See Special Notice on p. 388

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 3 p.m. on Wednesday, November 1st, in The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Mr. S. Ernest Melling, in the chair. The following papers were presented and discussed: "Some Experiences of Micro-biological Assays of Riboflavin, Nicotinic Acid and other Nutrient Factors," by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., and M. Meiklejohn; "A New Method for the Estimation of Micro-quantities of Cyanide and Thiocyanate," by W. N. Aldridge.

NEW MEMBERS

Miss Lilian Marjorie Chamberlin, B.Sc. (Lond.), A.R.I.C.; Harold Crossley, A.M.C.T., A.R.I.C.*; Basil Walter Drinkwater, B.Sc. (Lond.), A.R.S.M., A.R.C.S., F.R.I.C.; Benjamin Leonard Embrey; Henry Francis Frost, B.Sc. (Lond.), F.R.I.C.*; Alexander Eltringham Heron, A.R.I.C.*; Sydney Heslop, A.C.T.C. (Birm.); Eric Holt*; Thomas Edward Victor Horsley, B.Sc. (Lond.), A.R.I.C.; George Inskip, B.Sc. (Durham); Morris Boris Jacobs, B.S., M.S., Ph.D. (New York); John Thomas Jones, M.Sc. (Wales), A.R.I.C.; G. V. Joshi, M.Sc. (Bombay); Gavin Lawson, A.R.I.C., Ph.C.*; Alexander Joseph MacCallum†; Ian Peter MacEwan, B.Sc.Agric.; Kenneth Stuart McManus, A.R.I.C.; Arthur Charles Cecil Newman, B.Sc., Ph.D. (Lond.) A.R.C.S. D.I.C. F.R.I.C.*; Frank Albert Paine, B.Sc. (Lond.), A.R.I,C,; Israel Mordecai Rabinowitch, D.Sc., M.D., C.M., F.R.C.P.; Ronald Alfred Rabnott; Arthur John Radford, B.Sc. (Lond.), A.R.I.C.; Harold Percy Rooksby, B.Sc., F.Inst. P.; Max Salomon; Norman Thomas Simmons, A.R.I.C. F.Inst. Fuel; Juan Antonio Sozzi; George White, B.Sc. (Lond.), A.R.I.C.; Arthur Desmond Wright, B.Sc. (Leeds).

DEATHS

We deeply regret to have to record the deaths of Sir John Jacob Fox and Dr. Ernest Victor Suckling.

Micro Determination of Alkoxyl Values

By A. A. HOUGHTON, B.Sc., Ph.D., D.I.C., F.R.I.C., and H. A. B. WILSON

Introduction—Since the publication of the original method[1] for the micro-determination of alkoxyl values there have been numerous reports of erratic results and of the methods adopted to overcome the difficulties. Papers have been published on the subject *inter alia*[7] by Friedrich,[2,11] Clark,[3,9] Ware,[4] Vieböck and Schwappach,[5] Vieböck and Brecker,[6] Rigakos,[7] Bruckner,[8] Colson,[10] Chinoy,[12] Neumann,[13] Leiff, Marks and Wright,[14] Elek,[15] Samsel and McHard.[16]

One of us first became interested in the method about 7 years ago, when it was found that inconsistent results were sometimes obtained with carbohydrate derivatives. The aim was to provide a method which would give accurate results rapidly for routine analysis, and occupy the minimum of the analyst's time. The apparatus had to be robust, easy to handle and capable of running entirely unattended for an indefinite period. Only when these conditions had been fulfilled was the minimum sample on which an analysis could be carried out investigated. The method was worked out on carefully purified samples of phenacetin

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

and α-methyl mannoside, and checked for consistency with a sample of ethyl cellulose in film form. Vanillin, several times recrystallised, which was at first used, was found to yield inconsistent results, a fact also recorded by Pregl[1] and recently investigated by White.[17]

APPARATUS—The apparatus, developed from that of Clark,[9] and shown in the scale drawing (Fig. 1) was finally decided upon. The reasons for the various details are as follows.

