in common use is given in a second appendix.

Safety in Mines Research Board

THE DETERMINATION OF CARBON DIOXIDE IN MINE DUSTS

THE Coal Mines Regulations (M. & Q., Form 128, p. 8) prescribe that mine dust, sampled according to the Regulations, shall contain incombustible matter in a proportion depending upon the volatile matter of the coal.

	Not exceeding						Exceeding
Average volatile matter, %	20	22	25	27	30	32	35	35
Min. incombustible matter required, % ..	50	55	60	65	68*	70*	72*	75*

* The effect of the temporary amendment to Regulation 10 is to substitute the figure of 65%.

The Regulations also state (p. 10) that the carbon dioxide shall be determined as follows. "B (iii). A weighed quantity of the sieved dust shall be treated with dil. hydrochloric acid in a suitable apparatus, and the weight of carbon dioxide evolved from the dust shall be either (*a*) determined directly, or (*b*) calculated from the vol. of carbon dioxide evolved, or (*c*) determined by any other manner approved by the Board of Trade."

Form 128 also gives details of methods of: (i) volumetric determination by means of a calcimeter; (ii) volumetric determination by back titration with standard alkali soln.; (iii) gravimetric determination by means of a Schrödter flask; (iv) gravimetric determination by drying the gas evolved on boiling the dust with acid and passing it into a suitable absorbent in a weighed vessel (see also Paper 101, Safety in Mines Research Board).

The General Research Committee of the Monmouthshire and South Wales Coal Owners' Association in their Fourth Report (1942) publish a new and rapid method adapted from the procedure used by E. B. Hughes (*Chem. and Ind.*, 1942, **61**, 105) to determine carbon dioxide in baking powders by measuring the increase of pressure when the CO_2 is evolved in an evacuated space of fixed vol. An apparatus was constructed on these lines and found to give results that compared very favourably with those obtained by the other methods. The method has been tested in the laboratories of the Safety in Mines Research Board and in the Mining Equipment Testing Station of the Ministry of Fuel and Power, and the principle underlying it has been approved by the Board of Trade.

A simplified form of the apparatus is shown in Fig. 1. A is a reaction bottle (about 200 ml in capacity, fitted with a dropping funnel B and an outlet tube connected by stout rubber tubing with a drying tube C (containing calcium chloride or anhydrous silica gel) and a T-piece D, with connections to a mercury manometer E and a 3-way tap F which makes connection with either the atmosphere or a vacuum. (A simple water Sprengel pump is adequate.) The bottle A is immersed in water to keep the temp. constant. The manometer tube, which is about 18 in. by ¼ in., is fitted with a scale on which the carbon dioxide % can be read, in the following manner. Mark an arbitrary point *p* near the top of the manometer tube and bring the mercury to this point by suction; then close tap F. Drop 10 ml of a mixture of equal vols. of water and conc. hydrochloric acid (containing 2% of Perminal to ensure thorough wetting of the mine dust) into the bottle A. Mark the new level reached by the fall of the mercury in the manometer as the zero of the scale. Clean and replace the bottle after introducing 0·500 g of pure calcium carbonate, and evacuate the apparatus until the mercury in the manometer reaches the point *p*; then run in 10 ml of the acid mixture. When evolution of gas is complete mark the new level of mercury as 44%, the amount of CO_2 in calcium carbonate. Divide the scale evenly into 44 parts.

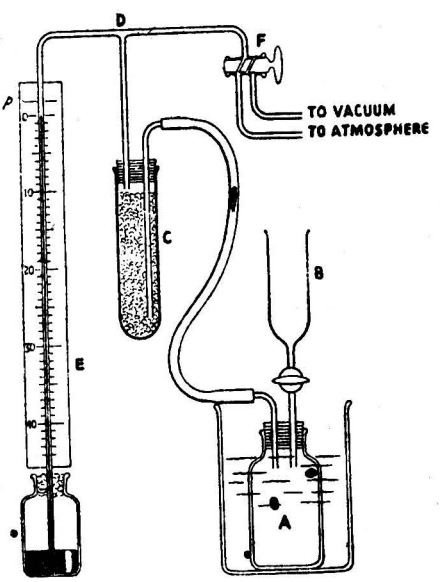

Fig. 1

For the determination of CO_2 in samples of mine dust use the same weight (0·500 g) as for the calibration. With calcium carbonate the reaction is complete almost as soon as the acid and dust have been shaken together, but magnesian limestone (commonly used as the incombustible dust in certain coal fields) and some other carbonates react more slowly than ordinary limestone. With such materials the reacting mixtures must be frequently shaken until the manometer reading is constant. Alternatively, the reaction vessel may be heated nearly to b.p. and then cooled to the original temp.; for this purpose, the bottle should be replaced by a flask and means taken to prevent loss of air and gas through the manometer.

In the Report of the General Research Committee (*supra*) an improved apparatus for more accurate work is described, and it is shown that it can be used for many other determinations in which the yield of gas can be measured. Thus it is suitable for the determination of urea, ammonium sulphate and carbides. Another probable use would be the evaluation of chloride of lime for A.R.P. and de-contamination work.

The Iron and Steel Institute

REVIEW OF THE WORK OF THE JOINT RESEARCH COMMITTEES, 1924–1943*

THIS review, initially prepared by Dr. W. H. Hatfield, F.R.S., summarises the work of the Joint Research Committees of the Iron and Steel Institute and the British Iron and Steel Federation. The Report opens with a complete list of the Special Reports already issued. It then, in an introductory section, describes the constitution and work of the four main Committees, each of which is dealt with in a separate section.

COMMITTEE ON THE HETEROGENEITY OF STEEL INGOTS—This Committee was originally established for the study of the problems of inclusions in steel and of the heterogeneity of steel ingots, but the scope now includes consideration of all matters affecting the structure and constitution of steel ingots. Some of the subjects have been entrusted to Sub-Committees for detailed investigation. They include *inter alia*, Pyrometry, Gases in Steel (O, H and N), successful methods for the determination of which have been evolved and are here outlined, Cleanness of Steel (including Count and Spectrographic Analysis) and Standard Methods of Analysis for Steel.

STANDARD METHODS—Reference procedures for the determination of sulphur, phosphorus and lead were first worked out to give results with a relative accuracy which might be not more than 0·005% and these have since been published by the British Standards Institution (B.S. No. 1121).

The Sub-Committee is now working on the development of methods for sulphur and phosphorus in highly alloyed steels. Two methods for the determination of tin in steel are under investigation—one based on nickel reduction and iodate titration, with controlled atmosphere, and the other depending on aluminium-antimony reduction.

To effect complete combustion of carbon in low-carbon ferro-chromium demands the use of finely divided samples, a temp. not less than 1100° C., and admixture of pure bar iron as well as of the use of additional flux, which may be strip lead or red lead.

It is hoped to establish methods for all the common steelmaking elements, which, when published as official British Standards, will provide a British equivalent of the official methods published by the American Society for Testing Materials.

This Section concludes with a full list (11 pages) of the Reports and Papers issued by the Committee.

THE ALLOY STEELS RESEARCH COMMITTEE—The work of this committee is complementary to that of the preceding committee (*supra*). An "arbitrary definition" of steels coming within the scope of the Committee's work is: "Carbon steels are regarded as steels containing not more than 1·5% of manganese and 0·5% of silicon, all other steels being regarded as alloy steels."

Among the investigations were the suppression of Phosphorus Brittleness, the Study of the Influence of Gases in Alloy Steels, Periodic Oscillation, Changes in Iron and Ferrous Alloys at Moderate Temperatures, Structure of Alloys, Corrosion and Fatigue.

Preparation of Pure Iron by Analytical Methods—A commercial pure iron was melted in a high-frequency furnace under very low pressure, excess of carbon or oxygen was removed by admitting hydrogen, and the product was melted *in vacuo*. The resulting iron contained: Ni, 0·06; Cr, 0·01; Cu, 0·03; lead, 0·02%; all other elements less than 0·02%. The iron was brittle.

The list of Reports and Papers issued by this Committee fills 4 pages.

THE STEEL CASTINGS RESEARCH COMMITTEE—The object of this Committee is to improve the quality of steel castings, with special reference to such defects as porosity, flaws, cracks, pits, etc. The list of Reports and Papers occupies 3 pages.

THE CORROSION COMMITTEE—The objects are to study the corrosion of ordinary steels as affected by variations in composition, methods of manufacture and conditions of use; also, to study corrosion problems arising from steam practice, including high temperature, and to investigate problems connected with rust-resisting and allied steels. The list of Reports and Papers occupies 5 pages.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Sugar in Apple Tissue. R. H. Leonard, R. C. Meade and R. B. Dustman (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 579–582)—Heat the weighed and sliced sample in a tared aluminium dish with a tight cover for 15 min. at 140° C. to soften the tissue, cool, transfer to the tared cup of a Waring Blendor with water and weigh to determine the water added. Comminute thoroughly in the Blendor and store as a bulk sample in the refrigerator. The advantages of using the Blendor instead of the hand mortar are its convenience and its saving of time. With mature, soft tissue the preliminary heating may be omitted. Extract 20–30 g in the Blendor with 1–2 g of calcium carbonate and *ca.* 150 ml of 95% alcohol. This results in the formation of a mixture containing more than 70% of alcohol and the separation of a flocculent ppt. Filter through paper on a Buchner funnel, return the paper and residue to the Blendor and extract again with *ca.* 100 ml of alcohol. Filter and wash the residue with alcohol until the filtrate measures 300 ml. Evaporate the filtrate on the water-bath with a little calcium carbonate to a vol. of 5–10 ml and dilute the syrupy residue with water to 100 ml. Clarification is not usually necessary. Determine reducing sugars, dextrose and laevulose by the combined procedure of Erb and Zerban (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**,

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246) and sucrose by the A.O.A.C. method (*Methods of Analysis*, 1940, 498, 504), after inversion with hydrochloric acid at room temp. Collect the cuprous oxide in a loose-bottom (Caldwell) crucible, place the crucible in a beaker containing 50 ml of ferric sulphate soln. (50 g of crys. ferric sulphate per litre, free from ferrous ions), dissolve the cuprous oxide, rinse and remove the crucible and add 20 ml of dil. sulphuric acid (1 + 7). Add 3 or 4 drops of a 0·2% soln. of diphenylamine in conc. sulphuric acid and titrate with potassium dichromate soln. (7·7135g per litre; 1 ml ≡ 10 mg of copper) until the liquid darkens. Add 5 ml of 85% phosphoric acid and 8 to 10 drops of the indicator. Dilute with water to 250 ml and complete the titration to the first permanent violet colour. If clarification of the syrupy residue of sugars is necessary, treat the soln. with 2 ml of sat. lead acetate soln., adjust the vol. to 100 ml and centrifuge. Remove lead from the clear liquid by centrifuging with sufficient dry powdered disodium phosphate. The results for cuprous oxide thus obtained are slightly higher than those obtained by applying the methods of the A.O.A.C. to apple tissue. The method is, however, sufficiently accurate for the determination of sugars in plant materials. A. O. J.

