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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

NORTH OF ENGLAND SECTION

THE Twentieth Annual General Meeting of the Section was held in Manchester on Saturday, January 27th, 1945. The Chairman (Mr. W. Gordon Carey) presided over an attendance of thirty. The Hon. Secretary presented the Report and Financial Statement, which were adopted.

Appointments were made as follows:—*Chairman:* H. M. Mason; *Vice-Chairman:* C. H. Manley. *Committee:* H. F. Bamford, W. Gordon Carey, W. F. Elvidge, C. J. House, J. G. Sherratt, R. W. Sutton. *Hon Auditors:* U. A. Coates, J. R. Walmsley. *Hon. Secretary and Treasurer:* Arnold Lees.

The following paper was read and discussed:—"A Note on the Determination of the Specific Gravity of Sterilised Milk," by Dr. G. H. Walker, B.Sc., F.R.I.C.

A meeting of the Section was held in Leeds on Saturday, April 14th, 1945. The Chairman (Mr. H. M. Mason) presided over an attendance of twenty-one. The following papers were read:—"Our Society," by W. Gordon Carey, F.R.I.C. (retiring Chairman); "The Peroxide Value of Oils and Fats," by H. Weatherall, F.R.I.C., and C. B. Stuffins, A.R.I.C.

PHYSICAL METHODS GROUP

A GENERAL Meeting was held at 3 p.m. on Thursday, May 3rd, at The Chemical Society's Rooms, Burlington House, London, W.1, with Mr. R. C. Chirnside in the chair. Mr. C. A. Bassett and Dr. D. C. Garratt were elected Honorary Auditors. The draft rules were approved by the meeting. A paper was read by Dr. H. W. Thompson on "Infra-Red Spectrography in Relation to Chemical Analysis."

MICROCHEMISTRY GROUP

A MEETING of the Microchemistry Group was held in conjunction with the Manchester and District Section of the Royal Institute of Chemistry on Friday, May 25th, at Manchester.

The Microchemical Laboratories at the Manchester College of Technology were visited, through the courtesy of Prof. J. Kenner, F.R.S. Apparatus for inorganic and organic semi-micro-analysis was on view and demonstrations were given, by the students, of inorganic analysis by drop methods and chemical microscopy.

The meeting was opened at the Grand Hotel, Aytoun Street, by the Chairman of the Group, Prof. H. V. A. Briscoe, who thanked Prof. Kenner for arranging the excellent demonstration of microchemical methods. Prof. Briscoe referred to the work of the Group Committee which had recently been instrumental in arranging for a census to be taken of all educational institutions in the country to find what provision was made for the teaching of microchemical methods. The policy of the Group Committee was to hold three meetings per annum, one to be held in London and the others at suitable provincial centres. The present meeting was the first provincial meeting of the Group, and the next would be held in Newcastle in the autumn.

The following papers were then read.

"Physical Methods used in Microanalysis," by Dr. Cecil L. Wilson. A review of recent micro-methods for the determination of viscosity, surface tension and refractive index was given.

"The Determination of Trace Amounts of Sulphur Dioxide, with Special Reference to the Determination of Sulphur in Ferro-Alloys," by Mr. G. Ingram.

After combustion of the sample in a quartz tube the sulphur dioxide produced is passed into hydrogen peroxide. Wire-form copper oxide is added to the sulphuric acid produced, the excess is removed by filtration and the copper in solution is determined by adding sodium diethyldithiocarbamate and measuring the colour produced in a Spekker Absorptiometer. The amount of copper is related to the sulphur and allows the percentage present in the original sample to be calculated.

"Micro-methods used in the Analysis of Cotton," by Miss M. Corner. A short review was given of micro-methods used in the examination of cotton and other textiles and, in particular, methods used for the detection and determination of metals present.

ANALYTICAL METHODS COMMITTEE

FORMATION OF A SUB-COMMITTEE ON VITAMIN ESTIMATIONS

THE Analytical Methods Committee has appointed a Sub-Committee on Vitamin Estimations to examine the methods that have been proposed for the determination of vitamins in foods, biological materials and medicinal preparations, and to put forward, when considered necessary, standardised procedures. The Sub-Committee is investigating in the first instance the determination of aneurine, riboflavin and nicotinic acid in (*a*) cereals and cereal products, (*b*) malt products, including malt extracts, (*c*) yeast and yeast products, and (*d*) meat extracts. The Chairman of the Sub-Committee is Dr. E. B. Hughes and Mr. Eric C. Wood has been appointed Honorary Secretary.

Three Panels have been formed by the Sub-Committee to carry out the investigations which must necessarily precede the formulation of standard procedures. Any individuals or institutions not at present represented on these Panels who feel that they are in a position to contribute to the advancement of the work are invited to communicate with Mr. Wood, Virol Ltd., Hanger Lane, Ealing, London, W.5.

DEATHS

WE deeply regret to have to record the deaths of the following members of the Society

John Evans Martin Onslow Forster Frederick Henry Newington

A Scheme for the Photometric Determination of Minute Amounts of Arsenic, Copper, Lead, Zinc and Iron (with Certain Other Metals) in Organic Compounds, *e.g.*, Medicinals

BY N. STRAFFORD, P. F. WYATT AND F. G. KERSHAW

(*Read at a meeting of the Microchemistry Group on January* 23, 1945)

INTRODUCTION—Several good methods exist for the determination of minute amounts of metallic impurities, more particularly arsenic,[1] lead[2] and copper,[3] with certain other metals, in organic compounds, and much study has already been devoted to the application of such methods to rapid routine work. Under pressure of war-time demands, however, these methods proved still much too tedious for routine testing of certain classes of compounds, *e.g.*, medicinals, in a busy laboratory. Thus, using the Metallic Impurities Committee's methods,[1,2,3] one senior assistant, with hard working, could test only about 12 samples per week for arsenic, lead and copper, together with determinations of zinc and iron by other methods. The problem as it affected us had been partly met by the use of the emission spectrograph,[4] but the method has several limitations: (1) direct arcing of the organic substance is quite useless, preliminary wet oxidation being found essential, (2) concentration on cadmium sulphide as carrier is required in order to obtain the necessary sensitivity for arsenic, (3) iron and zinc have to be determined by additional tests (colorimetric and turbidimetric, respectively). The main application of the method was as a limit test for very small amounts of arsenic, lead and copper (As_2O_3 up to 1 p.p.m., Pb and Cu up to 5 p.p.m. each), and it could not be recommended for the accurate determination of greater amounts in experimental samples. Thus, although by the use of the method it was found possible to increase the output to about 20 samples per week, the attendant difficulties led to a search for a more versatile method of high sensitivity and precision, which would enable all the required determinations to be made successively on a single portion of the sample, with reasonable speed.

The main scheme of analysis presented in this paper was, therefore, designed to enable photometric determinations of the five elements arsenic, copper, lead, zinc and iron to be carried out successively on a single 2-g sample of the organic material. It has been devised specially for medicinal products with a very low metal specification limit, but is readily adaptable to materials with a less rigid "heavy metals" specification. It should also be applicable to other organic materials, such as foodstuffs and biological samples.

By carrying the tests through in batches of 8 (7 samples and a reagent blank), one assistant is able to analyse more than 20 samples per week for the 5 elements named, or up to 30 samples if zinc is omitted.

The necessary precise separation of the individual elements is achieved by solvent extraction of the metals in the form of metallo-organic complexes, and the final determinations are carried out photometrically by means of the Spekker photoelectric absorptiometer. In particular, a new solvent extraction procedure for arsenic, which avoids the usual more tedious separation, by distillation as arsenious chloride followed by evolution as arsine, has been devised. The standard Spekker equipment, which employs a needle galvanometer in conjunction with a tungsten filament lamp and glass colour-filters, has been preferred to that which incorporates a more sensitive mirror galvanometer and the more selective Ilford gelatin filters. The first-named more robust type of equipment is better adapted to hard routine use, and for the purpose of the present (main) scheme is quite adequate.

The main scheme of analysis, for determination of the five elements copper, arsenic, lead, zinc and iron, is shown diagrammatically below. Possible interferences, with the types of organic compounds considered, are very few and unimportant, but an extension of the main scheme, which overcomes these interferences, and provides for determinations of bismuth, nickel and cadmium, if present, is also described later.

I. Main Scheme of Analysis

Wet decomposition—Decompose a 2-g sample in a modified 100-ml Kjeldahl flask with sulphuric, nitric and perchloric acids.

Extraction of Copper and Arsenic

Dilute the soln. and add hydrochloric acid to give minimum HCl concn. of 2 *N* (to prevent extraction of lead). Reduce arsenic to As^{III} with sodium iodide and sodium metabisulphite, and extract arsenic and copper with diethylammonium diethyldithiocarbamate in chloroform.

Carbamate Extract (*A*)

Copper—Clarify the extract with anhyd. sodium sulphate, and determine the optical density.

Arsenic—After detn. of copper decompose the extract with H_2SO_4-$HClO_4$-molybdate reagent and complete the decomposition under a "cold finger" condenser. Reduce the soln. with stannous chloride and determine the optical density of the resulting "molybdenum-blue" soln.

Acid Layer (*B*)

Extraction of lead and zinc—Evaporate the soln. to fuming to decompose carbamate and iodide. Dilute the residue, treat with citrate and metabisulphite, neutralise with ammonium hydroxide and extract with dithizone in toluene.

Dithizone Extract

Extract lead and zinc with *N*/10 hydrochloric acid.

Aqueous Layer (*C*)

Iron—Determine iron photometrically as the thioglycollate complex.

Hydrochloric Acid Extract of Lead and Zinc (*D*)

Lead—Add sodium citrate and then ammonium hydroxide and potassium cyanide. Extract lead with dithizone in toluene soln. Wash extract with the dilute ammonia-cyanide soln. and determine its optical density.

Zinc—After extraction of lead, treat the aqueous layer (E) with formaldehyde, and extract the zinc with dithizone in chloroform; wash the extract with dil. ammonium hydroxide and determine its optical density. Alternatively, determine zinc by extractive titration with the dithizone in chloroform soln.

Method

A. Reagents—(1) *Acids*—Nitric acid (sp.gr. 1·42); sulphuric acid, conc.; perchloric acid, 60%; hydrochloric acid, 5 *N*. All the acids must be freed from the last traces of metallic impurity by redistillation of AnalaR quality material from an all-glass (Pyrex) apparatus. Perchloric acid should be redistilled under reduced pressure. (2) *Ammonium hydroxide, 10 N*—Prepare by bubbling the gas, delivered from a cylinder of liquefied ammonia, first through a bubbler containing conc. ammonium hydroxide, and then into a Winchester coated on the inside with pure paraffin wax and containing 2 litres of redistilled water kept well cooled in ice and water. Continue passing the gas until a reagent of the desired strength is obtained. (3) *Solvents*—Chloroform, B.P.; toluene, pure. It is advisable to redistil in presence of lime. (4) *Diethylammonium diethyldithiocarbamate solns.*—(*a*) *Stock soln.*—Dilute 3·0 ml of redistilled diethylamine to 10·0 ml with chloroform, B.P., and add slowly with stirring 1·0 ml of redistilled carbon disulphide previously diluted to 10·0 ml with chloroform. Cool and preserve in a dark-coloured glass-stoppered bottle. (*b*) *Extraction reagent*—Dilute 5·0 ml of stock soln. to 100 ml with chloroform. This soln. is referred to hereafter

as "carbamate" extraction reagent. Prepare the stock soln. once a week, and the dilute soln. daily. (5) *Dithizone solns.*—(*a*) Approx. 0·008%, in toluene. Dissolve approx. 15 mg of diphenylthiocarbazone (dithizone) B.D.H., in 50 ml of redistilled toluene, and shake vigorously in a 100-ml separating funnel with 50 ml of water containing 2 ml of ammonium hydroxide (reagent 2). Reject the toluene layer, slightly acidify the aqueous layer with hydrochloric acid, 5 *N*, and extract with two 50-ml portions of redistilled toluene. Reject the aqueous layer, combine the toluene extracts, and wash with two 10-ml portions of water. Prepare this soln. freshly each day as required. (*b*) Approx. 0·008%, in chloroform. Prepare as (*a*), but substitute chloroform, B.P. redistilled for toluene throughout. (6) *Sodium citrate soln., approx.* 1 *M*—Dissolve 150 g of trisodium citrate (AnalaR) in water, add 0·5 ml of conc. ammonium hydroxide, dilute to 500 ml, and shake thoroughly with 25-ml portions of dithizone soln. (0·02% B.D.H. reagent in chloroform) until the last extract remains green and the aqueous layer becomes slightly yellow. Then add 5 ml of 20% AnalaR citric acid soln., and extract with 25-ml portions of chloroform until colourless. (7) *Potassium cyanide soln., approx.* 1·5 *M*—Dissolve 50 g of potassium cyanide, AnalaR, in the minimum amount of water, dilute to 100 ml, and repeatedly extract with 10-ml portions of dithizone soln. (0·02% B.D.H. reagent in chloroform) until the last extract remains green and the aqueous layer is tinged yellow. Extract the excess of dithizone with 10-ml portions of chloroform and dilute the extracted cyanide soln. to 500 ml with water. Provided that AnalaR cyanide is used, the reagent is suitable for use for at least 6 months after preparation. (8) *Iodide soln.*, 20%—Dissolve 20 g of pure sodium or potassium iodide in 100 ml of water, add 0·2 ml of ammonium hydroxide soln. and extract with 10 ml of carbamate extraction reagent (reagent 4*b*), shaking for 30 sec. Reject the chloroform layer, and wash with two 5 ml portions of chloroform. (9) *Acid molybdate soln.*—Mix exactly 250 ml of dilute sulphuric acid, 11 *N* (accurately standardised),

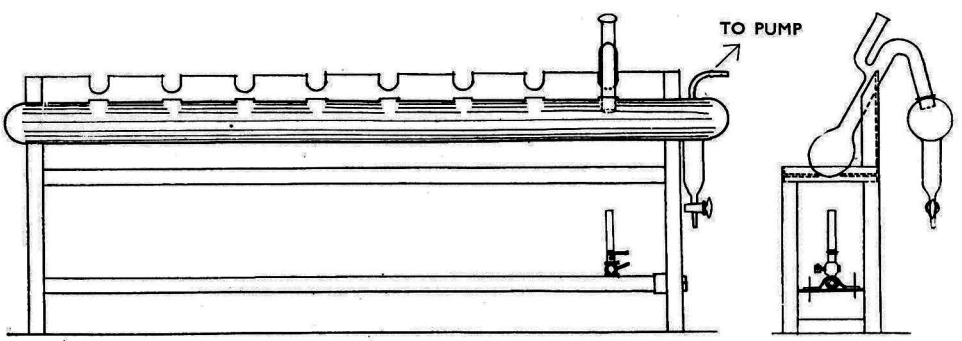

Fig. 1. Acid fume condenser and modified Kjeldahl flask.

Condenser—2½ in. diam. Orifices—1 in. diam.; 4½ in. between centres.
Flask—100 ml bulb capacity. Extension to neck ¾ in. to ⅞ in. external diam.

with 250 ml of ammonium molybdate soln., 7% in water. Filter into a litre measuring flask, wash with water, add exactly 250 ml of dil. perchloric acid, 3 *N* (accurately standardised), to the filtrate, and dilute to 1 litre at 20° C. with water. Mix well. (Five ml of this solution should require 17·5 ml of *N* sodium hydroxide, when titrated to methyl red indicator.) (10) *Sodium metabisulphite soln.*—5% in water, filtered. (11) *Dilute stannous chloride soln.* 0·4%—Dilute 1·0 ml of stannous chloride soln., 20% w/v in conc. HCl, to 50 ml with water. Prepare the dilute soln. freshly as required, and the 20% soln. weekly. (12) *Sodium sulphate*—Anhydrous AnalaR. (13) *Formaldehyde soln.*, 40% w/v. (14) *Standard metal solns.*—Prepare standard lead nitrate, copper sulphate, zinc sulphate, and ferrous sulphate solns. (and, if required, the further metal solns. used in the extended scheme), such that 1 ml ≡ 0·00001 g (10 μg) of metal, in the usual manner. Prepare also a standard sodium arsenite soln., such that 1 ml ≡ 0·00001 g (10 μg) of As_2O_3.

B. Apparatus—(1) 100-ml Kjeldahl flasks (Pyrex or Hysil), fitted with side funnel (carrying a protective cap) and extension to the neck (¾ inch to ⅞ inch ext. diam.) as shown in Fig. 1.* (2) Kjeldahl digestion rack and acid fume condenser (see Fig. 1). (3) 50-ml and

* The modified Kjeldahl flasks were made for us by Messrs. J. W. Towers & Co., Ltd., 44, Chapel Street, Salford, 3, Lancs.

100-ml conical flasks (Pyrex or Hysil). (4) Simple "cold finger" condensers, consisting of test tubes, 1·3 cm ext. diam. 7·0–7·5 cm long, with flanged mouths, to fit loosely 50-ml Hysil conical flasks. (5) 50-ml and 100-ml graduated cylindrical separating funnels, with well-fitting glass stopcocks and stoppers, and with the stems cut short to within ¼ inch to ½ inch of the stopcock barrel. (6) Hilger Spekker photoelectric absorptiometer (standard type H.454), fitted with tungsten filament lamp and needle galvanometer (Cambridge Unipivot) with set of glass filters (H.455) and 1-cm and 4-cm standard glass cells (for liquids). (7) Set of Ilford filters, Nos. 601–608 inclusive. (8) Suitable mirror galvanometer for use with the Ilford filters.

Note—Items (7) and (8) are required only for the extended scheme. It is an advantage to connect the needle and mirror galvanometers to the Spekker instrument *via* a change-over switch, so that either galvanometer can be put into the circuit as desired.

C. PROCEDURE—1. *Precautions*—Owing to the minute amounts of metals involved, special care must be taken to reduce the reagent blanks to as low a limit as possible and to avoid contamination during the test. All apparatus must be thoroughly cleaned with hot diluted hydrochloric acid (1+1), followed by thorough washing with distilled water, before use, and the described methods of preparation and purification of the reagents must be adhered to. All extractions must be carried out with smooth, rapid shaking (about 100 to 200 shakes per min.) and for the full time specified.

2. *Wet decomposition*—Introduce 2·00 g of the well-mixed sample, through the side funnel, into the dry 100-ml Kjeldahl flask (Fig. 1), place the flask in position on the digestion rack, and add a mixture of 6 ml of conc. redistilled nitric acid and 4 ml of conc. sulphuric acid. Warm cautiously until reaction commences, remove the flame until any initial vigorous reaction subsides, and then boil down rapidly until the soln. begins to darken. Next add nitric acid in small portions, heating after each addition until darkening again takes place, until finally the soln. fails to darken and is only pale yellow in colour (*ca.* 20 to 25 ml of nitric acid are usually required in all). Run in 0·5 ml of perchloric acid and a little nitric acid, and heat, with fuming, for about 15 min. Then add a further 0·5 ml of perchloric acid and heat for a few minutes longer. Allow to cool a little, add 10 ml of water, boil down to fuming, cool and add 5 ml of water. The soln. should be quite colourless at this stage. Again boil down until white fumes appear, and finally cool and dilute with 5 ml of water. During the whole of the above operations keep the funnel covered with its cap, except when adding reagents, to avoid risk of contamination.

Basic substances *e.g.*, methyl violet B.P., which are liable to deflagrate violently during the initial evaporation with acid, should be treated first with 10 ml of dil. (30%) nitric acid alone. After the initial vigorous reaction is over, decant the acid into a clean beaker, and wash the tarry residue with a little water (three or four 1-ml portions), adding the washings to the acid liquor. Add 4 ml of conc. sulphuric acid to the tarry residue, agitate to disperse the cake and introduce nitric acid, drop by drop, with warming if necessary, until vigorous reaction is over. Return the original acid liquor to the flask, boil down until the soln. begins to darken, and continue as described above. If no tarry cake forms, add 4·0 ml of sulphuric acid slowly to the cooled liquor in the flask and then boil down and proceed as before.[1]

Prepare a reagent blank soln. by putting 4 ml of conc. sulphuric acid, 20 ml of nitric acid, and 1·0 ml of perchloric acid in a 100-ml Kjeldahl flask, and boiling down to fuming. Fume for about 20 min., boil down twice with 5-ml portions of water and finally dilute with 5 ml of water.

3. *Extraction of copper and arsenic*—Transfer the "test" and "reagent blank" solns. *via* the side funnel, to 100-ml conical flasks, rinsing out the Kjeldahl flasks with two 1-ml portions of water. Place 15·0 ml of dil. hydrochloric acid, 5 *N*, in each Kjeldahl flask, heat to incipient boiling, swirl vigorously to wash the sides of the flask, and drain the acid into the corresponding conical flask, rinsing with two 1-ml portions of water. If, after the wet decomposition, the test soln. contains insoluble deposit or suspended matter, however small in amount, filter through a 7-cm filter paper into the conical flask, the subsequent washings, including the hot hydrochloric acid wash, being also passed in turn through the filter. If much residue is present, retain it as far as possible in the Kjeldahl flask, and boil for about 1 min. with the hydrochloric acid before the acid is drained through the filter.

Add to the contents of the conical flask 2·0 ml of iodide soln. (reagent 8) and warm to about 40° C., *i.e.*, as warm as can be comfortably borne by the hand. Add 0·5 ml of sodium metabisulphite soln. and transfer the soln. to a 50-ml graduated cylindrical separating funnel. Rinse out the flask with 1-ml portions of water until the volume of the soln. is 35 ml.

Introduce 5·0 ml of carbamate extraction reagent (reagent 4*b*) and shake the warm soln. vigorously for 40 sec., holding the stopper firmly in place; then carefully loosen the stopper to release the pressure. Allow to separate, and run the lower layer into a dry clean 25-ml separating funnel, taking care not to allow any of the aqueous layer to enter the stem of the first funnel. Wash the aqueous layer with 0·5 ml of chloroform, without mixing, and run the chloroform wash into the second funnel. Extract the aqueous soln. with a further 2·0 ml of carbamate reagent for 30 sec., separate, run the extract into the second funnel, and wash the aqueous soln. with chloroform as before. Add 10 ml of *N* sulphuric acid to the combined chloroform extracts and washings and shake for 5 sec.* Allow to separate, run the chloroform layer into a clean dry 10-ml measuring cylinder, wash the acid with 0·5 ml of chloroform and reject the acid. Add the chloroform washing to the main chloroform extract. Adjust the volume of the chloroform extract to 9·0 ml with chloroform, and drain into a clean dry 50-ml conical flask. Wash the cylinder with 1·0 ml of chloroform, add to the total extract, which now has a volume 10·0 ml (Extract A), 0·5 to 1·0 g of anhydrous sodium sulphate, and swirl gently until the supernatant liquid clears. Return the acid layer to the original 100-ml flask, rinsing the separating funnel with a little water, and reserve it (Soln. B). Treat the "reagent blank" in an exactly similar way to the test soln.

4. *Determination of copper*—The colour of the diethyldithiocarbamate extract A is a measure of the copper present. Fill a 1-cm cell with the extract, and determine the optical density on the Spekker absorptiometer, with No. 7 blue filters, against a "solution blank" consisting of 7 ml of carbamate extraction reagent and 3 ml of chloroform mixed in a 50 ml flask and clarified with anhyd. sodium sulphate. Determine the optical density of the "reagent blank" similarly, deduct its value from the test value, and read the copper content corresponding to the net value, on a standard curve established as follows. Measure 10 ml of water, 4 ml of conc. sulphuric acid, and 15·0 ml of hydrochloric acid, 5 *N*, into each of six 100-ml conical flasks. Add 0, 1·0, 2·0, 3·0, 4·0, 5·0 ml of standard copper soln. (1 ml ≡ 10 μg of Cu). Add 2·0 ml of iodide soln. to each, followed by 0·5 ml of metabisulphite soln., dilute to 30–35 ml and proceed exactly as described for the test. Determine the optical density of each of the extracts at 10 ml dilution, as before, but against the first solution (containing no added copper) as "solution blank." Plot a curve, with μg of copper as abscissae and optical densities as ordinates. The curve is rectilinear, a typical series of results being as follows:

Copper, μg	10	20	30	40	50
Copper (on 2-g sample), p.p.m.	5	10	15	20	25
Optical density	0·130	0·262	0·400	0·522	0·650

After determining the optical density, return the test and reagent blank solns. without loss to their respective flasks, and rinse out the cell with the minimum amount of chloroform, adding the rinsings to the main soln.

5. *Determination of arsenic*—Decant the extract A into a clean 50-ml conical flask, and wash the residue of sodium sulphate by decantation with three small portions of chloroform, taking care not to wash any particles of residue into the decanted soln. Reject the residue and add 2·0 (±0·02) ml of acid molybdate soln. (reagent 9) to the decanted soln. Insert a glass bulb into the mouth of the flask, cautiously evaporate the chloroform, and boil the soln. down on the hot plate until fuming accompanied by vigorous action begins. Immediately remove the flask, allow to cool a little and remove the bulb (without washing, since the acid must not be diluted). Insert a "cold finger" condenser filled with cold water, place the flask on the hot plate, and heat for 10 min. at such a temperature that a "blanket" of fumes fills the lower half of the flask and the condenser water finally attains a temperature of 80° C. (±5°) at the end of the heating period, when the flask is removed. Allow to cool a little, and add 4·5 ml of water from a pipette in the following manner. Raise the condenser, and rinse it from the top downwards with about half of the water, catching the rinsings in the flask. Then remove the condenser, and rinse down the sides of the flask with the remainder of the water. Place a glass bulb in the mouth of the flask, heat the soln. to b.p., and boil for 1 min. to remove most of the free chlorine present. Cool to 20° C. under the tap, remove the bulb, add 0·1 ml of sodium metabisulphite soln., and dilute to 5·0 ml in a 10-ml measuring cylinder. Drain the soln. back into the flask, add 0·5 ml of freshly prepared stannous chloride

* The washing with *N* sulphuric acid is introduced to prevent any possible interference by entrainment of phosphate, if present in the material under test. Even a trace of entrained phosphate would give, by reduction of the phosphomolybdate, a blue colour, and thus lead to fictitiously high results for arsenic.

soln. (reagent 11), with swirling, and leave for 10 min. Transfer the soln. (at 5·5 ml dilution) to a 1-cm cell, and determine the optical density on the Spekker instrument, using No. 1 red filters, against a solution blank consisting of 2·0 ml of acid molybdate soln. and 0·1 ml of sodium metabisulphite soln. diluted to 5·5 ml with water. Determine also the optical density of the reagent blank, treated similarly to the test, and allow for it. From the result find the arsenic content, by means of a standard curve obtained as follows. Prepare a standard series and extract with carbamate extraction reagent as described for copper, but in place of the standard copper soln. introduce 0, 0·1, 0·2, 0·5, 1·0, 1·5, 2·0, 2·5 ml of standard arsenite soln. (1 ml ≡ 10 μg of As_2O_3). Measure the optical densities, after continuing as for the test soln., in a 1-cm cell, with No. 1 red filters, against the first soln., containing no added arsenic, as solution blank, and plot a curve as usual. The curve is a straight line, a typical series of results being as follows:

As_2O_3, μg	1	2	5	10	15	20	25
As_2O_3 (on 2 g sample), p.p.m. ..	0·5	1·0	2·5	5	7·5	10	12·5
Optical density	0·017	0·037	0·100	0·199	0·303	0·398	0·502

After observing the optical density, check the acidity of each soln. as follows. Drain the soln. back into the flask and rinse the cell with a little water. Drain the cell on filter-paper before filling with the next soln. Dilute to about 20 ml, add 0·1 ml of methyl red indicator, and titrate with *N* sodium hydroxide. The colour change is from purple to green. The titre should be 6·75 (±0·1) ml, corresponding to a normality (at 5·5 ml dilution) of 1·23 (±0·02) *N*. If the acidity is outside this range, a correction may be applied (see later).

6. *Separation of lead and zinc*—Evaporate the acid soln. B, reserved after extraction of copper and arsenic, until no more iodine is evolved, and fuming occurs. This is conveniently done while the copper and arsenic are being determined. Allow to cool, dilute with 10 ml of water, add 2·0 ml of sodium citrate soln., 0·2 ml of sodium metabisulphite soln. and 0·2 ml of methyl red indicator. Almost neutralise with ammonium hydroxide (reagent 2), cool well, and continue the dropwise addition of ammonia until the colour just changes to pure yellow; then just acidify with hydrochloric acid, 5 *N*. Transfer the cold soln. to a 50-ml separating funnel, and extract the indicator with two 5-ml portions of toluene. Run the aqueous layer back into the flask and wash the toluene layer with 1 or 2 ml of water, without mixing. Reject the toluene extracts, return the aqueous soln. to the funnel, add 0·5 ml of ammonium hydroxide and 2 ml of toluene, and then (from a 10-ml burette) 1·0 ml of dithizone in toluene soln. (reagent 5*a*). Shake for 15 sec., and, if the dithizone extract does not change colour immediately to bright pink, add ammonium hydroxide, drop by drop, with shaking, until the dithizone reacts. Continue adding dithizone soln., 1 ml or other convenient increment at a time, until with the last addition the colour changes from bright pink to a slightly duller more purplish shade, due to the presence of unchanged dithizone. Run the lower layer back into the flask, wash the toluene layer with 1 or 2 ml of water, and reserve the aqueous soln. for the determination of iron (soln. C).