In order that the apparatus might run unattended, it was necessary that there should be a perfectly steady passage of carbon dioxide through the receiver G and that bumping should be avoided. This was achieved by applying the carbon dioxide from a Kipp's apparatus at an approx. constant pressure through a jet K designed to pass about 6 ml of gas per min. To prevent stoppage of the jet by moisture from the Kipp's apparatus, a calcium chloride tube H was introduced. Bumping was prevented partly by the weighing spoon and partly by the flow of carbon dioxide through C to the bottom of the flask. The weighing spoon was designed to sit conveniently on the hooks of a micro-balance and not to

TABLE I—PERCENTAGE RECOVERY OF ALKYL HALIDE AFTER VARIOUS TIMES WITH VARIOUS QUANTITIES OF ALKOXYL COMPOUNDS

	Alcohols, 10 mg								Phenacetin			Vanillin	Ethyl cellulose		
Time min.	Methyl	Ethyl	*iso*-Propyl	*iso*-Butyl	*n*-Propyl	*sec.* Butyl	*n*-Butyl	*tert*-Butyl	5 mg	20 mg	40 mg	20 mg	10 mg	50 mg	20 mg
5	55·7	23·0	—	—	10·8	—	—	—	15·9	23·0	15·3	1·2	24·1	25·4	—
10	96·6	80·4	35·6	—	20·6	—	6·1	—	48·2	48·3	45·4	48·6	87·3	74·7	40·0
15	99·7	—	—	37·7	31·6	14·3	—	10·0	72·4	68·5	64·6	83·4	98·3	97·5	—
20	99·9	99·5	86·4	—	38·6	—	16·3	—	83·0	80·5	75·5	95·6	—	99·1	91·9
25	—	99·8	—	—	48·5	—	—	—	89·0	88·2	84·6	99·0	—	99·8	—
30	—	—	99·3	72·8	56·6	45·8	27·1	12·5	92·0	93·5	91·1	99·5	—	100·1	—
35	—	—	—	—	—	—	—	—	94·9	97·0	94·3	100·0	—	—	—
40	—	—	99·8	—	77·5	—	37·4	—	96·6	98·9	97·5	—	—	—	99·0
45	—	—	—	98·1	—	76·4	—	14·7	97·9	99·5	98·6	—	—	—	—
50	—	—	—	—	97·1	—	—	—	98·8	100·0	99·4	—	—	—	—
55	—	—	—	—	—	—	—	—	99·5	—	100·1	—	—	—	—
60	—	—	—	99·5	99·8	98·5	55·2	16·8	—	—	—	—	—	—	100·0
70	—	—	—	100·0	100·0	—	61·8	—	—	—	—	—	—	—	—
75	—	—	—	—	—	99·6	—	18·8	—	—	—	—	—	—	—
80	—	—	—	—	—	—	68·6	—	—	—	—	—	—	—	—
90	—	—	—	—	—	100·0	75·3	—	—	—	—	—	—	—	—
100	—	—	—	—	—	—	85·1	—	—	—	—	—	—	—	—
110	—	—	—	—	—	—	93·5	—	—	—	—	—	—	—	—
120	—	—	—	—	—	—	99·1	—	—	—	—	—	—	—	—
130	—	—	—	—	—	—	99·7	—	—	—	—	—	—	—	—
140	—	—	—	—	—	—	100·1	—	—	—	—	—	—	—	—