Determination of Cacao Tannin. E. C. Humphries (*Nature*, 1943, **152**, 569)—Duthie (ANALYST, 1938, **63**, 27) found that the filtrate from the cinchonine tannate method gave a ppt. on heating under reflux with formaldehyde and hydrochloric acid (Stiasny reagent). He suggested that the amount of this ppt. is a measure of catechin and similar phenolic compounds. Expts. in which freshly picked cacao beans were minced and allowed to autolyse showed that the amount of tannin, extracted with 40% acetone and pptd. by cinchonine sulphate, decreased progressively with time, and that the Stiasny precipitate of the filtrate decreased proportionally. A Stiasny determination on an aliquot part of the original tannin soln. yielded a ppt. approx. equal to the sum of the cinchonine tannin and the residual Stiasny ppts. These results suggest that the cinchonine sulphate combines with part of the tannin molecule, or that two substances are present, one yielding a ppt. with cinchonine sulphate and the other a Stiasny ppt. and that both substances decrease proportionally with time. The latter explanation seems the less likely. The amount of tannin found by the Löwenthal (A.O.A.C.) method was found to run parallel with that of the residual Stiasny determination, whilst the amount of the total substances oxidisable by potassium permanganate closely followed the changes in the amount of total Stiasny ppt., and the amount of reducing substances not fixed by gelatin followed the changes in the amount of tannin pptd. by cinchonine sulphate. It thus appears that with cacao, the cinchonine sulphate reacts with one part of the tannin mol., whilst gelatin reacts with the other. Stiasny determinations on the filtrate from solns. of cacao tannin treated with gelatin and sodium chloride confirmed this. The values obtained corresponded with the amount of tannin pptd. by cinchonine sulphate from the original solns. Hitherto the results obtained by the 2 above methods have shown little agreement, and the present observations offer an explanation and throw fresh light on the structure of the cacao tannin mol. The methods may be brought into agreement, so far as cacao is concerned, by taking into account the total Stiasny ppt., the residual Stiasny ppt. and the cinchonine sulphate ppt. in the one method, and the total reducing substances, the reducing substances fixed by gelatin and the reducing substances not fixed by gelatin in the other. Certain non-tannin substances oxidised by permanganate are present in the cacao bean, but these are very small in amount compared with the amount of tannin. These methods may be applied to the determination of tannin in other plant materials if the amount of non-tannin reducing material is known. J. G.

Palisade Ratios for the Official Drugs of the Labiatae Family. G. Ullrich (*Amer. J. Pharm.*, 1943, **115**, 196–206)—The palisade ratio (see Wallis and Dewar, *Quart. J. Pharm.*, 1933, **6**, 347) of the following were investigated—*Scutellaria lateriflora*, *Mentha piperita*, *Mentha spicata* (*viridis*), *Nepeta cataria*, *Thymus vulgaris*, and *Salvia officinalis*. The dried leaves were soaked in water until pliable, and segments, 2–3 mm square, were cut from the base, apex, margin and centre, cleared in a soln. of 50 g of chloral hydrate in 20 ml of water until almost transparent (4–15 days), mounted with Berlese Mountant (Wallis and Forsdyke, *Quart. J. Pharm.*, 1938, **11**, 700; distilled water, 10 ml; dextrose, 5 g; acacia, 8 g; chloral hydrate, 74 g; glacial acetic acid, 3 ml; in that order) under cover-slips, and examined at × 450 with direct light. All those palisade parenchyma cells whose half area or more came within the boundaries of the epidermal cells were counted, and counts were made under epidermal cells of approx. the same size and situated away from veins. Leaves of *Thymus vulgaris* were mounted whole, being too small to divide, while no determination was possible with *Salvia officinalis*, owing to the undulating hairy epidermis. The average ratio for each species was finally obtained as the result of 100 determinations (*viz.*, 5 leaves of each species, 4 sections from each leaf, 5 determinations of each section) which are set out in tables. These average values are: *Scutellaria lateriflora*, 6·4 (4·0–9·5); *Mentha piperita*, 6·3 (4·2–9·2); *M. spicata*, 6·2 (4·0–9·2); *Nepeta cataria*, 4·7 (2·5–6·5); *Thymus vulgaris*, 3·2 (1·0–5·7). It is concluded that the last two can be distinguished from each other and from any of the first three by a determination of the average palisade ratio, but that it is not possible to distinguish between the first three. J. A.

***Gigartina Decipiens* from New Zealand as Substitute for Irish Moss** (*Bull. Imp. Inst.*, 1943, **41**, 163–165)—The % composition of a dried New Zealand seaweed, *G. decipiens*, was: moisture, 14·3; proteins, 11·1; sol. in (*a*) cold and (*b*) hot water, 44·2 and 63·0; ash, 15·4. The arsenic (as As_2O_3) and lead contents were 4 and 2 p.p.m. respectively. Darkening on heating at 100° C. was at practically the same rate as that of Irish moss. The seaweed gave a fairly stiff jelly at a concn. of 6% equal to that of Irish moss at 4%. In composition it is of the same type, but appreciably superior in proteins (Irish moss protein *ca.* 7%). For use in brewing, manufacturers prefer Irish moss to contain not more than 2 p.p.m. of arsenic. Brewery trials were made by boiling portions of sweet wort with hops together with (*a*) no addition, (*b*) Irish moss and (*c*) *G. decipiens*. For this clarification of wort, the seaweed was superior to the Irish moss. The 4 p.p.m. of arsenic, though not harmful, is above the legal limit (1·42 p.p.m.). Like Irish moss, however, the seaweed could be diluted to the required standard by admixture with suitable material. E. B. D.

Biochemical

Critique of the Foreman Method for the Estimation of the Dicarboxylic Acids in Protein Hydrolysates. K. Bailey, A. C. Chibnall, M. W. Rees and E. F. Williams (*Biochem. J.*, 1943, **37**, 360–372)—The lime-ethanol method of Foreman (*Biochem. J.*, 1914, **8**, 463) for the estimation of aspartic and glutamic acids in protein hydrolysates, including the various modifications introduced by other workers, has been investigated. It has been shown that during treatment with lime the cystine undergoes partial dismutation to the sulphinic or sulphonic acids which are pptd. by the ethanol together with the calcium salts of the dicarboxylic acids and small amounts of cystine, tyrosine and serine. Since the calcium salts of these dismutation products are very insoluble and interfere with the estimation of aspartic acid as copper aspartate, it is desirable to prevent their formation. This is accomplished by removal of cystine as the cuprous mercaptide of cysteine before the hydrolysate is made alkaline with lime. To a portion of the hydrolysate, containing about 9 g of nitrogen per litre, maintained at 45–50° C., add slowly a suspension of cuprous oxide (washed free from inorganic ions and dried with alcohol and ether) in 500 ml of water, with rapid stirring, until an excess of cuprous oxide is present and the supernatant liquid is blue. Filter off the resulting ppt. and treat the filtrate by the lime-ethanol method of Foreman. The method has been further improved by introducing a second lime-ethanol treatment of the final mother liquor, since this was shown to contain both aspartic and glutamic acids, the calcium salts of which are appreciably soluble. It is believed that the results obtained by this modified procedure are accurate within *ca.* 2%. The possibility of applying solubility corrections to the dicarboxylic acid results from one lime-ethanol treatment has been investigated; by this method higher results have been obtained for casein and horse and cattle haemoglobin than those recorded in the literature. F. A. R.

Estimation of Amino Acids by the Solubility Product Method. S. Moore and W. H. Stein (*J. Biol. Chem.*, 1943, **150**, 113–130)—The estimation of certain amino acids, such as leucine, is difficult owing to the absence of characteristic functional groups. A method has now been developed for the estimation of amino acids and other substances which form sparingly soluble dissociable salts. The procedure is illustrated by reference to leucine, which forms such salts with 2-bromotoluene-5-sulphonic acid. Two aliquot portions of the soln., each containing A mM* of *l*-leucine are taken. To one is added a weighed amount of leucine-2-bromotoluene-5-sulphonate and, after equilibrium has been reached, the amount of salt (S_1 mM) which has gone into solution is measured. The leucine salt is dissolved to the point of saturation against the common ion effect of the A mM of leucine initially present. To the second aliquot portion R mM of 2-bromotoluene-5-sulphonic acid and a weighed amount of leucine sulphonate are added, and the solubility (S_2 mM) of the leucine salt is determined in this soln. at the same temp. In this second estimation the common ion effect of the added sulphonic acid further lowers the solubility of the salt. The total amount of sulphonic acid present in the first soln. is S_1 mM, and the total amount of leucine is $(A + S_1)$ mM. and the solubility product K_1 is therefore $S_1(A+S_1)$. Similarly, the solubility product K_2 in the second reaction is $(R + S_2)(A_1 + S_2)$. If the solubility product is constant under both conditions, as with leucine, then $K_1 = K_2$ and the amino acid in the sample $A = \frac{S_1^2 - S_2(R+S_2)}{(R+S_2) - S_1}$. If the values of K are not identical, as with glycine, a factor F, such that $K_1 = FK_2$, must be introduced, and the amino acid in the sample is represented by

$$A = \frac{S_1^2 - FS_2(R+S_2)}{F(R+S_2) - S_1}.$$

Thus to estimate the amount of amino acid an accurate estimation of the values of S_1, S_2 and R is required. Briefly, the apparatus used consists of a small flask, the mouth of which fits loosely into a small filter with a sintered glass plate, both these vessels being made to fit a centrifuge tube, where they are held in place by rubber washers. Micro-analytical technique must be employed for drying and weighing these vessels, counterpoise bottles being used in preference to brass weights. Aliquot parts of the amino acid solns. are pipetted into two flasks, and then the sulphonic acid is weighed into one flask and amino acid salt into both flasks. To obtain equilibrium between the liquid and solid phases in the flasks, the inverted centrifuge tubes are rotated in an inclined position in a cold room. The centrifuge tubes are then transferred to a centrifuge cup with a sheath of ice to maintain the temp. at zero during centrifuging, and the tubes are centrifuged to effect filtration of the mixture. The tubes are then allowed to reach room temp. and are removed, and the flasks, filters and filter plates are weighed before and after drying. A correction is applied for the weight of soln. adhering to the ppt. and the values so obtained are used to calculate the value of A, as described above. Like *l*-leucine, phenylalanine forms a sparingly sol. salt with 2-bromotoluene-5-sulphonic acid, but it does not interfere unless present in more than an equimolar proportion; tryptophan and histidine rarely interfere. For the estimation of glycine, 5-nitronaphthalene-1-sulphonic acid is satisfactory, the estimation being carried out for preference at 0° C. in *N* hydrochloric acid-methyl cellosolve (30%) soln. Hydroxyproline and arginine will interfere if present in more than 2 and 1·5 equivalents respectively, but phenylalanine, histidine and lysine do not interfere. The method has been used to estimate leucine in egg albumin, and glycine in silk fibroin, and the recoveries of pure amino acid added to the hydrolysates were quantitative in each instance. To secure satisfactory results it is essential to follow closely the experimental details given in the original paper which should, therefore, be consulted. F. A. R.