Add 10 ml of *N*/10 hydrochloric acid to the toluene extract in the funnel, shake for 30 sec., separate, run the acid extract into a clean 50-ml conical flask and wash with 1 or 2 ml of water. Repeat the extraction with a further 10 ml of *N*/10 acid, and add the acid wash to the first acid extract (soln. D). Then reject the toluene layer, which should now be clear green in colour, unless nickel or cobalt is present.

7. *Determination of lead*—Add to soln. D 0·5 ml of sodium citrate soln. and 0·5 ml of ammonium hydroxide (reagent 2), followed by 0·5 ml of potassium cyanide soln. (reagent 7). Transfer to a 50-ml separating funnel and rinse the flask with a little water. To the soln. add 2 ml of toluene and 0·5 ml of dithizone in toluene (reagent 5*a*), and shake well. If the toluene layer becomes bright pink, continue the addition of dithizone soln., 0·5 ml at a time, until the toluene layer develops a slightly duller tint, and the lower aqueous layer a slight yellow tint, due to a small excess of free dithizone. Then run the lower (aqueous) layer into a clean 100-ml flask, wash the dithizonate layer with 1 or 2 ml of water, and return the toluene layer to the original 50-ml flask. Return the aqueous layer to the funnel and shake with 2 ml of toluene. If all the lead is extracted, the toluene layer will be pale purplish-green, and the aqueous layer pale yellow. Return the aqueous layer to the 100-ml flask and add the toluene layer, after washing with a little water, to the main dithizone in toluene extract. Reserve the aqueous soln. for the determination of zinc (soln. E).

The combined toluene extracts should have a volume of less than 10 ml, since the amount of dithizone required to extract all the lead is usually less than 3 ml. Return the toluene

soln. to the funnel, add 10 ml of dilute ammonia-cyanide soln. (2 ml of ammonium hydroxide and 1 ml of potassium cyanide soln. per 100 ml of metal-free water), and shake until no change in colour occurs (about 10 sec.). Allow to separate completely, and run off and reject the ammonia wash, which should be only very pale yellow in colour. If it is deeply coloured give the toluene soln. a second ammonia-cyanide wash. Finally wash with 2 ml of water. Separate any small amount of water retained in the funnel as completely as possible, dry the stem of the funnel with filter-paper, run the toluene extract into a dry 10-ml measuring cylinder and rinse the funnel with a little toluene. Dilute to the 10-ml mark with toluene, mix and transfer to a dry 50-ml conical flask. Cork the flask to prevent evaporation, swirl the solution and leave for a few min. to ensure that any small amount of water collects in a drop at the bottom. If the solution is then not perfectly clear, filter it through a dry 9-cm paper into another dry flask.

Measure the optical densities of the test solution and of the reagent blank solution (similarly obtained) on the Spekker instrument, in a 1-cm cell, using blue-green filters No. OB2, against a solution blank of pure toluene. Find the lead content corresponding to the net optical density from a standard curve established as follows. Measure 0, 0·5, 1·0, 2·0, 3·0, 4·0, 5·0 ml of standard lead soln. (1 ml ≡ 10 μg of Pb) into 50-ml flasks, add to each 20 ml of $N/10$ hydrochloric acid, and continue as described for the determination of lead in the test soln. (beginning of Sec. 7). Having prepared the washed and clarified lead dithizonate in toluene extracts, at 10·0 ml dilution, determine the optical density of each soln. in turn against the first soln., containing no added lead, as solution blank, in a 1-cm cell, using blue-green filters No. OB2. Plot a curve as usual. This is virtually a straight line, a typical series of results being as follows:

Lead, μg	5	10	20	30	40	50
Lead (on 2-g sample), p.p.m...	2·5	5	10	15	20	25
Optical density	0·077	0·153	0·305	0·450	0·597	0·745

8. *Determination of zinc*—Transfer soln. E to the separating funnel and shake for 10 to 15 sec. with 5 ml of chloroform. Reject the chloroform layer, and add to the aqueous layer 0·5 ml of formaldehyde soln. and 0·5 ml of ammonium hydroxide soln. Then add dithizone in chloroform soln. (reagent 5*b*), 0·5 ml at a time, with shaking, until the colour of the extract changes from bright pink to a slightly duller shade. Run the lower layer into the original flask, and wash the aqueous layer with a little chloroform. Add 2 ml of chloroform and 0·5 ml of dithizone soln. to the aqueous layer, shake for 10 sec. and allow to separate. The chloroform layer should be purplish green in colour and the aqueous layer show a yellowish tint, if extraction is complete. Add this extract to the main extract, and reject the aqueous layer. Transfer the pink dithizonate in chloroform soln. to a separating funnel, dilute to 15 ml with chloroform (used to rinse the flask), add 10 ml of dilute ammonia wash (2 ml of ammonium hydroxide in 100 ml of water), and shake until no further change in colour occurs (about 5 sec.). Separate, reject the ammonia layer after washing with a little chloroform, and repeat the washing operation. Dry the stem of the funnel with filter-paper, and run the chloroform layer into a dry 25-ml measuring cylinder. Wash the ammonia layer with a little chloroform, and add the wash to the main extract. Adjust the volume of the soln. in the cylinder to 20·0 ml, transfer it to a dry corked 50-ml flask, swirl vigorously, and leave for a few min. If the soln. does not clear, filter through a dry filter-paper. Determine the optical densities of this soln. and of the similarly obtained "reagent blank" soln., on the Spekker instrument, using a 1-cm cell and blue-green filters No. OB2. Read the μg of zinc present corresponding to the observed readings, and obtain the net μg of zinc present by difference, using a calibration curve prepared as follows.

Take a suitable number of clean 50-ml conical flasks, and add to each 20 ml of $N/10$ hydrochloric acid, 0·5 ml of sodium citrate soln., 0·5 ml of ammonium hydroxide, 0·5 ml of potassium cyanide soln., and finally 0·5 ml of formaldehyde soln. Transfer each soln. in turn to a separating funnel, add 2 ml of chloroform, and then dithizone in chloroform soln., 0·5 ml at a time, with shaking, until a slight excess is present, to remove any traces of zinc present in the reagents. Reject the extract, and wash the aqueous layer with a little chloroform. Return the extracted aqueous solns. to their respective flasks, and add to each a suitable amount of standard zinc soln. (1 ml ≡ 10 μg of Zn), to give a series increasing by at most 5 μg increments (see example below). Transfer the aqueous solns. in turn to the separating funnel, and extract with dithizone in chloroform soln. exactly as described for the test and finally dilute the washed extract to 20·0 ml with chloroform. Determine the optical

density of each soln. in turn, against the first soln., containing no added zinc, as solution blank, using a 1-cm cell, and blue-green filters No. OB2. Construct the graph as usual. A typical series of results is the following:

Zinc, μg	2·5	5	10	15	20	25	30	35	40	50
Optical density ..	0·094	0·175	0·311	0·433	0·540	0·628	0·695	0·750	0·795	0·850

9. *Determination of iron*—Add to the aqueous soln. C, reserved after extraction of lead and zinc, 1·5 ml of hydrochloric acid, 5 *N*, transfer the soln. to a separating funnel, and shake with 5 ml of toluene. Return the soln. to the 100-ml flask and reject the toluene layer after washing with a little water, boil for 5 min., cool to room temp., add 1·0 ml of thioglycollic acid, 10%, followed by 2·5 ml of ammonium hydroxide soln., dilute to 50·0 ml and mix. Determine the optical densities of the test soln. and of the reagent blank soln. (similarly obtained) on the Spekker instrument, using a 4-cm cell, and blue-green filters No. OB2. Establish the standard curve required as follows. Place in each of a series of 100-ml conical flasks 15 ml of water, 4 ml of sulphuric acid, 2·0 ml of sodium citrate soln., and known amounts of standard iron soln. (1 ml ≡ 10 or 100 μg of Fe, whichever is more convenient), as indicated below in the table of typical results. Add 0·2 ml of methyl red indicator, nearly neutralise with ammonium hydroxide, cool, and make just alkaline with ammonia. Add 1·5 ml of hydrochloric acid, 5 *N*, extract the indicator completely with 5-ml portions of toluene, boil the aqueous solns. for 5 min. and cool. Add 1·0 ml of thioglycollic acid, 10%, and 2·5 ml of ammonium hydroxide, dilute to 50·0 ml, mix, and determine the optical density of each soln. in turn, against the first soln. (containing no added iron) as solution blank, in a 4-cm cell, using blue-green filters No. OB2. A typical series of results is as follows:

Iron, μg	20	40	60	80	100	200
Iron (on 2-g sample), p.p.m.	10	20	30	40	50	100
Optical density	0·089	0·180	0·271	0·360	0·445	0·885

Discussion of Method

(*a*) *Arsenic*—Kahane and Pourtoy[5] have shown that large losses of arsenic can occur during wet decomposition unless fully oxidising conditions are maintained throughout. We have found that even with the much smaller amounts of arsenic dealt with here a considerable proportion can also readily be lost. For this reason a 100-ml Kjeldahl flask fitted with a condenser dipping into 5 ml of water contained in a boiling-tube surrounded with ice and water, as used for arsenic distillations,[1] was first adopted. After decomposition the distillate was transferred to the flask, and the whole was boiled down to fuming with the condenser removed, before treating with perchloric acid. Although we still consider this the safest method of preventing loss of arsenic, it proved too tedious and cumbersome for routine use. The alternative method described is reliable if care is taken not to let the residue char too much, and a small but definite excess of nitric acid, or finally, perchloric acid, is maintained in the soln. The apparatus shown in Fig. 1 is found to be very convenient and effective for condensing the acid fumes, and the modified design of Kjeldahl flask affords a fuller protection from contamination than the usual open type.

For separation of the arsenic, the sodium ethyl xanthate reagent of Klein and Vorhes[6] was first tried, but proved unsatisfactory in the following respects: (1) it was relatively troublesome to prepare, (2) it tended to discolour rapidly and deposit insoluble matter, (3) extraction of arsenic was slow, and not quite complete (*e.g.*, with 10 μg of As_2O_3, about 8·5 μg were recovered). It is suspected that the presence of alcohol in the reagent retards extraction. Sodium diethyldithiocarbamate also reacts with arsenious ions, but was found similarly unsatisfactory, and it was considered that the dithiocarbamate of an organic base should give better results. Finally, diethylammonium diethyldithiocarbamate was found to meet all the requirements of easy preparation, according to the reaction

$$2NH(Et)_2 + CS_2 \rightarrow S{=}C\begin{matrix}S.NH_2(Et)_2\\ N(Et)_2\end{matrix},$$

high stability, and high solubility in chloroform alone, while, most important of all, extraction of As^{III} is complete; under the proper conditions the amount left unextracted is certainly less than 0·2 μg of As_2O_3. Quinquevalent arsenic, however, is left untouched, so that complete reduction to the tervalent state is essential. A wide range of acidity is permissible; extraction was found to be complete over the range 1 *N* to 10 *N* in sulphuric acid.

The arsenic extract was at first decomposed with bromine water[6] and Milton and Duffield's procedure[7] applied directly to the resulting soln. This generally gave good results,

but annoying discrepancies occurred which were traced to a slight turbidity in the soln., or to greasiness due to traces of organic matter separating on the side of the flask, or to incomplete oxidation to the quinquevalent state. For this reason the more drastic method of treatment given was adopted. The presence of a little perchloric acid does not interfere with either the initial reduction and extraction of arsenic or the final reduction to "molybdenum blue." In the latter reduction it appears to have a stabilising effect and results in a lower "reduction blank." The use of a cold finger condenser is necessary to obtain adequate control of the acidity, and to ensure that there is no loss of arsenic during decomposition. The intensity of blue colour obtained for a given amount of arsenic varies with the acidity[7]; thus the following results were obtained for solns. containing 25 μg of As_2O_3, with varying acidity, at 5·5 ml dilution.

Soln. No.	1	2	3	4	5	6	7	8
Observed Spekker reading								
(1) test (25 μg of As_2O_3)	0·462	0·499	0·533	0·547	0·566	0·595	0·613	0·630
(2) blank	0·032	0·040	0·043	0·045	0·052	0·057	0·062	0·067
Net optical density	0·430	0·459	0·490	0·502	0·514	0·538	0·551	0·563
Ml of *N* sodium hydroxide required	7·35	7·1	6·85	6·75	6·65	6·4	6·25	6·1
Normality (5·5 ml dilution)	1·34	1·29	1·25	1·23	1·21	1·16	1·14	1·11

In the method the strength of the acid molybdate soln. has been adjusted to give a final acidity in the test soln. of 1·23 ($\pm$0·02) *N* (see Sec. 5). If the titration of the test soln. shows that the acidity falls outside this range, a correction can be applied by means of the above table.

The amount of carbamate extraction reagent specified (5 ml + 2 ml) will extract at least 25 μg of As_2O_3. For larger amounts of arsenic increase the amounts of reagent used and of the final dilution accordingly. Thus for 25 to 100 μg As_2O_3, use 10 ml + 5 ml of carbamate extraction reagent, dilute to 20 ml with chloroform for the copper determination, evaporate with 4 ml of acid molybdate reagent, dilute the acid soln. to 10·0 ml, reduce with 1·0 ml of 0·4% stannous chloride soln. and determine the optical density at 11·0 ml dilution. Typical Spekker results for this range of arsenic content are as follows:

As_2O_3, μg	blank	25	50	70	90	110
Optical density (net)	(0·050)	0·250	0·509	0·707	0·892	1·090

In an effort to eliminate the reduction blank altogether addition of chlorate was tried,[8] but this caused such rapid fading that accurate Spekker observations were impossible.

(*b*) *Copper*—Copper is rapidly and completely extracted as the characteristically coloured diethyldithiocarbamate complex. To accelerate reduction and extraction of arsenic, the soln. is extracted warm, and as the extract cools it becomes turbid owing to the separation of traces of water. Provided that sufficient care is taken in separating the chloroform and aqueous layers, the amount of anhyd. sodium sulphate specified is sufficient to absorb the water and clarify the extract. The addition of about 20% by vol. of absolute ethyl or methyl alcohol will also clarify the extract, but this method was discarded because fading of the colour sometimes resulted, and because subsequent evaporation of alcohol in presence of perchloric acid was considered a potential source of danger from explosion.

(*c*) *Lead*—In presence of sulphuric acid alone lead may be partly or wholly extracted with arsenic and copper by the carbamate reagent, but if hydrochloric acid is present in a concn. of at least 2 *N* the lead remains wholly in the acid layer, and the subsequent extraction from ammoniacal citrate-cyanide soln. provides a practically specific and highly sensitive method of determination.[9] A soln. of dithizone in toluene is found to have certain advantages over the usual soln. in chloroform or carbon tetrachloride; the excess of dithizone used in the extraction tends to enter the aqueous layer almost completely, instead of contaminating the lead dithizonate layer, while the optical density curve obtained is practically rectilinear, whereas that obtained with chloroform solns. showed marked convexity.

(*d*) *Zinc*—Presence of cyanide completely inhibits the extraction of zinc by dithizone, but on adding formaldehyde the excess of cyanide is converted into formaldehyde cyanohydrin and the zinc cyanide is decomposed. Under these conditions zinc is quantitatively extracted from the slightly ammoniacal soln. as the dithizonate. The photometric determination as dithizonate is extremely sensitive, and the amount of zinc present is frequently too great for its convenient application. In such event an extractive titration of the zinc with a standardised dithizone in chloroform soln. is preferable, as follows. After addition of formaldehyde, add 2 ml of hydrochloric acid, 5 *N*, to acidify the soln., and extract the residual dithizone with 5 ml, followed by two 2-ml portions, of chloroform. The chloroform layer

should be green at first, then colourless. Add 0·1 ml of bromothymol blue indicator, and then ammonium hydroxide until the indicator becomes blue, plus 0·3 to 0·4 ml in excess. Add 2 ml of chloroform and titrate with dithizone in chloroform soln., approx. 0·008%, added at first in any convenient increments of 1 to 5 ml. Shake vigorously after each addition and discard the lower layer at convenient intervals, until it is judged that most of the zinc has been extracted. Finally complete the titration in increments of 0·1 ml plus 1 to 2 ml of chloroform, until the last increment, instead of changing the colour to bright pink, leaves it green or purplish green. It is best to titrate the "reagent blank" soln. first, then to standardise the dithizone soln. against 5·0 or 10·0 ml of standard zinc soln. (1 ml ≡ 10 μg Zn) added to the extracted "reagent blank" soln., and finally to titrate the test soln.

(*e*) *Iron*—Iron is retained throughout in the aqueous soln. and since all interfering metals, except manganese, which is also quantitatively retained, are extracted in the previous operations, the thioglycollic acid method provides a specific method of determination. The interference due to manganese, in the small amounts likely to be present, is quite negligible.

(*f*) *Interferences*—Although the scheme has been found to provide in practice a virtually specific method of determination of the five metals considered, for the type of material examined, one or two of the common metals interfere, and while it has not been considered necessary to provide for their elimination in the main scheme, a few notes regarding the means of overcoming such interference, and of including the determination of some of these metals in the scheme if desired, may be acceptable.

(i) *Dithiocarbamate extraction stage*—Under the conditions of the method bismuth and mercury are quantitatively extracted with arsenic^{III} and copper by the carbamate reagent. Mercury does not colour the extract, but if present in sufficient amount may interfere with the determination of arsenic, by giving a turbidity at the final reduction stage, on addition of stannous chloride. Bismuth gives a yellow coloured compound, and therefore interferes with the copper determination. The colour is not nearly so intense as that of the copper complex, about 40 μg of bismuth being required to give an extract of the same optical density as that provided by 1 μg of copper under the same conditions. Hence, if the amount of bismuth present does not exceed the copper content, the error introduced in the copper determination cannot be more than about +2·5%, which may be considered negligible for most purposes. The presence of an appreciable amount of bismuth is indicated by the appearance, on addition of sodium iodide, of a yellow colour which is not discharged by metabisulphite. Since quinquevalent arsenic is not extracted by the carbamate reagent, bismuth, mercury and copper can be separated from it by extraction as diethyldithiocarbamates before reduction, the arsenic being reduced and extracted subsequently. In absence of a reducing agent part of the iron is extracted with the bismuth, imparting to the extract an olive-brown colour, and must be removed by repeating the extraction in presence of iodide and metabisulphite. The iron-free extract is decomposed, bismuth is separated from copper by extraction as dithizonate in presence of cyanide, and the copper is finally extracted from the residual solution, after addition of formaldehyde, by means of carbamate reagent. The recovered iron is subsequently added to the main soln. after separation of arsenic.

(ii) *Dithizone extraction stage*—The only common metals that interfere with the determination of lead are bismuth and tin^{II}. Bismuth has already been removed, while tin apparently remains throughout in the stannic condition, and is not extracted by dithizone, for no interference has been found from amounts of tin up to 500 p.p.m. The metals extracted at this stage, apart from lead and zinc, are nickel, cobalt and cadmium. Treatment of the dithizone extract with $N/10$ hydrochloric acid removes lead, zinc and cadmium, nickel and cobalt dithizonates being retained in the toluene layer. Although Sandell[10] states that nickel dithizonate is partly decomposed by very dilute hydrochloric acid, we find no appreciable trace of nickel in the acid layer under the given conditions. In the extended scheme (see later), the hydrochloric acid is buffered with a little sodium citrate, to give a *p*H of about 1·5, as an added precaution against loss of nickel, while permitting rapid extraction of zinc, etc. To separate zinc from cadmium, advantage is taken of the fact that whereas zinc dithizonate is readily decomposed by N sodium hydroxide, cadmium dithizonate is reasonably stable.

EXTENDED SCHEME OF ANALYSIS—Based on the observations outlined above, the following extended scheme enables all interferences to be overcome, and determinations of bismuth, nickel and cadmium to be included.

II. Extended Scheme

Arsenic, Copper, Lead, Zinc, Iron, Bismuth, Nickel and Cadmium

(All interferences of common metals—up to 500 p.p.m. of each—eliminated)

Wet Decomposition

Decompose 2 g of the sample in a modified Kjeldahl flask with sulphuric, nitric and perchloric acids.

Extraction of Copper and Bismuth (Hg, noble metals)

Dilute the soln. and add hydrochloric acid to give the min. HCl conc. of 2 *N*, as in Scheme I. Copper, bismuth (Hg, etc.) extracted cold with carbamate reagent. Some iron is also removed, but arsenic remains in the quinquevalent condition and is not extracted.

1st Carbamate Extract

Decompose the extract with $H_2SO_4+HClO_4$, add iodide and metabisulphite, and re-extract Bi, Cu, etc., with carbamate reagent.

Acid layer (A1) contains residual iron. Add it to acid soln. B2.

Carbamate extract—Decompose with $H_2SO_4+HClO_4$, and dilute.

Bismuth—Treat soln. (A2) with citrate and metabisulphite, neutralise, and add cyanide to prevent extraction of Cu, Hg, etc. Extract Bi with dithizone in toluene soln., wash the extract with dil. KCN soln., and determine optical density.

Alternatively, for larger amounts of Bi, decompose the dithizone extract and determine Bi by extraction as iodobismuthous acid.

Copper—Extract Bi with dithizone. Add formaldehyde to the aqueous layer, and extract copper with carbamate reagent. Determine the optical density of the extract as in Scheme I.

Acid Layer

Reduce arsenic with iodide and metabisulphite and extract with carbamate reagent as in Scheme I.

2nd Carbamate Extract (B1)

Arsenic—Evaporate chloroform, add acid molybdate reagent, and complete the determination of arsenic as in Scheme I.

Acid Layer (B2)

Evaporate acid layer (Al added) to fuming, and treat as acid layer B in Scheme I. Extract lead, zinc, nickel, cadmium and cobalt with dithizone in toluene soln., as before.

Dithizone extract

Extract lead, zinc and cadmium with *N*/10 HCl-citrate soln.

Aqueous layer

Determine iron as in Scheme I.

Dithizone layer (B3)
Ni (Co)
Nickel

Decompose soln. with H_2SO_4, HNO_3 and $HClO_4$. Add citrate, bromine water and ammonium hydroxide, followed by dimethylglyoxime soln. Determine the optical density at 25 ml dilution, in 4-cm cell, using blue filters No. 7.

HCl extract (B4)
(Pb, Zn, Cd)
Lead

Treat acid extract with citrate, ammonium hydroxide and cyanide, extract and determine lead as dithizonate, as in Scheme I.

Extraction of zinc and cadmium—After removal of lead, treat the soln. with formaldehyde, and extract Zn and Cd with dithizone in chloroform soln.

Determination of Zinc and Cadmium

Wash the extract with dil. ammonium hydroxide, and determine the optical density of the soln. of mixed Zn and Cd dithizonates ai 20·0 ml dilution in 1-cm cell, using Ilford green filters No. 604. Treat a 10-ml portion of the extract wtth *N* sodium hydroxide to decompose Zn dithizonate, and measure the optical density of the residual Cd dithizonate, using the same filters No. 604. Cadmium is thus determined, and the zinc content is obtained by difference from the two optical density readings.

Method

A. Separation and Determination of Arsenic, Bismuth and Copper—After wet decomposition, dilution and addition of hydrochloric acid, extract the acid soln. directly in the cold with 10 ml, followed by 5 ml, of carbamate extraction reagent, shaking for 30 sec. each time. (If sodium iodide has already been added, add a few drops of nitric acid, evaporate to fuming to remove iodine, digest with 0·5 ml of perchloric acid, add 10 ml of water and evaporate to fuming again, then dilute with 10 ml of water, add 15 ml of hydrochloric acid, 5 *N*, cool and extract). Separate, shake the acid layer with 5 ml of chloroform for 10 to 15 sec., and add the chloroform wash to the main extract. Next reduce the arsenic in the acid layer by adding 2 ml of sodium iodide soln. and warming. Add 0·5 ml of sodium metabisulphite soln., extract with 5 ml, followed by 2 ml, of carbamate reagent, and determine the arsenic in this second carbamate extract (B1) as before (Sec. 5, p. 236). Reserve the acid layer (B2).

Transfer the 1st carbamate extract, containing copper, bismuth, etc., and part of the iron, to a 50-ml flask, add 2 ml of diluted sulphuric acid (1+1) and 0·5 ml of perchloric acid,

evaporate the chloroform, heat to fuming, and then strongly for 10 min. Dilute with 15 ml of water, boil for 1 min., cool, add 1·0 ml of sodium iodide soln. and 0·2 ml of sodium metabisulphite soln. and re-extract with 10 ml followed by 5 ml of carbamate reagent. Add the residual acid layer (A1) to the main acid soln. (B2). Decompose the carbamate extract with 2 ml of diluted sulphuric acid (1+1) and 0·5 ml of perchloric acid, as before, and dilute with 10 ml of water (Soln. A2). Boil for 1 min., add 1·0 ml of sodium citrate soln., 0·2 ml of sodium metabisulphite soln. and 0·1 ml of bromothymol blue indicator, and render just alkaline with ammonium hydroxide. Cool thoroughly, just acidify with hydrochloric acid, 5 *N*, and add 0·5 ml of potassium cyanide soln, which should change the colour of the indicator to blue again. Extract the bismuth with dithizone in toluene soln., as described for lead (Sec. 7). Do not add ammonia, as bismuth dithizonate is less stable than the lead compound in presence of free ammonium hydroxide; thus Hubbard's procedure[11] gives slightly incomplete separation. Mercury and copper remain in the aqueous layer. Not all the mercury will be present, for some will have been lost by volatilisation during the initial wet decomposition; hence the determination of mercury is not considered. To determine copper, extract the aqueous layer 2 or 3 times with chloroform to remove all the excess dithizone, then add 1·0 ml of formaldehyde soln. with mixing, followed by 1·0 ml of ammonium hydroxide (extraction is incomplete from acid soln. under the given conditions). Extract the copper with 5 ml, followed by 2 ml, of carbamate reagent, dilute the extracts to 10·0 ml with chloroform, clarify with anhydrous sodium sulphate, and determine the optical density as described in Sections 3 and 4, pp. 235, 236). The same Spekker calibration curve applies.

If the amount of bismuth is small it may be determined as dithizonate. Wash the bismuth dithizonate in toluene extract with two 10-ml portions of dil. potassium cyanide soln. (1 ml of potassium cyanide reagent in 100 ml of water), shaking for 5 to 10 sec. each time. Wash the toluene layer twice with 5 ml of water, without mixing, dilute the washed extract, which is orange in colour, to 10·0 ml with toluene, and determine the optical density in a 1-cm cell, using blue No. 7 glass filters. Establish a standard curve by adding known amounts of standard bismuth soln. (1 ml ≡ 10 μg of Bi) to 2 ml of diluted sulphuric acid (1+1) and 0·5 ml of perchloric acid, evaporating to fuming, diluting, and continuing as for the test soln. (A2). A typical series of results is as follows:

Bismuth, μg	5	10	20	30	40	50
Optical density ..	0·112	0·231	0·460	0·676	0·869	1·045

For larger amounts of bismuth (say 50 to 250 μg of Bi) we prefer a procedure based on Haddock's method,[12] whereby the yellow compound formed by bismuth with iodide in acid soln. is extracted with a mixture of amyl alcohol and ethyl acetate. To apply this method, transfer the dithizone extract to a conical flask and evaporate most of the toluene, so that the vol. is reduced to about 10 ml. Add 2 ml of diluted sulphuric acid (1+1) boil off the toluene completely, add 0·5 ml of nitric acid and 0·2 ml of perchloric acid, evaporate to fuming, and fume strongly for 10 min. Add 10 ml of water, evaporate to fuming, cool, add 10 ml of water, boil for 1 min., add 0·1 ml of sodium metabisulphite soln., and boil for a further 1 min.* Cool thoroughly, add (with swirling) 3·0 ml of a mixture of sodium iodide soln. and hypophosphorous acid (2 vol. of 20% sodium iodide soln. and 1 vol. of 30% hypophosphorous acid), and transfer to a 25-ml separating funnel, adjusting the volume with wash water to 15 ml. Add 5 ml of amyl alcohol and ethyl acetate mixture (3+1 by vol.), shake for 10 to 15 sec., separate, run the lower layer back into the flask, and transfer the solvent layer to a dry 10-ml measuring cylinder. Do not attempt to wash the extract, or it will become turbid, but separate the two layers as sharply as possible. Run 4 ml of mixed solvent through the funnel into the flask containing the acid layer, and return the mixture to the funnel, shake, separate, reject the lower acid layer, and add the solvent layer to the first extract in the measuring cylinder. Adjust the vol. to 10·0 ml with mixed solvent, and drain into a dry 50 ml conical flask. Swirl the soln., and allow any traces of aqueous soln. to collect in a drop at the bottom. Determine the optical density in a 1-cm cell, using blue filters No. 7. Establish a standard curve by extracting known amounts of bismuth with

* In Haddock's procedure, hypophosphorous acid is apt to reduce sulphur dioxide to hydrogen sulphide, with resulting formation of some Bi_2S_3. Subsequent addition of iodide usually slowly converts all the bismuth into iodobismuthous acid, but a turbidity due to free sulphur then forms in the soln. For this reason we prefer to (1) remove all but a trace of sulphur dioxide by boiling, (2) mix the hypophosphorous acid and iodide reagents before adding them to the test soln.

dithizone in toluene as described for the dithizone method, and then treat the dithizone extracts as described above, finally determining the optical density of the iodobismuthous acid extracts in amyl alcohol-ethyl acetate mixture. A typical series of results is as follows:

Bismuth, μg	15	30	50	100	150	200	250
Net optical density ..	0·042	0·080	0·133	0·263	0·394	0·520	0·640

The optical density should be determined without undue delay.