spill its sample when in a vertical position. Further, the lower capillary boiling-tube portion (Clark) is of such length that the sample is readily washed out. To allow for the rapid expansion of air when A is heated, a second jet, K_1, and a two-way tap J are provided to give a flow of carbon dioxide of 1 ml per min. As it was found that the draughts caused violent volume fluctuations, it became desirable to boil the mixture strongly in A with a hot, mantled micro-burner. This in turn made the water condenser D necessary. The condenser is as close as possible to the ground-joint with A to reduce to a minimum the hot vapour space which can be affected by draughts. The apparatus must be swept out with carbon dioxide. The bulbs L and C are to allow for a small amount of sucking back; C is so arranged chat it drains completely into A. Only water was found necessary in the trap E, which is conveniently at right angles to the plane of the diagram. The receiver G contains 5 ml when filled to the top of the spiral. The pitch of the spiral is satisfactory at about 12 turns in 10 cm, when the bubbles take about 6 sec. to rise. Fewer turns increase the rate of ascent of the bubbles, and more do not provide sufficient room for liquid to flow down as the bubbles flow up. The bottom of the centre tube M and the receiver G are ground together and then a small groove is cut in the ground bottom surface of M, thus ensuring that the bubbles are perfectly uniform in size every time the receiver is assembled. The receiver is attached to the rest of the apparatus by the springs or rubber bands F. As either sort tends to be attacked by bromine vapour, fairly frequent replacement is necessary. The ground-joint between A and D is

inclined to leak with continued use and this is ascribed to attack by hydriodic acid, which has been observed by Samsel and McHard[16] to attack glass wool. The apparatus is supported by two clamps, one at K and the other at the neck of A. It is found convenient to have two flasks and receiver units for each apparatus. Since it was found that rubber, in air saturated with methyl iodide, took up 5% of its weight in 16 hr., all rubber connections and bungs in contact with the alkyl halide have been rigorously avoided.