* mM = millimoles = molecular weight in milligrams.

Identification of Small Amounts of Organic Compounds by Distribution Studies. Application to Mepacrine. L. C. Craig (*J. Biol. Chem.*, 1943, **150**, 33–45)—Mepacrine can be readily estimated by colorimetric or fluorimetric procedures, the latter being the more sensitive. It is not specific, however, and closely related compounds, such as might occur in metabolism experiments, exhibit similar fluorescent properties. A method of identifying small amounts of mepacrine by measuring the distribution coefficient of the drug between two immiscible liquids has been developed. Transfer 25 ml of an ethylene dichloride soln. containing

20–100μg of mepacrine, the fluorescence of which has been determined, to a centrifuge separating funnel and add 20 ml of 0·001 *N* cacodylic acid buffer soln. (half neutralised with sodium hydroxide). Shake and adjust to 24° ± 1° C., centrifuge and withdraw a little of each layer for measurement of fluorescence. Calculate the concn. of mepacrine in μg per ml by means of concentration-fluorescence curves, determined experimentally in aqueous and ethylene dichloride solns. The ratio of the concns. in the ethylene dichloride and aqueous solns. is termed the "apparent distribution constant" K_a. Return the soln. quantitatively to the separating funnel, add 5 ml of methyl alcohol and repeat the procedure, thereby obtaining another distribution constant. Add further vols. of methyl alcohol, making measurements after each addition, until the two phases become completely miscible. Plot the series of "apparent distribution constants" against the vol. % of water in the aqueous phase; the resulting curve is characteristic for mepacrine. Other acridine compounds closely related to mepacrine gave different curves and, moreover, mixtures of such substances with mepacrine gave curves that could readily be distinguished from that of pure mepacrine. When dog urine containing a known amount of mepacrine was treated by the above procedure, a curve substantially identical with that obtained with pure mepacrine was obtained. A different curve was obtained when water % was plotted against the "calculated distribution constants" (K_c). This constant is the ratio between the ethylene dichloride concn. and the aqueous concn. calculated by subtracting the amount of mepacrine in the ethylene dichloride layer from the total amount present. The discrepancy between the actual and calculated distribution constants is due in part to a quenching effect of the buffer and in part to the fact that the observed fluorescence is referred to the standard concentration-fluorescence curve of the base in ethylene dichloride instead of in water. To obtain satisfactory results it is necessary to compare, under exactly the same conditions, the curve given by the test sample with that of an authentic specimen of the substance suspected to be present. The method gives results with 20–30μg of mepacrine, but by using smaller cells in the fluorimeter 1/10 of this amount, or even less, could be employed. The method was used to determine whether changes occurred when mepacrine had been administered to patients.

F. A. R.

Colorimetric Estimation of Acetoin and Diacetyl. E. Stotz and J. Raborg (*J. Biol. Chem.*, 1943, **150**, 25–31)—The method depends on the formation of nickel dimethylglyoxime and estimation of the nickel by a sensitive colorimetric method. Diacetyl and acetoin in amounts above 10μg can be recovered to the extent of 84–88%, and as little as 5μg can be detected qualitatively. Add a 3-ml sample of blood to 6 ml of water, and then 3 ml of 10% sodium tungstate soln. and 3 ml of 0·75 *N* sulphuric acid. Leave for 10 min. and centrifuge for 15–20 min. Put 7 ml of the filtrate into a 25-ml Pyrex test-tube and add 1 ml of 10 *N* sulphuric acid and 2 ml of 50% ferric chloride soln. Mix and immerse in a boiling water-bath for 15 min., inserting a clean rubber stopper in the mouth of the tube at first lightly and then firmly. Transfer 9 ml of the soln. to the flask of an all-glass distillation apparatus and put into a 15-ml graduated Pyrex centrifuge tube, which serves as receiver, 0·5 ml of 10% hydroxylamine soln. neutralised to *p*H 7·4 (phenol red as indicator), 0·5 ml of 20% sodium acetate soln. and 0·2 ml of 0·5% hydrated nickel chloride soln. With the end of the condenser dipping into the nickel solution, collect sufficient distillate to give 4·5 ml, then remove the condenser and wash it with a final 0·5 ml of distillate. Stopper the tube, immerse it in a water-bath at 55–60° C. for 30 min., then cool and leave it in the refrigerator for 12 hr. If no crystals are formed, less than 5μg of acetoin is present. To any crystals that separate add 5 ml of wash soln. consisting of a satd. soln. of nickel dimethylglyoxime which has been shaken with an excess of octyl alcohol and then filtered. Centrifuge the tube for 5 min., shake gently to submerge any floating ppt. and re-centrifuge. Withdraw as much of the supernatant liquid as possible, add 10 ml of the wash soln. without disturbing the precipitate, and again centrifuge. Remove the supernatant liquid and repeat the washing procedure. Add 2 ml of 0·2 *N* hydrochloric acid, shake and immerse the tube in a boiling water-bath. When the ppt. has dissolved, add 0·6 ml of 0·1% (by vol.) bromine water, followed, if it is decolorised, by 0·4 ml or more until the colour persists for at least 30 sec. Cool, add 2–3 drops of conc. ammonia and dilute the soln. to 5 ml. Add 1 ml of a 1% soln. of dimethylglyoxime in 95% alcohol by forcible ejection from a pipette, followed by distilled water to the 10-ml mark, and measure the colour of the resulting clear soln. in a photoelectric colorimeter at 460mμ, with water as blank. Prepare a standard soln. by treating 2 ml of a nickel chloride soln. (2·5μg of Ni per ml) with bromine, decolorising and developing the colour as described above. Diacetyl can be determined by directly distilling a tungstic acid filtrate of blood into the hydroxylamine and nickel mixture. If both acetoin and diacetyl are present, both are measured by the acetoin procedure; diacetyl can then be determined separately, and the acetoin obtained by difference. 1μg of nickel ≡ 3·07μg of acetoin or 2·93μg of diacetyl.

F. A. R.

Ether Extraction Method for the Estimation of Blood Phenols. E. G. Schmidt (*J. Biol. Chem.*, 1943, **150**, 69–73)—One difficulty in estimating blood phenols is due to the very small concn. of true phenols present, and another is removal of blood proteins without loss of phenolic substances. Dilute 12 ml of oxalated blood with 24 ml of water in a 100-ml centrifuge tube and after several min. add 12 ml of 10% sodium tungstate soln. and then, gradually, 12 ml of 0·75 *N* sulphuric acid. Stir for 15 min. and centrifuge for a further 15 min. The coagulum so obtained carries with it about 24% of the true phenols. Transfer a measured quantity of the supernatant liquid to an all-glass extraction apparatus, and add 1 ml of 10 *N* sulphuric acid. Add freshly distilled ether until the ethereal layer overflows into the return tube and attach the condenser. Put 3 ml of ether into the receiver, attach this to the extraction apparatus and partly immerse it in water at 70–75° C. Extract continuously for 2 hr., adjust the contents of the receiver to 3 ml and dilute the ethereal extract with 5 ml of 95% alcohol. Transfer 5-ml portions of standard solns. containing 0·001–0·005 mg of phenol in alcohol to test-tubes each containing 3 ml of ether. To each tube add 2 ml of water, 1 ml of diazotised *p*-nitroaniline (add 25 ml of a soln. of 1·5 g of *p*-nitroaniline in 500 ml of water containing 40 ml of conc. hydrochloric acid to 1·5 ml of 5% sodium nitrite soln.) and 3 ml of 5%

sodium carbonate soln. Mix, match the colours in a colorimeter, and divide the result by 0·76 to correct for the phenolic substances adsorbed by the pptd. proteins. To estimate conjugated phenols, remove most of the ethereal layer from the extract, decant the residue into a flask and evaporate the ether. Heat the aqueous soln. (*p*H 1) for 1 hr. under reflux and re-extract the hydrolysate with 3 ml of ether for 2 hr. Then proceed as described above. F. A. R.

Sulphonamiduria: A Simple Test for its Detection. E. Bogen (*U.S. Naval Med. Bull.*, 1943, **41**, 1185; *Amer. J. Pharm.*, 1943, **115**, 267)—The lignin test is recommended as a reliable and convenient field test for the determination of sulphonamides in urine. Place 1 drop of the sample on a piece of wood-pulp paper and add 1 drop of hydrochloric acid; a yellow to orange colour is produced roughly proportional to the amount of sulphonamide present: deep yellow, 0·05%; orange-yellow, 0·1%; orange, 0·5% or more. Sulphanilamide, sulphapyridine, sulphapyridine sodium, sulphathiazole, sulphathiazole sodium, sulphadiazine and sulphaguanidine give positive reactions, but the method is inapplicable in presence of prontosil or neoprontosil owing to their colour. Aniline, benzidine, hydrazine, naphthylamine, *o*-tolidine, *p*-dimethylaminobenzaldehyde, *p*-aminobenzoic acid, sulphanilic acid, procaine, benzocaine and larocaine* give a similar colour, but these have not been encountered in urine in sufficient concn. to cause interference. Inorganic substances, alcohols, aldehydes, acids, alkaloids, hormones or vitamins do not react. J. A.