B. Separation and Determination of Dithizone Extractable Metals—After removal of copper, bismuth, etc., and separation of arsenic, evaporate the combined acid layers (A1 and B2) to fuming, treat as acid layer B in Scheme I, and extract with dithizone in toluene soln. (see Sec. 6, p. 237), iron being determined as previously (Sec. 9, p. 239).

After separating the dithizone in toluene extract, add to it a further 20 to 25% excess of dithizone in toluene soln. and separate the lead, zinc and cadmium by extracting with two 10-ml portions of hydrochloric acid and citrate soln. (2·5 ml of trisodium citrate soln., 1 *M* (reagent 6), mixed with 100 ml of *N*/10 hydrochloric acid), shaking for 30 sec. each time. Reserve the combined acid extracts (soln. B4). The residual toluene layer contains all the nickel and cobalt. To determine nickel, add 2·0 ml of diluted sulphuric acid (1+1) to the toluene extract, contained in a 50-ml conical flask, evaporate the toluene completely, add 0·5 ml of nitric acid and 0·2 ml of perchloric acid, evaporate to fuming and heat strongly for 10 min. Allow to cool, add 15 ml of water, boil for 2 min., cool, add 0·5 ml of sodium citrate soln., 1·0 ml of saturated bromine water, and ammonium hydroxide soln. until the yellow colour is just completely discharged. Cool to about 20° C., add 2·0 ml of ammonium hydroxide, 2·0 ml of dimethylglyoxime soln. (0·2% in ammonium hydroxide, 5 *N*) and mix. Add a further 0·5 ml of saturated bromine water, dilute to 25·0 ml, mix, and allow to stand for 15 min. Determine the optical density in a 4-cm cell, using blue filters No. 7. To establish a standard curve, add known amounts of nickel to 2·0 ml of diluted sulphuric acid (1+1) and 0·2 ml of perchloric acid, evaporate to fuming, dilute and continue as above. A typical series of results is as follows:

Nickel, μg	5	10	15	20	30	40	50
Optical density	0·140	0·265	0·390	0·520	0·760	0·975	1·190

The curve is therefore not quite rectilinear.

Determine lead in the reserved hydrochloric acid extract B4 as described in Scheme I (Sec. 7), and then determine zinc and cadmium in the residual citrate-cyanide layer as follows. Add 0·5 ml of formaldehyde soln. and 2·0 ml of hydrochloric acid, 5 *N*, and extract the residual dithizone with small portions of chloroform. Render the soln. slightly alkaline with ammonium hydroxide, extract zinc and cadmium with a slight excess of freshly prepared dithizone in chloroform soln., and wash the extract by shaking for 5 to 10 sec. with two successive 10-ml portions of dil. ammonium hydroxide soln., 0·2 *N*. Dilute the washed soln. of zinc and cadmium dithizonates to 20·0 ml with chloroform, and reserve 10 ml in a clean dry 50-ml conical flask for the determination of cadmium. Determine the optical density of the remaining 10 ml in a 1-cm cell. For this determination use Ilford Spectrum green filters No. 604, since it is necessary to employ filters which give a rectilinear curve for both zinc and cadmium dithizonates. The same tungsten filament lamp is used, but the more sensitive "spot" galvanometer is required. After determining the optical density of the soln. of mixed zinc and cadmium dithizonates, determine that due to cadmium dithizonate alone as follows. Transfer the reserve 10 ml of soln. to a 25-ml separating funnel, and shake for 15 sec. each time with 10 ml of *N* sodium hydroxide soln., followed by two 5-ml portions of sodium hydroxide soln. diluted with 5 ml of water. Run the chloroform layer into a dry flask, rejecting the sodium hydroxide layer each time, then return the chloroform layer to the funnel, and shake with 10 ml of water. Determine the optical density of the washed pinkish-orange cadmium dithizonate in chloroform soln. in a 1-cm cell, using Ilford green filters No. 604, as before. From the result determine the cadmium content by means of the standard curve, remembering that the original dilution of the cadmium soln. was 20·0 ml. Determine the zinc content by difference, thus: From the standard zinc curve find the number of μg of zinc corresponding to the observed optical density of (*a*) the soln. of mixed zinc and cadmium dithizonates, (*b*) the soln. of cadmium dithizonate alone. Then the difference between (*a*) and (*b*) gives the zinc content in μg of zinc.

Observed optical density data for standard cadmium and zinc solutions are given in Table I, which also includes similar data obtained by us for the other metals considered, using Ilford gelatin filters and a mirror galvanometer with the tungsten filament lamp.

TABLE I

OPTICAL DENSITIES, USING ILFORD COLOUR FILTERS

		Metal							
	μg	Cu	As_2O_3	Pb	Zn	Fe	Bi*	Ni	Cd
	5	—	0·118	—	0·298	—	—	0·138	0·145
	10	0·100	0·242	0·302	0·596	0·055	0·328	0·262	0·295
	15	—	0·352	—	0·90	—	—	0·378	0·435
	20	0·202	0·470	0·610	1·20	—	0·655	0·475	0·58
	25	—	0·588	—	1·50	—	—	—	0·73
	30	0·303	—	0·915	1·79	0·164	0·99	0·670	0·88
	40	0·404	—	1·218	—	—	1·32	0·855	—
	50	0·504	1·18	1·523	—	0·267	1·63	1·02	1·44
	100	—	—	—	—	0·535	—	—	—
	200	—	—	—	—	1·06	—	—	—
Dilution, ml	..	10	5·5	10	20	50	10	25	20
Cell, cm	..	1	1	1	1	4	1	4	1
Filter No.	..	602	608	604	604	604	602	602	604
Filter colour	..	blue	red	green	green	green	blue	blue	green

* Bismuth as dithizonate.

Note on Spekker observations—Certain discrepancies obtained at first in optical density readings were traced to rapid loss of solvent by evaporation. Hence, when the coloured solutions (particularly in volatile organic solvents) have been made up to their final volume for observation of the optical density on the Spekker absorptiometer, they should be preserved in tightly-corked flasks or vials, or in glass-stoppered cylinders, and during the determination of optical density the cell should be kept covered with a glass plate.

ACCURACY OF THE METHOD—Tests on the recovery of known amounts of metals added to certain medicinals gave the following results (Table II).

TABLE II

RECOVERY OF ADDED METALS*

Medicinal used	Mepacrine hydrochloride				*p*-Aminobenzenesulphonamide									
Test No.	1		2		3		4		5		6		7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Bi	—	—	—	—	—	—	—	—	15	14·7	250	243	100	104
Hg	—	—	—	—	—	—	—	—	50	—	50	—	200	—
Cu	—	4·7	5	5·3	—	2·5	30	32·4	10	10·0	50	49·6	35	35·9
As_2O_3	—	0·2	15	15·3	—	0·1	5	5·2	2	2·2	8	8·1	18	17·9
Pb	—	2·6	10	9·8	—	0·5	20	20·0	10	10·9	30	28·9	20	20·6
Zn	—	4·5	15	14·9	—	1·1	5	5·1	25	23·9	15	14·5	5	5·2
Ni	—	—	—	—	—	nil	—	—	10	8·5	50	49·1	30	28·8
Co	—	—	—	—	—	—	—	—	50	—	—	—	50	—
Cd	—	—	—	—	—	nil	—	—	15	15·1	5	5·6	25	24·5
Fe	—	37	200	205	—	26	25	24	50	49·8	10	10·3	150	154
Mn	—	—	—	—	—	—	—	—	22	—	550	—	110	—
Sn	—	—	—	—	—	—	—	—	—	—	—	—	500	—
Sb	—	—	—	—	—	—	—	—	—	—	500	—	—	—
P_2O_5	—	—	—	—	—	—	—	—	—	—	—	—	500	—

* A, amount added in μg. B, amount found in μg.

Notes—(1) All values given are net values, after deduction of the appropriate blanks, and where metals have been added (Tests 2, 4, 5, 6 and 7) the amounts of metals found in the pure medicinals have been allowed for.

(2) Tests 1, 2, 3, 4 were carried out according to the main scheme; tests 5, 6, 7 by the extended scheme.

(3) Bismuth in test 5 was determined as dithizonate; in tests 6 and 7 by the iodide method.

The reproducibility of the results, as shown by a large number of routine tests, is of the order indicated in Table III.

The data given in Tables II and III indicate that the methods given provide an effective means of separation and specific determination of the metallic impurities considered, in compounds such as medicinals which contain only small amounts of inorganic matter (other than the alkali metals). The degree of accuracy obtainable may be considered highly satisfactory for the purpose of routine control of quality.

TABLE III

REPRODUCIBILITY OF RESULTS

Metal	Range p.p.m. on 2-g sample	Reproducibility p.p.m. on 2-g sample
Copper, Cu	(*a*) 0 – 10	±0·5
	(*b*) 10 – 25	±1
Arsenic, as As_2O_3 ..	0 – 12·5	±0·5
Lead, Pb	(*a*) 0 – 10	±0·5
	(*b*) 10 – 25	±1
Zinc, Zn	0 – 25 (titrimetric or photometric)	±1
Iron, Fe	(*a*) 0 – 50	±2
	(*b*) 50 – 250	±5
Nickel, Ni	0 – 25	±1
Bismuth, Bi		
(*a*) dithizone ..	0 – 25	±1
(*b*) iodide ..	25 – 125	±5
Cadmium	0 – 25	±1

SUMMARY—A scheme is presented for the successive determination of minute amounts of copper, arsenic, lead, zinc and iron in a single 2-g sample of organic material. It has been devised principally for application to organic compounds, such as medicinals, with a very low metal specification limit, but is sufficiently flexible to be adaptable to materials with a somewhat higher metal content. The interferences of other common metals, supposed present in amounts up to 500 p.p.m., have been investigated. Under the given conditions the method is found to be specific for arsenic, lead and iron. Bismuth interferes with the determination of copper; cadmium is included with the zinc. Conditions are described for eliminating these interferences, and for determining bismuth, nickel and cadmium if desired. Data are given regarding the accuracy and reproducibility of the results obtained.

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The Volumetric Determination of Sodium in Blood and Serum

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The techniques described for the determination of sodium in serum include those of Kramer and Gittlemann (1924),[1] Butler and Tuthill (1931),[2] Weinbach (1935),[3] and King, Haslewood, Delory and Beall (1942).[4] The need for a rapid and accurate procedure for the analysis of a series of specimens arose in this laboratory, and the first three of the above methods have been critically examined. Kramer and Gittleman's technique, depending on pptn. of sodium from the ashed serum as the pyroantimonate in alcohol and subsequent determination of this iodimetrically failed to give satisfactory results. The Butler and Tuthill method consists in gravimetric determination of sodium uranyl zinc acetate pptd. from 1 ml of wet-oxidised serum, and, although it was found to yield excellent results, it was considered unsuitable for serial analysis because of the time and attention required for complete oxidation with nitric and sulphuric acids. This drawback was particularly evident when whole blood was to be analysed. It was decided therefore to investigate the accuracy of the alkalimetric method of determining the triple salt pptd. from deproteinised blood and serum. The principle of this method has been outlined by Weinbach,[3] who also described details for its application to biological materials. Critical examination has shown that it is subject to a number of objectionable features, which are discussed below. A simple modification has been devised which, besides being economical in time and material, yields results of sufficient accuracy for studies of the electrolyte distribution between blood cells and plasma.

Experimental

Alkalimetric Titration of Triple Salt—The reaction of the triple salt with alkali might be expected to proceed according to the equation assumed by Weinbach, *viz.*

$$(UO_2)_3ZnNa(CH_3COO)_9 6H_2O + 8NaOH = 3UO_2(OH)_2 + Zn(OH)_2 + 9CH_3COONa + 6H_2O$$

It has been found, however, that the stoichiometric relationship between the two is dependent on the conditions of the titration. When very dilute solns. of the triple salt are titrated with 0·02 *N* sodium hydroxide, as recommended by Weinbach, they do give results approximating to the above equation (Table I), but the end-point with phenolphthalein is transient and difficult to determine with accuracy. Further expts. showed that the titration value under these conditions varied with the temperature of the soln. and that a reasonably sharp and permanent end-point was obtained only at 30–45° C. (Table II).

Table I

Titration of Triple Salt Dissolved in 70 ml of Water at 32° C. with 0·02 *N* Sodium Hydroxide

Triple salt, mg	4·0	8·0	12·0	16·0
Titre, ml	1·03	2·01	3·09	4·16
Litres *N* alkali equiv. to 1 g-mol. of triple salt	7·9	7·8	8·05	8·0

Table II

Effect of Temperature of Triple Salt Solution on Alkali Titre

8·0 mg of T.S. in 70 ml of water and 0·5 ml of phenolphthalein titrated to first faint pink with 0·02 *N* NaOH

Temp. °C.	Titre ml of 0·02 *N* NaOH	End-point colour	Litres of *N* NaOH equiv. to 1 g-mol of triple salt
10	1·75	not permanent	6·72
15	1·82	,, ,,	7·00
20	1·88	,, ,,	7·22
25	1·98	,, ,,	7·61
30	2·04	fades slowly	7·85
35	2·12	permanent	8·15
40	2·11	,,	8·10
45	2·13	,,	8·19

When, however, stronger solns. of the triple salt are titrated with 0·1 *N* NaOH, the stoichiometric relationship is quite different (Table III). This can be explained by a reaction

between the amphoteric $UO_2(OH)_2$ and NaOH, for it has been found from separate volumetric analysis of uranyl acetate under similar conditions that 1 g-mol. of this salt is equiv. to 2·33 litres of *N* sodium hydroxide.

TABLE III

TITRATION OF TRIPLE SALT IN 5 ML OF WATER WITH 0·1 *N* NaOH

Amount of triple salt	Titre ml of 0·1 *N* NaOH	Litres of *N* NaOH equiv. to 1 g-mol. of triple salt
10 mg	0·60	9·2
30 ,,	1·74	8·9
40 ,,	2·28	8·8
50 ,,	2·88	8·9
60 ,,	3·46	8·9
75 ,,	4·30	8·8

From Table III is derived the relationship that 1 ml of 0·1 *N* sodium hydroxide is equiv. to 17·3 mg of triple salt, *i.e.*, to 0·259 mg of Na as triple salt, and that under the conditions described the equation for the reaction more closely approximates to

$$(UO_2)_3ZnNa(CH_3COO)_9 6H_2O + 9NaOH = NaHUO_4 + 2UO_2(OH)_2 + Zn(OH)_2 + 9CH_3COONa + 7H_2O$$

than to Weinbach's equation. From the practical viewpoint, the advantage of titrating a strong soln. of triple salt is the much greater sharpness and permanence of the end-point; it can, moreover, be carried out with precision even in artificial light, and is not affected by any ordinary variation in the temperature of the solution.

PRECIPITATION OF TRIPLE SALT FROM BLOOD FILTRATES—The removal of proteins from blood by trichloroacetic acid prior to determination of sodium has been criticised by Van Slyke *et al.* (1927),[5] and by Oberst (1935),[6] because of possible unequal distribution of the sodium between the coagulum and the aqueous phase. In some preliminary expts. on this point, I have found that provided that a sufficiently high dilution is effected (1 in 10 for plasma and 1 in 20 for blood) accurate values are attainable.

Weinbach has recommended addition of 95% alcohol to the mixture of blood filtrate and uranyl zinc acetate reagent to complete the pptn. of triple salt. It has been observed here, however, that alcohol depresses the solubility of the reagent itself to a small extent, and therefore this practice is likely to lead to high results. To avoid this error one can simply concentrate the blood filtrate to small bulk prior to adding the precipitating reagent in accordance with Butler and Tuthill's procedure.

Prior to alkalimetric determination of the triple salt, it is obviously essential to remove all traces of uranyl zinc acetate reagent. The very concentrated supernatant layer was decanted by Weinbach after centrifuging, and the tube and ppt. were drained for a few minutes before washing with one 10-ml portion of acetone previously saturated with triple salt and filtered. This single centrifugal wash he claimed to be sufficient, but in my experience the technique gives high results, most probably due to incomplete washing. On the other hand, multiple centrifugal washing is undesirable because of a tendency for the fine ppt. to creep down the sides of the inverted tube during drainage.

In view of the uncertainties and difficulties of Weinbach's method the following simple modification, which has been in use here for several months with very satisfactory results, is recommended for speed and accuracy.

Reagents required—(1) 0·1 *N* sodium hydroxide; (2) 1% w/v soln. of phenolphthalein in alcohol; (3) 15% w/v trichloroacetic acid; (4) uranyl zinc acetate reagent; (5) redistilled alcohol saturated with triple salt. The last two reagents are prepared as described by Peters and Van Slyke.[7]

Method—To 1 vol. of material add 7 vols. of water for the analyses of plasma or serum or 17 vols. for whole blood, followed by dropwise addition of 2 vols. of trichloroacetic acid. Shake vigorously, and, if necessary, warm to 60–70° C. to complete coagulation of protein. Filter through a small Whatman filter No. 42. Measure 2 ml of the clear colourless filtrate from serum (or 4 ml of blood filtrate), into a hard-glass test-tube and evaporate gently to a single drop. Add 5 ml of freshly-filtered uranyl zinc acetate reagent and stir for 2 or 3 min. with a thin glass rod. Leave at room temp. for about 1 hr., and then filter through a small sintered-glass funnel (Jena 12 G 3 or its equivalent) fitted into a filter test-tube, applying slight suction when necessary. Thoroughly rinse the sides of the tube in which pptn. was carried out with four 2-ml lots of freshly-filtered alcohol reagent, thereby transferring the ppt. quantitatively to the filter. Drain the ppt. by slight suction at each washing. Place the funnel in a second

clean filter test-tube and dissolve the triple salt by adding five 1-ml lots of water and applying suction after each addition. Add 1 drop of indicator to this soln., and titrate with 0·1 *N* sodium hydroxide from a 5-ml micro-burette graduated in 0·01 ml, to the first shade of pink (T ml). Correct the vol. of sodium hydroxide soln. required by subtracting the volume for a blank analysis of the reagents employed (t ml). Sodium in mg per 100 ml of material = (T–t) × 0·259 × 500.

This procedure has been critically examined by analyses of solutions of sodium chloride, of blood, and of mixtures of the two. Some specimen analyses are recorded as an indication of the accuracy attainable (Table IV). The results of a few comparative analyses by the present method, by Butler and Tuthill's method, and by Weinbach's method are recorded in Table V

TABLE IV

ANALYSIS OF SODIUM CHLORIDE SOLUTIONS, BLOOD, ETC., AND RECOVERY EXPERIMENTS

Specimen analysed, equiv. to	Na added mg	Sodium content by analysis mg	Amount of added sodium found mg
Standard Na solns. containing 0·317 mg ..	nil	0·321	—
0·396 „ ..	„	0·398	—
0·475 „ ..	„	0·480	—
0·554 „ ..	„	0·559	—
0·634 „ ..	„	0·624	—
0·712 „ ..	„	0·712	—
Serum in quadruplicate, 0·2 ml	„	0·663	—
	„	0·665	—
	„	0·668	—
	„	0·663	—
Blood in duplicate, 0·2 ml	„	0·378	—
	„	0·370	—
Defibd. plasma in duplicate, 0·2 ml ..	„	0·622	—
	„	0·632	—
Serum, 0·2 ml	nil	0·676	—
	0·197	0·870	0·194
	0·394	1·075	0·399
Serum, 0·2 ml	nil	0·650	—
	0·079	0·725	0·075
	0·158	0·810	0·160
	0·237	0·891	0·241
	0·316	0·960	0·310

TABLE V

COMPARATIVE ANALYSES BY THE PROPOSED METHOD, BY WEINBACH'S AND BY BUTLER AND TUTHILL'S METHOD

Material analysed	Na content by proposed method mg	Na content (Weinbach) mg	Na content (Butler and Tuthill) mg
Standard NaCl solns. containing			
0·197 mg Na	0·199	0·207	—
0·393 „ „	0·396	0·415	0·394
0·590 „ „	0·600	0·622	—
Plasma A	314 per 100 ml	324 per 100 ml	312 per 100 ml
B	308 „ „ „	318 „ „ „	312 „ „ „
C	306 „ „ „	319 „ „ „	304 „ „ „
D	304 „ „ „	325 „ „ „	307 „ „ „

SUMMARY—A simple method for the determination of sodium in whole blood or serum is described, in which the sodium is pptd. as sodium uranyl zinc acetate from the deproteinised material, the amount of triple salt being determined by micro-titration with alkali. A previously published method on the same general principles by Weinbach is the subject of critical examination, and specimen analyses by the two methods are recorded for comparison.

The proposed technique is economical in time and material, and provides results of the same degree of accuracy as are attainable by the original gravimetric procedure of Butler and Tuthill.

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Polarographic Determination of Lead in Brasses and Bronzes

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INTRODUCTION—The conventional method for the determination of lead in brasses and bronzes involves its separation as sulphate. For complete pptn. of lead sulphate, the soln. must stand for at least 1 hr.[1] Since it is always advisable to remove lead before going on with a complete analysis of brasses and bronzes, the routine analysis of these alloys is a slow process. A direct polarographic method of determining lead was therefore attempted with the view of speeding up the routine analysis of these alloys.

PRELIMINARY CONSIDERATIONS—For the determination of lead in brass Hohn[2] separated the lead from the copper as basic lead carbonate and finally determined it polarographically, using a base electrolyte of saturated ammonium chloride soln. Mnich,[3] however, found that this method gave low results unless the ppt. was allowed to stand for 24 hr. before filtration.

Alternatively, copper can be separated from lead by reduction to the metallic state by sodium hypophosphite. The lead can then be determined polarographically on the filtrate, using a saturated ammonium chloride solution as the base electrolyte.

The methods so far suggested involve chemical separations because copper interferes with the lead curve in the base electrolytes chosen. Nickelson[4] has shown that lead in Ledloy (free-cutting steel containing 0·15 to 0·30% of lead) can be determined directly, and so the possibility of a direct determination of lead in copper base alloys was investigated.

Copper forms with cyanide a complex ion whose half wave potential is well outside the normal polarographic working region for lead, which in cyanide medium produces a curve with a half-wave potential of −0·72 volt.[5] The type of curve obtained under these conditions was found unsuitable for accurate quantitative work and attempts to improve the curve by addition of a saturated ammonium chloride soln. proved unsatisfactory, as the decomposition potential of lead was too near to zero potential.

So far, the removal of dissolved oxygen, which interferes with polarographic curves, was effected by passing hydrogen through the soln. Since sodium sulphite can be more conveniently used to remove dissolved oxygen in alkaline media, the effect of adding potassium cyanide to the cyanide medium was investigated. This base electrolyte gave a much improved curve for lead, but it was found necessary to control the concentration of sodium hydroxide to obtain the best results. The lead curve had a half-wave potential of −0·2 volt with respect to a mercury pool anode (−0·7 volt with respect to S.C.E.). Using this curve for lead, the following methods were developed for the determination of lead in brasses and bronzes.

METHODS—I. DETERMINATION OF LEAD IN BRASSES AND BRONZES (LEAD > 1·0%)—Dissolve 1·0 g of the sample in 5 ml of nitric acid (sp.gr. 1·42) and 10 ml of water. Boil off nitrous fumes, cool, and make up to 50 ml. Pour a quantity through a dry Whatman No. 40 filter-paper into a dry beaker to remove tin.

Pipette 10 ml of this soln. into a dry beaker and add the following solns. accurately from burettes in the order given—(1) 10 ml of sodium sulphite solution (50 g in 100 ml of water); (2) 8 ml of a 13% potassium cyanide soln.; (3) 10 ml of 10 *N* sodium hydroxide; (4) 2 ml of a 0·2% soln. of gelatin in water.

Pour a quantity of this well-mixed soln. into a polarograph cell, add a pool of mercury to form the anode, and place the cell in a thermostat at 25° C. for 10 min. Record the

polarogram, using the sensitivity of 10 micro amperes of the Tinsley Polarograph. Range 0 to −1·0 volt.

II. Determination of Lead in Brasses and Bronzes (Lead < 1·0%)—Dissolve 2·0 g of the sample in 10 ml of nitric acid (sp.gr. 1·42) and 10 ml of water. Boil off nitrous fumes. Cool and make up to 50 ml. Pour a quantity through a dry Whatman No. 40 filter-paper into a dry beaker to remove tin.

Pipette 10 ml of this solution into a dry beaker and add the following solutions accurately from burettes in the order given—(1) 10 ml of sodium sulphite soln. (50 g in 100 ml of water); (2) 16 ml of a 13% potassium cyanide soln.; (3) 10 ml of 10 *N* sodium hydroxide; (4) 2 ml of a 0·2% soln. of gelatin in water.

Record the polarogram using the sensitivity of 4 micro amperes of the Tinsley Polarograph. Range 0 to −1·0 volts.

Results—To 1·0 g portions of pure copper, each dissolved in 5 ml of nitric acid (sp.gr. 1·42) and 10 ml of water, a standard lead acetate soln. was added to give amounts of lead ranging from 1·0 to 5·0%. Method I was carried out on these solns., with the results shown in Table I. The graph of the results was a straight line passing through the origin.

Table I

Lead, %	Height of curve in divisions (average of 3 determinations)
1·0	8·0
2·0	16·5
3·0	24·5
4·0	32·0
5·0	40·0

To 2·0 g portions of pure copper, each dissolved in 10 ml of nitric acid (sp.gr. 1·42) and 10 ml of water, a standard lead acetate soln. was added to give amounts of lead ranging from 0·20 to 1·0%. Method II was carried out, with the results shown in Table II. The graph of the results was a straight line passing through the origin.

Table II

Lead, %	Height of curve in divisions (average of 3 determinations)
0·20	8·5
0·40	17·0
0·60	25·5
0·80	34·0
1·0	42·5

The effects of all the other elements normally occurring in brasses and bronzes were then studied. In expts. in which these elements were present in both small and large amounts the same linear relationship was obtained with the graph passing through the origin, except that when manganese was present in amounts greater than 1·0% interference occurred owing to pptn. of manganese hydroxide. These methods were then applied to a wide variety of copper base alloys other than those containing more than 1·0% of manganese, with the results shown in Table III.

Table III—Elements, %

Type of alloy	Cu	As	Fe	Ni	Sn	Zn	Mn	Ag	Pb Chem.	Pb Polarog.
Leaded brass ..	56·87	0·02	0·10	0·05	0·16	Rem.	—	—	3·09	3·08, 3·10, 3·07
Gun metal	Rem.	0·08	0·60	0·07	7·03	6·82	—	—	2·55	2·57, 2·58, 2·53
Cast phosphor bronze	,,	0·02	0·02	0·02	7·95	4·37	—	—	0·29	0·27, 0·27, 0·28
,, ,, ,,	,,	0·02	0·02	0·01	8·35	2·87	—	—	0·12	0·13, 0·12, 0·13
Brass with nickel ..	73·0	—	0·32	0·74	1·0	Rem.	—	—	1·73	1·70, 1·76, 1·69
,, ,, Fe and Mn	57·5	—	0·44	—	1·06	,,	0·85	—	1·52	1·52, 1·52, 1·53
Lead bronze	72·15	—	0·46	—	2·08	—	—	—	25·45	25·36, 25·41*, 25·41*
,, ,, ...	Rem.	—	—	—	—	—	—	0·95	25·48	25·60*, 25·43*, 25·53*

* Sensitivity of 40 micro-amperes used for these determinations.