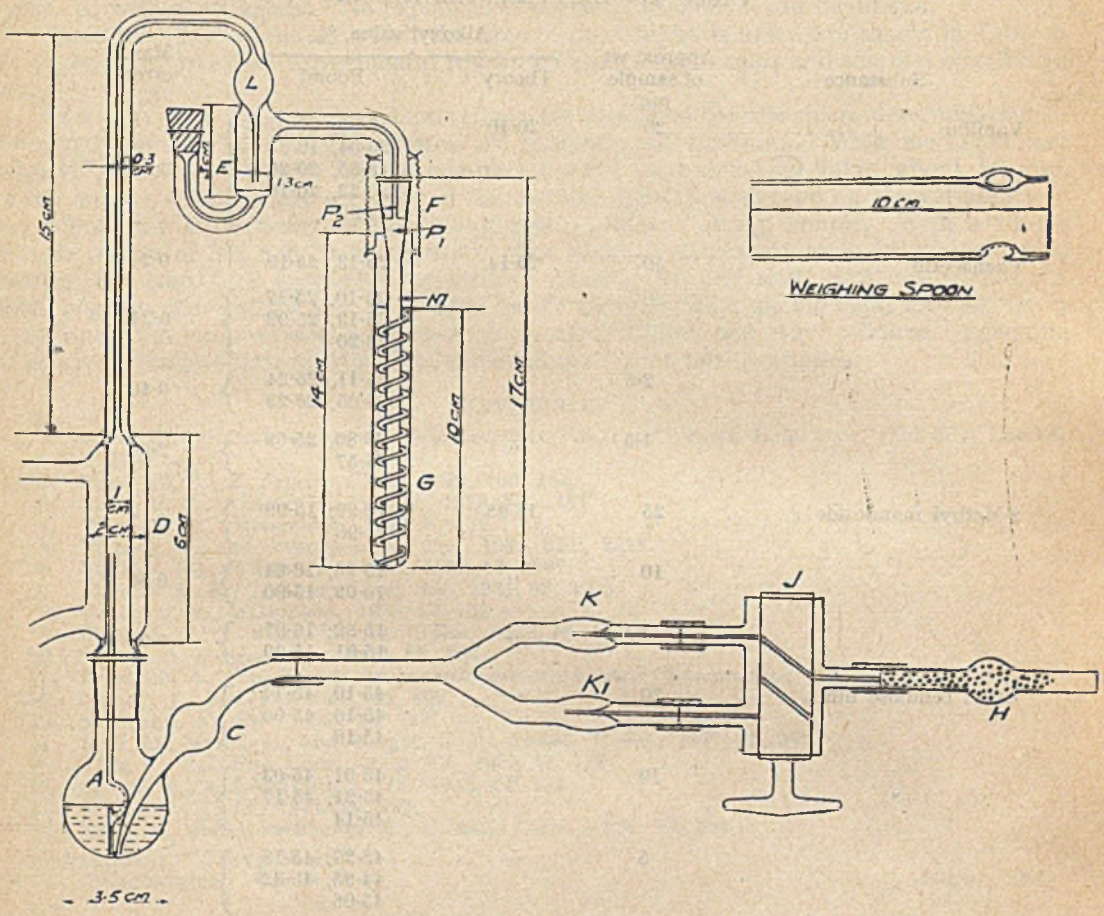

Fig. 1

PROCEDURE—Place 5 ml of pure hydriodic acid (b.p. 127° C.) and 2·5 ml of B.P. liquefied phenol in the boiling-flask.* Introduce a little water into the trap, fit the condenser in position and boil the reaction mixture for about 1 hr., meanwhile passing carbon dioxide at the rate of 6 ml per min. If new reagents are being used, make a blank determination at this stage; repeatable blanks of 0·04 ml of $N/100$ thiosulphate[17] can be obtained. About 10 determinations can normally be made before changing the acid and re-filling the trap.

Put into the receiver 5 ml of bromine soln., prepared by dissolving 17·6 g of potassium

* The hydriodic acid may be made by evaporating 1 litre of titration residues to 200 ml, cooling, treating with 50 ml of conc. sulphuric acid (which drives off the bromine), cooling and treating with a soln. of 20 g of potassium dichromate in the minimum of water. The fine ppt. of iodine is filtered off, thoroughly washed and suspended in water, and hydrogen sulphide is passed in until the liquid is straw-yellow and no obvious iodine remains. The sulphur is filtered off, and the liquid is boiled under reflux in a stream of carbon dioxide to remove hydrogen sulphide. The hydriodic acid is then distilled, and the fraction boiling at 127° C. is retained and redistilled. The distillate is treated with sufficient 50% hypophosphorous acid to discharge any iodine coloration.

hydroxide in 227 ml of glacial acetic acid and adding 2 ml of iodine-free bromine. Weigh sufficient of the sample to give titres between 5 and 10 ml with the chosen concn. of thiosulphate soln., in the weighing spoon, and introduce it into the reaction flask. Replace the condenser, turn on carbon dioxide at the rate of 1 ml per min., and put a hot mantled microflame under the flask. When its contents boil, after about 1 min., increase the flow of carbon dioxide to 6 ml per min. for an appropriate time. Then remove the receiver and rinse the

TABLE II—EXPERIMENTAL RESULTS

Substance	Approx. wt. of sample mg	Alkoxyl value, % Theory	Alkoxyl value, % Found	Max. error %
Vanillin	20	20·40	19·60, 20·07 20·04, 19·92 19·85, 20·20 20·22, 20·19 19·92	2·0
Phenacetin	50	25·14	25·13, 25·19	0·2
	20	,,	25·10, 25·17 25·13, 25·09 25·20	0·24
	2·5	,,	25·11, 25·24 25·05, 25·23	0·40
	1·5	,,	25·80, 25·69 25·57	2·64
α-Methyl mannoside	25	15·98	15·99, 15·99 15·96	0·12
	10	,,	15·96, 16·04 16·02, 15·90	0·50
	5	,,	15·82, 16·07 15·91, 15·99	0·96
Ethyl cellulose film	20	—	45·10, 45·14 45·16, 45·06 45·16	
	10	—	45·01, 45·03 45·21, 45·17 45·14	
	5	—	45·27, 45·18 44·95, 45·32 45·06	
n-Butyl cellulose, fibrous	40	—	12·52, 12·50	
	10	—	12·49	

contents with water into 5 ml of a soln. of 250 g of sodium acetate per litre, contained in a 100-ml conical flask. Discharge the bromine colour with 90% formic acid, remove the flask to another room, add a few drops of 10% sulphuric acid and a few crystals of potassium iodide (AnalaR), and titrate the soln. by means of a micro-burette.