Quantitative Method for the Estimation of Uroporphyrin in Pathological Urines. C. Rimington (*Biochem. J.*, 1943, **37**, 443–447)—It has been found that kieselguhr adsorbs both coproporphyrin and uroporphyrin from dil. hydrochloric acid solns. while rejecting almost entirely the dark brown-red non-porphyrin pigment. By using suitable buffers for elution the coproporphyrin can be separated from the uroporphyrin, but it has not yet been found possible to separate the I and III isomers of either pigment. The method can be used for the quantitative estimation of uroporphyrins. Add hydrochloric acid to a known vol. (1–2 ml) of urine containing 5–50μg of porphyrin until the concn. of acid is 1%. Dilute with 1% hydrochloric acid to 5 ml and leave overnight. Add 0·7 g of acid-washed kieselguhr, shake for 1 min. and centrifuge for 5 min. Discard the supernatant liquid, provided that it shows only a greenish-blue fluorescence unmixed with red in ultra-violet light. Wash the kieselguhr twice with 1% hydrochloric acid and once with water, and elute the coproporphyrin by 3 successive washings with 7-ml portions of 0·1 *N* sodium hydroxide. Dilute the combined eluates to 25 ml, leave them overnight, and then dilute a suitable vol. of the clear soln. with 0·1 *N* sodium hydroxide so that the final porphyrin concn. is approx. 0·25μg/ml. Measure the concn. fluorimetrically as previously described (*Biochem. J.*, 1943, **37**, 137); the result gives the total porphyrin. Coproporphyrin is estimated in a portion of the eluate or in the original urine by the acetic acid and ether extraction method and fluorimetric analysis and, by subtraction, the quantity of uroporphyrin is calculated. This method is preferred to a two-stage elution and separation of the two prophyrins by means of buffers. Recovery of added uroporphyrin was quantitative and the error of the method is believed to be less than 10%. F. A. R.

* *Abstractor's note*—Larocaine is 3-diethylamino-2:2-dimethylpropyl-*p*-aminobenzoate.

Spectrophotometric and Biological Assay of Vitamin A in Oils. N. H. Coy, H. L. Sassaman and A. Black (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 441–443)—A new U.S. Pharmacopoeia reference cod liver oil (No. 2) for biological assay of Vitamin A has been introduced. Spectrophotometric assay on freshly opened samples, supplied over a period of 2 years, showed that no significant variation of the value of $E^{1\%}_{1\,cm}$ was obtained among the samples tested, each test being carried out within the stated safe date. In addition, 3 samples were selected for stability tests over periods up to 1 year. After several months a fall in potency of about 14% was suggested by the spectrophotometric measurements. On the old reference oil, U.S.P. No. 1 (Coy *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 74), a loss of this order of magnitude was recorded in as many weeks. The average conversion factor for the No. 2 oil, computed on the claimed content, 1700 U.S.P. per gram, is 2280 for the unsaponifiable fraction and 2000 for the whole oil. The conversion factors, computed on biological and spectrophotometric measurements on 32 cod-liver oils and 61 oils of higher vitamin A potency show a decrease of from 20 to 14 % compared with the values computed when the older standard reference oil No. 1 was used in biological assays. This decrease in the conversion factors may be due to the difference in stability of the two reference oils. B. S. C.

Direct Method for the Estimation of N-Methyl Derivatives of Nicotinic Acid in Urine. H. P. Sarrett (*J. Biol. Chem.*, 1943, **150**, 159–164—It has recently been shown that N-methyl nicotinamide is a metabolite of nicotinic acid, and a direct method of estimating both trigonelline and N-methyl nicotinamide in urine is now described. Put 1–5 ml of urine into a Pyrex test tube calibrated at 10 ml and dilute with water to *ca.* 9 ml. The best results are obtained when 50–150μg of trigonelline are present. Add 0·3 ml of 11 *N* sodium hydroxide and heat in a boiling water-bath for 30 min. Cool, dilute to the 10-ml mark and add 3·3 ml of lead nitrate soln. (25 g in 95 ml of water) to each tube. Shake, add a drop of phenolphthalein (0·5% in 50% alcohol) and, if the soln. is still alkaline, a little powdered lead nitrate. Shake, centrifuge and decant the supernatant liquid into another test-tube. Remove the excess of lead by adding solid tripotassium phosphate to alkalinity and, after 5–10 min., centrifuge. Put 4 ml of the clear soln. into a large Pyrex test-tube graduated at 30 ml and add 16 ml of 95% alcohol and 4 ml of 11 *N* sodium hydroxide. Shake, close the mouth of the tube with a glass bulb, and heat at 75–80° C. for 45 min. Cool, add 3·7 ml of conc. hydrochloric acid, again cool and, after mixing, add another drop of phenolphthalein, then make the soln. just acid by dropwise addition of 5 *N* hydrochloric acid and then just alkaline with 0·5 *N* sodium hydroxide. Dilute to 30 ml and centrifuge. Put 10 ml of the clear soln. in an Evelyn colorimeter tube and add 1 ml of benzidine soln. (1 g in 25 ml of 95% alcohol and 75 ml of 0·75 *N* hydrochloric acid). Mix and read the colour within 25–30 min., using filter No. 490. The colour reaches a maximum in 20 min. and remains stable for an additional 20 min., after which it fades very slowly. As

reagent blank, use 4 ml of water taken through the alcoholic alkaline hydrolysis and treated in the same manner as the test soln. The preliminary hydrolysis in 0·35 *N* alkali converts N-methyl nicotinamide into trigonelline and this is then hydrolysed to nicotinic acid by the stronger alkali. The recoveries of trigonelline and N-methyl nicotinamide added to urine varied between 93 and 107% of the expected value. F. A. R.

Microbiological Assay Method for Aneurine. C. F. Niven and K. L. Smiley (*J. Biol. Chem.*, **1943, 150**, 1–9)—Microbiological assay methods previously described for aneurine have generally involved using an organism that responds to either cleavage product alone or in combination. The present method, in which *Streptococcus salivarius* is used, is specific for aneurine, and as little as 0·00001μg can be detected quantitatively per ml of medium. Cocarboxylase is approx. 40% more active than aneurine itself calculated on a molecular basis. Stock cultures of *Streptococcus salivarius* are maintained as stab cultures on a meat infusion with 1% of tryptone, 0·1% of glucose, 0·2% of K_2HPO_4, 1·5% of agar, and excess of calcium carbonate. After growth the stab cultures are kept in the refrigerator until required. For assays, the culture is carried directly from the agar stab into the basal medium to which 0·01μg of aneurine per 10 ml has been added, and the tubes are incubated at 37° C. for 24 hr. The basal medium has the following composition: 10% casein hydrolysate (vitamin-free casein hydrolysed with sulphuric acid and then neutralised with baryta), 0·5 g; 3% aneurine-free yeast extract, 0·3 g; glucose, 1·0 g; phosphate buffer (0·4*M*, *p*H 7·4), 10 ml; salt soln. (10 g of NaCl, 0·8 g of $MgSO_4$, 40 mg of $FeSO_4.7H_2O$, and 12 mg of $MnCl_2$ in 100 ml of distilled water), 1·0 ml; sodium thioglycollate, 10 mg; uracil, 0·5 mg; nicotinic acid, 50·0μg; riboflavin, 50·0μg; calcium pantothenate, 50μg; biotin (methyl ester), 0·1μg; water to 100 ml. Traces of aneurine in the casein hydrolysate are removed with Norit (20 g/100 g of casein) at *p*H 3·0. To prepare the aneurine-free yeast extract, autoclave 6 g of Difco Bacto-yeast extract in 200 ml of water for 15 min., adjust the *p*H to 3·0 and shake with 10 g of fullers' earth for 30 min. Filter, adjust the filtrate to *p*H 1·0 and again autoclave for 15 min. Cool, add a second 10-g portion of fullers' earth and shake overnight. Again filter, add 1·5 g of K_2HPO_4, adjust to *p*H 7·4, again autoclave for 15 min., then filter and adjust the vol. to 200 ml. Prepare sufficient of the basal medium in double the concn. necessary in the final preparation and adjust the *p*H to approx. 7·4. Transfer 5-ml quantities to 6-in. bacteriological test-tubes, 16 mm. in diam. and free from scratches. Add water to each tube so that the final vol. after addition of the test substance will be 10 ml. Sterilise the tubes by autoclaving and then add aseptically a standard vitamin soln. (dissolve 10 mg of aneurine in 100 ml of 0·1 *M* acetate buffer, *p*H 4·5, sterilise by autoclaving for 15 min. and dilute this stock soln. aseptically with sterile distilled water to a concn. of 0·001μg/ml) in such quantities that the tubes contain 0, 0·1, 0·2, 0·4, 0·7, 1·0 and 2·0mμg per tube (10 ml). To other tubes add samples for assay estimated to contain 0·1 to 0·6mμg of aneurine. Add 0·5 ml of inoculum to 10 ml of sterile saline and inoculate each tube of medium with 1 drop of the suspension thus obtained. Mix and incubate at 37° C. for 24 hr. It is preferable to close the tubes with plastic or aluminium caps rather than with cottonwool plugs. Measure the turbidity in each tube with a photoelectric colorimeter; or, alternatively, titrate with 0·05 *N* sodium hydroxide, the acid formed using bromothymol blue as indicator. The results thus obtained agree very well with those obtained by the use of *Phycomyces blakesleeanus* and with those recorded in the literature. The recovery of aneurine added to whole wheat flour, dried tomatoes and dried turnip tops was 100–124%. F. A. R.

Apparent Vitamin C in Walnuts. R. Melville, F. Wokes and J. G. Organ (*Nature*, **1943, 152**, 447–448)—The apparent vitamin C contents of 21 samples (representing 5 different species) were estimated by Mapson's visual method (*cf.* ANALYST, **1943, 68**, 285) and by the authors' visual and potentiometric methods (not described, *cf. id.*, 286). With certain exceptions (under further investigation) all 3 methods were in reasonable agreement. Results for (*a*) total vitamin C (mg per 100 g) and (*b*) apparent vitamin C (as % of total vitamin C) are, respectively: *Juglans regia*, (*a*) 1390–2968, (*b*) 19–50; *J. nigra*, (*a*) 950–2660, (*b*) 23–51; *J. rupestris*, (*a*) 1022–1606, (*b*) 5–28; *Carya ovata*, var. *pubescens*, (*a*) 700, (*b*) 12. The apparent vitamin C is not due to tannin, since the results were not appreciably affected by removal of this constituent. In general the vitamin contents found, and the distribution of the vitamin in the various parts of the plant, are in agreement with results of other workers, after allowing for variations due to seasonal causes. The proportion of apparent vitamin C in the total vitamin C is often much higher in the husk than in the central tissues, and this appears to be associated with a higher *p*H. J. G.