The chemical determinations of lead were made by pptg. as sulphate and finishing as lead molybdate.

These results verify the conclusion that the other elements normally present in brasses and bronzes do not interfere with the polarographic lead determination. With regard to the interference when manganese is present in large amounts, it was found that ammonium citrate soln. retards the pptn. of manganese hydroxide sufficiently to prevent it from interfering with the lead determination. The following methods were therefore developed for such brasses.

MODIFIED METHODS OF DETERMINATION OF LEAD IN BRASSES AND BRONZES IN WHICH MANGANESE EXCEEDS 1·0%—METHOD I—LEAD > 1·0%—Proceed as described in Method I (p. 250) up to the stage "Pipette 10 ml of this soln. into a dry beaker," but after adding 10 ml of sodium sulphite soln. add 2 ml of ammonium citrate soln. (100 g of citric acid dissolved in 50 ml of water and treated with 100 ml of conc. ammonia), before the remainder of the reagents.

Record the polarogram using a sensitivity of 10 micro amperes of the Tinsley Polarograph. Range 0 to −1·0 volts.

METHOD II—LEAD < 1·0%—Proceed as described in Method II (p. 251) to the stage "Pipette 10 ml of this soln. . . . ," but after adding 10 ml of sodium sulphite soln. add 2 ml of ammonium citrate soln. before the remainder of the reagents.

Record the polarogram, using a sensitivity of 4 micro-amperes of the Tinsley Polarograph. Range 0 to −1·0 volts.

RESULTS—To 1·0 g portions of pure copper, each dissolved in 5 ml of nitric acid (sp.gr. 1·42) and 10 ml of water, a standard manganese soln. was added to give 4·0% of manganese, and varying amounts of a standard lead acetate soln. were added to give amounts of lead ranging from 1·0 to 5·0%. Modified Method I was carried out with the results shown in Table IV. The graph of the results was a straight line coinciding with the graph obtained by carrying out modified Method I on synthetic mixtures containing no manganese.

TABLE IV

Lead, %	Height of curve in divisions (average of 3 determinations)
1·0	8·5
2·0	17·0
3·0	25·5
4·0	33·5
5·0	42·0

To 2·0 g portions of pure copper, each dissolved in 10 ml of nitric acid (sp.gr. 1·42) and 10 ml of water, a standard manganese soln. was added to give 4% of manganese. Varying amounts of a standard lead acetate soln. were added to give proportions of lead ranging from 0·20 to 1·0%. Modified Method II was used, with the results shown in Table V.

TABLE V

Lead, %	Height of curve in divisions (average of 3 determinations)
0·20	8·75
0·40	17·25
0·60	26·0
0·80	34·25
1·0	43·0

On plotting these points linear relationships were obtained, the graphs passing through the origin. These graphs coincided with those obtained by means of the above methods on synthetic mixtures containing no manganese.

These methods were then applied to a variety of copper base alloys with a high percentage of manganese, and the results shown in Table VI were obtained.

TABLE VI—Elements, %

No. of sample	Cu	As	Fe	Ni	Mn	Sn	Zn	Pb Chem.	Pb Polarog.
1	56·87	0·02	0·10	0·05	4·0	0·16	Rem.	3·07	3·04, 3·05, 3·09
2	57·33	0·02	0·15	0·13	4·05	0·56	,,	2·10	2·10, 2·13, 2·08
3	56·28	0·02	0·24	0·10	4·31	0·72	,,	2·03	2·01, 2·03, 2·04
4	Rem.	0·02	0·02	0·02	3·0	7·95	4·37	0·27	0·27, 0·28, 0·26

These results confirm the conclusion that ammonium citrate inhibits the pptn. of manganese hydroxide sufficiently to prevent interference with the polarographic lead determination.

DISCUSSION OF RESULTS AND CONCLUSIONS—For all this work a drop time of 1·5 sec. was used. The solns. were maintained at 25° C. and 2 volts were applied across the main potentiometer of the Tinsley Polarograph.

The lead figures given in the Tables represent the results by several workers and show the reproducibility of the polarographic determination. An agreement within ±0·01% with figures obtained by standard chemical methods was achieved for the brasses and bronzes containing a low percentage of lead, whilst agreement within ±0·03% was achieved for those with lead contents up to 4%. For bearing metals containing amounts of lead ranging from 20 to 28% agreement within ±0·15% can be attained provided that there is no doubt about the homogeneity of the original samples. By this method a single determination can be carried through in 20 min., showing considerable saving in time as compared with the chemical method with an equivalent accuracy.

The Director of Scientific Research, Admiralty, has granted permission to publish this paper. The help and co-operation of the Staff of the Bragg Laboratory are gratefully acknowledged.

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BRAGG LABORATORY
SHEFFIELD, 9

November, 1944

The Photometric Determination of Lead in Brass

BY R. E. OUGHTRED

FOR the determination of lead in brass, Fisk and Pollak[1] have described a rapid procedure which entails the separation of the lead as chromate. The ppt. is dissolved and the determination is finished volumetrically with standard ferrous ammonium sulphate and potassium dichromate. To compensate for solubility errors the lead factor for the dichromate is obtained by a control determination carried out on a standard lead soln. Considerations of the solubility of the ppt. and the small titration values impose a lower limit (1% on a 1·5 g sample) on the amount of lead which can be estimated satisfactorily by this method.

These drawbacks can be overcome and the range of the determination extended to zero if (i) virtually complete pptn. of the lead is achieved and (ii) the determination is subsequently finished absorptiometrically (after a method of Jones[2]).

METHOD—The details of the method used in this laboratory are as follows. For brass containing only small amounts of tin, treat 3·75 g with 20 ml of conc. nitric acid and evaporate to low bulk, dilute, digest and filter off the metastannic acid on a pulp pad. Wash with 1% nitric acid and make up the filtrate and washings to 250 ml. Pipette a 100-ml fraction into a 300-ml beaker and treat dropwise, while stirring, with saturated sodium carbonate soln. to produce a permanent ppt.; clear with a few drops of glacial acetic acid and then add 10 ml in excess. Heat to boiling and precipitate with 40 ml of saturated potassium dichromate soln., adding the reagent dropwise, with stirring, until pptn. begins. Boil for 3 min. and cool by immersing the beaker in running water for 15 min. Filter through an asbestos pulp pad and wash with cold 5% acetic acid followed by cold water. Pipette 20 ml of dil. nitric acid (2 vols. of HNO_3 sp.gr. 1·42; 3 vols. of water, previously boiled and cooled) into the beaker in which the pptn. was made and pour over the pad, collecting the filtrate in a 100-ml volumetric flask. Should any lead chromate remain undissolved, return the nitric acid soln. to the beaker *via* the pad, warm slightly, and repeat the extraction. When all is dissolved rinse the beaker and pad with cold water. Mix the contents of the flask by swirling, cool and make up to 100 ml with water. Fill a 2-cm cell with the well-mixed soln. and measure the absorption in the Spekker absorptiometer (instrument setting: water-to-water 1·00; filters, violet 601).

The maximum drum reading corresponds to *ca.* 3% of lead on the basis that the 100 ml of final soln. represents 1·5 g of the sample, but lower percentages up to 1·5% can be

measured in 4-cm cells. Should the lead content exceed 3%, take a smaller aliquot of the filtrate from the tin. If the tin content is so high as to make filtration of the metastannic acid difficult, reduce the initial sample wt. to 1·5 g and adjust the vol. of the filtrate to 100 ml. Without further fractionation treat the soln. with dichromate as described above.

Construction of calibration curves—Dissolve 0·9 g of pure lead in 40 ml of diluted nitric acid (1+1), boil to expel nitrous fumes, cool and make up to 1 litre. Deliver quantities ranging from 50 to 5 ml by decrements of 5 ml into 300-ml beakers, make up to 100 ml with water, and proceed exactly as described above. Plot [1·00 minus drum reading] against % of lead. The absorption of 20 ml of the nitric acid solvent (diluted to 100 ml) does not differ from that of water; consequently the curves may be produced to pass through the origin.

RESULTS—Table I compares results given by this method with those obtained by means of established procedures.

TABLE I

Brass No.	Lead, %		
	Titration method	Photometric method	Lead sulphate method
1	2·44	2·41	2·39
2	2·01	1·95	1·98
3	2·78	2·78	2·71
4	0·98	—	0·98
5	1·46	—	1·44
6	1·73	1·74	1·72
7	—	0·53	0·55
8	—	0·37	0·35
9	—	0·14	0·17
10	—	0·03	0·04

The results in column two were obtained by pptng. the lead as described in the photometric method and applying the stoichiometric lead factor of the $N/10$ dichromate to the subsequent titration by the method of Fisk and Pollak. Since the values so obtained are in agreement with those yielded by the lead sulphate method, it can be inferred that the pptn. of lead is substantially complete. To test the speed of pptn. of small amounts of lead, in a particular instance, the solns. were allowed to cool for various periods after pptn. of the lead chromate. The following results (Table II) obtained from the calibration curve, indicate that practically complete separation of the lead is effected after the beaker has stood in running water for 15 min.

TABLE II

Time of cooling	Lead, %
15 min.	0·21
1 hr.	0·20
2 ,, ...	0·23
5 ,, ...	0·21
22 ,, ...	0·24

Notes—(1) In absence of interfering elements the metastannic acid ppt. may be used in routine work for the determination of tin. (2) The time for the lead determination is approx. 45 min. (3) The yellow colour is stable for at least 12 hr. (4) The asbestos for the filter pads should, after use, be returned to a stock reserved for the purpose.

SUMMARY—A procedure has been devised whereby minute quantities of lead in brass may be accurately determined. It involves (1) virtually complete pptn. of the tin as metastannic acid; (2) pptn. of the lead as chromate from the filtrate, as in the method of Fisk and Pollak; (3) solution of the lead chromate in nitric acid and absorptiometric determination of the lead.

This method has been developed in the laboratories of Messrs. A. Reyrolle & Co., Ltd. I wish to thank the Directors for permission to publish the paper, and Mr. E. G. Elphinstone for his assistance with the experimental work.

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CHEMICAL LABORATORIES
A. REYROLLE & CO., LTD.
HEBBURN-ON-TYNE.

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Notes

APPLICATION OF THE SILVER REDUCTOR TO THE MICRO-VOLUMETRIC DETERMINATION OF IRON

THE determination of iron by micro-volumetric methods involving the use of the silver reductor[1] has been described in the literature during the past ten years.[2,3,4] The quantities of iron considered are usually greater than 1·0 mg, the reductors vary in size up to that of the macro apparatus,[1] and the volumes of solution titrated range from 20·0 to 100·0 ml.

The apparatus and procedure described here were devised for the determination of 1·0 mg (or less) of iron with an accuracy of between 1·0 and 5·0%.

APPARATUS—(1) *The reductors*—The reductor and titration vessel in Fig. 1 is suitable for the determination of 0·1 to 1·0 mg of iron in a final vol. of *ca.* 5·0 ml. The smaller apparatus in Fig. 2 was designed for

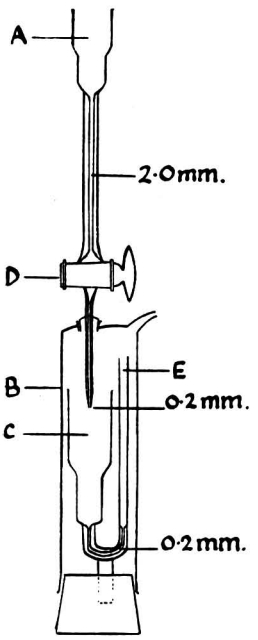

Fig. 1. ½ actual size

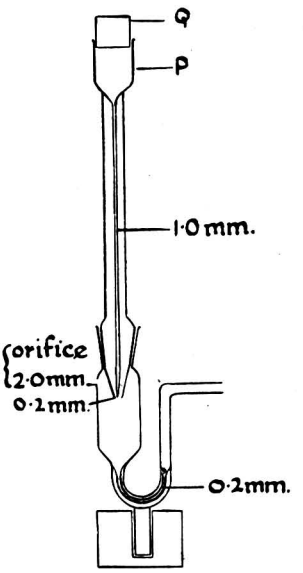

Fig. 2. ⅜ actual size

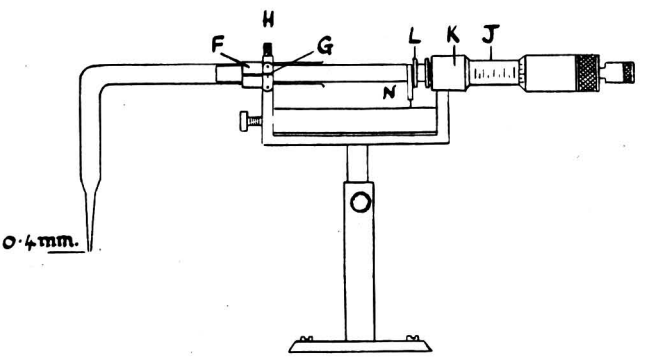

Fig. 3. ½ actual size

the reduction and titration of less than 0·1 mg of iron in about 0·2 ml of solution. The silver for these reductors is prepared as described by Walden, Hammett and Edmonds,[1] and separated by sieving to give fractions of convenient particle size. For the larger column about 0·25 g of the fraction passing a No. 18 B.S.S. sieve, and retained on a No. 36, is required. About 0·1 g of the fraction passing a No. 52 B.S.S. sieve and retained on a No. 72, is suitable for the smaller reductor. When the column of silver becomes discoloured over a length of about 1·0 cm, the exhausted metal should be replaced by fresh silver of the appropriate grain size. This may be done by dissolving out the silver chloride in conc. ammonia soln., washing the column with water and *N* hydrochloric acid in succession, and introducing the required amount of silver (a few mg) to restore the original length of the column.

(2) *The micro-burette*—As shown in Fig. 3, this is a slightly modified form of the instrument described by Hadfield.[5] The micrometer-head J, the burette barrel, and the spring-loaded piston are held rigidly in an adjustable support. Removal of the burette for cleaning or filling is facilitated by the provision of a hinged collar F, fastened by a screw H. The instrument can be used for the accurate delivery of volumes not exceeding *ca.* 0·4 ml.

PROCEDURE—(*a*) *Determination of* 0·1 *to* 1·0 *mg of iron*—Fill the reductor (Fig. 1) with sufficient *N* hydrochloric acid just to cover the top of the silver column. Evacuate the jacket B by means of the water-pump, and then admit carbon dioxide. Transfer the sample soln. to the cup A, evacuate the vessel B, and adjust the tap D until the sample soln. flows from the reductor at the rate of *ca.* 0·3 ml per min. (one drop every 8 sec. approx.). Rinse the sample container with three separate portions of 0·5 ml of *N* hydrochloric acid, and pass each wash soln. through the reductor. Admit carbon dioxide to the vessel B, remove the titration vessel C, and connect the tube E to the carbon dioxide generator. Add the required volume of indicator (accurately measured), and titrate with standard ceric sulphate soln. The stream of carbon dioxide passing through the tube E, serves to stir the soln. (*ca.* 5·0 ml) and to hinder atmospheric oxidation.

(*b*) *Determination of less than* 0·1 *mg of iron*—Fill the smaller reductor (Fig. 2) with *N* hydrochloric acid, and sweep out the titration vessel with carbon dioxide. Transfer the sample soln. to the cup P and insert the rubber bung Q. Drive the soln. through the reductor at the rate of *ca.* 0·3 ml per min. by carefully "screwing" the bung into the cup P. Rinse the sample container as already described for the larger quantities of iron, using about 0·05 ml of *N* hydrochloric acid for each washing. Titration and stirring of the soln. (vol. *ca.* 0·5 ml) are effected as already described.

DETAILS OF TESTS WITH THE TWO REDUCTORS—Numerous determinations of quantities of iron ranging from 0·005 to *ca.* 1·0 mg have been carried out to test the apparatus and procedure. A stock soln. of pure ferric chloride in *N* hydrochloric acid was prepared and its iron content was determined by a volumetric macro method. Suitable weight dilutions of this standard were made, and weighed volumes (0·5 to 0·2 ml) of these were taken for analysis. Various indicators were examined, *e.g.*, N-phenyl-anthranilic acid, phenanthroline-ferrous-iron ("Ferroin") and αα′-dipyridyl.[2] For the determinations of less than 0·1 mg of iron, "Ferroin" indicator is preferred, and for quantities between 0·1 and 1·0 mg, αα′-dipyridyl provides an excellent indicator. Determinations of iron in refractory materials have also been made and the results compared with the values obtained by the corresponding macro method. For these analyses the samples (10·0 to 20·0 mg) were heated with 200 mg of sodium carbonate at 900° C., acidified with diluted hydrochloric acid (1+1) to give solutions normal with respect to hydrochloric acid, and transferred (together with suspended silica) to the cup of the larger reductor. The suspended silica was retained by a plug of glass wool inserted above the column of silver.

The more rapid method of fusion with sodium peroxide in a nickel crucible was abandoned on account of the interference of nickel with the end-point. The smaller reductor has also been applied with success to the determination of iron present as impurity in organic compounds, when the amounts of iron did not exceed about 0·03 mg in the quantity of sample available.

RESULTS OF TEST EXPERIMENTS—(*a*) *Determination of iron in solutions of pure ferric chloride*—

Taken, mg	0·0059	0·0047	0·0116	0·0119	0·298	0·651	1·312
Found, ,, ...	0·0058	0·0046	0·0121	0·0122	0·292	0·636	1·321

Note—*N*/1000 ceric sulphate was used for the first four expts. and *N*/10 ceric sulphate for the remainder.

(*b*) *Determination of iron in refractories* (*using N*/10 *ceric sulphate*)—

	Fe_2O_3, %	
	Macro method	Micro method
Fire-brick No. 1	3·70	3·66, 3·68
,, ,, 2	5·23	5·21, 5·17
Bureau of Standards Sample No. 76	2·38 (aver.)	2·20, 2·30

PREPARATION OF INDICATOR SOLUTIONS—

"*Ferroin*"—Dissolve 7·42 mg of *o*-phenanthroline monohydrate in 25·0 ml of 0·0005 *M* ferrous sulphate soln. Volume used in each titration = 0·05 ml + 0·1 ml of 6 *N* sulphuric acid.

αα′-*Dipyridyl*—Dissolve 0·25 g in 50·0 ml of water, and dilute to 100·0 ml with conc. ammonia soln. (sp.gr. 0·880). Volume used in each titration = 0·05 ml.

N-*Phenyl-anthranilic acid*—Dissolve 10·66 mg in 10·0 ml of 0·1% sodium carbonate soln. and dilute to 100·0 ml. Volume used in titrations = 0·2 ml + 0·1 ml of conc. sulphuric acid.

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February, 1945

THE ARSENIC CONTENT OF GRAIN DRIED DIRECTLY WITH FLUE GAS

THE now widespread use, especially in connection with combine harvesters, of grain driers, in which flue gases are drawn or blown directly through the grain, has raised the question whether there is any risk of arsenical contamination of the dried grain.

In order to be able to answer this question, 7 samples of grain dried under widely different conditions in farm driers, with coke of varying arsenic content, were obtained and the arsenic contents of fuel and grain

determined. Suitable solutions prepared from the samples in the manner described in the footnote* were examined by the modification of Gutzeit's method given in the British Pharmacopoeia under General Methods of Testing.

The results showed that in no instance did the dried grain (wheat, barley or oats) contain more than 0·2 p.p.m. of arsenic (as As_2O_3) despite the fact that the arsenic contents of the various lots of coke used extended fully over the range ordinarily found in this fuel, *viz.*, 0 to 80 p.p.m. The figure 0·2 p.p.m. referred to is well below the limit of 1·43 p.p.m. (1/100 grain per lb.) in solid foodstuffs which the Royal Commission on Arsenical Poisoning (1901) recommended should render the vendor liable to prosecution, no matter whether the foodstuff be habitually consumed in large or small quantities or whether taken by itself or mixed with other substances.

This satisfactorily low arsenic content in the dried grain might reasonably have been expected, despite the findings of the Royal Commission on Arsenical Poisoning that the drying of malt (which in this sense may be regarded simply as very damp grain) with direct flue gas from coke of high arsenic content could lead to serious arsenical contamination. Figures, quoted by the Brewers' Export Committee before the Royal Commission on Arsenical Poisoning, for 138 representative samples of malted barley showed that eight contained 1·8 to 3·7 p.p.m., the remainder having less than 1·43, and the malt dust (often principally rootlets and culms), amounting to 0·5 to 2% of the malt, might contain up to 286 p.p.m. From our tabulation (not reproduced here) it appears that with anthracite and coke the ratio of arsenic content to that of the dried mealt ranged from 5 to 32, a rough mean being 18. Since the weight of the dried malt is five or more times that of the fuel used, it appears from the evidence as a whole that about one-third of the total arsenic in the fuel entered the malt.

In modern farm grain drying by means of direct flue gas, however, the conditions on balance should favour a greater escape of arsenic. On the one hand, there is likely to be less opportunity for flue dust to settle out before reaching the grain, or for arsenic to be "absorbed" by the plant (factors represented to the Royal Commission as helping to reduce arsenical contamination of the product dried in the kiln). On the other hand:

(1) The thickness of layer of grain through which the flue gases pass is less; not more than, say, 6 in. in the farm grain drier ("f.g.d.") as against 12 in. in the malt kiln ("m.k.").

(2) The flue gas temperature is a little lower; it rises to 200° F. or more in the m.k. as drying proceeds, but on current recommendations should not exceed 150° F. in the f.g.d.

(3) The wind speed is very much greater; 40 or more ft. per min. in the f.g.d., as against about 5 ft. per min. in the m.k.

(4) The grain in the f.g.d. will not remain cold nearly so long as the wetter malt in the m.k., although it must be remembered that the Royal Commission's expts. showed that dry malt absorbed about half as much arsenic as wet malt.

(5) Owing to the much lower water content to be evaporated, the amount of fuel burned per unit weight of grain in the f.g.d. will be much less than in the m.k. A round figure for the coke consumption in grain driers using direct heat is ½ cwt. per ton of grain handled, for a moisture fall of about 6%. In extreme instances, say, with careless operation and with very damp grain involving a moisture fall of, *e.g.*, 10%, it is conceivable that the figure might become 1 cwt. of coke per ton of grain. This compares with the use, in malting, of 3 to 4 cwt. of coke per ton of malt. Assuming the absorptive power of the grain in the f.g.d. to be only half that of the malt, this means that the arsenic content of the output from the f.g.d. is never likely to be more than one-sixth that of the m.k. for any given fuel. On this reasoning, therefore, the farm-dried grain is never likely to have an arsenic content of more than 1/30 (1/6 × 1/5) of that of the fuel, and it may reasonably be expected not to have more than about 1/100 (or 1/6 × 1/18, the mean ratio).

Coke is the solid fuel chiefly used in grain drying, and, since this does not usually appear to contain more than 40 p.p.m., the arsenic in farm-dried grain may reasonably be expected not to exceed 0·4 p.p.m. The results of the actual tests made may be regarded as satisfactory confirmation of this expectation based upon an analysis of the Royal Commission's findings.

We are indebted to Mr. W. H. Cashmore of the National Institute of Agricultural Engineering, York, for supplying us with several of the samples of grain and coke used in our tests.

CEREALS RESEARCH STATION
MINISTRY OF FOOD
ST. ALBANS

C. R. JONES
E. C. DAWSON

May, 1945

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* *Coke*—The sample (several pounds) was roughly broken, and sub-sampled by repeated quartering to 6 or 8 oz., which was then ground to a fine powder. Five g were ignited at moderate red heat with 3 g of arsenic-free lime until the carbon was removed. The residue was extracted twice with 25 ml of diluted hydrochloric acid (AnalaR) (1+5) by boiling and decanting through a filter, and then washed with hot water. The cooled filtrate and washings were made up to 100 ml. Four ml of this soln. were used for the B.P. test together with the usual 14 ml of brominated HCl (As-T), 40 ml of water and a few drops of stannous chloride soln., As-T. A reagent blank was carried through from the beginning in the same way. Standard stains were prepared by adding to each 4-ml portion of the "blank" soln. suitable quantities of dil. soln. of arsenic, *e.g.*, 0·2 ml (≡ 10 p.p.m. of As_2O_3), 0·5 ml, 1·0 ml (≡ 50 p.p.m.), 1·5 ml, etc.

Wheat—The sample was roughly ground and 10 g were placed in a 500-ml Kjeldahl flask with 10 ml of conc. sulphuric acid (AnalaR) and 5 ml of conc. nitric acid (AnalaR, redistilled). Heat was applied, very cautiously at first, and more nitric acid was added in 5-ml portions at intervals, until a clear, pale straw-yellow soln. was obtained and all nitrous fumes and excess of nitric acid were driven off. The cooled liquid was transferred to the Gutzeit apparatus with 40 ml of water and a little stannous chloride soln., As-T, was added; no hydrochloric acid was necessary. Two blanks were treated in the same way, receiving the same quantity of nitric acid, and to these were added 0·2 ml and 0·4 ml respectively of dil. soln. of arsenic, AsT. These yielded the standard stains of 0·2 p.p.m. and 0·4 p.p.m.

THE PHOTOMETRIC ANALYSIS OF COPPER-BASE ALLOYS
III. THE DETERMINATION OF NICKEL

NICKEL is a frequent constituent of copper-base alloys and it was therefore thought desirable to develop a method for its photometric determination which fits into the composite method for the determination of iron and manganese used in this laboratory.[1]

Haywood and Wood[2] have recently adapted Vaughan's method for nickel in steel[3] to its determination in copper-base alloys, but, as this method is strictly limited to alloys containing not more than 4% of nickel, it would be of little use in this laboratory, and it was necessary to develop a method that could be applied to copper-base alloys in general without restriction as to nickel content.

Copper causes an interference in the dimethylglyoxime method, but this can be limited by a control of the amounts of reagents used and be measured by a standard-blank technique.

REAGENT CONTROL—With limited amounts of dimethylglyoxime the copper interference is proportional to the amount of reagent present. A series of tests on samples of a complex aluminium bronze containing 4 to 6% of nickel showed that, with the sample size and conditions of reading given below, the optimum amount of dimethylglyoxime is 5 mg. With this amount the nickel colour is fully developed within 7 to 9 min. from the addition of the reagent and is stable for 30 to 35 min. The copper blank is of the order of 15 scale divisions, an amount easily allowed for in analysis.

Following Vaughan's recommendation,[3] iodine solution is used as the oxidising agent. Some tests were made with ammonium persulphate soln., but it did not give a sufficiently stable nickel colour. The optimum amount of iodine to use with the amount of dimethylglyoxime recommended above is 24 mg. With less, colour development is retarded and may never reach a stable maximum; with more, the nickel colour fades too rapidly.

These amounts of reagents are based on the use of a 1-ml sample of the initial alloy soln.[1] A larger sample cannot be taken for low-nickel alloys, as quite different amounts of the reagents would then be required.

LIGHT FILTERS—To enable the sample taken for colour development to be as small as possible and, hence, to minimise the copper interference, the filters used in the absorption measurement should be those that give the maximum response to variations in the nickel colour. Ilford 601 Spectrum Violet filters are the most sensitive.

As, in addition, these filters give a straightline calibration graph, they are to be preferred to the 602 Spectrum Blue filters formerly recommended.[2,3]

MEASUREMENT OF THE COPPER BLANK—Dimethylglyoxime is slightly unstable in ammoniacal soln,[4] and hence the effective amount added in a given vol. of soln. will decrease as the solution ages. It is therefore necessary to measure the blank with each batch of determinations if the reagent is made up in bulk. This is readily done by the following procedure.

Calibrate the absorptiometer with a pure nickel salt soln. in the normal manner with a neutral filter setting of 0·15,[5] using 2-cm glass cells and Ilford 601 filters. Test a suitable copper-base alloy sample of known nickel content by the procedure given below and determine the neutral filter setting at which its observed reading is the same as its "theoretical" reading (*i.e.*, based on its nickel content only). The difference between this setting and 0·15 gives the copper blank. If convenient, standard-blank solution may be prepared in bulk.

The bulk of the samples requiring analysis in this laboratory contain 4 to 6% of nickel and 75 to 85% of copper, but many alloy types contain significantly different amounts of these metals. With high-nickel alloys a smaller sample is taken for colour development, but it is necessary to run a separate standard-blank for these, as the copper blank does not decrease in direct proportion to the decrease in the amount of copper present. Low-nickel alloys can be read in 4-cm cells; it may be assumed that the copper-blank is twice that with the 2-cm cells.