Prior to the titration another determination may be begun in a cool flask with another receiver. It is not claimed that the procedure makes unnecessary the use of higher concns. of hydriodic acid or of various solvents, such as propionic anhydride, recommended by other authors as appropriate in special circumstances.

DISCUSSION—An investigation of the titration part of the analysis showed that the end-point sensitivity in a bromine-free atmosphere without starch in good light was consistent at 0·01 ml of $N/100$ thiosulphate. In artificial light without starch figures of 0·05, 0·03 ahd 0·04 ml were obtained. In artificial light with starch the sensitivity again became 0·01 ml consistently. When the titration was carried out in the same room as the rest of the expt. the bromine in the atmosphere gave blanks of 0·07, 0·04, 0·10, 0·05 and 0·09 ml of

$N/100$ thiosulphate. A reagent blank in a bromine-free atmosphere was consistent at 0·04 ml of $N/100$ thiosulphate, and the total experimental blank in a bromine-free atmosphere ranged from 0·14 to 0·28 ml of $N/100$ thiosulphate (5 tests). This blank was shown to be due to hydriodic acid mist passing the trap and could be filtered out with cotton or glass-wool plugs; the total experimental blank then fell consistently to 0·04 ml of $N/100$ thiosulphate. All attempts to introduce either cotton-wool or glass-wool plugs during the actual determination gave low results. The lost alkyl halide could not be recovered by sweeping the apparatus through with carbon dioxide, nor after continually running did plugs become saturated with alkyl halide. No explanation of this phenomenon can be offered.

RESULTS—The results on which the time of an analysis is based are shown in Table I. In Table II are recorded experimental results with pure compounds and samples of cellulose ethers.

SUMMARY—An apparatus and procedure are described for the micro-determination of alkoxyl groups. The method requires the minimum of attention. With methoxyl and ethoxyl groups an analysis can be completed every hour and only takes about 20 min. of the analyst's time. The accuracy of the method appears to depend on the variability of the blank, the latter being relatively insignificant with a 10-mg sample. With a 1·5-mg sample, errors of 3% can be anticipated. The blank is attributed to hydriodic acid mist passing the trap. All successful methods of filtering out the mist gave low results. A table is given showing the course of alkyl iodide recovery from all the eight alcohols up to butyl and from various sized samples of phenacetin, vanillin and ethyl cellulose. A second table gives results obtained with different weights of some pure materials.

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"ROWANHURST," GROVE ROAD
BEACONSFIELD, BUCKS.

August, 1944

"Lead Prints" for the Detection of the Presence and Segregation of Lead in Steels and Brasses*

By B. S. EVANS, M.C., M.B.E., D.Sc., F.R.I.C.

Since lead, added to steel and brass to improve machinability, is disseminated through the mass of the metal in the form of minute discontinuous particles, it is possible to reveal its presence and distribution by means of prints on paper applied to the surface of the metal and containing reagents which can be made to yield coloured lead compounds. Many such methods are in use, the great majority of which rely on the formation of brown lead sulphide. Such a method can yield admirable prints where the lead content is high or where it is highly segregated; with low, evenly distributed, lead contents, however, I have found it very uncertain, such uncertainty being bound up with the fact that it is easy to get a greenish-grey print from the carbon in the specimen and that, if an alkaline conversion to sulphide has been used, there is probably some greenish-black ferrous sulphide mixed with the lead, of the complete removal of which it is difficult to be sure. If, on the other hand, an acid conversion is used, this necessitates passing hydrogen sulphide through the paper, an operation of some difficulty with large prints. In the process described below the sulphide conversion has been abandoned and in its place conversion to the scarlet dithizone compound has been adopted; this colour is so distinctive that there is no possibility of confusion with other substances. The lead chromate colour (sometimes used) is also distinctive, but far too faint to be visible when the content is low.

It is usual in printing methods to use gelatin paper (*e.g.*, "fixed" photographic paper). Initial trials by the qualitative method given below failed to give any result whatever with this paper. This difficulty has now been overcome (*vide infra*), but for the detection of lead the method of using filter-paper is quicker and simpler. For the demonstration of segregation, on the other hand, the gelatin paper should be used; the surface is far better for photographic reproduction and the minute points of lead are shown as dots instead of rings or smudges, as they tend to be on the filter paper; also, where large surfaces are to be handled, the robustness of the gelatin paper is a valuable asset.