Neurospora Assay for Pyridoxine. J. L. Stokes, A. Larsen, C. R. Woodward and J. W. Foster (*J. Biol. Chem.*, **1943, 150**, 17–24)—Microbiological assays using *Lactobacillus* are not satisfactory for the estimation of pyridoxine, owing to the response of the organism to pseudo-pyridoxine. Use has now been made of an X-ray-induced mutant of the mould *Neurospora sitophila*. The growth of the mould is estimated by weighing the dried mycelium, and the procedure is therefore applicable to highly coloured or turbid solns. The pyridoxine content of meat and cereals estimated by the use of this mould agree well with the results of animal assays. Stock solns. of the mutant No. 299 of *Neurospora sitophila* are grown on Sabouraud agar slants (maltose 38 g, Bacto-peptone 8 g, Bacto-malt extract 2 g, agar 20 g, water 1 litre) at 30° C. After sporulation the cultures are stored in the refrigerator until required. The basal medium has the following composition, this soln. having twice the concn. of the final soln.: sucrose, 30 g; ammonium tartrate, 10 g; KH_2PO_4, 5 g; $MgSO_4.7H_2O$, 1 g; NaCl, 0·2 g; $CaCl_2$, 0·2 g; $FeCl_3$, 10 mg; $ZnSO_4.7H_2O$, 2 mg; biotin, 8μg; distilled water, 1 litre. Transfer 5-ml quantities of basal medium to 50-ml conical flasks, add 0·5, 1·0, 1·5 and 2·0 ml of the extract to be tested and dilute the contents of each flask to 10 ml. Prepare a similar set of flasks containing 0·1 to 1·0μg of pure pyridoxine, and blanks containing no pyridoxine. Autoclave the flasks at 15 lb. for 15 min., cool and inoculate with 1 drop of spore suspension made by transferring spores with a platinum loop from the agar slant into 10 ml of sterile water. Incubate the flasks at 30° C. for 5 days and then steam at 100° C. for 5 min. and remove the mycelium with a stiff wire needle. Press the mycelium between paper, roll into a

small pellet and dry at 100° C. for 2 hr. Weigh the mycelium to the nearest mg and calculate the pyridoxine content from a standard curve. Insoluble materials for assay are ground or finely chopped, and a 1–5 g sample is mixed with 40 ml of *N* hydrochloric acid and autoclaved at 15 lb. for 1 hr. to liberate combined pyridoxine. Filter, if necessary, and dilute the extract to 50 ml. Any aneurine in the extract must be destroyed, as the test organism responds to this vitamin. To 10 ml of extract add 0·7 ml of 10 *N* sodium hydroxide and then 5 ml of freshly prepared 1% sodium sulphite soln. Adjust the *p*H to 7, steam at 100° C. for 30 min. and add sufficient 2% hydrogen peroxide to destroy the excess of sulphite. Adjust the vol. to 20 ml and use the resulting soln. for assay. The results obtained with different levels of test sample agreed closely, indicating absence of stimulatory or inhibitory substances. Excellent duplicates were obtained, and recoveries of pyridoxine added to biological materials were quantitative within the limits of experimental error. With the exception of aneurine, none of the known vitamins or growth factors interfered. The mould does not respond to pseudo-pyridoxine.

F. A. R.

Rapid Penicillin Assay. U. Wilson (*Nature*, **1943, 152**, 475–476)—Existing methods require 12–18 hr., a serious disadvantage in studying the production of penicillin, because the rapid change in *p*H which occurs under certain conditions makes it essential that harvesting should take place with min. delay if serious loss of activity is to be avoided. The rapid method now suggested is also economical of space and materials, it is accurate, and it does not require special precautions as to sterile conditions. It is based on the inhibition by penicillin of the growth of a suitable group-*A* β-haemolytic *Streptococcus*; a suspension of washed sheep's blood cells is used as indicator. Dilute a soln. of the pencillin to be tested until it is equiv. to *ca.* 1 unit per ml. Measure out (from a micrometer syringe) 10 portions covering the range 0·20–0·10 ml into test-tubes (8 × 1 cm.) containing 1 ml of nutrient broth. Add to each tube 0·2 ml of a suspension containing 500–700 × 10^6 organisms per ml, and 0·8 ml of a 5% suspension of the sheep's blood cells. Mix the contents of each tube, by inverting it, and incubate them in a water-bath at 37° C. for 3·0–3·5 hr. Centrifuge, and read the tubes for haemolysis; a suitable standard and controls are included in each run for comparison purposes. The most critical factor in the test is the maintenance of the culture in such a condition that the suspension of the organism does not contain any preformed haemolysin. This is achieved by growing the culture on plain agar, and washing off with broth immediately before use. J. G.

Agricultural

Determination of Sulphur Residues from Sulphur Application on Citrus Foliage. F. A. Gunther, R. L. Beier and J. P. Ladue (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 574–575)—Store the sample (*ca.* 125 leaves) in a 2-qt. capped Mason jar at 0° C. Before analysis, remove the leaves from the jar with forceps and determine their surface area photoelectrically (Gunther and Barnhart, "Photoelectric Device for Measuring Surface Areas of Irregular Planar Objects," in preparation). Shake the sample in the jar for 2 min. with 100 ml of recently distilled carbon disulphide. Decant the liquid through a Buchner filter and repeat the extraction with 100 ml of carbon disulphide for 1 min., decanting the liquid through the same filter. Rinse the jar and funnel carefully with 50 ml of carbon disulphide and make the vol. of the filtrate and rinsings up to 250 ml. To 3 g of fusion mixture (8 parts of potassium hydroxide, 1 part of potassium nitrate and 0·3 part of water) in a 20-ml silver crucible add exactly 5 ml of the carbon disulphide extract. Evaporate the solvent with gentle heat under reduced pressure and fuse the residue cautiously over a shielded micro-burner, stirring with a silver or platinum wire. When effervescence has ceased, heat strongly until the fused liquid is clear, dissolve the cooled residue in 10 ml of water and neutralise the soln. to phenolphthalein with 6 *N* hydrochloric acid. Filter the soln. and wash the filter with four 50-ml portions of water, dilute the filtrate to 300 ml and add 1 ml of 6 *N* hydrochloric acid. Add 0·1 *N* barium chloride drop by drop to the boiling soln., with constant stirring, until pptn. is complete, add 10 ml in excess and keep the liquid hot for 1 hr. Collect the barium sulphate in a Gooch crucible, wash it free from chloride ion and dry for 1 hr. at 160°–180° C. Blank determinations should be made with the solvent and with untreated leaves. Expts. showed that 10–200μg of sulphur per sq.cm. of leaf surface can be determined rapidly and accurately. The method is subject to a positive error due to co-pptn. from the excessive amounts of salts formed by neutralisation and to a negative error due to presence of forms of sulphur insol. in carbon disulphide. A. O. J.

Value of Horse Chestnuts as Food for Pigs. R. Braude (*Nature*, **1943, 152**, 571–572)—When 10% of horse chestnuts, either dried and ground, or boiled, or soaked, are added to a fattening mixture, the pigs consume them and no toxic effects are observed. The food value of such chestnuts, however, is very small, mainly because of the unpalatable, thick, hard shell which the pigs tend to reject, provided that they can separate it from the rest of the meal. When the shell is removed, the decorticated soaked chestnuts are able to replace satisfactorily 10% of wheat feed in a balanced fattening meal. Boiling the chestnuts before feeding reduces their nutritive value, some of the nutrients being dissolved and drained off with the liquid. Even after boiling the shell remains objectionable to the animals. Although the amount of decorticated and prepared chestnuts can be increased to about 20% of the ration, none of the methods of preparation merits recommendation for practical application, because of the difficulties in storing raw chestnuts and the labour and cost of preparation. The dried horse chestnut meal has no nutritive value, and the pigs show a definite dislike to the meal mixture containing it. Whatever the nutritive value of the nut itself, the presence in the meal of the ground shell completely masks it. The question of the nutritive value of decorticated, but otherwise untreated, horse chestnut meal remains open (*cf.* Ministry of Agriculture Bull. No. 124; Temperton, ANALYST, 1943, **68**, 382).

J. G.

Organic

Determination of Halogen in Organic Compounds. P. K. Winter (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 571–574).—The sample is burned in a stream of halogen-free combustible gas at a small