METHOD

(*a*) *Reagents*—(i) *Dimethylglyoxime*—Dissolve 0·50 g of dimethylglyoxime in 500 ml of approx. 50% v/v ammonium hydroxide (sp.gr. 0·880). Dimethylglyoxime is not readily "wetted" by ammonium hydroxide and solution is facilitated by presence of a trace of a suitable detergent (*e.g.*, Lissapol L.S. powder, as supplied by I.C.I. Ltd.). Alcohol should not be used to assist solution as this tends to form a precipitate when the reagent is added to the nickel-iodine solution. When solution is complete, add 500 ml of 10% w/v A.R. citric acid.

(ii) *Iodine solution*—Dissolve 24 to 24·5 g of A.R. iodine in water with 30 to 35 g of potassium iodide. make up to 1 litre and store in amber glass bottles.

(*b*) *Procedure*—Prepare the initial alloy soln. as already described.[1] If a manganese determination is not required, hydrochloric acid or *aqua regia* may be used to ensure complete solution of the sample.

Place a 1-ml aliquot of the initial soln. in a 100-ml graduated flask and add successively 40 to 80 ml of water, 1 ml of iodine soln. and 10 ml of dimethylglyoxime reagent. Make up to 100 ml with water and mix well.

Measure the absorption with a Spekker Absorptiometer between 10 and 30 min. after addition of the dimethylglyoxime, using Ilford 601 filters. Determine the neutral filter setting which allows for the copper blank, and use this to control the setting during reading.[5]

RESULTS AND DISCUSSION—This method has been in routine use in this laboratory for nearly a year and has given results of satisfactory accuracy. Table I gives a selection of some typical results.

TABLE I NICKEL, %

	1	2	3	4	5	6	7	8	9	10
Spekker ..	0·52	0·58	1·98	2·60	3·67	4·45	5·30	5·50	14·78	17·68
Chemical ..	0·50	0·62	1·96	2·58	3·70	4·43	5·33	5·54	14·87	17·60

A feature both of this method and of that for iron is the use of small aliquots. This speeds up the work, as it avoids taking intermediate dilutions of the initial solution and, provided reasonable care be taken,

significant pipetting errors are not introduced. The use of a standard blank eliminates systematic errors, and hence it is the *reproducibility* of delivery and not the absolute accuracy of a given pipette which is significant.

This method was primarily intended for the analysis of alloys containing about 5% of nickel, the amounts of reagents, absorptiometer setting, etc., being based on this. If alloys with a different nickel content form the bulk of samples requiring analysis, it would be desirable to modify the amounts, etc., accordingly.

Thanks are due to the Chief Chemist of Langley Alloys Ltd., Mr. W. T. Edwards, A.R.I.C., for advice and criticism and to Mr. E. Lee, B.Sc., for invaluable help with the checks.

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April, 1945

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A SIMPLE APPARATUS FOR HANDLING STANDARD SOLUTIONS OF BROMINE IN POTASSIUM BROMIDE

(*Read at the Meeting, May* 2, 1945)

For the quantitative use of bromine it has usually been the practice in the past to employ bromate-bromide mixtures together with the appropriate quantities of acid to liberate free bromine equivalent to the bromate. The adoption of this procedure is due largely to the difficulties of handling solutions of bromine in potassium bromide (which assists in stabilising the soln.) owing to the volatility of the bromine. This last reagent was, however, employed by Linstead and Mann,[1] using 40% potassium bromide soln.

The use of the bromate-bromide mixture may be unsuitable in certain circumstances, owing, for example, to the pptn. of the bromate of an organic base, or a need to maintain approximate neutrality of the soln. undergoing bromination. For these reasons it was thought that the reagent might be unsuitable for an examination of the bromination of certain of the aromatic diamidines, notably propamidine (4 : 4′-diamidino diphenoxypropane) and stilbamidine (4 : 4′-diamidino stilbene), which it was desired to undertake, and it was decided to employ the bromine-potassium bromide reagent. In view of the satisfactory results which the method devised for handling the reagent has given, even at summer temperatures in Khartoum, it appeared desirable to publish a short account of it. As the high concn. of potassium bromide employed by Linstead and Mann would almost certainly have caused troublesome pptn. of bromides of the diamidines a much lower concn. of potassium bromide was used.

The apparatus consists of a wide-mouth Winchester quart bottle (A) through a cork in the mouth of which passes obliquely a 25-ml burette, so that the top of the burette nearly touches the bottom *and the side* of the Winchester. This Winchester is used in the inverted position, and the cork is protected from the action of the bromine by a massive plug of paraffin wax, which is melted and allowed to solidify *in situ*. A glass tube passes through the cork and centrally up the Winchester until it nearly touches the bottom. The tube has two right-angled bends outside the Winchester, the first to take the tube clear of the burette tap and the second to enable it to pass through a waxed cork in the mouth of a second, similar, Winchester (B), which is in the upright position. The tube should reach to within about 1 or 2 inches of the bottom of B. The cork in B is also fitted with a short glass tube, bent at right-angles, for connecting with bellows, and for admission of air during use. The bromine soln. of approx. the desired strength is poured into B and is then forced into A through the glass tube by pressure from the bellows, the burette tap being open to permit escape of air from A. In this way A can be nearly filled with the soln., and provides a substantial reservoir, from which there can be no significant loss of bromine by volatilisation, provided that any soln. removed therefrom is replaced by brominated air of the same concn. as that in equilibrium with the soln. After A has been filled the level of soln. in B is reduced until it is just clear of the end of the tube, and the concn. of bromine in the air space in B is adjusted visually for each series of determinations, by "pouring" in bromine vapour, until it is approx. the same as in the air space above the soln. in A. By tilting the apparatus to one side the burette is filled with bromine soln., and from the burette any desired volume can be run, an equal vol. of brominated air being drawn into A from near the bottom of B, and unbrominated air into B near the top. In this way fair constancy of the strength of the bromine soln. in A is maintained. The burette jet carries an extension tube so that the bromine soln. is run into the bromination flask or bottle near the bottom, thus minimising transfer losses. Owing to the difficulty of reading the burette accurately in its inclined position, and through the glass and the bromine soln., the exact quantity of reagent used on each occasion is weighed. Potassium iodide soln. can be introduced into the flask through the neck without loss of bromine, by cooling; or, when a thick-walled bottle is used, can be contained in a sealed bulb which is broken by shaking after the desired period of contact with the bromine. The liberated iodine is titrated with thiosulphate soln. in the usual way. If the bromine soln. is run direct into potassium iodide soln. the titration figure is slightly higher than if it is run into a little water (10 to 20 ml) and potassium iodide added subsequently, but the difference in titration figures, using 25 ml of 0·025 *N* solns., is of the order of only 0·15 ml of thiosulphate soln. The bromine soln. is standardised under conditions as close as possible to those existing in the actual bromination expt., and a small correction in the titration figure is made, the magnitude of which is determined by the amount of excess of bromine present after bromination is complete.

It has been found that, if the bromine is run into a substantial vol. of water (50 ml or more), there is a significant effective loss of bromine through interaction with the water. For this reason the vol. of the soln. being brominated should be kept as low as possible, but, if it is necessary to use larger volumes on account of low concns., the difficulty can be overcome by acidifying the soln. with about 1 ml of 2 *N* hydrochloric acid per 50 ml of soln. before adding the bromine. The bromine losses with considerable vols. of water could be reduced by using water redistilled from alkali and acid, but were still significant, in spite of the fact that the bromine reagent itself is somewhat acid on account of hydrolysis.

It has been observed that the diamidines so far encountered all tend to form complexes with free bromine and iodine, which have low solubilities in water and can, under suitable conditions, separate as solids, occasionally crystalline; sometimes these complexes are induced to redissolve only with considerable difficulty, thus interfering with the back-titration of the excess of halogen. For this reason the excess of bromine reagent over that required for the bromination should normally be kept low.

Results obtained in the titration of varying quantities of bromine-bromide reagent in presence of the stated volumes of water, according to whether potassium iodide is added before or after the bromine-bromide reagent, are given in Table I. The bromine soln. was made up in 2% potassium bromide soln., and the strength of the thiosulphate was 0·0281 *N*.

TABLE I

Expt.	Wt. of Br soln. g	Vol. of water ml	Vol. of thiosulphate soln. reqd. (*a*) KI then Br ml	(*b*) Br then KI ml	Difference ml	Difference %
1	25·25	125	20·70, 20·69, 20·62 (mean 20·67)	20·02, 20·00, 19·99 (mean 20·00)	0·67	3·5
2	10·17	125	8·31	7·56	0·75	9
3	1·74	125	1·41	0·97	0·44	30
4	25·28	125	20·48	19·98	0·50	2·4
5	25·28	nil	—	20·50	—	—
6	25·28	10	—	20·43	—	—
7	3·02	nil	2·35	2·31	0·04	—
8	3·02	125	2·31	2·01	0·30	13

Note—Expts. 4 to 6 were carried out two days after Expts. 1 to 3, and the strength of the bromine soln. had changed slightly. Expts. 7 and 8 were carried out 10 days later with the same soln. The water used in Expt. 8 had been freshly redistilled from alkali and from sulphuric acid. In Expt. 4 the extension tube dipped into the soln. in the flask.

Many determinations of the bromine absorption of stilbamidine hydrochloride dihydrate by the above method have now been carried out in connection with a detailed examination of the photochemical decomposition of the compound in solution. Under favourable conditions there is no difficulty in obtaining results well within 1% of the theoretical figure (0·428 g of Br per 1 g). The method has also been used for following up the bromination of propamidine.

I am grateful to the Director, Sudan Medical Service, for permission to publish this note.

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WELLCOME CHEMICAL LABORATORIES
SUDAN MEDICAL SERVICE, KHARTOUM

A. J. HENRY

April, 1945

Ministry of Food

STATUTORY RULES AND ORDERS*

1945—No. 460. The Canned Fruit and Vegetables Order, 1945. Dated April 27, 1945. Price 3d.

This is a consolidating Order, replacing and in substance repeating the provisions of the Canned Fruit and Vegetables Order, 1944, and its amendments. Consequent upon the coming into force of the Labelling of Food (No. 2) Order, 1944, the former requirements as to labelling bottled fruit have been omitted. New conditions relating to the canning of vegetables have been introduced (pars. 8-12 of Schedule I, Part II), *viz.*,

8. Peas shall be packed in liquid containing not less than 2·3% by weight of cane or beet sugar, and not less than 1·25% nor more than 2·5% by weight of salt.

9. Spinach purée shall contain not less than 0·1% and not more than 0·6% of salt, expressed as % of the canned product.

10. (i) Macedoine shall be packed in a brine containing not less than 1·25% nor more than 2·5% by weight of salt and shall contain the following vegetables in the following proportions, *expressed as % of the drained canned product.*

Carrot (diced), *min.* 25%; *max.*, 45%. Turnips (diced) or swedes (diced), *min.* 10%; *max.* 35%. Peas, fresh or soaked, *min.* 15%; *max.* 36%.

(ii) Macedoine may also contain any of the following (*but no other*) vegetables; if present they shall comply with the following proportions, *expressed as % of the drained canned product.*

Beans (soaked), white or flagelot, *min.* 10%; *max.* 20%. Potatoes (diced), *min.* 10%; *max.* 35%. Stringless beans, *min.* 10%; *max.* 20%. Celery, *min.* 4%; *max.* 8%.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

11. Beans in tomato sauce shall be of the pea or haricot type, boiled or steamed, or steamed and oven-baked. Apart from sugar, spices and other seasonings, the sauce shall be prepared only from tomatoes or tomato products. The canned product shall contain not less than 5% of sugar by weight (expressed as invert sugar) and not less than 28% of solid matter when dried for 16 hr. at 98° C.
12. All vegetables referred to in *Schedule I* other than those mentioned in pars. 8, 9, 10 and 11 shall be packed in a brine containing not less than 1·25% and not more than 2·5% by weight of salt.

1945—No. 577. Directions, dated May 18, 1945, supplementary to the Feeding Stuffs (Rationing) Order, 1943 (S.R. & O., 1943) (No. 1498), II, p. 849). Price 1d.

The main purpose of these Directions is to add National Horse Feed Mixture to the list of feeding stuffs for which horse feed ration documents are available. The Directions, dated October 23, 1943, supplementary to the Feeding Stuffs (Rationing) Order, 1943, are revoked. *In the main these Directions have been rendered obsolete by the issue of new ration documents.*

— **No. 627. The Dried Egg (Control of Use) Order, 1945. Dated May 29, 1945.** Price 1d.

This Order, *which came into force on June* 11, prohibits, except under licence, the use of dried egg by way of trade or business in the manufacture (i) of certain specified foods, *viz.*, ice-cream, synthetic cream and baker's cream filling and (ii) of products sold in a wet state for use in the manufacture of those foods. The Order also requires manufacturers who include dried egg in a dry mixture intended for the manufacture of any specified food to declare the fact in a prescribed form on the container.

The intention of the Order is to provide against conditions occurring in which bacteria might multiply; and licences issued under the Order will prescribe the methods of processing to be adopted for this purpose.

Standard Methods for Testing Petroleum and its Products*

THE sixth edition of this work represents a revision of the volume published in 1944. It opens with the report of the Standardisation Committee in which the principal changes introduced by the various Sub-Committees are detailed.

Sampling and Measurement—In the sections on the sp.gr. of gases the Chancel Flask and Schilling Effusion methods have been included. A slight alteration has been made in the apparatus for Sampling Petroleum and Products (Sec. 8 (*a*)).

Liquefied Petroleum Gases, Gasoline, Kerosine and Light Distillates—New methods have been added for Hydrogen Sulphide, Mercaptan Sulphur, and Sulphur by the Lamp Method. Minor alterations have been made in the methods for determining the Aniline Point, and the Smoke-Point of Kerosine, and there are some changes in the Bomb Method of determining Sulphur.

Gas Oil, Diesel Oil and Fuel Oil—Petroleum spirit replaces ethyl alcohol for the extraction of Asphaltenes. There are several alterations in the sections on the determination of the Calorific Value (Gross).

Engine Tests—The methods for the Knock Rating of Motor Fuels and of Aviation Fuels are revised.

Lubricants—Recommendations affecting the Conradson Method and Ramsbottom Method for Carbon Residue are made. Minor modifications are made in the descriptions of the methods for the Drop Point of Greases, the Oxidation Test for Lubricating Oil, and the determination of Sulphur (Corrosive).

Asphaltic Bitumen—The following B.S.I. methods are included. No. 598—1940. Recovery of Asphaltic Bitumen from Asphalts. No. 598—1940. Bitumen Content and Water Content of Mixtures. No. 803—1938. Electric Strength of Asphaltic Bitumens.

There are two new methods: Acidity (Neutralisation) Value of Asphaltic Bitumens. Fluxing Value of Flux Oils.

The following methods have been withdrawn. Sulphur in Asphaltic Bitumen and Flux Oils. Acidity of Tar/Asphaltic Bitumen Mixtures.

Petroleum Waxes—In determining Colour by means of Lovibond Tintometer the cell for scale or paraffin wax may be heated by means other than electrical.

Derived Petroleum Chemicals—The limits of precision for Acetate Content of Solvents and Halogen Content have been increased. The Wijs solution for determining Iodine Value must be freshly prepared and have a freezing-point not below 15·2° C. The period of contact of the oil and iodine soln. is increased from 30 min. to 1 hr. Limits of precision have been increased to 4%.

Apparatus—Alterations have been made to facilitate the manufacture of the apparatus for Carbon Residue (Ramsbottom Method). Small alterations and additions are made in the descriptions of the apparatus for a number of other methods. Attention is directed to the fact that sintered glass crucibles are now commercially available with porosities ranging from 200–500μ to 0·7–3μ.

* Sixth Edition. Pp. 539. Published by the Institute of Petroleum, London, W.1. 1945. Price 15s.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Rapid Determination of Starch. Factors for Starches and Comparison with Acid and Enzymic Hydrolysis Methods. J. P. Nielsen and P. C. Gleason (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 131–134)—In previous work (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 176; ANALYST, 1943, **68**, 220) a rapid and reasonably accurate method for determining starch in fresh, frozen and canned vegetables, based on colour development with iodine, was described. Although it was recognised that unit amounts of starch from different sources yield different intensities of colour with iodine, data concerning these differences were not available. The present work establishes factors for the calculation of starch in various vegetables with potato starch as standard, compares results by the proposed method with those by enzymic action or acid hydrolysis, describes minor changes in procedure that increase accuracy, and describes the application of the method to dehydrated foods. The samples included fresh, frozen, dehydrated and canned vegetables. If the sample is dehydrated, soak 10 to 20 g for several hr. with enough water to cover the sample. Re-hydration may be hastened by heating at 60° C. for 2 hr., replacing water lost by evaporation. Add a wt. of water equal to the wt. of the sample and the re-hydrating water, and disintegrate in an electric food blender, preferably adding half of the water and running the blender at low speed, then adding the remainder of the water and blending at max. speed. Certain raw wet-ground products with large starch grains (*e.g.*, potato) are difficult to sample because the starch settles rapidly and frothing may occur. These difficulties can be minimised by the use of as little water as possible and by adding a few drops of amyl alcohol. The % of starch in the sample will determine the dilution necessary to obtain a colour within the range of the photoelectric colorimeter. The starch content of soya beans, snap beans and most kinds of squash averages *ca.* 1%; that of green peas 5%; that of lima beans, potato and sweet potato, 10–20%. Starch concns. up to 2·5 mg per 50 ml in a colorimeter tube of *ca.* 1·25-cm diam. give colour intensities that obey Beer's law. Pea starch gives a colour that is *ca.* twice as intense as that obtained with the same wt. of most other starches; therefore the value for the upper limit of the range should be halved. The method is not satisfactory for woody tissue and tissue of low starch content; for these a dilution of less than 25 ml after treatment with perchloric acid is necessary. In the method previously described 1 ml of water was added to a 3-g sample before the starch was solubilised with 72% perchloric acid. More consistent results are obtained and local hydrolysis of starch is minimised if the perchloric acid is diluted with this water before solubilisation, which is complete in 5 to 7 min. As a rule the starch may stand with the perchloric acid for 30 min. with little loss. The concn. of the sodium hydroxide soln. used to neutralise the aliquot before development of the colour has been reduced from 6 *N* to 2 *N* because prolonged exposure to high alkalinity lowered the amount of starch found by *ca.* 10% when 5 drops of 6 *N* alkali were added in excess of neutrality.

To establish the "factors" for various starches, based upon pure potato starch as standard, the influence of maturity and variety of the plants was studied, 50 to 100 g of starch with 3 ml of water being solubilised and the colour formed with iodine measured. The "factor" is log 100/colorimeter reading of 1 mg of potato starch in 50 ml, divided by log 100/colorimeter reading of 1 mg of other starch in 50 ml, the instrument being adjusted to read 100 with the reagent blank. The % of starch, calculated as potato starch equivalent, is multiplied by the established factor for the product in question. For Lima beans the factor ranged (according to variety) from 0·99 to 1·06, for peas from 0·53 to 1·18, for potatoes from 0·99 to 1·02, for sweet potatoes from 1·2 to 1·3, and for squash from 1·10 to 1·12. For wheat the factor was 1·26, for soya 1·06, for snap beans 1·00, and for eucalyptus leaves 1·04. With these factors and a curve for pure potato starch the starch content of any of the vegetable products mentioned can be obtained. Owing to the difficulty of obtaining pure starch from soya beans, snap beans and eucalyptus leaves the factor was obtained in a different manner. The result of a starch analysis by the proposed method was calculated into the potato starch equivalent. Starch was then determined in the same sample by an enzymic method, and the factor was obtained by dividing the latter result by the potato starch equivalent. It was not found possible to prepare sweet corn starch free from glycogen, and the factor for this starch could not be determined. Corn starch is said to have the same amylose content as potato starch. The factor would then be 1·00 on the assumption that corn amylose gives the same amount of colour per unit wt. with iodine as does potato amylose. Since the starch enzymes hydrolyse glycogen, the enzymic method could not be used. The red colour from glycogen in the proposed method would however not cause nearly so much error as would the sugar from glycogen in the enzymic method. The purity of the starches used was checked by acid hydrolysis and subsequent determination of sugar, with concordant results for all types of starches.

Special precautions must be observed with material containing active amylases. Material to be stored before analysis must be frozen and kept at −18° C., or, when practicable, may be heated rapidly in boiling water to inactivate the enzyme. Alternatively, the material may be disintegrated immediately with cold water and the perchloric acid added to the weighed portion to prevent enzymic acition. Of the samples studied, only sweet potato and carrot contained very active amylases. During storage the starch of apples is converted into sugar, but when the apples are disintegrated an inhibitor is released which prevents hydrolysis of the starch. A correlation found to exist for peas between starch content and tenderometer readings suggests that starch determinations by the proposed method may be used for determining maturities of processed material, the tenderometer being applicable only to raw material.

A. O. J.

Identification of some Barbiturates. R. N. Castle and C. F. Poe (*J. Amer. Chem. Soc.*, 1944, **66**, 1440–1442)—The substituted benzyl derivatives of four new barbituric acids recently introduced into medicine have been prepared and their m.p. determined. The compounds examined were Cyclopal (5-cyclopentenyl-5-allyl-barbituric acid), Delvinal (5-ethyl-5-(1-methyl-1-butenyl)-barbituric

acid, Seconal (5-allyl-5-α-methylpropylcarbinyl-barbituric acid) and Sigmodal (5-*sec*amyl-5-β-bromallyl-barbituric acid), and the *p*-chlorobenzyl, *o*-chlorobenzyl, *p*-bromobenzyl, *o*-bromobenzyl and *p*-nitrobenzyl derivatives were prepared. *General method*—Dissolve 0·04 g-mol. of sodium carbonate and 0·02 g-mol. of the barbiturate in the minimum quantity of boiling water and add 0·04 g-mol. of the substituted benzyl bromide previously dissolved in a vol. of alcohol twice that of the water used. Heat the mixture under reflux for 1½ hr. when the *p*-chloro- and *p*-bromo-benzyl derivatives are present, 3 hr. for the *o*-chloro- and *o*-bromobenzyl derivatives and 30 min. for the *p*-nitrobenzyl compounds. Filter off the product, wash well with water to remove any unchanged barbiturates, alkali carbonate, alkali halide or mono-substituted derivative that may be formed and recrystallise 3 times from chloroform. The m.p. (° C. corr.), determined by the usual capillary tube method and with the Maquenne block apparatus, were as follows:

	Cyclopal		Delvinal		Seconal		Sigmodal	
	block	tube	block	tube	block	tube	block	tube
p-Chlorobenzyl ..	110·5	122[a]	71	72·5	102	103	91	92
o-Chlorobenzyl[b] ..	101·5	103	101·5	103	—[c]	—[c]	103	105
p-Bromobenzyl ..	116	119	75·5	77	110	111·5	92	95
o-Bromobenzyl[b] ..	108·5	110	100·5	102	94	98	108	115
p-Nitrobenzyl ..	181·5[d]	185[d]	131·5	132·5	158	163	172	178

a Shrinks at 110·5° C., melts sharply at 122° C.
b In some instances *o*-chloro- and *o*-bromobenzyl derivatives separate as oils and only crystallise on standing in the refrigerator.
c The *o*-chlorobenzyl seconal derivative could not be made to crystallise.
d Decomposition.

The *p*-nitrobenzyl derivatives are recommended for the identification of the barbiturates, since they are easy to prepare and purify and the m.p. cover a wide range of temperature. A table is reproduced giving the m.p. taken from the literature of various derivatives of a number of barbituric acids. J. A.

Simple Tests for Identification of Sulphonamides. A. B. Sample (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 151–152)—When solns. of the alkaline salts of sulphonamides are treated with solns. of copper sulphate or cobalt nitrate coloured ppts. are formed, but, since the copper compounds exhibit a series of colour changes which at some stages are the same for different sulphonamides, it is advisable to confirm the results either by timing the colour changes or by applying the cobalt nitrate test. To 5 ml of *ca.* 0·1 *N* sodium hydroxide add small amounts of the sulphonamide until no more will dissolve and filter the soln. To the clear filtrate add 3 drops of a 15% soln. of copper sulphate pentahydrate or of a 15% soln. of cobalt nitrate hexahydrate and note the appearance of the ppt. and the time (approx.) of each colour change, comparing the results with the results of tests either made with known specimens simultaneously or tabulated previously. It is important to avoid presence of free sodium hydroxide by taking more of the unknown material than is soluble in 5 ml of alkali and removing the excess by filtration. The blue ppt. of copper hydroxide should be observed as a precaution against mistaking it for the copper sulphanilamide ppt. The copper sulphanilamide reaction may be checked as follows. To the reaction mixture containing the ppt. add 5 ml of 30% sodium hydroxide soln. followed by 5 ml of 5% potassium cyanide soln. Copper hydroxide and copper sulphanilamide both dissolve in sodium hydroxide soln., forming blue solns. When potassium cyanide is added the sulphanilamide soln. becomes yellow to amber, whereas the copper hydroxide soln. becomes colourless. The copper and cobalt ppts. of other sulphonamides react similarly. Sodium sulphanilamide yields a ppt. of large clear plates with cerium nitrate which may be useful for microscopical identification. The tests with copper sulphate and cobalt nitrate are not applicable to material insol. in alkali (*e.g.*, sulphaguanidine) and it is thus advisable to add the unknown material to the alkali in small amounts until its solubility is indicated. Sulphonamides insol. in alkali must be tested by such tests as those of Calamari *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 534; ANALYST, 1942, **67**, 365). It is recommended that the analyst should make his own descriptions of the colour changes with known material. The colour changes for nine sulphonamides are given in a table, from which the changes undergone by sulphapyridine are quoted here as an example—Copper sulphate: yellow ppt. (immediate), light green ppt. (1 sec.), bright green ppt. (10 sec.), grey-green ppt. (1 min.), light yellow brown ppt. (5 min.), light brown ppt. (15 min.), no change (30 min.). Cobalt nitrate: pinkish-white ppt. (immediate), light violet pink ppt. (30 sec.), pink-white ppt. (3 min.), pink-white sediment and pink supernatant (5 min.), no change (30 min.).

A. O. J.

Biochemical

Simple Gravimetric Method for the Determination of Urinary Potassium. S. Freeman and M. W. Burrill (*J. Biol. Chem.*, 1945, **157**, 287–295)—The method involves the pptn. of potassium as the silver cobaltinitrite after removal of interfering substances by ashing in presence of excess of sulphuric acid. Evaporate 10 ml of urine with 2 ml of 10 *N* sulphuric acid to a syrupy consistence on a steam-bath. Put the dish in a cold muffle furnace and ash overnight at 510–540° C. Cool, dissolve the ash in water and transfer the soln. to a 50-ml volumetric flask and make up to volume. Filter 25 ml of the soln. through a No. 40 Whatman filter-paper, warm the filtrate and a quantity of freshly-prepared silver cobaltinitrite reagent (add 1 ml of 40% silver nitrate soln. to 10 ml of sodium cobaltinitrite soln. prepared as described by Peters and van Slyke, "*Quantitative Clinical Chemistry, Methods*," Baltimore, 1932, 726) to 18–20° C. and pipette 10 ml of the reagent, followed by 10 ml of the filtered ash soln. into a weighed, stoppered

filter. Stir for 1 min., cover with a watch-glass and leave for ½ hr. at room temp. Filter off the ppt. and wash with the aid of suction, using three 10-ml portions of water, two 10-ml portions of 95% alcohol and two 10 ml portions of ether. Remove the filter carefully from the suction flask and dry in a vacuum desiccator; after 30 min. weigh the filter. Multiply the weight of the ppt. by the factor 0·1166 to obtain the amount of potassium present. With solns. containing only small amounts of potassium (1 mg) or large amounts (14–20 mg) other factors are necessary to obtain satisfactory results. F. A. R.

Estimation of Error in the Volhard Analysis for Chloride by Titration in Acetic Acid. D. S. McKittrick and C. L. A. Schmidt (*Arch. Biochem.*, 1945, **6**, 273–276)—The volumetric determination of chloride has the disadvantage that adsorption of silver ions on the ppt. tends to make the results high. Several modifications have been proposed to overcome the difficulty, and it is now claimed that satisfactory results can be obtained by carrying out the titration in 40 to 65% acetic acid soln. The end-point shows no fading in 24 hr., and it is therefore believed that under these conditions silver chloride is less soluble than silver thiocyanate. Chloroform may be added to produce a second liquid phase in order to facilitate coagulation of the silver chloride. The method was used to estimate chloride in urine and blood filtrates; in both instances recoveries were theoretical. Where, as with some urines, the solns. are so deeply coloured as to interfere with the matching of the end-points, the colour of the blank may be brought to that of the urine by addition of a few drops of bromophenol blue indicator. F. A. R.