Detection—Rub down the specimen to a bright surface with emery paper and etch it with nitric acid (the attack should be allowed to proceed until it is vigorous or even slackening). Hold the specimen under a running tap and well rub it with the finger to remove loose carbon as far as possible; then, without touching the prepared surface further, rinse the specimen in acetone and allow it to dry. Cut a piece of thick, close-grained, filter-paper to the required size, immerse it in a mixture of equal parts of conc. acetic acid and 10% solution of chromium trioxide (CrO_3), allow it to drain for a few sec. with its lower edge touching a piece of filter-paper and apply it to the surface of the specimen, smoothing it down with the fingers to remove air bubbles but taking care not to let it move relative to the surface. During the smoothing operation, pressure applied should be the maximum consistent with not rubbing away too much of the paper. Finally, press it down very firmly all over with a folded sheet of filter-paper, still taking care to avoid lateral movement. Allow the paper to remain in position for 5 min.; then, holding it by one corner, strip it off and transfer it to a beaker containing 10% acetic acid. The iron, etc., dissolves out in brown clouds, and the paper should be allowed to remain in the liquid, with occasional swirling, until this process seems to be complete. Wash the paper, like a photographic print, in a stream of cold running water for 2 or 3 min., at the end of which time it should be perfectly white (there may be pale lemon-yellow spots of lead chromate but all brown should have been removed). Prepare a mixture in the proportion of 50 ml of a 1% soln. of potassium cyanide and 5 ml of a 0·1% soln. of dithizone in chloroform; shake vigorously, place in a dish or beaker and transfer the paper to it. Leave for 15 min. with occasional swirling, then wash it again in cold water for a few min.; finally spread the paper on a sheet of glass and allow it to dry at the ordinary temperature and out of direct sunlight. The presence and distribution of lead in the surface is indicated by red spots on the white background of the paper.

The method was tried on (i) six plain carbon steels containing 0·33, 0·25, 0·23, 0·21, 0·20, 0·17% of lead respectively; (ii) a segregated steel ("No. 4") of high lead content; (iii) an edge-segregated billet.

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* Communication from the Armament Research Department (formerly the Research Department Woolwich).