jet beneath a chimney through which the products of combustion are carried by a stream of air through a gas-washing bottle containing a soln. of sodium carbonate or hydroxide (0·2 *N*) and sodium arsenite (0·1 *N*). The halide in the absorption liquid is then determined by a modified Volhard procedure. Two forms of apparatus are required: one for solids and liquids boiling above 100° C. and the other for low-boiling liquids or condensable gases. For solids and high-boiling liquids the apparatus consists of a U-tube made of 9-mm glass tube except for the greater part of one limb, which is of 1-mm capillary tube and which forms the jet. The sample is placed in the bend of the tube, in which it may be weighed. The wide limb of the tube serves as inlet for the fuel gas. An ordinary glass adapter of suitable size serves as the chimney which fits over the jet and is connected through an absorption bottle with suction apparatus drawing a controllable stream of air past the jet and through the absorption apparatus. The U-tube is enclosed to a height slightly above that of the jet in an electrically heated crucible furnace, which is used to melt and vaporise the sample. For low-boiling liquids an H-shaped tube is used, one vertical limb being of wide tubing and the cross arm and the other limb of capillary tubing. The wide limb is sealed at its lower end and closed at its upper end by a stopper carrying a capillary tube reaching to the bottom. This capillary tube is connected by rubber tubing and a Y-connection with the lower end of the capillary limb, the upper end of which is the jet. The remaining orifice of the Y-tube is connected with the supply of fuel gas. The weighed sample is contained in a small bulb with a long sealed-off capillary neck which can be broken by manipulation of the capillary tube when the bulb rests at the bottom of the wide limb of the H-tube. According to the volatility of the sample, the base of the wide limb is immersed in a cooling bath or is heated gently, and the vapour is swept out through the cross arm by the stream of fuel gas entering by the long capillary tube. The gas is burned at the jet, which is enclosed in a chimney connected with an absorption bottle, as previously described. The flexible tubes connecting the long capillary tube with the Y-tube and the Y-tube with the jet should carry screw clips to regulate the flow of gas, which may be the ordinary coal gas supply if it gives a sufficiently low blank value. Spark ignition points, above the jet and worked from an induction coil, are a convenience but are not essential. The tube from the chimney to the absorption flask should terminate in a sintered glass dispersion disc and the flask should contain *ca.* 50 ml of the carbonate-arsenite soln. When burning of the sample is complete, the chimney and removable parts of the absorption apparatus are rinsed, and the rinsings are added to the absorption liquid, which is then treated with 0·1 *N* silver nitrate until a ppt. of silver arsenite indicates an excess, 2 ml of conc. nitric acid, 0·5 g of ferric sulphate and 3 ml of nitrobenzene for every 10 ml of 0·1 *N* silver nitrate present. The liquid is stirred until the ppt. coagulates, and the excess of silver nitrate is titrated with 0·05 *N* potassium thiocyanate. The method gave results of satisfactory accuracy with a number of organic compounds containing chlorine, bromine or iodine. Two solid compounds (3,5-dinitrobenzoyl chloride and 2,6-dibromoquinone-chloroimide) gave low results, probably owing to retention of halide by the carbonaceous residue. Compounds containing fluorine gave low results. Reaction of hydrofluoric acid with the glass of the chimney and absorption tubes appeared to be the only reason for this, and the possibility of using collecting apparatus of other material is being investigated. A. O. J.

Determination of Combined Formaldehyde in Organic Compounds and in Cellulose Formals. C. L. Hoffpauir, G. W. Buckaloo and J. D. Guthrie (*Ind. Eng. Chem., Anal. Ed.*, **1943, 15**, 605–606)—Organic compounds containing combined formaldehyde yield the formaldehyde quantitatively when hydrolysed with sulphuric acid. Distillation can thus be avoided and the formaldehyde determined in the hydrolysate by the method of Blaedel and Blacet (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 449; ANALYST, 1941, **66**, 431) in a photoelectric colorimeter, in which the magenta colour with Schiff's reagent is measured. Correct results are obtained even in presence of other aldehydes, and 0·01–0·10 mg of formaldehyde may be determined in 5 ml of the sample. With 12 *N* sulphuric acid a constant maximum yield of formaldehyde is reached in 16 hr. or less from cellulose formals at room temp., but 16 days are required to obtain theoretical yields from trimethylene-*d*-mannitol and similar compounds. At 90° C. these compounds, as well as the cellulose formals, give the theoretical amount of formaldehyde in 2 hr. Further heating of the cellulose formals causes darkening of the solns., resulting in lower values. Heating at 90° C. for 2 hr. is thus recommended as a general procedure, but for cellulose formals treatment overnight with 12 *N* sulphuric acid is satisfactory. The amount of sample used should yield *ca.* 0·05 mg of formaldehyde in 5 ml of the hydrolysate, and it is advisable to set up a series of standards containing from 0·01 to 0·10 mg of formaldehyde in 5 ml and to develop the colour in them at the same time as in the unknown solns. by adding 6 ml of 10 *N* sulphuric acid and 10 ml of the fuchsin-sulphite soln. To prepare this reagent, dissolve 0·5 g of fuchsin in 500 ml of water, add 5·15 g of sodium bisulphite, leave for 15 min., add 17 ml of 6 *N* hydrochloric acid and leave for at least 3 hr. before using. Allow the reaction tubes to stand for 2 to 2·5 hr., during which time the colour given by higher straight-chain aldehydes and their polymers will fade completely, leaving the colour due to formaldehyde almost unaffected. Plot the transmission against mg of formaldehyde to construct the calibration curve. The formaldehyde soln. used for preparation of the standards may be standardised by pptn. with dimedone (Yoe and Reid, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 238; ANALYST, 1941, **66**, 391). Crystalline formals of known composition were prepared from the corresponding alcohols and 20–40 mg were dissolved in 1 litre of 12 *N* sulphuric acid. Portions of the soln. were placed in glass-stoppered flasks in a constant temp. oven at 90° C., and, after 2 hr., 5-ml aliquot portions of the cooled soln. were placed in the tubes of an Evelyn colorimeter provided with filter No. 565 (Corning Glass Co.). To each were added 6 ml of water (6 ml of 10 *N* sulphuric acid to the standards) and 10 ml of the fuchsin-sulphite reagent. After 2–2·5 hr. the transmission was determined. With cellulose formals *ca.* 0·1 g of the sample was treated with exactly 100 ml of 12 *N* sulphuric acid for 2 hr. at 90° C. or at room temp. overnight, and the formaldehyde in 5-ml aliquot portions was determined as described. Solution of the sample was not necessary for correct results. Cellulose formals of known composition were not available, but the results agreed well with those obtained by

distilling the formaldehyde by the method of Wood (*J. Soc. Chem. Ind.*, 1933, **52**, 331) followed by its determination by pptn. with dimedone (Yoe and Reid, *loc. cit.*). A. O. J.

Effect of Citrate on the Rotation of the Molybdate Complexes of Malate, Citramalate and *Iso*citrate. H. A. Krebs and L. V. Eggleston (*Biochem. J.*, 1943, **37**, 334–338)—The rotation of *iso*citric acid, like that of other α-hydroxy acids, is greatly increased by ammonium molybdate. When this observation was applied to the polarimetric estimation of *l*(-)malic acid in plant extracts it was found that addition of citric acid further increased the dextro-rotation of the malate-molybdate complex. Thus, $[\alpha]_D^{20°}$ was + 716° for *l*(-)malic acid in presence of ammonium molybdate and + 1340° for *l*(-)malic acid in presence of ammonium molybdate and citrate. Citric acid also increased the rotations of the molybdate complexes of citramalic and *iso*citric acids. The rotatory powers of various mixtures of hydroxy acids in presence of ammonium molybdate were therefore determined at various dilutions and various temperatures. The interference by citrate with the estimation of malic or *iso*citric acids in presence of ammonium molybdate can be overcome by increasing the citrate concn. to about 0·1 *M*. Under these conditions the rotations are approx. proporcional to the conc. of hydroxy acid. Mix 4 ml of the soln. to be examined, which should be neutral, with 1 ml of *M*-trisodium citrate, 0·5 ml of acetic acid and 4·5 ml of 29% ammonium molybdate soln. and measure the rotation. Calculate the concn. of hydroxy acid from data obtained by measuring the rotation of similar solns. containing known amounts of the hydroxy-acid. The method has the advantage that the sensitivity of the polarimetric method is increased thereby. F. A. R.

Selective Monoreduction of Aromatic Dinitro-compounds by Alkaline Sulphides and by Acid Stannous Chloride. H. H. Hodgson (*J. Soc. Dyers & Col.*, 1943, **59**, 246–247)—αβ-Dinitro-compounds in the naphthalene series, having the two substituent groups on different nuclei, are reduced by acid reducing agents, such as stannous chloride in a mixture of glacial acetic acid and hydrochloric acid, first to β-nitro-α-naphthylamines and then to the diamines, whilst sodium or ammonium sulphide reduces the dinitro-compounds to the α-nitro-β-naphthylamines and then ceases to react. When the two nitro-groups are on the same nucleus, both stannous chloride and alkaline sulphides produce a mixture of α-amino-β-nitro- and β-amino-α-nitro-compounds, with the former greatly predominating; 1.2-dinitronaphthalene, however, is reduced by stannous chloride to 1-nitro-2-naphthylamine. Di-α-nitro-compounds, *e.g.*, 1·5- and 1.8-dinitronaphthalene, are reduced by stannous chloride to the diamino-compounds; the 1.5-compound gives 5-nitro-1-naphthylamine with alkaline sulphides, whilst the 1.8-compound is not attacked. The theory put forward to explain this selectivity holds also for the partial reduction of picric acid to picramic acid by alkaline sulphides, and for the reduction of 2.4-dinitrotoluene to 2-nitro-4-aminotoluene by stannous chloride and to 2-amino-4-nitrotoluene by alkaline sulphides. E. M. P.

Rapid Colorimetric Method for the Estimation of Glycols in Air. H. Wise, T. T. Puck and H. M. Stral (*J. Biol. Chem.*, 1943, **150**, 61–67)—The method consists in passing the air through water and analysing the soln. by quantitative oxidation with acidified potassium dichromate, the green colour of the resulting chromic ion being used to measure the concn. of glycol. Bubble the air at a rate of 20–30 litres per min. through two boiling-tubes connected together in series, each tube being calibrated at 15 ml and containing 12 ml of water. Measure the vol. of the air with the aid of a flow-meter. For propylene glycol use a 50-litre sample, and for triethylene glycol a 300-litre sample. Remove the boiling-tubes, rinse the delivery-tubes and the inside of the flowmeter and adjust the vol. in each boiling-tube to the mark. Transfer aliquot portions containing not more than 2·4 mg of glycol to Pyrex test-tubes and slowly add 10 ml of potassium dichromate soln. (2·0 g in 20 ml of water made up to 1 litre with conc. sulphuric acid). Heat the tubes in a boiling water-bath for 15 min., closing the mouth of each with a glass bulb. Cool and compare the colour of the sample with standards, prepared in the same way, containing 0·2–2·4 mg of glycol in steps of 0·2 mg. If comparison is made with the aid of a photoelectric colorimeter fitted with a No. 620 glass filter, a calibration curve can be used. Visual matching gives an accuracy of ± 0·1 mg and a photoelectric colorimeter an accuracy of ± 0·05 mg. F. A. R.