Leucine, Valine and *Iso*leucine Content of Meats. B. S. Schweigert, I. E. Tatman and C. A. Elvehjem (*Arch. Biochem.*, 1945, **6**, 177–184)—Leucine and valine were estimated microbiologically by the method of Schweigert, McIntire, Elvehjem and Strong (*J. Biol. Chem.*, 1944, **155**, 183) and *iso*leucine by a similar method using the same basal medium with omission of the appropriate amino acid. The animal tissues were hydrolysed by autoclaving with 2 *N* hydrochloric acid for 5 hr. The amounts of valine, leucine and *iso*leucine in animal tissue protein averaged 5·2, 7·7 and 5·7% respectively, the results being remarkably uniform in different animal tissues. A higher percentage of amino acids was found in cooked meats, but this was satisfactorily accounted for by the loss in weight of the meat during cooking. Of the fresh tissues, liver, veal and beef muscle tissues were the best sources of these amino-acids. Retention of the amino-acids during cooking ranged from 86 to 106% with an average of 97%. F. A. R.

Amino Acids yielded by Yeast, Sunflower Seed Meal and Sesame Seed after Hydrolysis of the Fat-free Tissue. R. J. Block and D. Bolling (*Arch. Biochem.*, 1945, **6**, 277–279)—The following methods were used: nitrogen by the micro-Kjeldahl method; arginine, histidine, and lysine by a small-scale modification of the Kossel-Kutscher method following hydrolysis with 8 *N* sulphuric acid; tyrosine and tryptophan by Lugg's adaptation of the Millon reaction, using 5 *N* sodium hydroxide for hydrolysis; phenylalanine by a modification of the Kapeller-Adler procedure with either 5 *N* sodium hydroxide or 7 *N* sulphuric acid for hydrolysis; cystine by the Folin or Fleming-Vassel method following hydrolysis with a 1 : 1 mixture of 20% hydrochloric acid and 90% formic acid; methionine by the McCarthy–Sullivan method following hydrolysis with 6 *N* hydrochloric acid; threonine by oxidation to acetaldehyde following hydrolysis with 6 *N* hydrochloric acid; leucine, *iso*leucine and valine by a modification of the microbiological methods of Shankman (*J. Biol. Chem.*, 1943, **150**, 305) and McMahan and Snell (*Id.*, 1943, **152**, 83) after autoclaving with 3 *N* hydrochloric acid at 15 lb. pressure; glycine by the Zimmermann–Patton *o*-phthaldialdehyde reaction following hydrolysis with 6 *N* hydrochloric acid. For references to the chemical methods, see Block and Bolling, "*The Amino Acid Composition of Proteins and Foods. Analytical Methods and Results.*" C. C. Thomas, Springfield, Ill., (1945), and "*The Determination of Amino Acids.*" Burgess Publishing Co., Minneapolis, Minn. (1942). The following results were obtained for the amino acid content (%) of various proteins. (Calculated to 16·0% of nitrogen.)

	Yeast (de-bittered brewer's)	Sun-flower seed	Se-same seed	Chop-ped beef	Case-in
Arginine ..	3·1	8·2	8·7	7·7	4·1
Histidine ..	3·3	1·7	1·5	2·9	2·5
Lysine ..	7·1	3·8	2·8	7·2	7·5
Tyrosine ..	3·8	2·6	3·5	3·4	6·4
Tryptophan ..	1·2	1·3	1·8	1·3	1·2
Phenylalanine	4·5	5·7	8·3	4·9	5·2
Cystine ..	1·1*; 1·2	1·4*; 1·6	1·3*	1·3	0·4
Methionine ..	2·7	3·4	3·1	3·3	3·5
Threonine ..	5·5	4·0	3·6	5·4	3·9
Leucine ..	7·3	6·2	7·5	7·7	12·1
*Iso*leucine ..	6·0	5·2	4·8	3·0	6·5
Valine ..	5·3	5·2	5·1	3·5	7·0
Glycine ..	—	—	9·3	—	—

* Fleming-Vassel method.

F. A. R.

Biuret Reaction of Proteins in Presence of Ethylene Glycol. J. W. Mehl (*J. Biol. Chem.*, 1945, **157**, 173–180)—In the method previously proposed for the estimation of proteins by the biuret reaction, pptn. of cupric hydroxide often leads to difficulties. These are avoided by the use of ethylene glycol, which forms a compound with copper soluble in alkali; the solution reacts with protein, to give the biuret colour. The absorption spectrum of a soln. of the protein-copper complex has a maximum at 545 $m\mu$ and, if allowance is made for the absorption at this wave-length of the copper-ethylene glycol complex, the absorption is almost directly proportional to the protein concn., although the value of $E^{1\%}_{1\,cm}$ decreases somewhat with increasing protein concn. The protein-copper complex has a greater absorption in the ultra-violet region of the spectrum than at 545 $m\mu$, and by measuring the absorption at 320 $m\mu$ instead of at 545 $m\mu$, greater sensitivity can be achieved, as the correction due to residual copper is considerably lower at this wave-length.

For work in the visible region, use a reagent prepared as follows. Mix 100 ml of ethylene glycol, 40 ml of 60% sodium hydroxide soln. and 50 ml of 4% $CuSO_4 5H_2O$ soln., dilute to 400 ml, heat until pptn. is complete and then filter and add sufficient sodium hydroxide to make the final conc. 10 to 11%. The reagent is stable for several months. For work in the ultra-violet region, prepare a similar but more dilute soln. containing 0·012% of copper, 10% v/v of ethylene glycol and 6·3% of sodium hydroxide.

Both reagents contain a considerable excess of glycol and more copper can be added without pptn.; the concn. of alkali can also be varied over a rather wide range. Use 10 ml of either reagent and make the final volume 25 ml. The protein soln. should have a concn. not exceeding 0·3% for work in the visible and 0·03% for work in the ultra-violet region. For most proteins the colour should be read after 2 hr., but plasma proteins invariably begin to develop a turbidity after 1 hr., and the colour of these should therefore be measured between 25 and 45 min. after addition of the reagent. To correct for the absorption of the ethylene glycol-copper complex, subtract 1·57 times the optical density at 750 $m\mu$ from the optical density at 545 $m\mu$.

F. A. R.

Microbiological Method for the Estimation of Aspartic Acid and Serine. J. L. Stokes and M. Gunness (*J. Biol. Chem.*, 1945, **157**, 651–659)—Aspartic acid and serine are essential for the growth of *Lactobacillus delbrückii* LD 5, which can be used for their estimation microbiologically. Stock cultures are carried on a medium containing 1 g of glucose, 0·5 g of Bacto-peptone, 0·6 g of anhydrous sodium acetate, Salts A and B in half the concn. given below and 1·5 g of agar per 100 ml of medium at *p*H 6·8. Store the cultures in a refrigerator and sub-culture each month. Prepare inocula for assay by transferring a small amount of growth from the stab culture to a centrifuge tube containing 8 ml of the same medium but without agar. Incubate for 16 to 24 hr. at 37° C., centrifuge, wash the cells with water, and resuspend in 20 ml of water.

The basal medium has the following composition. *dl*-Leucine, 100 mg; *dl-iso*leucine, 100 mg; *dl*-valine, 100 mg; *l*(-)cystine, 100 mg; *dl*-methionine, 100 mg; *l*(-)tryptophan, 100 mg; *l*(-)tyrosine, 100 mg; *dl*-phenylalanine, 100 mg; *dl*-glutamic acid, 100 mg; *dl*-threonine, 100 mg; *dl*-alanine, 100 mg; *dl*-aspartic acid, 100 mg; *l*(+)lysine, 50 mg; *l*(+)arginine, 100 mg; *l*(+)histidine, 100 mg; *dl*-serine, 100 mg; *l*(−)proline, 100 mg; *l*(−)hydroxyproline, 100 mg; *dl*-norleucine, 100 mg; glycine, 100 mg; glucose, 10 g; sodium acetate (anhydrous), 3 g; adenine, 10 mg; pantothenic acid, 100 μg; riboflavin, 100 μg; nicotinic acid, 100 μg; pyridoxamine, 100 μg; biotin, 0·05 μg; folic acid, 0·5 μg*; Salts A: K_2HPO_4, 250 mg; KH_2PO_4, 250 mg; Salts B: $MgSO_4$, $7H_2O$, 100 mg; NaCl, 5 mg; $FeSO_4.7H_2O$, 5 mg; $MnSO_4.4H_2O$, 5 mg. Adjust to *p*H 6·8 and add distilled water to 250 ml.

Put 5 ml of this medium, omitting either aspartic acid or serine, into each of a number of test-tubes. For the estimation of aspartic acid, add to one set of tubes, in duplicate, amounts ranging from 0 to 4 ml of a standard soln. of *l*-aspartic acid containing 200 μg per ml, and to another set of tubes, 1·0, 1·5, 2·0, 2·5 and 3·0 ml of the soln. to be tested. Dilute the contents of each tube to 10 ml, plug with cotton-wool and autoclave at 15 lb. for 13 min. Inoculate with 1 drop of the suspension described above, incubate at 37° C. for 72 hr., and titrate with 0·1 *N* sodium hydroxide, using bromothymol blue as indicator. Serine is estimated in exactly the same way, using a standard soln. containing 100 μg of *dl*-serine (equivalent to 50 μg of *l*-serine) per ml. Although *L. delbrückii* can utilise the *d*- and *l*- isomers of aspartic acid equally well, standard curves prepared from *l*- and *dl*-aspartic acid are not superimposable in the upper regions, and *l*-aspartic acid should therefore be used as standard. With serine, on the other hand, only the *l*-isomer is utilised, so that the racemic compound, which is more readily available, can be used as standard. Protein hydrolysates are best prepared by autoclaving 300 mg of protein with 3 ml of 10% hydrochloric acid in a sealed tube at 15 lb. pressure for 10 hr. Satisfactory duplicates were obtained in the assay of both acids, and recoveries of the acids added to proteins prior to hydrolysis were quantitative within the limits of experimental error, which was estimated to be ± 15%. F. A. R.

* "Potency 40,000."

Specificity of Leucine, *Iso*leucine and Valine Requirements of *Lactobacillus arabinosus* 17-5. D. M. Hegsted (*J. Biol. Chem.*, 1945, **157**, 741–746)—Isomers and derivatives of leucine, *iso*leucine and valine were tested for their ability to replace these amino acids in the nutrition of *Lactobacillus arabinosus*. *d*(−)Leucine, acetyl-*dl*-leucine, *dl*-leucylglycine and sodium α-keto*iso*caproate partly and glycyl-*l*(+)leucine completely replace *l*(+) leucine for the organism. *l*(+)Allo*iso*leucine partly and calcium *dl*-α-hydroxy-β-methyl-*n*-valerate almost entirely replace *l*(+)*iso*leucine. The only compounds which could replace *l*(+)valine were calcium *dl*-α-hydroxy*iso*valerate and α-keto*iso*-valeric acid, and these were only partly effective.

F. A. R.

Estimation of Tryptophan in Human Urine. A. A. Albanese and J. E. Frankston (*J. Biol. Chem.*, 1945, **157**, 59–68)—The method previously described (*J. Biol. Chem.*, 1942, **144**, 563) for protein hydrolysates has been adapted for use in the estimation of tryptophan in urine. The colour reaction is specific for the indole nucleus, and no interference is therefore encountered from histidine, tyrosine or phenols, as it is with other colorimetric methods. In applying the method to urine, however, two difficulties were encountered. First, other indole derivatives are present in urine, and secondly the tryptophan content of normal urine is low. These difficulties were overcome by extraction with ether, which removes interfering indoles without removing tryptophan, and by pptng. the amino acid with acid-mercuric sulphate.

Collect 24-hr. specimens of urine in 2-litre brown bottles containing 50 ml of 15% hydrochloric acid and 1 ml of 10% alcoholic thymol soln. Carry out the assay immediately at the end of the collection period. *Isolation technique*—Run, at the rate of 1 drop per sec., 50 ml of urine through a column of activated Permutit (10 g) in a 150 × 15 mm tube, plugged with coarse glass wool and fitted with a piece of rubber tubing and pinch-clip to regulate the flow. The filtrate should give a negative reaction with Nessler's reagent. To 20 ml of the treated urine add 1 ml of 50% sulphuric acid and shake in a separating funnel with 20 ml of ether. Transfer the aqueous layer to a 50-ml conical centrifuge tube containing 10 ml of acid-mercuric sulphate reagent (10 g of mercuric sulphate in 100 ml of 5% v/v sulphuric acid). Leave at 4° C. for 2 hr. and centrifuge for 10 min. at 3,000 r.p.m. Decant the supernatant soln. and dissolve the ppt. in the minimum (*ca.* 1 ml) of acid reagent (made by mixing 60 ml of 40% trichloroacetic acid and 40 ml of conc. hydrochloric acid). Adjust to 5 ml so that 1 ml of the final soln. contains approx. 1 mg of tryptophan.

Colorimetric technique—Put 2-ml aliquots of the final soln. into graduated Klett-Summerson colorimeter tubes and add 0·3 ml of 3% sodium nitrite

soln. and 0·1 ml of 10% acetic acid. Shake intermittently for 10 min. and then add, in succession, 0·3 ml of 1% potassium persulphate soln., 0·5 ml of a 1% thymol soln. in 95% ethanol and 5 ml of acid reagent. Immerse in a boiling water-bath for 5 min. and cool in an ice-bath for 5–10 min. During the cooling the reagent mixture separates into two layers. Remove all but about 0·3 ml of the upper layer by means of a capillary pipette and dilute the coloured lower layer to the 5-ml mark with glacial acetic acid. Evaluate the colour with a No. S 54 filter and compare with that of a similarly treated tryptophan standard (1 ml of a 0·1% soln.). Run a reagent blank and subtract the reading from that of the unknown and the standard. Since the relation between concn. and colour is not strictly linear, the results are best calculated from a calibration curve. Recovery tests indicated the necessity of a correction factor to compensate for the solubility of the tryptophan-mercury compound; under the conditions described this was equivalent to 0·78 mg of tryptophan per 100 ml of urine. F. A. R.

Colorimetric Estimation of Urea. R. M. Archibald (*J. Biol. Chem.*, 1945, **157**, 507–518)—The following method is based on the use of α-*iso*-nitrosopropiophenone mentioned in a previous communication (*J. Biol. Chem.*, 1944, **156**, 121). Prepare blood filtrates according to the method of Somogyi (*J. Biol. Chem.*, 1930, **87**, 339), Fujita and Iwatake (*Biochem. Z.*, 1931, **242**, 43) or Miller and van Slyke (*J. Biol. Chem.*, 1936, **114**, 583) or dialysates by the method of Hamilton and Archibald (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 136). Dilute the filtrate or dialysate 10-fold if the blood urea level is normal, put 2-ml aliquots into reaction tubes and add 5·0 ml of water. For urine, if the sample contains more than 0·5% of protein add to 5 ml an equal vol. of acetate buffer (10 g of hydrated sodium acetate and 10 ml of glacial acetic acid in 1 litre), giving a *p*H of 4 to 5, heat to 100° C. and centrifuge. Dilute the centrifugate 25- to 500-fold (and untreated urines containing less than 0·5% of protein 50- to 1000-fold), according to the volume of urine excreted per min. so as to contain *ca.* 1 to 3 μg of urea per ml. Put 2 ml of the diluted urine into a reaction tube and add 5·0 ml of water.

Into similar reaction tubes put 0, 2, 4 and 6 ml of standard urea soln. containing 1·07 μg per ml and 7, 5, 3 and 1 ml of water, respectively. To each tube add 5 ml of a mixture of conc. sulphuric acid (1 vol.), syrupy phosphoric acid (3 vols.) and water (1 vol.) and 0·40 ml of 4% alcoholic α-*iso*-nitrosopropiophenone soln. Stopper the tubes and immerse in a boiling water-bath in a dark room. Heat for 1 hr., cool in water at room temp. with complete exclusion of light and, after 15 min., evaluate the colour in a colorimeter at a wavelength of 540 mμ with the optical density of the reagent blank adjusted to zero. Of a large number of substances tested, the only ones that gave a red colour similar to that given by urea were alloxantin and uric acid in relatively high concns., and parabanic acid, alloxan, alloxan treated with potassium cyanide, biuret, allantoin and protein. Of these substances, the only one likely to cause interference is allantoin, and this does not result in serious errors. The urine urea values obtained by the method were on the average 2·8% higher than those obtained by the manometric urease method; the discrepancy was somewhat less with blood. Although the colorimetric method cannot at present be recommended when a high degree of accuracy is required, the error is generally much smaller than that involved in measuring the rate of urine flow, and the method is therefore probably sufficiently accurate for most clinical purposes. F. A. R.

Rapid Colorimetric Method for the Estimation of Tryptophan in Proteins and Foods. M. J. Horn and D. B. Jones (*J. Biol. Chem.*, 1945, **157**, 153–160)—The estimation of tryptophan in proteins by means of *p*-dimethylaminobenzaldehyde frequently gives high values when the free tryptophan is used as standard. Consistent results can be obtained, however, if it is borne in mind that with peptides the maximum colour develops either at 85° C. or at room temp., whereas with the free amino acid it develops only at room temp. The method of Shaw and McFarlane (*Canad. J. Res., Sect. B.*, 1938, **16**, 361) is inapplicable to the hydrolysis of materials containing carbohydrates, as the soln. darkens with the sulphuric acid used, and the procedure of May and Rose (*J. Biol. Chem.*, 1922, **54**, 213), involving the use of conc. hydrochloric acid, has the disadvantage that several days are required for maximum colour development. On the other hand, alkaline hydrolysis cannot be used because, if carbohydrates are present, coloured solns. are formed. It has now been found that overnight digestion with papain gives satisfactory results, closely agreeing with those obtained by microbiological assays (Wooley and Sebrell, *J. Biol. Chem.*, 1945, **157**, 141).

Free tryptophan—Put a soln., containing not more than 0·1 mg of tryptophan, into a colorimeter tube and add 0·5 ml of a 5% soln. of *p*-dimethylaminobenzaldehyde in conc. hydrochloric acid. Dilute to 2 ml with water, add 5 ml of conc. hydrochloric acid, shake and, after 5 min., add 2 drops of an old 5% sodium nitroprusside soln. or one drop of a 0·2% sodium nitrite soln. Evaluate the blue colour in an Evelyn colorimeter after 2 min., using filter 540. Prepare a calibration curve by treating in the same way a series of tubes containing 0·1 to 1·0 ml of a soln. containing 10 mg of tryptophan per 100 ml.

Tryptophan in casein—Put 1 to 3 g portions of the material, depending on its nitrogen content, into a conical flask, add 35 ml of 0·05 *N* sodium hydroxide, 10 ml of papain soln. (prepared by shaking 2 g of commercial papain for 2 min. with 100 ml of water and filtering) and 10 drops of 5% sodium cyanide soln. Stopper the flasks and incubate overnight at 70° C. Add a small quantity of Celite, shake, filter, wash the Celite with water, and dilute the combined filtrate and washings to 100 ml. Estimate the tryptophan content of the soln. by the method described above. The following values were obtained for the tryptophan content (%) of moisture-free proteins; arachin, 0·90; casein, 1·20; cottonseed globulin, 1·29; lactalbumin, 1·72; lima bean globulin, 1·01; ox muscle, 1·30; phaseolin (navy bean), 0·50; maize germ (defatted), 0·23; cottonseed flour, 0·70; peas (black-eyed), 0·23; peanut flour, 0·56; soya-bean flour, 0·62; wheat (whole), 0·16; wheat germ (defatted), 0·30. F. A. R.

New Method for the Determination of Tyrosine and its use in Determining the Tyrosine Content of Edestin, Casein and Tobacco Mosaic Virus. L. E. Thomas (*Arch. Biochem.*, 1944, **5**, 175–180)—Gerngross, Voss and Herfeld (*Ber.*, 1933, **66B**, 435) observed that a purple-red colour was formed when a hot soln.

containing tyrosine and α-nitroso-β-naphthol was treated with conc. nitric acid, but they were unable to make the method quantitative, as the colour was unstable. It has now been observed that the colour fades much more slowly if dilute nitric acid is used and the acidity is increased by means of conc. hydrochloric acid. Put 5 ml of a soln. containing 0·08 to 0·30 mg of tyrosine into each of two test-tubes and add 1 ml of a soln. of α-nitroso-β-naphthol in 95% alcohol (120 mg per 100 ml) to one of the tubes and 1 ml of 95% alcohol to the other. To each tube add 2 ml of conc. hydrochloric acid (sp.gr. 1·19) and 1 ml of nitric acid (prepared by diluting 12 ml of conc. nitric acid, sp.gr. 1·42, to 100 ml with water). Mix and shake in a boiling water-bath for exactly 47 sec., immediately place in a water-bath maintained at 20–30° C., shake for 1 min. and then read the colour of the soln. containing nitroso-naphthol in a photoelectric colorimeter within 8 min of its removal from the cooling bath. Use the soln. in the other tube as the blank instead of distilled water. Almost theoretical results were obtained with a mixture of tyrosine and 18 other amino acids. The only other amino acid occurring in proteins to give a colour is diiodotyrosine; this gave considerably less colour than tyrosine. To estimate tyrosine in proteins, hydrolyse by refluxing with 20% hydrochloric acid using 15–20 ml of acid per g of protein. Transfer the hydrolysate to a volumetric flask, make up to vol. and filter to remove insoluble humin. Tyrosine added to such protein hydrolysates was quantitatively recovered. F. A. R.

Effect of Various Lighting Conditions on Riboflavin Solutions. L. J. DeMerre and W. S. Brown (*Arch. Biochem.*, 1944, **5**, 181–190)—In view of the possible destruction of riboflavin by light during its fluorimetric estimation, a study was made of the rate of destruction under various lighting conditions. Aqueous solns. of *p*H 3·7, 6·3 and 9·0 and solns. in a mixture of *n*-butyl alcohol, pyridine and acetic acid were investigated. In aqueous solns. of *p*H 3·7 no destruction occurred in diffused daylight over a period of 2 hr., whereas in sunlight almost complete destruction occurred in 20 min. A similar soln. was not affected by a 150-watt electric light at a distance of more than 50 cm. but appreciable loss occurred within 1 hr. at a distance of 30 cm. Red and green light were less harmful than violet light. Ultra-violet light from a mercury-vapour lamp caused some loss even at a distance of 50 cm and almost complete destruction in 20 min. at a distance of 30 cm. Somewhat greater loss of riboflavin resulted in the soln. of *p*H 6·3 in visible, but not in ultra-violet, light. A systematic study at *p*H 9·0 was not made. In the butyl alcohol mixture riboflavin was moderately stable when exposed to artificial light at a distance of 100 cm, but was rapidly destroyed at 30 and 50 cm. More rapid destruction occurred with green, red and violet light and less rapid destruction with ultra-violet light than in aqueous soln.

F. A. R.

***Lactobacillus* Assay Method for *l* (+) Glutamic Acid. J. C. Lewis and H. S. Olcott** (*J. Biol. Chem.*, 1945, **157**, 265–285)—The stock culture of *Lactobacillus arabinosus* 17–5 is carried in yeast extract—glucose—agar stab cultures, which are transferred monthly, being incubated at 30° C. for 24 hr. and then stored in the refrigerator. Prepare the inoculum with the basal medium described below, in which ordinary casein hydrolysate replaces the glutamic acid-free hydrolysate. Transfer the organism from the stab culture to this medium, incubate for 20 hr. at 30° C., centrifuge the cells and resuspend in sterile 0·9% saline. The basal medium has the following composition:

	%
Glucose	1·0
Glutamic-acid-free acid-hydrolysed casein	0·5
l-Cystine	0·01
l-Tryptophan	0·01
Sodium acetate trihydrate	1·0
,, chloride	1·0
Potassium monohydrogen phosphate trihydrate	0·05
Potassium dihydrogen phosphate	0·05
Magnesium sulphate heptahydrate	0·02
Ferrous sulphate heptahydrate	0·001
Manganous sulphate tetrahydrate	0·001

	p.p.m.
Adenine	10
Guanine	10
Uracil	10
Aneurine hydrochloride	0·5
Riboflavin	0·2
Calcium pantothenate	0·1
Nicotinic acid	0·5
Pyridoxine hydrochloride	0·5
Biotin	0·01
p-Aminobenzoic acid	0·01

To prepare the casein hydrolysate, heat under reflux 500 g of technical casein for 12 hr. with 5 litres of conc. hydrochloric acid and remove as much of the hydrochloric acid as possible by repeated evaporation to a thick paste *in vacuo*. Dilute the hydrolysate to 1·5 litres, filter, adjust to *p*H 2·9 with 15 *N* sodium hydroxide (about 200 ml) and autoclave for 4 hr. at 125° C. Filter and extract continuously for 48 hr. with ethyl acetate. Repeat the autoclaving and extraction until all the glutamic acid is removed; 4 or 5 treatments will be required, followed by a final autoclaving. The rate of removal of pyrrolidonecarboxylic acid can be estimated, if desired, by determining the amino-nitrogen content of the ethyl-acetate-soluble fractions before and after reconversion of the pyrrolidonecarboxylic acid to glutamic acid by acid hydrolysis.

Mix the basal medium immediately prior to use, adjust the *p*H to 6·8 to 6·9 with *ca.* 12 ml of 4 *N* sodium hydroxide and transfer 5-ml portions to Pyrex test-tubes. Add suitable quantities of standard *l*(+)glutamic acid soln. and of the solns. to be assayed, and make the volume in 4 *N* sodium hydroxide and transfer 5-ml portions to Pyrex test-tubes. Add suitable quantities of standard *l*(+)glutamic acid soln. and of the solns. to be assayed, and make the volume in each tube up to 10 ml with distilled water. The standard solns. should contain from 130 to 400 μg per tube and the assay samples 150 to 250 μg of *l*(+)glutamic acid. Plug the tubes with cotton-wool, autoclave at 15 lb. pressure for 10 to 15 min., cool and inoculate, keeping the amount of inoculum as constant as possible from tube to tube by adding 3 drops of the saline suspension from a hypodermic syringe. Incubate at 30° ± 0·5° C. for 64 hr., autoclave and titrate with 0·1 *N* sodium hydroxide, using bromothymol blue as indicator. Calculate the results from a standard curve. Hydrolyse proteins by heating under reflux with 20% hydrochloric acid at 120–125° C. for 24 hr. and neutralising with sodium hydroxide

before use. The recovery of *l*(+)glutamic acid added to casein hydrolysate ranged from 98 to 102% of the theoretical. F. A. R.

Use of *Neurospora* for the Estimation of Choline and Biotin in Milk Products. A. Z. Hodson (*J. Biol. Chem.*, 1945, **157**, 383–385)—The method of Horowitz and Beadle (*J. Biol. Chem.*, 1943, **150**, 325) for choline gave satisfactory results when applied to dairy products. The mould was grown in 50-ml conical flasks into which were put 5 ml of double-strength medium, the soln. to be tested, and sufficient water to make a total of 10 ml. Cultures were incubated for 5 days. The Permutit step could be omitted without serious disadvantages. Replicate results agreed within ±20%, and recoveries of choline added to 8 samples of milk ranged from 85 to 102%, with an average of 96%. The average choline content of 10 samples of fresh milk was found to be 149 mg per litre. The value obtained by Engle (*J. Nutrition*, 1943, **25**, 441) by a chemical method was 147 mg per litre. The "choline-less" strain of *Neurospora crassa* was also used for biotin assays on milk and other dairy products. For this purpose the biotin in the medium was replacd by 20 mg of choline per litre of the double-strength medium; the Permutit step was also omitted. Assays on 10 samples of fresh milk indicated a content of 30 to 40 mμg per ml, in good agreement with the results of Lampen, Bahler and Peterson (*J. Nutrition*, 1942, **23**, 11), who used *Clostridium butylicum*. F. A. R.