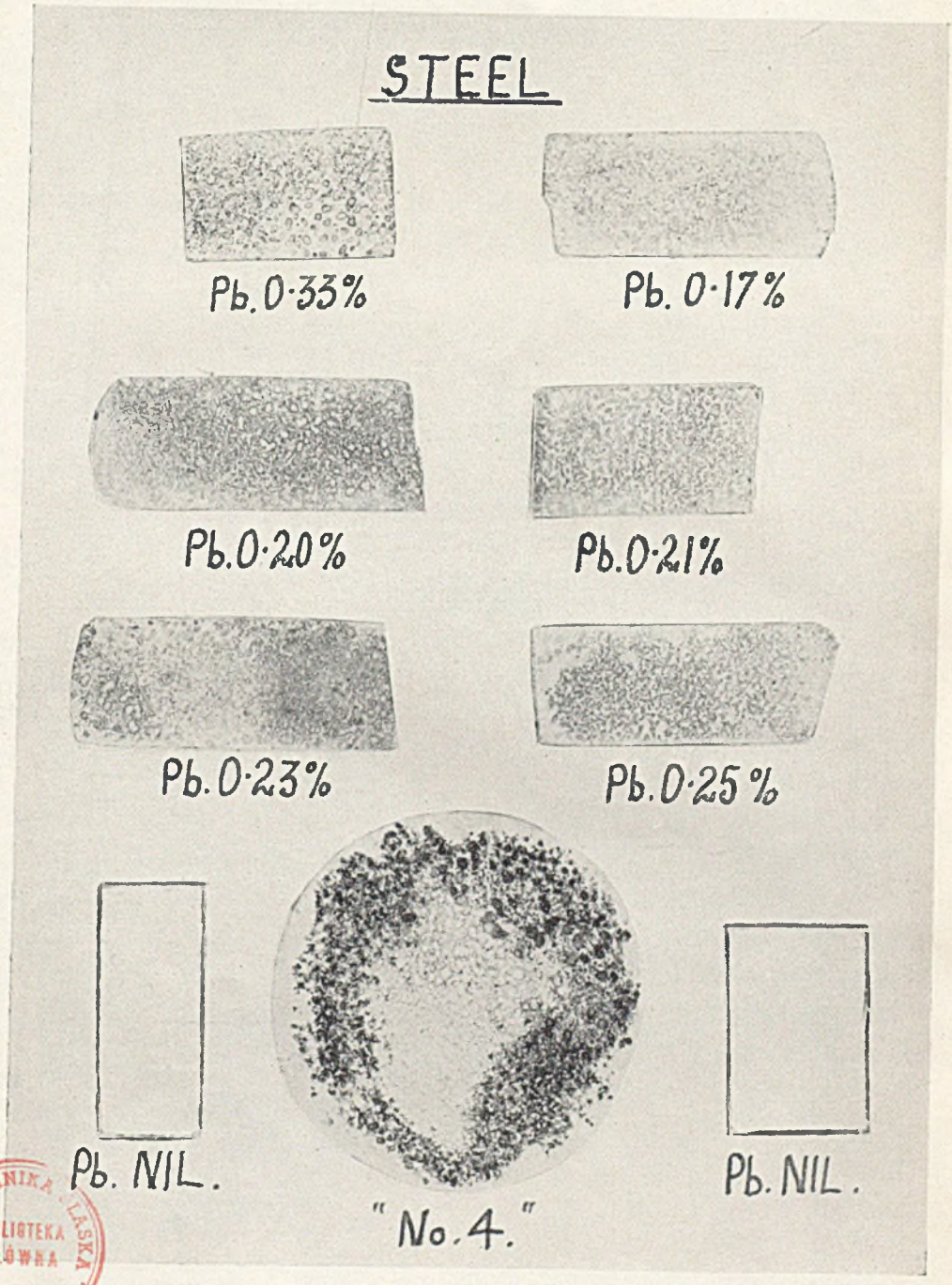
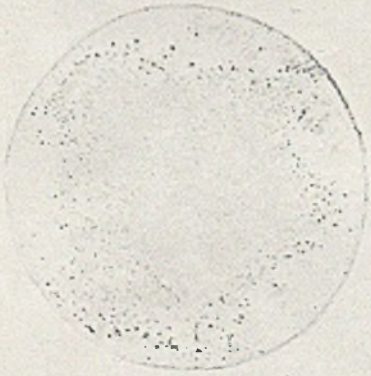


Plate I
"Lead Prints" of leaded steel.

STEEL

SEGREGATED BILLET

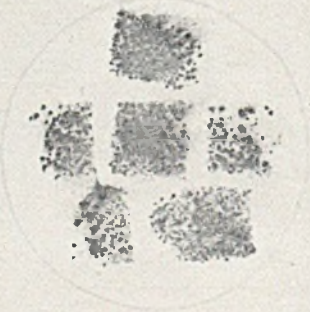
BRASS



60:40. Pb. 1·84%

60:40. Pb. 3·0%

60:40. Pb. NIL.



GUN METAL
Pb. 4·18%

Plate II

"Lead Prints" of leaded steel and brass.