Determination of Benzene in Presence of Cyclohexane. R. L. Bishop and E. L. Wallace (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 563–565)—The method is a more rapid and practical procedure developed from the method of Corson and Brady (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 531) for determining small amounts of benzene in cyclohexane by measuring the heat of reaction of the benzene with nitrating acid. In the original work two important conditions were noted, *viz.*, the composition of the nitrating acid and the variation in reaction heat due to changes in the initial or starting temp. of the reaction. The apparatus consists of a glass vacuum flask (2 × 10 in.) closed by a stopper carrying the glass shaft of a motor stirrer (1600 r.p.m.), a thermometer of length 18 in. and range 45°–100° F. and a funnel. The lower part of the flask fits into a felt-lined container. Weigh 50 g of sample in a 4-oz. tared bottle and place this and the bottle of nitrating acid (2 vols. of 69–70% nitric acid mixed with 1 vol. of 95·5% sulphuric acid) in a constant temp. bath at 68° F. Pipette 50 ml of nitrating acid into the flask, fit the flask to the stirring device and, after 3 min., start the motor. After 1 min. record the initial temp. (*ca.* 70° F.). Stop the motor and pour the sample from the 4-oz. bottle into the flask through the funnel and start the motor. Take readings after 1, 2, 3, and 5 min. The difference between the temp. after 3 min. and the initial temp. is a measure of the benzene content of the sample, which is determined from a calibration curve prepared from known mixtures of benzene and cyclohexane. Expts. showed that nitration is complete in 2 min. and that changes in the design of the apparatus or the speed of stirring or any other changes tending to cause less effective mixing reduce the precision of the method. Slight variation in the composition of the nitrating acid is permissible. In one instance small amounts of acetone and alcohol, occurring as impurities in the cyclohexane and benzene mixture, were successfully removed by extraction with water and subsequent drying of the mixture over calcium chloride. An appreciable amount of water would cause a rise in reaction temp., but a mixture of

cyclohexane and benzene contains only 0·01% of moisture when saturated at room temp. The method may be applied to the determination of benzene in ethylene dichloride and in propylene dichloride, but separate calibration curves are required for each solvent. A. O. J.

Determination of Surface Oxidation of Bituminous Coal. J. A. Radspinner and H. C. Howard (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 566–570)—A number of methods for determining the extent of surface oxidation of bituminous coals were examined qualitatively, *viz.*, sorption of electrolytes, *p*H of aq. suspensions, sorption of basic organic compounds, carboxyl group determination, oxidation-reduction potential, rate of oxidation, rate of drying, and thermal decomposition of the surface complex. Of these, only two—carboxyl group determination and thermal decomposition of surface complex—appeared to justify detailed examination. Samples of 3 typical coals, *viz.*, Pocahontas No. 3, Pittsburgh, and Illinois No. 6, were oxidised artificially at 75°, 100° and 125° C. for varying periods in specially designed apparatus and under such conditions that the extent of oxidation was definitely known. (1) The procedure for determining the carboxyl groups on the surface of the oxidised coal is essentially that of Ubaldini and Siniramed (*Inst. Ind. Chem. Fuels, Polytechnicum*, Milan, 1932–3, **4**, 333) for determining the equiv. wts. of naturally occurring humic acids and is based on the decomposition of calcium acetate by the acid groups and subsequent determination of the resulting acetic acid by liberation of carbon dioxide from calcium carbonate. The finely ground sample of coal was heated under reflux with 1 g of calcium carbonate and 100 ml of 3% calcium acetate soln., and the carbon dioxide evolved was swept by a stream of purified nitrogen through drying tubes and was absorbed in Ascarite. With slightly oxidised coals it was necessary to add 10 ml of a 1% soln. of a wetting agent (*e.g.*, Aerosol or Nekal BX) to the reaction mixture. In calculating the carboxyl groups the amount of carbon dioxide evolved by the unoxidised coal was deducted from the value for the oxidised sample. The results showed that only a fraction of the total fixed oxygen reacts as carboxyl group, and that this fraction varies greatly, ranging from a few units % for the Pocahontas up to 40% for the Illinois coal. There is, however, for each coal a general linear relation between the oxygen found as carboxyl and the total fixed oxygen. (2) It is known that the gaseous products obtained from the thermal decomposition of partly oxidised coals contain larger proportions of the oxides of carbon than do those from virgin coals. To collect the gaseous products quantitatively, *ca.* 10 g of coal were placed in the bottom of a small molecular still and this was evacuated through a multiple stage mercury diffusion pump backed by a Toepler pump which delivered the gases evolved into a mercury-filled gas sampling tube. The collected gas was measured and analysed in an Orsat apparatus. The coal sample was covered with a 200-mesh nickel screen to prevent spirting, the condenser of the still was filled with a mixture of dry ice and ethylene chloride to freeze out water and hydrocarbons, and heat was applied by means of an electrically heated aluminium block. The temp. was kept at 350° C. for 3 hr. with continuous evacuation. This method proved to be a more sensitive means of determining surface oxidation, since a much larger fraction of the oxygen was evolved as a measurable product (oxides of carbon). Modification of the method to include determination of the water evolved might increase the sensitivity considerably. A. O. J.

Estimation of Vegetable Matter in Scoured Wool. M. Lipson (*J. and Proc. Roy. Soc. New South Wales*, 1943, **76**, 225–228; *J. Text. Inst.*, 1943, **34**, 598A)—Thoroughly wet at room temp. 20–30 g of scoured wool containing vegetable matter in a soln. of 500 ml of 6% hydrogen peroxide, 25 ml of 5% copper sulphate and 8 ml of 5% sodium bicarbonate; optimum *p*H, 4·2. Heat at b.p. for 5 min., with stirring, boil for 2 min., with stirring, remove the degraded wool, squeeze and wash with water. Open the wool out by hand and stir for 2 min. in 600 ml of 1% anhydrous sodium carbonate soln. at 95–100° C.; this dissolves the wool completely. Filter, wash, dry and weigh the intact vegetable matter, which has no noticeable change in appearance, but which loses weight during recovery; a table shows the percentage recoveries of common types of vegetable matter introduced into burr-free wool. The method should be applicable also to the determination of cotton in cotton and wool mixtures, and its use for removing wool from mixtures with other fibres is being investigated. E. M. P.

Determination of Sulphur in Rubber. C. L. Luke (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 602–604)—A semi-micro method consisting in oxidation of the organic matter, reduction of the sulphate to sulphide by means of hydriodic and hypophosphorous acids, distillation of hydrogen sulphide, and titration of the distillate. For full working details reference should be made to the original. W. R. S.

Determination of Mercury in Rubber. C. L. Luke (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 597–599)—Treat 0·02–0·1 g of the finely-divided sample with 0·3 g of potassium persulphate and 2 ml of fuming sulphuric acid at the lowest point in a tilted conical 125-ml flask provided with a well-fitting 80-cm. dry air condenser. Heat with a Tirril semi-micro burner. With rubber compounds containing mercury and iodine, keep the flame low at the outset of the digestion; the upper half of the condenser must remain cold throughout the operation, and white fumes should issue slowly from the tube. When the organic matter is destroyed, cool, rinse down the condenser with 15–20 ml of nitric acid (1 : 1), detach it and rinse the joint, cover the flask with a watchglass and boil very gently on a hot plate until the vapour is free from iodine and no insoluble iodides of mercury remain. Evaporate to about 10 ml, dilute to 30–50 ml, cool, add permanganate soln., drop by drop, till pink, and decolorise with a drop of hydrogen peroxide; then add ferric alum indicator, and titrate carefully with 0·01 *N* ammonium thiocyanate. Deduct a blank. W. R. S.

Peat Wax from Chatham Islands, New Zealand (*Bull. Imp. Inst.*, 1943, **41**, 157–162)—Wax from Chatham Islands peat, examined at the Imperial Institute, had the following % composition—loss at 100° C., 3·2; (A) insol. and (B) sol. in boiling alcohol, 42·4 and 54·4; (X) sol. in light petroleum (b.p. 80–100° C.), 74·0; insol. in benzene, 0·3; (Y) sol. in cold ether, 21·9; ash, 0·4. Other

results were: acid value, 33·3; sap. val., 129·8; m.p. (open tube method), 69° C.; sp.gr. (18° C.), 1·033. (A) contained 21·8% (*a*1) of petrol-insol. "asphaltic" matter, m.p. not below 360° C., and 20·6% (*a*2) of petrol-sol. waxy matter, m.p. 83° C. (B) consisted of 37·1% (*b*1) waxy matter, m.p. 71·5° C., and 17·3% (*b*2) resinous product, m.p. 54° C. Acid vals. were (*a*1) 6·1, (*a*2) 26·3; (*b*1) 51·4 and (*b*2) 65·9, and sap. vals. were (*a*1) 151·8; (*a*2) 107·6; (*b*1) 114·8 and (*b*2) 128·6. This sample, said to have been "extracted by wax-works benzol" and to represent "*ca.* 10% of air-dried wt. of peat," is similar to a sample of peat wax extracted in 1926 with benzene at the Imperial Institute from peat received from New Zealand. Its appearance and composition are said to resemble those of montan wax, but the m.p. is *ca.* 10° C. lower, "asphaltic" content *ca.* 11% higher, and it is less compatible with paraffin wax, with which it is miscible in the ratio of 2 of peat wax to 3 of paraffin wax; miscibility decreases rapidly with increasing content of paraffin wax. The high content of "asphaltic" matter and, to some extent, the resinous matter, are responsible for the less favourable properties of peat wax. Probably reduction of these two fractions by an altered method of production would give satisfactory results; further research is required. In the 1926 investigation at the Imperial Institute, benzene was selected as the most suitable solvent economically for peat-extraction; no work was done to determine the proportion of "asphaltic" matter in waxes extracted by various solvents. Whilst the present dark brown, brittle wax is considered worth *ca.* 80–85% of the value of montan wax, refined and bleached peat wax might be superior in value. E. B. D.