Spectroscopic Determination of Vitamin A in Fish Liver Oils. W. S. Metcalf (*Nature*, 1945, **155**, 575–576)—The custom of expressing the vitamin A content of a fish liver oil, which has been determined spectroscopically, in I.U. per g is criticised on the ground that the factor used for converting the value of $E^{1\%}_{1cm}$ 328 mμ to I.U. per g may be uncertain to the extent of 30% to 100% (see Morton, *Ann. Rev. Biochem.*, 1942, **11**, 365; Hume, *Nature*, 1943, **151**, 535). The value of $E^{1\%}_{1cm}$ 328 mμ is a physical property of an oil and may be determined to within ±2% on a suitable instrument, and it is suggested that one way out of the difficulty would be to express the vitamin A content in mg per g of oil, since $E^{1\%}_{1cm}$ 328 mμ can be converted into this by the factor $\frac{1000}{E \text{ pure vitamin A}}$. Tables are presented to show that for pure vitamin A alcohol, E = 1780 ± 50, and for unsaponified oil E = 1750 ± 50, should be used for the factor. It is stated that the chief error in spectroscopic assay is irrelevent absorption at 328 mμ, and, while much of this can be removed by saponification, some vitamin A is lost in the process (Jones and Haines, ANALYST, 1943, **68**, 8), whence the spectroscopic assay of vitamin A is subject to greater error that the 2% usually allowed for E measurements, so that an error of 3% in the E value does not invalidate it. It is urged that the results of spectroscopic assay of fish oils be expressed in mg per g of oil and that, for the convenience of analysts and as an indication of the basis of the estimation, the value of $E^{1\%}_{1cm}$ 328 mμ be also stated. J. A.

Riboflavin in Beer and in the Brewing Process. J. W. Tullo and W. J. Stringer (*J. Inst. Brewing*, 1945, **51**, 86–97)—The riboflavin content of a number of samples of beer has been determined by the microbiological method of Snell and Strong (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 346). Subsequent modifications to the original procedure of Snell and Strong, have not been found to be any improvement. The results obtained are summarised in a table. *Stouts*—Twelve samples showed a riboflavin content ranging from 0·25 to 0·85 μg per ml (av. 0·49 μg) and from 0·51 to 1·36 μg per 100 ml per degree of original gravity (av. 1·07). *Ales*—Fourteen samples showed from 0·25 to 1·18 μg per ml (av. 0·49) and from 0·86 to 1·45 μg per 100 ml per degree of original gravity (av. 1·06). *Lagers*—The riboflavin in 7 samples ranged from 0·22 to 0·45 μg per ml (av. 0·32) and from 0·50 to 0·95 μg (av. 0·73) per 100 ml per degree of original gravity. Each sample of beer was assayed six times, each assay being in triplicate at each of three concn. levels. The error is therefore less than 10%. The level of riboflavin has been followed in various stages of the brewing process by the same method, and it is shown that there is possibly a small loss during mashing. On boiling with hops no loss is indicated, but it has been found that hop resins, added to the culture tubes in ethereal soln., exert an inhibitory effect on the response of *L. helveticus* to riboflavin. The hops used contained 3·5 μg riboflavin per g, and at the rate of 2 lb. per barrel would have contributed less than 0·02 μg per ml to the hopped wort. At 6 lb. per barrel, 0·05 μg per ml could be contributed by the hops, but the fact that unhopped wort and wort hopped at 6 lb. per barrel show the same average riboflavin content is attributed to the effect of the hop resins. It is considered that at present-day hop rates, the effect of the hop resins on the assay is negligible, but at higher hop rates a preliminary extraction of the beer with ether would be necessary. It is further shown that there is no loss of riboflavin due to boiling in a copper vessel. During top fermentation there is an increase in riboflavin content of over 100%. Beer contains twice as much as the original wort, while the yeast crop contains from 10 to 20 times as much as the pitching yeast. This ability to synthesise riboflavin in hopped wort is shown to be possessed by several strains of *Saccharomyces cerevisiae*.

The extraction of riboflavin from malt has been studied, and it has been found that non-flavin substances which stimulate *L. helveticus* in presence of riboflavin are extracted by acid. These stimulants can be removed from the acid extract by adjusting to *p*H 4·5, leaving overnight and filtering. Digestion with water is considered to be the best method for the extraction of malt. Cheldelin, Eppright, Snell and Guirard (*Univ. Texas Publ.* No. 4237, p, 15) recommend enzymic extraction for the liberation of riboflavin from plant and animal tissues and, since it has been stated (Andrews, *Cereal Chem.*, 1943, **20**, 16) that the diastase present in the grain is sufficient for this process, it is further recommended that the extraction be carried out at 60° C. The results of riboflavin determinations on malt, using various methods of extraction, are reproduced in a table; they show that the same results are obtained by aqueous digestion at 60° C. as by acid extraction and pptn. at *p*H 4·5, within the limits of experimental error. J. A.

New Colorimetric Reaction of Vitamins D_2 and D_3 and their Provitamins. A. E. Sobel, A. M. Mayer and B. Kramer (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 160–165)—The reaction occurs when glycerol dichlorohydrin or a related compound is added to vitamins D_2 and D_3 in presence of acetyl chloride or other acid halides. With calciferol

a yellow colour appears immediately, changes to green in 1 min., reaches a maximum in 15 min. and remains stable at 625 $m\mu$ for several hr. With ergosterol a faint pink colour forms immediately, becomes orange in 15 min. and later a fluorescent green. With 7-dehydrocholesterol no colour appears for several min., then a faint pink colour forms and becomes more intense in 24 hr. No colour is produced with cholesterol. At 625 $m\mu$ ergosterol has less than 4% and 7-dehydrocholesterol less than 0·3% of the absorption produced by vitamins D_2 and D_3. Cholesterol has no absorption. The antimony trichloride reaction for vitamin D has the advantage of a higher extinction coefficient at the wavelength of max. absorption, but the new reaction has the advantages of stability of both the reagent and the colour formed and the differences in the shape of the absorption curves for vitamin D, ergosterol and 7-dehydrocholesterol, the max. absorption being at 625 $m\mu$ for vitamin D and between 500 and 525 $m\mu$ for the two provitamins. With antimony trichloride these maxima are very close together near 500 $m\mu$. The new reaction is also more specific for vitamin D, since there is no reaction with cholesterol and less interference from 7-dehydrocholesterol and ergosterol at 625 $m\mu$.

The reagent is glycerol-1,3-dichlorohydrin (Eastman Kodak's practical grade); for qualitative tests treat a trace of the sterol in 1 ml of solvent with 1 or 2 ml of reagent. Of a number of solvents investigated chloroform proved to be the best. When the reagent is purified by treatment with active charcoal it no longer forms a colour with any of the sterols mentioned, but upon addition of acetyl chloride or certain other organic acid chlorides or inorganic chlorides the reagent regains its activity. The fact that the green colour developed with calciferol disappears upon addition of thionyl chloride may furnish a clue to the nature of the colour. For quantitative work chloroform was found to be the best solvent, giving the most intense colour with all proportions of reagent and solvent. Although in the investigation the colours were developed in the dark, daylight appears to have no significant effect. Max. absorption was obtained with all solvents when calciferol was dissolved in 1 ml and 4 ml of the reagent were added. After 15 min. the intensity of the colour remains practically constant but, since ergosterol interfered after 30 min., the colour with calciferol was read after 15 min. Max. absorption with calciferol was reached at 400–425 $m\mu$ with a second peak at 625 $m\mu$, which was chosen as the most convenient wavelength because it is the wavelength of min. absorption of ergosterol and 7-dehydrocholesterol. The colours produced in presence of acetyl chloride are more intense, chloroform again being the best solvent. The min. absorption for the colour with calciferol occurs between 500 and 550 $m\mu$. There is a maximum at 550 for ergosterol and at 500 $m\mu$ for 7-dehydrocholesterol and a minimum for ergosterol and 7-dehydrocholesterol at 625 and 450 $m\mu$.

In the qualitative test the 3,5-dinitrobenzoates of vitamins D_2 and D_3 give the same reaction as calciferol but somewhat more slowly. Ergosterol-3,5-dinitrobenzoate behaves like the free sterol. The 3,5-dinitrobenzoates of 7-dehydrocholesterol and cholesterol resemble cholesterol by giving no colour in 24 hr. and only a faint greenish colour in 2 or 3 days. Quantitatively the dinitrobenzoate of calciferol has *ca.* one-sixth of the absorption of the free vitamin, and the dinitrobenzoate of ergosterol *ca.* one-half of the absorption of the free sterol. Like the free sterols the dinitrobenzoates of ergosterol and 7-dehydrocholesterol show marked differences in the colours produced, but the absorptions of the two vitamins derived from these sterols differ only slightly.

The reaction in presence of acetyl chloride is well suited for quantitative work especially when the effects of varying proportions of acetyl chloride, reagent and solvent are determined and tabulated. For example, with 0·5% of acetyl chloride and a solvent—reagent ratio of 4 to 1 no colour is given by 7-dehydrocholesterol at 625 $m\mu$ and conditions are suitable for determining ergosterol or calciferol in presence of 7-dehydrocholesterol. At a ratio of 3 to 2 with 1% of acetyl chloride at 625 $m\mu$ ergosterol has less than 4% and 7-dehydrocholesterol less than 0·3% of the colour intensity of calciferol. Conditions are then suitable for determination of calciferol in presence of the other two sterols. Ergosterol may be determined with a ratio of 2 to 3 and with 4% of acetyl chloride at 500 $m\mu$. Under the conditions found best for differentiation of calciferol and ergosterol (3 to 2 solvent—reagent ratio and 1% of acetyl chloride) the dinitrobenzoates of 7-dehydrocholesterol and cholesterol gave no colour even in amounts up to 10 mg, whereas the dinitrobenzoates of ergosterol and the two vitamins gave a distinct colour similar to that of the free sterols. This is in marked contrast to the effect of using only 1,3-dichlorohydrin, with which the dinitrobenzoic esters give only *ca.* 20% of the colour given by the equiv. amount of the free sterols. The reaction with glycerol-1,3-dichlorohydrin is thought to be the first reaction differentiating between ergosterol and 7-dehydrocholesterol. A Coleman 11 Universal spectrophotometer is recommended for the quantitative work, and the glycerol-1,3-dichlorohydrin, if not colourless, should be redistilled *in vacuo*.

A. O. J.

Water

False Residual Chlorine Values produced by Insoluble Manganese and the Effect of *Actinastrum* in a Water Supply. G. G. Schaut (*Amer. J. Pharm.*, 1944, **116**, 446–459)—Treatment of a sample of raw river water with *o*-tolidine reagent indicated the apparent presence of chlorine, and the colour produced could readily be matched with the usual standards. When the water was filtered the filtrate did not answer to the test, but when the filter-paper was moistened with the *o*-tolidine reagent a greenish-yellow colour immediately developed. Since nitrites would have occurred in the filtrate and since the iron content of the water was insignificant, manganese was proved to be the cause of the reaction. Further expts. showed that manganous compounds do not react with *o*-tolidine but that manganic compounds simulate chlorine. Substitution of acetic acid for hydrochloric acid in the *o*-tolidine reagent produced an even more intense colour, and the possibility of formation of free chlorine by reaction between the manganic compounds and hydrochloric acid was excluded. Expts. with water from various points along the Schuylkill River showed that the total manganese content bore no relation to the intensity of the colour produced with *o*-tolidine, but that there was a definite linear relation between the insoluble manganese content and the colour measured in terms of its chlorine equivalent. It has been suggested that free chlorine in treated water containing manganese could be measured by reaction with *o*-tolidine before and after boiling, the difference

being equivalent to the free chlorine removed by boiling. If, however, a large proportion of the manganese is present in the manganous state, especially in alkaline water, some is oxidised during the boiling to the manganic state, and the colour may then be more intense than before boiling. The oxidation of small amounts of manganese by residual chlorine (0·1 to 0·2 p.p.m.) is a slow reaction. Stored, filtered, chlorinated water containing soluble manganous compounds will ultimately give a colour with *o*-tolidine owing to slow oxidation of the manganese not removed by filtration. Other methods tried for overcoming the interference of manganese were filtration and centrifuging (2000 r.p.m.). Both methods were equally effective, but the use of filter-paper (Whatman No. 42) has the disadvantage that the filter-paper has a high chlorine demand, and a filtering medium that has no demand or a demand that is easily satisfied was sought for. The Sedgwick–Rafter plankton filter was ultimately adopted. This consists of a ½-in. depth of washed sea sand supported by a small piece of bolting cloth in a glass funnel of 250-ml capacity graduated in 50 ml. The inherent chlorine demand of this device is rapidly satisfied. *Method*—Pour 250 ml of water into the filter-funnel and allow 100 ml of the filtrate to run to waste. Add 100 ml more of the sample and collect 100 ml of filtrate for manganese determination by the bismuthate method. Collect the next 50 ml in a Nessler glass, add 0·5 ml of *o*-tolidine reagent and match the colour, which is due to free chlorine, with the usual standards. During the summer months of some years the river water was abnormal in that free carbon dioxide was absent and the dissolved oxygen content rose from 40–50% saturation to 100%. This suggested presence of algae and it was found that one organism of the *Chlorophyceae* group was always present in excessive numbers when the oxygen saturation approached 100%. The organism is a variety of *Actinastrum*, closely resembling but not identical with the variety of Lagerheim (G. M. Smith, "*Fresh Water Algae of the United States*," 1st Ed., p. 521, McGraw-Hill Book Co. Inc., New York and London, 1933). It consists of coenobes of 4, 6 or 8 elongated cells radiating in all directions from a common hub, the individual cells being of *ca.* 5 μ in diam. and *ca.* 25 μ long. No doubt the high oxygen content accompanying the presence of this organism renders some of the soluble manganese insoluble. During the occurrence of this organism in the river fishermen reported a reluctance in the fish to bite and believed that something in the water made their mouths sore. Expts. with minnows in an aquarium fed from the river confirmed the observation that the fish refused to feed, but the injury sustained by the fish is not permanent, since they ate freely when transferred to stabilised tap water. The disturbing substance is probably nascent oxygen produced by the organism. Relatively large amounts of manganese do not appear to be necessary for the existence of *Actinastrum*. A. O. J.

Determination of Fluoride in Water. W. L. Lamar (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 148–149)—This colorimetric method employs a zirconium-alizarin reagent which differs from those previously reported in being stable indefinitely. The fluorine content of 100-ml samples of water may be determined within 0·1 p.p.m. if less than 500 p.p.m. of sulphate and less than 1,000 p.p.m. of chloride are present. *Reagents*: *Indicator soln.*—Dissolve 0·184 g of zirconyl nitrate dihydrate in 25 ml and filter. Add this soln. to 50–100 ml of water and slowly stir in 0·037 g of alizarin monosodium sulphonate dissolved in 25 ml. Make up to 500 ml, mix well, and add 500 ml of sulphuric acid (2·10 to 2·12 *N*). The indicator is ready for use in about one hour. *Standard sodium fluoride soln.*—Stock soln., 0·2210 g per litre. Standard soln. (1 ml ≡ 0·01 mg of F), 100 ml of stock soln. diluted to 1 litre. *Method*—Transfer to Nessler tubes 100-ml samples of the water and suitable quantities of standard sodium fluoride soln., each diluted to 100 ml. Add 10 ml of indicator soln., mix, and compare the colours after 1 hour, or preferably after the tubes have been left overnight. When the alkalinity of the sample exceeds 100 p.p.m. as $CaCO_3$ (122 p.p.m. of $Ca[HCO_3]_2$) neutralise with 0·164 *N* nitric acid and dilute tests and standards to 105 ml before adding the indicator. This procedure is suitable for samples containing 0·0 to 1·6 p.p.m. of fluorine. Errors introduced by the presence of sulphate, chloride and unneutralised bicarbonate are as follows, the units being p.p.m.

Sulphate	Fluoride error (plus)	Chloride	Fluoride error (minus)	Bicarbonate	Fluoride error (minus)
200	0·03	400	0·02	100	0·03
300	0·05	600	0·05	200	0·08
400	0·08	800	0·08	300	0·12
500	0·10	1000	0·10	400	0·17
600	0·12	2000	0·17	500	0·21
1000	0·20	—	—	—	—

L. A. D.

Agricultural

Quantitative Estimation of DDT and of DDT Spray or Dust Deposits. F. A. Gunther (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 149–150)—Zeidler (*Ber.*, 1874, **7**, 1180) and Fischer (*Id.*, 1874, **7**, 1191) reported that dichlorodiphenyltrichloroethane [2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane] readily lost a mol. of hydrogen chloride per mol. of parent compound when boiled with alc. potassium hydroxide, and Brand and Busse–Sundermann (*Id.*, 1942, **75B**, 1819) showed that 50 ml of 0·5 *N* alc. potassium hydroxide completely dehydrohalogenated 5 g of DDT within 3 min. under reflux, with formation of 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene. Since one and only one chloride ion is liberated from each mol. of DDT, this may be determined by pptn. with silver nitrate and the excess of silver determined by the Volhard procedure. Strip the treated fruit, leaves or other material with benzene, either by shaking in a Mason jar or by washing individually with a stream of benzene from a wash bottle. Filter the soln. through cotton-wool or glass-wool and collect the filtrate in a standard taper 500-ml Erlenmeyer flask. If preliminary expts. have shown the presence of inorganic chloride sol. in benzene, extract the benzene soln. with several portions of water. Evaporate the soln. nearly to dryness on an electric plate at 80° C. with the aid of a current of air, avoiding a rise of temp. above 95° C., at which temp. DDT may decompose. Add 50 ml of *N* alc. potassium hydroxide without wetting the ground joint, fit a standard taper condenser to the flask, and gently heat the mixture under reflux on the same hot plate for exactly 30 min. Remove the hot plate, and through the condenser add 50 ml of water, 50 ml of *ca.* 2 *N* nitric acid and an additional 50 ml of water, in that order. Remove the condenser, add *ca.* 5 ml of nitrobenzene and exactly 20 ml of 0·1 *N* silver nitrate.

Stopper the flask with a standard taper plug and shake vigorously for a few sec. Rinse down the stopper and the sides of the flask with water, add 3 ml of *ca. N* ferric nitrate indicator, and titrate the excess of silver ion with 0·1 *N* potassium thiocyanate. The amounts of reagents specified are most convenient for determination of 2 to 625 mg of DDT, but the range may readily be increased by increasing the amount of standard silver nitrate used, and with micro-burettes and weaker standard solns. the method may be applied to the determination of less than 2 mg. Benzene dissolves *ca.* 45% of its wt. of DDT at room temp. and will not dissolve any appreciable amount of the inorganic halides expected to occur in insecticidal sprays or dusts containing DDT. Although benzene is an excellent solvent for the hydrocarbon oils employed as carriers in most spray mixtures, the oil carried over into the final titration mixture is of no consequence. Technical DDT contains polymers and condensation products of chloral and possibly traces of chlorobenzene, but these cannot interfere under the specified conditions. A. O. J.

Leaching of Soluble Salts from Alkali Soils without Affecting the Exchangeable Bases. M. Y. Shawarbi and A. G. Pollard (*J. Soc. Chem. Ind.*, 1945, **64**, 31)—In determining the exchangeable bases in alkali soils it is often necessary to remove a relatively large proportion of soluble salts without disturbing the exchangeable bases. Salgadi and Chapman (*Soil Sci.*, 1931, **32**, 199) washed 10-g samples of soil with 200 ml of 50% aq. alcohol, but with alkali soils some hydrolysis of sodium clay appears to occur. Expts. with two samples of soil showed that alcohol of not less than 70% concn. must be used if hydrolysis is to be avoided. With 70–80% and 90% alcohol sodium ceases to be leached when a minimum amount of 400 ml of alcohol is used. With less concentrated alcohol the amount of sodium leached progressively increases, thus indicating slow continuous hydrolysis of the sodium clay. Satisfactory results have since been obtained by washing 10-g samples of soil with 400 ml of 70% alcohol in seven or eight successive portions. A. O. J.

Organic

Method of Classification of Petroleum Waxes. A. Kinsel and J. Phillips (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 152–156)—The classification of petroleum waxes into crystalline and amorphous waxes is almost obsolete, and it is generally understood that amorphous waxes do not exist. The assumption that crystalline waxes are hard and brittle and that the so-called amorphous waxes soft and plastic is not necessarily true, since, for example, hard and dry ceresine wax is the same as amorphous wax of petroleum. Crystallographic analysis does not distinguish the two types clearly, the crystalline form being greatly affected by impurities. In general, waxes of low mol. wt. are more crystalline than those of high mol. wt. In course of work on petroleum waxes it was noted that on solidification the contraction of macro-crystalline waxes was different from that of micro-crystalline waxes. Closer study of the contractions showed that whereas an oil has only one type of contraction on cooling, three types of contraction are involved when a wax is cooled from above to below its m.p. When wax is cooled from 200° F. to its m.p. it behaves like an oil, and its contraction (K_l) depends upon its mol. wt. only. There is no distinction between macro- and micro-crystalline waxes. The contraction (K_c) occurring during the change from liquid to solid depends not on the mol. wt. but on the crystallisation tendency of the wax, and this type of contraction increases with increasing crystallinity. The contraction (K_s) of the solid wax from its m.p. to 80° F. probably depends primarily on the mol. wt. and does not measure its crystallinity. The total contraction K_t is the sum of these values, but macro- and micro-crystalline waxes could be most sharply distinguished by measurement of K_c only. This could be done in two ways. The contraction of the wax could be determined at its m.p., or K_l and K_s could be determined for each individual wax and subtracted from the total contraction, thus yielding K_c by difference. Samples of commercial waxes (100 g) of widely divergent properties were allowed to cool at the rate of 10° F. per hour in a thermostatically controlled bath, and the average % contraction per °F. was noted for temperatures ranging from 200° F. to *ca.* 5° F. above the m.p. of each sample. The results show that the value 0·04 can be used for the calculation of K_l for all waxes, with probably a max. error of ±0·005. K_s, however, had no such constant value. All the samples yielded large values for K_s immediately below their m.p., and these values decreased as the temp. was lowered. The large contraction immediately below the m.p. is due not simply to thermal contraction but also to change in the structure of the solid wax. Hence it would be incorrect to subtract from K_t the average values found for K_s, since these values include the contraction due to structural changes. This part of the contraction should be included in K_c, but cannot readily be obtained experimentally. However, an ideally amorphous wax would contract in the solid state as if it were a liquid, *i.e.*, K_s would be constant and identical with K_l. Thus the contraction of the waxes should be studied through the same temperature range with respect to their m.p. and not through the same absolute range, and the range should be wide enough to ensure that all structural contraction has ceased. In all expts. the contraction was determined from 10° F. above to 50° F. below the m.p. The "crystallinity index" is defined as $10K_c$, where $K_c = K_t - (K_l$ per° F. $\times$ 10 + K_s per °F. $\times$ 50) or, since K_s per ° F. can be taken as equal to K_l per °F. and K_l per °F. is 0·04 for all waxes, $10K_c = 10(K_t - 2{\cdot}4)$. To determine K_t, pour exactly 100 ml of the wax, heated to 10° F. above its determined m.p.. into a 100-ml mixing cylinder which has been heated to the same temp. Allow it to cool for 2 hr. and then for 2 hr. in a water-bath kept at 50° F. below the m.p. As the wax cools it will form a cavity at the centre. often covered with a thin layer of wax. Pierce the layer with a pointed glass rod and add from a burette a 50% aq. soln. of glycerin to the 100-ml mark. Apply a slight reduction of pressure to liberate trapped air and re-adjust the liquid level to the mark. The vol. of liquid added is K_t. With a number of samples of commercial waxes the crystallinity index of true micro-crystalline waxes ranged from 65 to 80 of semi-crystalline waxes from 90 to 103 and of macro-crystalline waxes from 109 to 117. There is thus no sharp line of demarcation between the micro- and macro-crystalline waxes. Addition of amorphising agents (pour-point depressants) to macro-crystalline waxes renders them more or less micro-crystalline, and this was confirmed by a reduction in the value of the crystallinity index. Similarly, removal of amorphising agents from crude micro-crystalline wax raised its

crystallinity index from 67 to 82. Specifications for high-grade micro-crystalline waxes demand such qualities as good cohesion at low temperatures without flaking or cracking. Tests for these qualities are not easy to make, and it is suggested that the crystallinity index could be used to replace or supplement such tests. The cohesion of waxes is related to their crystallinity index somewhat in the following manner: >100, flaking; 100–110, cracking; 90–100, slight cracking; <90 acceptable. Crystallinity index can also be used to detect adulteration of micro-crystalline wax with commercial paraffin (macro-crystalline) wax, since a mixture of both tends to have a crystallinity index higher than the arithmetical average. A. O. J.

Precise Method for Isolation of High Polymers. F. M. Lewis and F. R. Mayo (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 134–136)—A procedure is described for isolating polymers from monomers and solvents in a form convenient for handling and analysis. Previous workers have either pptd. polymers as a powder, using suitable mixtures of solvents, or have assumed that solvents and monomers could be removed from the massive polymer by vacuum distillation with or without preliminary pptn. of the polymer or addition of inhibitors. Expts. showed that the powder method is adequate but not always possible or convenient, and that the distillation method is generally inadequate for rapid work and precise results. The main difficulty in these methods is that the rate of separation depends upon diffusion of the solvent through relatively large masses of hard polymer. This difficulty is largely avoided by the following procedure. Dissolve the polymer in 8 to 10 times its wt. of benzene and quickly freeze the soln. Sublime the benzene from the polymer without melting the solvent or sintering the polymer. The polymer is left as a soft, fluffy, very porous solid from which remaining traces of volatile matter are rapidly removed and which breaks up easily for transference and analysis and dissolves readily in solvents. Other solvents that can conveniently be sublimed could doubtless be used. Samples (*ca.* 2 g) of polystyrene were dissolved in 20 g of benzene in 125-ml Erlenmeyer flasks, and the solns. were frozen in "dry ice." The flasks were then transferred to an ice-bath and kept at 0° C. and 1 mm pressure. Eight to ten hr. were required for removal of most of the benzene, during which the rate of loss of solvent was almost linear. Removal of solvent was then completed at a higher temp. still under reduced pressure. The higher the temp., the more quickly constant wt. is attained, and the upper temp. limit which may be used is determined by the temp. at which the polymer sinters and loses its porous structure. Polystyrene sinters slowly at 100° C., the porous cake shrinking to *ca.* half its original vol. in 10 hr., but no depolymerisation has been detected in as long as 2 weeks at 100° C. and 1 mm pressure. The method is reproducible, giving the same result when the polymer is re-dissolved in benzene and the process repeated. Pyrex Erlenmeyer flasks are recommended because they give large flat cakes of polymer. The less volatile the monomer present, the more important is it to re-ppt. the polymer from soln. two to three times before the final soln. in benzene is made up. After most of the benzene has been removed at 0° C. the product should stand at room temp. for 1 to 3 hr. before heat is applied to reduce the tendency of the remaining small amount of solvent to cause sintering. Since the whole procedure is carried out at reduced pressure, no difficulties arise from oxidation or moisture adsorption, although some products containing chlorine have sometimes lost hydrogen chloride. Microscopical examination of the unsintered product shows a sponge-like structure with inter-connecting cells of 10–50 μ diameter with walls 5–10 μ thick. Rough calculations suggest a surface area of *ca.* 2000 sq.cm. per g of polymer. Moisture adsorption amounts to 2 or 3 mg on polymer and flask together on humid days, but can be eliminated by admitting dried air to the evacuated flasks and storing them in a desiccator before weighing. A. O. J.

Identification of Melamine and Urea Resins in Wet Strength Paper. R. W. Stafford, W. M. Thomas, E. F. Williams and N. T. Woodberry (*Paper Trade J.*, 1945, **120**, 19th Ap., *T.A.P.P.I. Sect.*, 155–160)—Wet strength properties in paper are produced by parchmentising, or by addition of proteins, viscose, natural gums or plastics (*e.g.*, melamine-, urea- or phenol-formaldehyde types). Procedures for general and specific methods of identification of members of the above plastics groups are described. The former include hand inspection; odour on burning; elementary qualitative analysis (sodium fusion method); determination of reduction in wet-strength after hydrolysis for 0 to 160 min. in excess of boiling 0·25% aluminium sulphate soln. (if melamine is present the initial reduction is much less than with urea plastics). The specific methods include the Jorissen phloroglucinol test for formaldehyde; the biuret and Millon tests for proteins; the Schmidt ammonium nitromolybdate pptn. test for glue in presence of casein. These tests are applied to extracts of the paper in water, acid or alkali, as appropriate. A specific staining test for melamine resins depends on the fact that papers containing them are more substantive to certain acid dyes than are untreated papers. (*a*) Dye square samples of paper (side, 1·5 in.) and also a sample of an untreated paper of a similar type, for 5 min. at room temp. by immersion, with stirring, in a 0·1% soln. of Calcoacid Alizarin Blue S.A.P.G. (American Cyanamid Co.) in 0·1% sulphuric acid, using 200 ml of dye soln. per g of paper. Wash the specimens well in three 500-ml portions of cold water or until the surface of the control sheet is free from adsorbed dye. This procedure is also carried out (*b*) using a soln. of the dye in 1·0% acid at the b.p., and (*c*) also after pre-treatment of the samples with 200 ml/g of 0·25 *N* sodium hydroxide at the b.p. for 5 min., followed by thorough washing. With these 3 procedures, respectively, melamine resins give (i) a blue, (ii) intense blue and (iii) intense blue stain; urea resins, (i) blue, (ii) pale blue or colourless, (iii) pale blue or colourless; proteins, (i) blue, (ii) blue of variable intensity, (iii) colourless; control, (i) colourless, (ii) colourless, (iii) colourless. The effectiveness of the test may be enhanced when more than one additive is present, by making the tests in succession on the same sample. Xanthydrol condenses with urea and thiourea giving cryst. compounds of well-defined chemical and optical properties; melamine does not. Photomicrographs of dixanthyl urea, of dixanthyl thiourea and of melamine (which may be pptd. in the free state as a result of hydrolysis) are reproduced, and their orientation and other optical properties are described. Stir 1 to 2 g of paper (in small pieces) with a mixture of 40 ml of glacial acetic acid and 15 ml of water for 30 min. at just below the b.p., filter, cool to 25° C., and filter again if any ppt. forms (wash the filter with a little acetic acid). To 45 ml

of clear filtrate, slowly add 5 ml of an 8% soln. of xanthydrol in methanol. The ppt. may be identified by reference to crystals produced similarly from urea or thiourea. If desired, filter off the ppt., wash with ethanol, dry for 1 hr. at 100° C., and weigh. The wt. × 0·143 gives the approx. wt. of urea. The remainder of the extract may be evaporated and examined for melamine. If difficulty occurs in obtaining the ppt. in a suitably crystalline state, recrystallise it from a mixture of dioxan with 40% of water. J. G.