Inorganic

Benzoin as Fluorescent Qualitative Reagent for Zinc. C. E. White and M. H. Neustadt (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 599–600)—Organic compounds that co-ordinate with metallic ions often have a hydroxyl and a carboxyl group adjacent to or near oue another, and, in an examination of compounds of this type, it was found that benzoin is an excellent qualitative reagent for zinc. A freshly prepared soln. of benzoin gives better results than an older one, and, since this is probably due to oxidation in the older soln., sodium dithionite (hydrosulphite, $Na_2S_2O_4$) is added as reducing agent to the reaction mixture. The fluorescence with zinc appears in an alkaline soln. in presence of magnesium hydroxide and is more lasting when a small amount of silicate is present. Probably the adsorbing agent is a basic magnesium silicate. Make the test soln. (containing 0·1 mg or more of zinc ion) neutral or slightly alkaline with sodium hydroxide and filter. To the filtrate, which should measure 5–15 ml, add *ca.* 1 ml of each of the following reagents in the order stated—sodium hydroxide and sodium silicate soln. (prepared by mixing 100 ml of 2·5 *N* sodium hydroxide with 0·6 ml of 35% sodium silicate soln.); sodium dithionite soln. (1 g per 25 ml of water); benzoin soln. (0·3 g of benzoin in hot 95% alcohol); magnesium nitrate soln. (2 g of magnesium ion per litre). Shake the mixture and after 1–2 min. observe its appearance under the U.V. lamp. A green fluorescence between 465*mμ* and 570*mμ* indicates presence of zinc. For concns. of less than 0·1 mg of zinc a blank test should be made and the colour observed through green cellophane. The fluorescence increases during the first few min. and fades slowly, disappearing in several hours. Boron and beryllium both give a fluorescence with benzoin, that of boron being in the range 471*mμ*–570*mμ* and that of beryllium 493*mμ*–570*mμ*. Both fluoresce before magnesium hydroxide is added, and, if the test soln. is examined at this point, they will not be confused with zinc. After addition of magnesium hydroxide, zinc can be distinguished from beryllium by comparison with standards. The boron fluorescence is not apparent in concns. < 1 part in 100,000. Antimony, which gives a purplish fluorescence that completely masks the green of the zinc, can be as easily removed as the sulphide. Of the cations left in soln. after treatment with sodium hydroxide, only the platinum metals, mercury, silver and gold cause difficulty by reduction to metallic ppts. by the dithionite. These must therefore be removed. Anions of molybdenum, vanadium, tellurium and selenium interfere by reduction, and coloured anions (*e.g.*, chromate and permanganate) mask the fluorescence. Silicate, phosphate, carbonate and arsenate in decreasing order seem to intensify the fluorescence of zinc, although they do not cause fluorescence when alone or with other elements. The test is sensitive to 10*μ*g of zinc or a concn. of 1 p.p.m. It was not found possible to isolate the fluorescent compound. A. O. J.

Spot Test for Detection of Manganese in Steel. Anon. (*Metal Progress*, 1943, **43**, 746–747)—As a qualitative test, allow 1 drop of dil. nitric acid (1 : 1) to act on the cleaned steel surface. When the reaction has stopped, soak a piece of filter-paper *ca.* 1/32 to 1/16 sq.in. in the drop. To the paper, placed in the depression of a white tile, add 2 drops of dil. nitric acid (1 : 1), 2 drops of 0·4% silver nitrate soln. and 1 drop of sat. ammonium persulphate soln., and mix with a glass point. The development of a reddish to a reddish-violet colour in 5–10 min. indicates manganese. For steels containing 2% or more of manganese the smaller size of absorbing paper is preferred, as the larger size may absorb more manganese than can be oxidised by the amounts of reagents normally used. The test is capable of giving an indication of the manganese content of the steel in comparison with that of standard steels when equal amounts of steel soln. are used. Differences in manganese content of the order of 0·5% may be readily distinguished, and differences of 0·2%, with experience. Cobalt interferes.

Another method, capable of quantitative application (R. G. Townsend, *id.*, 1943, **44**, 275) is to place a small piece of filter-paper on the clean steel surface and drop on to it 3 drops of dil. nitric acid (1 : 1). When the reaction has ceased, roll the filter-paper on a glass rod and transfer it to a test-tube. Add 5 ml of water and a few drops of conc. sulphuric acid. Heat to boiling, add 5 drops of 0·8% silver nitrate soln. and 10 drops of 3% ammonium persulphate soln. and boil until the permanganate colour develops (about 30 sec.). S. G. C.

Determination of Average Atomic Weight of Rare-Earth Mixtures. G. L. Barthauer, R. G. Russell and D. W. Pearce (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 548–549)—The authors favour the determination of the ratio $R_2O_3 : C_2O_3$ in the oxalates by ignition to oxide together with permanganate titration, with standardisation against a pure sample of the preponderant earth in the mixture.

Standardisation against sodium oxalate results in a positive error in the atomic weight, possibly due to co-pptn. of nitrato-oxalate. Dissolve 1 g of rare earth (standard alongside unknown) in 7 ml of 15 *N* nitric acid diluted to 50 ml. Ppt. the boiling soln. (100 ml) by slow addition of a hot soln. of oxalic acid (10 g in 50 ml). Wash the ppt. twice by decantation with 100-ml portions of hot water, collect and return it to the beaker, and decant twice as before. Transfer the ppt. to a beaker, pour off the clear liquid, and dry at 110° C. for 8–12 hrs. Accurately weigh duplicate 0·2-g. portions of the partially dehydrated oxalate, place in a cold electric muffle, and heat to constant weight at 900° C. Dissolve another two portions of oxalate (0·15 g) in 20 ml of warm 10 *N* sulphuric acid, dilute to 100 ml, heat to 90° C. and titrate with 0·04 or 0·025 *N* permanganate. The rare-earth mixture should be free from ceria; praseodymia and terbia, which form higher oxides, introduce an element of uncertainty in the determination. Further work is in progress. W. R. S.

Identification of Sulphides in Nickel and Nickel Alloys. A. M. Hall (*Amer. Inst. Mining and Metallurgical Eng. Tech. Publ.*, No. 1584, 1943)—Sulphur in nickel forms films of nickel sulphide between the nickel grains and this impairs the malleability and ductility of the metal. Additions of manganese or of magnesium improve or restore the ductility by converting nickel sulphide into manganese sulphide, which tends to form relatively harmless intercrystalline particles, or into magnesium sulphide which appears as detached particles in the body of the nickel grains. Other sulphides may also be present in alloys, *e.g.*, copper sulphide in copper-nickel (Monel) or chromium sulphide in nickel-chromium-iron (Iconel). Identification involves microscopic examination of polished sections; tungsten-mercury arc light was used, direct and under polarising conditions, with magnifications of 500 to 1500; the polished surfaces were also treated with reagents to aid identification; "sulphur printing" was also used. *Nickel sulphide* is practically indistinguishable from nickel in the polished alloy, but may be revealed by immersing the specimen for a few sec. in 2% nitric acid in alcohol, when it appears pale yellow in direct light, or black in polarised light. *Manganese sulphide* shows a grey colour, or black in polarised light. *Magnesium sulphide* also appears grey, but gives irregular effects in polarised light. It may be distinguished from manganese sulphide and other sulphides by immersing the specimen for 20 min. in distilled water, which attacks magnesium sulphide. These two sulphides are revealed on a sulphur print, whereas the sulphides of nickel, copper and chromium are not. (In sulphur printing, the standard technique for iron and steel was used except that a stronger acid solution was required, *i.e.*, 10% by wt. sulphuric acid). *Copper sulphide* shows various shades of grey or bluish grey, or black in polarised light. *Chromium sulphide* appears pale grey (bluish fringe with apochromatic objective; reddish fringe with fluorite objective) or black in polarised light. S. G. C.

Physical Methods, Apparatus, etc.

Stability of the Nickelous-Ammonia Colour System. J. P. Mehlig and R. E. Kitson (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 606)—In developing a photometric method for the determination of nickel in steel with ammonia, Ayres and Smith (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 365; ANALYST, 1939, **64**, 845) found no change in the colour of the system after 150 hr. Mehlig made a spectrophotometric study of this method (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 289; ANALYST, 1942, **67**, 309) and found no evidence of colour change after 4 weeks. The 6 solns. that were used in the spectrophotometric test, containing 100, 200, 300, 500, 1000 and 1500 p.p.m. of nickel in 1·5 *M* ammonium hydroxide, have been re-measured after standing a further 55 weeks in glass-stoppered Pyrex bottles in diffuse light. The spectral transmission curves are very similar to those originally obtained on the freshly prepared solns. From the transmission at 582*mμ* the apparent concn. of nickel was calculated. The apparent change in concn. of the nickel averaged 3·0%. Such marked stability makes possible the use of a series of permanent standards; these must be kept tightly stoppered to prevent loss of ammonia and are best kept in Pyrex bottles to reduce to a minimum the action of ammonia on the glass. B. S. C.

Review

ORGANIC REAGENTS FOR METALS. By the Staff of the Research Laboratory of Hopkin & Williams, Ltd. 4th Edition. Pp. 176. London: Hopkin & Williams, Ltd. 1943. Price 4s. post free.

During recent years many new applications have been found for reagents forming co-ordination compounds, and many papers published on revised technique for the older and better known. All the more important and generally useful of such developments appear to have been included in this revised edition of Messrs. Hopkin and Williams' already well-known book. Five new reagent monographs appear for the first time and two have been excluded since the last edition.

Comparison with the previous edition shows that revision has been thorough and that the same spirit of discrimination and selection that marked their previous work is still shown by Mr. W. C. Johnson and his colleagues.

Two features distinguish this edition from its forerunners, namely, the large number of selected references to original papers (more than 100 from THE ANALYST) and a reference

table to colorimetric qualitative reactions in the end cover; this shows in concise form the reagent necessary, interfering elements to be removed and any special conditions to ensure specificity, in testing for twenty-one metals. These pages will probably be amongst those most often consulted.

The appreciative foreword by Drs. A. D. Mitchell and A. M. Ward that first appeared in the second edition has also been revised. Coming from such well known workers in this subject it leaves a reviewer with very little to add except to congratulate all concerned in the production of the book; in its artistic water-proofed cover it is equally fitted for either the library shelf or the laboratory bench.

F. L. OKELL

ANALYTICAL METHODS COMMITTEE

The following Reports may be obtained direct from the Editor of THE ANALYST, The Close, Weedon, Aylesbury, Bucks. (**not through Trade Agents**), at the price of 1s. 6d. each to Members of the Society, 2s. each to non-Members. Remittance must accompany the order, and be made payable to "The Society of Public Analysts."

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Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
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Report No. 2. Determination of Unsaponified Fat in Soaps.
Report No. 3. Determination of Free Alkali in Soaps.
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Report No. 5. Determination of Rosin in Soaps.

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THE Editor welcomes Papers and Notes for insertion in THE ANALYST, whether from members of the Society or non-members. They are submitted to the Publication Committee, who decide on their suitability for insertion or otherwise.

Authors and prospective authors are reminded that, owing to the paper shortage, all contributions to the journal must be condensed as far as possible.

The Publication Committee have recently issued a circular containing Advice to Authors on the writing of Papers for THE ANALYST. This can be obtained on application to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3. All Papers submitted will be expected to conform to the recommendations there laid down and any that do not may be returned for amendment or rejected.

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