Inorganic

Sensitive Test for Silver. P. S. Baker and J. H. Reedy (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 268–269)—Acidify the test soln. with dil. nitric acid, warm, and filter off any ppt. (tungstic acid, silica, etc.). Treat the filtrate with dil. hydrochloric acid, and chlorine soln. or dil. nitric acid if mercurous or lead ions are present, and warm. Collect the ppt. in a Hirsch funnel, wash it free from mercury and lead, dry by suction, and treat it with 1 or 2 drops of iodomercurate reagent; if the ppt. becomes orange-red, silver chloride is indicated. The reagent is prepared by shaking 24 g of mercuric iodide with 100 ml of warm *M* potassium iodide soln., and leaving the liquid to clear. Conversely, chloride and bromide can be detected by pptn. with silver nitrate and application of the above test. The cause of the colour is uncertain. W. R. S.

Sulphamic Acid as an Aid in Copper Electrolysis. L. Silverman (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 270–271)—Urea, which is added to remove oxides of nitrogen from the electrolyte, has several disadvantages. Its action is slow, it neutralises acid, and it may give a dark coating on the cathode. Sulphamic acid is free from these objections, and has been found to promote deposition and give adherent deposits of copper and lead peroxide. Addition of 0·25 to 0·5 g of sulphamic acid in 10% aqueous soln. 15 min. before interrupting the electrolysis is recommended. W. R. S.

Determination of Copper in Aluminium Alloys. S. Weinberg (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 197)—Dissolve 1 g of drillings in 20 ml of 70% perchloric acid, 5 ml of water and 5 ml of conc. nitric acid, warming gently after the initial vigorous reaction. Addition of a further 5 ml of water often helps to complete the solution. Add 4 ml of conc. nitric acid and 2 ml of diluted sulphuric acid (1 + 1) and boil to expel nitrogen oxides. Dilute to 150 ml and electro-deposit the copper on to a platinum gauze cylindrical cathode (1¾ in. diam. and 2 in. long), using a platinum wire spiral anode, and stirring by passing a current of air through the soln. Set the current initially at 2 to 3 amp. and electrolyse for 30–40 min. without further adjustment. Completeness of deposition may be tested by the use of sodium sulphide soln. Elements, *e.g.*, bismuth, which are also deposited under the given conditions are rarely encountered. Chromium, manganese and nickel may be determined in the residual electrolyte. L. A. D.

Mode of Occurrence and Detection of Lead in Lead-bearing Steels. W. E. Bardgett and R. E. Lismer (*Iron and Steel Institute*, Advance copy, March, 1945)—As little is known about the mode of occurrence and distribution of lead in leaded steels, an investigation has been made in the course of which methods of detecting the lead have been examined. High sulphur and manganese-molybdenum wrought steels and 0·25% carbon—1% manganese steels have been studied. Lead occurs in a variety of ways. Segregation is found near the bottom of ingots as streaks of metallic lead containing particles of sulphide, oxide and steel. Other segregated streaks consist of oxide and sulphide particles in a matrix of oxide or silicate, and lead is also found as metal and oxide in sulphide and silicate inclusions. Normal microscopic methods fail to reveal lead except in inclusions, but electrolytic etching in ammonium acetate soln. reveals particles, believed to be lead, in the structure, and an electrographic printing method shows lead distribution clearly. On heating polished sections of steel, lead is exuded on to the surface; the temperature at which exudation is observed may be above or below the m.p. of lead, some having been noted at 30° C. with a segregated manganese-molybdenum steel billet. Beads exuded from a specimen heated to 240° C. are found to contain *ca.* 98–99% of lead.

Electrolytic etching—Place the specimen, face upward, on the bottom of a stainless-steel bath containing ammonium acetate soln. (50 g of crystals per litre of water). Connect the positive terminal of a 6 V. accumulator to the bath and the negative terminal to a platinum wire loop extending across the surface of the specimen. Hold the wire 1/4 in. from the sample and move it backwards and forwards for 1 min. Alternatively, connect the bath to the negative terminal, attach the specimen to the positive terminal, and hold it face downward 1/4 in. from the bottom of the bath for 1/2 min. A sharp brown stain forms on the lead-containing areas. Non-leaded steels have not shown any such stains when etched in this way, except rolled high-sulphur rapid-machining quality steel.

Electrographic method—Soak gelatin-coated paper in 10% aqueous ammonium acetate soln., remove the excess and place the paper with the gelatin uppermost, on a double layer of blotting-paper soaked in the same soln. The blotting-paper rests on a flat aluminium plate connected to the negative terminal of a battery. Press a polished face of the specimen firmly on to the paper and connect to the positive battery terminal. Pass a small current for about 2 min., applying about 2V. per sq.in. Immerse the paper in 5% ammonium acetate soln. for ½ min., wash, and then develop the print with a dilute soln. of hydrogen sulphide. This developing agent is preferred to sodium sulphide, potassium iodide, potassium chromate or dithizone. L. A. D.

Assay of Lead and Sodium Azides. J. W. Arnold (*Ind. Eng, Chem., Anal. Ed.*, 1945, **17**, 215–217)—Azides react with ammonium hexanitratocerate—

$$Pb(N_3)_2 + 2(NH_4)_2Ce(NO_3)_6 \rightarrow Pb(NO_3)_2 + 4NH_4NO_3 + 2Ce(NO_3)_3 + 3N_2.$$

Stir up 54·8 g of the ceric salt with 60 ml of strong nitric acid and 40 of water, followed by 100 ml of 4% nitric acid; stir continuously and slowly dilute to 1000 ml. Standardise the soln. against sodium oxalate by adding an excess to 0·3000 g of oxalate in 75 ml of *M* sulphuric acid, and titrating back with 0·1 *N* ferrous sulphate soln. in 6 *N* sulphuric acid (*o*-phenanthroline ferrous complex indicator).

Lead azide—Weigh 0·3000 g on a small paper scoop and transfer to a 250-ml conical flask containing a little water. Add 50 ml of water, and 50 ml of cerium soln. while gently agitating the

flask; rinse down its sides at the end. Add a drop of indicator and titrate back with ferrous salt, the colour changing from pale green to reddish-yellow. At the end-point the acidity should be molar, and the calculated amount of sulphuric acid (if any) be added near the end-point. A blank determination is made. The ferrous soln. is standardised against the stabler ceric soln.

Sodium azide—Dissolve 0·3000 g of dried salt in 100 ml of water in a conical flask, etc., exactly as above. W. R. S.

Colorimetric Determination of Traces of Nickel. E. Passamaneck (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 257–258)—The nickel is pptd. with dimethylglyoxime, and the ppt. is dissolved in pyridine, giving a yellow to orange-red soln. In a 50-ml beaker evaporate almost to dryness an aliquot portion of the hydrochloric acid soln. (20–50 μg of Ni for a Duboscq micro-colorimeter, or 2 to 30 μg for a photoelectric colorimeter). Dissolve the residue in 2 ml of water, add 1 ml of potassium citrate soln. (0·5 g per ml) and 5 drops of strong ammonia, mix in 1·6 ml of 1% alcoholic glyoxime soln., and set aside overnight. Collect the ppt. in a No. 2 Gooch crucible containing asbestos, wash the beaker with four 2-ml portions of hot water, and dry the crucible for not more than 1·5 hr. at 100° C. in the pptn. beaker. Transfer the asbestos to the beaker and rinse the sides of the crucible with a few drops of pyridine. Filter by suction into a small graduated tube standing in a suction flask and wash with small amounts of pyridine, collecting 3 to 5 ml of filtrate for the Duboscq, or 10 ml for the photoelectric, colorimeter. Compare the tint with that of a standard pyridine soln. containing 0·06 mg of nickel per ml ($\equiv$ 0·295 mg of glyoxime ppt.). The ppt. is obtained from a pure nickel soln.; it is collected by suction, washed, and dried at 100° C. All the water used should be double-distilled from all-Pyrex vessels. A blank assay must be run under identical conditions.

W. R. S.

Colorimetric Determination of Aluminium in Aluminium Steel. C. H. Craft and G. R. Makepeace (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 206–210)—The reagent is a soln. of 0·1 g of ammonium aurintricarboxylate (aluminon) in water, with 10 ml of 10% benzoic acid in methanol, diluted to 100 ml; it is set aside 3 days before using. The steel should contain not more than 2% of chromium. Dissolve 0·5 g in 5 ml of 8 *N* nitric, and 5 ml of 60% perchloric, acid (the latter dehydrates silica). Evaporate nearly to dryness, add 25 ml of water and 10 ml of 12 *N* hydrochloric acid, boil and filter. Evaporate the filtrate to less than 20 ml, transfer to a separator, rinsing the beaker with enough 6 *N* hydrochloric acid to complete 20 ml, then with a further 10 ml of 12 *N* acid, and finally with 30 ml of *iso*propyl ether. Shake well, allow to settle, draw off the aqueous layer into another separator; wash the ethereal layer with 2 ml of 8 *N* hydrochloric acid, adding this to the aqueous fraction. Repeat the extraction twice with 10-ml portions of ether. Boil the aqueous fraction free from ether, evaporate with 5 ml of perchloric and 5 ml of 6 *N* nitric acid until heavy fumes appear. Cool and add 25 ml of water, filtering again if any silica is observed. Neutralise to litmus with ammonia, dissolving any ppt. which may form with just enough 6 *N* hydrochloric acid, and dilute to a suitable bulk (0·002 to 0·008 mg of Al per ml). Transfer 5·00 ml to a Pyrex vessel cleaned with chromic acid, add 15 ml of reagent, heat on a steam-bath for 10 min., and set aside in the cold for 10 min. Dilute to 100 ml and compare the transmission in a Coleman spectrophotometer (Model 11) at 525 mμ with that of water; a curve may be used. All the factors affecting the accuracy of the method are discussed in great detail. W. R. S.

Determination of Lithium in Silicates. F. R. Bacon and D. T. Starks (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 230–232)—The periodate method for lithium (ANALYST, 1943, **68**, 229) was applied in the determination of the alkalis in spodumene (0·5 g) and glass (1 g) after decomposition with hydrofluoric and sulphuric acids. The insoluble portion (if any) of the residual sulphates consists of barium sulphate. The sulphate soln. is treated with 2 ml of hydrochloric acid and pptd. in small bulk with excess of ammonia, and the ppt. is dissolved in a minimum of acid and re-pptd. without addition of ammonium chloride. The combined filtrate is treated with 8-hydroxyquinoline, and heated with excess of ammonia to coagulate the ppt., containing the magnesium, manganese, some calcium and the remaining aluminium. The balance of the lime is removed by double oxalate pptn., and the ammonium salts by evaporation first with *aqua regia* and then with fuming sulphuric and perchloric acids. The filtered soln. is evaporated in platinum, and the residue is ignited to constant weight, giving the mixed alkali sulphates. The soln. of the sulphates is used for the volumetric determination of lithium as periodate. Potassium is determined in a separate portion, and sodium is computed by difference. W. R. S.

Modified Methyl Yellow Indicator for Direct Titration of Sodium Carbonate. W. R. Carmody (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 141–142)—The indicator soln. contains 0·8 g of methyl yellow and 0·04 g of methylene blue in a litre of alcohol and is recommended for high precision titrations of sodium carbonate solns. with 0·5 or 0·2 *N* hydrochloric acid. About 0·1 ml is used for each 100 ml of soln. at the end-point; the indicator is, however, not added until the titration is more than half completed, as methyl yellow fades rapidly in alkaline soln. In alkaline soln. the indicator is yellow-green; as the end-point is approached the soln. becomes straw-coloured, and the actual end-point is taken as the point when a trace of pink is first observed. Under a particular set of titration conditions used the titrated soln. was not completely saturated with carbon dioxide. The resulting error was found to be nil with 0·5 *N* acid and a correction of only $-0·02$ ml in about 50 ml was required when using 0·2 *N* acid. A temperature difference of 5° from the standard 20° C. introduced an error of about 1 part in 2000 when using 0·2 *N* solns. L. A. D.

Determination of Portland Cement in Drilling Muds and Soil—Cement Mixtures. R. G. Mihram and B. Brown (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 156–158)—In oil-field work it is frequently necessary to determine the portland cement content of drilling mud to ascertain if cement (which causes flocculation of the clay particles) is present, so that the mud may be chemically re-conditioned for use as drilling fluid, or to discover if a cement sample is contaminated with drilling mud. Recently the use of soil–cement in road building has demanded a simple and rapid method for determining the cement content of soil–cement mixtures. Six methods with three variations

of one of them were investigated. In all expts., except those with neat cement slurry blanks, weighed amounts of cement were added to weighed samples of drilling mud. The solid matter of the drilling mud was determined upon a separate sample by drying to constant wt. at 110° C., so that results could be based upon the total solid matter present. In a method proposed by the cement manufacturers sulphate is determined as barium sulphate in an aq. extract of the sample. Cement contains *ca.* 2% of calcium sulphate added in the manufacture as a retarder. Since the calcium sulphate content is low and variable, blank determinations on both soil and cement are necessary. Filtration is slow and the method is tedious and not accurate. Cement contains *ca.* 21% of silica, which may be determined in combined extracts made with hydrochloric acid and sodium hydroxide soln. Blank determinations with the soil or mud are essential and with the cement desirable, filtration is tedious, and the accuracy of the method is not high. Calcium may be determined in the filtrate from the silica determination. Cements usually contain *ca.* 65% of calcium oxide and in this method blank expts. are not always necessary, but filtration again is slow and the accuracy is not high. Silica determination after fusion of the sample with sodium carbonate provides a good method where time is of no consequence. Blank determinations with the soil are necessary and with the cement desirable. Silica determination after evaporating the sample with perchloric acid affords a rapid but not highly accurate method. Three basically similar variations of a volumetric method were investigated. In the "Indicator" variation add excess of 0·5 *N* hydrochloric acid to 1 g of the dried sample. Boil the mixture, cool, boil again, and dilute to *ca.* 150 ml. Add a few drops of mixed bromocresol green and methyl red indicator and titrate the excess of hydrochloric acid to a purple or green end-point with 0·2 *N* sodium hydroxide. Phenolphthalein may be used as indicator and is more accurate, but the end-point is more difficult to see. The reaction is $2H+CaX_2=Ca^{\cdot\cdot}+H_2X_2$, where X represents an equiv. of the slightly soluble acid supposed to occur in cement or an equiv. of oxygen. In the "*p*H Meter" variation treat the sample exactly as in the previous method, but use 1·0 *N* sodium hydroxide for the back-titration and a Beckman *p*H meter to obtain the end-point (which was found to be at *p*H 4·5) by plotting *p*H against ml of sodium hydroxide. In the "High *p*H" method cover the sample with water and obtain the *p*H of the suspension. Add excess of standard hydrochloric acid and treat the mixture exactly as in the "Indicator" method. Back-titrate the liquid to the *p*H of the water suspension. In all three variations blank determinations with the soil or mud are essential and with the cement desirable. The volumetric method is more rapid and more accurate than any of the other methods. Expts. showed that the phenolphthalein indicator method or back-titration to the initial end-point is more accurate than back-titration to the mixed indicator end-point. With cement-contaminated mud it makes no difference which indicator is used, since a deviation of 1 or 2% is permissible. Soil-cement mixtures require the phenolphthalein indicator method or back-titration to the original *p*H. The cement in such mixtures ranges from 4 to 16%, and results will be within 0·2% of the actual values. With the lower *p*H end-point, results will be within 0·6% of the actual values. Choice of method thus depends upon the accuracy required. In presence of hydrated cement blank determinations are necessary, since hydrated cement contains less than 65% of calcium oxide. A. O. J.

Microchemical

Micro-determination of Certain Alkaloids and other Bases by Photometric Turbidimetry. G. C. Kyker and D. P. Lewis (*J. Biol. Chem.*, 1945, **157**, 707–716)—An investigation was made of the specificity of quinine estimations by the silicotungstate method, and a variety of substances, including other alkaloids, antimalarials and vitamins, were tested. Hydroquinidine, cinchonidine, cinchonine, quitenine, totaquinine, nicotine and aneurine responded qualitatively and quantitatively like quinine, whilst cocaine, novocaine, narcotine, strychnine, egg and serum albumin, and proteose-peptone responded at slightly higher concns., and quinoline at still higher concns. Quitenine, aneurine, cocaine and the proteins were not extractable, and narcotine, nicotine and novocaine were extracted incompletely. At relatively high concns. antipyrine, morphine, caffeine, pyridine and choline also produced turbidities with silicotungstic acid. The composition of the silicotungstates of many of these substances was determined. F. A. R.

Physical Methods, Apparatus, etc.

(Photoelectric) Determination of Titanium in Corrosion-Resistant Steels. O. Milner, K. L. Proctor and S. Weinberg (*Ind. Eng. Chem. Anal. Ed.*, 1945, **17**, 142–145)—The colour produced by the action of hydrogen peroxide is assessed in a photoelectric instrument after partial separation of the titanium. *Reagents—Acid mixture*—310 ml of conc. hydrochloric acid, 595 ml of water and 95 ml of conc. sulphuric acid. *Cupferron soln.*—6 g in 100 ml of water, freshly prepared and filtered before using. (To preserve the solid reagent it is recommended that a small bag of lump ammonium carbonate be kept in the container.) *Standard titanium soln.*—Heat 3·68 g of air-dried recryst. potassium titanium oxalate with 8 g of ammonium sulphate and 100 ml of conc. sulphuric acid and boil for 5 to 10 min. Cool, dilute to 1000 ml and filter. One ml contains approx. 0·5 mg of Ti, the exact content being determined gravimetrically by taking 100-ml portions, diluting to 250 ml, precipitating with ammonia, filtering, igniting and weighing. *Method*—Dissolve 5 g of the sample (for a titanium content of <0·05%) in 80 ml of acid mixture and evaporate almost to dryness. Dilute to 150 ml with hot water, stir until all soluble salts dissolve, and cool below room temp. in running water. Add filter-paper pulp and, drop by drop, cold cupferron soln., stirring continuously, until the ppt. is deep red-brown (3 to 5 ml of reagent soln. are usually required). Stir thoroughly, allow to settle for 2 or 3 min. and filter on a fine filter-paper containing some pulp, washing with cold diluted sulphuric acid (1+19). Place the paper in the pptn. beaker, add 25 ml of conc. nitric acid, 10 ml of conc. sulphuric acid and 10 ml of 70% perchloric acid and evaporate to "fumes" of sulphur trioxide. Cool, dilute to 75 ml, cool again, and add 25% sodium hydroxide soln. until the soln. is just alkaline. Gradually add 0·5 g of sodium peroxide, heat and boil for 10 min. Filter and wash with dilute ammonia

Soln. (1 vol. of conc. soln. in 100). Place the paper in the beaker, add 15 ml of nitric acid, 15 ml of sulphuric acid and 5 ml of perchloric acid, and again evaporate to "fumes." Cool, add 75 ml of water, warm to dissolve the salts, add 3 ml of sulphurous acid and boil for 5 min. to expel the excess of sulphur dioxide. Add 10 drops of nitric acid and boil for a further minute or two. Cool, filter and dilute exactly to 100 ml. Take 10 ml (with a Klett-Summerson instrument using "test-tube" cells) and measure the optical absorption, using a blue filter with max. transmission at 420 $m\mu$. Add 3 drops of 3% hydrogen peroxide and measure again. From the difference between the two readings calculate the titanium content. If molybdenum and vanadium are absent omit the sodium peroxide separation; up to 0·05% of molybdenum causes a titanium error $>$0·001% and need not generally be removed, but vanadium has a greater effect. Chromium and nickel are not completely separated by a single cupferron pptn. and, as the residual amount varies, it is essential to make the two readings on every sample and not to use the first measurement on one sample as a "blank" for a series. Magnesium, silicon, copper, tungsten, zirconium, aluminium and tin do not interfere. Beryllium interferes, apparently by forming a compound with ferric iron in presence of hydrogen peroxide. To prevent this error, remove the iron by the ammoniacal tartrate method of Cunningham (*Id.*, 1938, **10**, 233) after the final destruction of organic matter. Remove excess of hydrogen sulphide, reprecipitate the titanium with cupferron, digest with nitric-sulphuric-perchloric acid mixture and continue as above. The method may be used with a suitable amount of sample with steels containing $>$0·05% of titanium, and with cast iron, and steel of various alloy composition. It is immaterial if some of the titanium-bearing material does not dissolve in the original acid attack of the sample, as in no instance has any titanium remained in the insol. residue after the "fuming" processes.

L. A. D.

Analysis of Aluminous Ore, using Spark Spectra. J. R. Churchill and R. G. Russell (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 24–27)—To assist in the study of mineral sources of aluminium other than bauxite, a rapid spectrographic method has been developed which is satisfactory for grade sorting and preliminary testing of ores. A suitable sample is ground to pass 100-mesh and the loss on ignition at 1100° C. is determined. A sample of the residue is then analysed semi-quantitatively by the conventional d.c. arc method in order to provide an estimate of the total amount of elements present other than aluminium, silicon, iron and titanium; this total is generally less than 5%. For the quantitative analysis, a pellet is made from a mixture of 1 part of the sample, 5 parts of sodium fluoride and 12 parts of de-ashed natural graphite. The sodium fluoride reduces the effects of variation in the state of chemical combination of the elements in different samples. The special graphite powder is available in spectroscopic purity. The spectrum of the pellet is then excited by normal spark technique using a pure graphite counter electrode. The ratios of silicon, iron and titanium to aluminium are determined from calibration curves prepared, using synthetic standard samples. Errors are minimised by the use of standards having compositions approximating to those of the sample under test. For example, the standards contain small quantities of calcium or magnesium if the preliminary arc test shows these elements to be present. From the three ratios so determined, and the preliminary figures for loss on ignition and total other elements present, the proportion of alumina in the sample can be calculated. B. S. C.

Spectrophotometric Determination of Traces of Copper with Rubeanic Acid. E. J. Center and R. M. MacIntosh (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 239–240)—The reagent is a 0·1% soln. of rubeanic acid (dithio-oxamide) in "200-proof ethyl alcohol"; it is stable for 2 to 3 months. The acetate buffer is prepared from 400 ml of glacial acetic acid, 400 g of C.P. ammonium acetate, and 200 ml of water. To an aliquot portion of soln., free from mineral acid (0·025–0·2 mg Cu), in a stoppered test-tube having a 50-ml mark add 2·5 ml of buffer, water to 49·5 ml, and 0·5 ml of reagent. Stopper and invert the tube several times. Read the transmission within 2–3 min. at 650 or 400 $m\mu$ in a Coleman spectrophotometer, using distilled water as a blank. All the water and reagents used should be tested by blank runs. The method was used in the analysis of potable water. W. R. S.

New Method for the Polarographic Determination of Nitrate. I. M. Kolthoff, W. E. Harris and G. Matsuyama (*J. Amer. Chem. Soc.*, 1944, **66**, 1782–1786)—Tokuoka's method for the polarographic determination of nitrate, which is carried out in presence of a large excess of lanthanum, has been studied and shown to hold only over a narrow range of nitrate concns. An alternative method has been developed, based on the reduction of nitrate in the presence of uranyl or uranous salts. This method holds for a much wider range of nitrate concns., provided that the ratio of uranyl to nitrate is above a certain minimum. Another advantage of the new procedure over the lanthanum method is that small amounts of sulphate do not interfere.

B. S. C.

Standard Testing Methods for Light Fastness in the Paper Industry. H. A. Lips (*Paper Trade J.*, 1945, **120**, 15 Mar., *T.A.P.P.I. Sect.*, 108–110)—Previous work is reviewed, and a method for standardising fading lamps is proposed. A control (*e.g.*, a piece of paper or cloth, coloured with suitable dyestuffs) is exposed for 20 hr. (or other suitable period), with the sample, in the lamp in question. It is then matched against one of a series of permanently coloured plastic chips, which illustrate the results of exposure for 12, 14...26 hr. in the standard fading lamp. Then the time of exposure which the sample has undergone is given, in "standard hours" by the chip which provides the match. The method may also be used for rating exposures to sunlight and for correlating these with exposures made in fading lamps. The standard lamp to be selected should be one that will represent the average degree of colour change shown by the control after exposure for 20 hr. each time in 50 or more lamps. J. G.

Review

A TEXTBOOK OF DAIRY CHEMISTRY. By E. R. LING, M.Sc., F.R.I.C., A.R.C.S. 2nd Ed. Vol. I: Theoretical. Pp. vii + 196. London: Chapman and Hall, Ltd. 1944. Price 13s. 6d.

During the years since the first edition of this work was published in 1930, dairy chemistry and its technical application in the dairy industry have made much progress. These developments are reflected in the new edition; it has been completely rewritten and considerably enlarged, and now gives a more advanced treatment to a wider field, with the theoretical and practical aspects of the subject in separate volumes. It is virtually a new work.

The Volume I under review deals with the theoretical aspects. It provides, within a total of 190 pages, an excellent account of the constituents of milk, some physical properties of milk, the composition of milk, the effects of heat on milk, the action of milk on metals, dairy detergents, chlorine sterilisation, cream, butter, margarine, cheese, annatto, soft curd milk, condensed milk, milk powder, and dairy by-products. The subject matter is brought right up to date and accompanied by many references to the original literature. It is presented in such a manner as to enable the salient facts to be appreciated by those with only an elementary knowledge of chemistry and physics; whilst for others there are the appropriate formulae and equations. The author has very successfully selected the most useful and reliable data on each subject and wisely omitted the older material where it is no longer of value. It is for this reason that so much is given in a small compass, and that it is unusually free from error.

Here is a volume which is much more than a compilation of chemical properties and quantitative data; it is well written and the reader's interest is maintained, and his knowledge broadened, by the desirable manner in which the author enlivens his basic facts with an account of their particular significance, be it from the technological, legal, or nutritional point of view. Appreciation of the modern scope of dairy chemistry is shown by the inclusion, for example, of outlines of the latest studies of hydrolytic and oxidative deterioration of milk fat, of the influence of hormones on the composition of milk, and of the theory of rennet action; and by the consideration of such topics as dairy detergents, cream neutralisation, and the flavour of butter. Even the effect of air raids on the composition of milk is briefly discussed, and the hope expressed "that there will be no need further to study this particular factor."

This little book is strongly recommended to all who are interested in milk and milk products. It will undoubtedly appeal greatly to students taking courses in dairying and agriculture and to those engaged in the technical branches of the dairy industry. It is clearly printed, attractively bound, and good value at 13s. 6d. S. J. ROWLAND

FORMATION OF A GROUP DEALING WITH BIOLOGICAL METHODS OF ANALYSIS

IN pursuance of the policy for the formation of Groups for special branches of analytical chemistry, the Council has decided to form a Group to be known as the Biological Methods Group. The objects of the Group will be to promote discussion of all aspects of the design, technique and computation of quantitative assays in which measurements of the response of living organisms, or parts thereof, to the substance assayed provide data from which the result is calculated. The Group should be of special interest to members of the Society concerned with vitamins, hormones, insecticides, chemotherapy, industrial toxicology and the toxicology of new drugs, pharmacology and statistics. The Group will deal with methods of analysis and not with substances as such.

Members of the Society who wish to become members of the Group are asked to notify the Hon. Secretary of the Society, 7–8, Idol Lane, London, E.C.3. Members who have already notified Mr. E. C. Wood need not make further application.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g in 10 ml of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis,*" Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

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