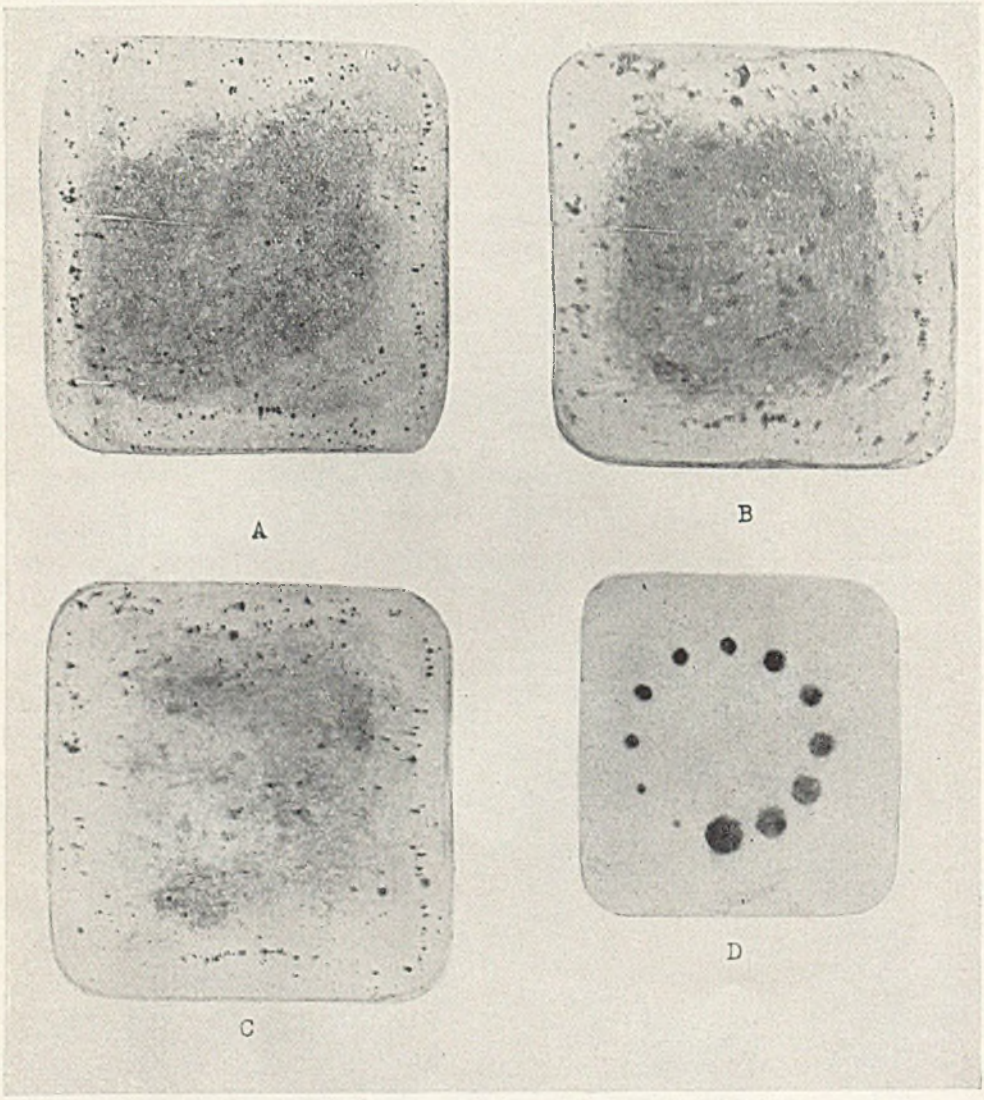


Plate III

Demonstration of Segregation.

A, B and C are successive gelatin prints taken, with intermediate polishing and etching, from a section of a bar of leaded steel at right angles to the direction of rolling. The same essential pattern can be traced throughout.

D is the gelatin print of a plate of plain carbon steel into which has been inlaid a circle of lead discs of gradually decreasing diameter. This synthetic sample shows that the prints give an accurate picture of the lead present.

Photographs of all these prints are shown (Plates I and II). The intensities of the "plain carbon" steel prints are roughly, but not exactly, in the order of the lead content. Sulphide prints taken of the two segregated steels show exactly the same patterns.

A number of plain carbon steels and alloy steels free from lead but containing one or more of the following elements: Al, Cu, Cr, Mo, Mn, Ni, Se, Si, Ti, V and W, were also printed. In none of these was anything shown, the paper remaining white. Photographs of two of these are included (Plate I), the printed area being outlined in black to show its extent.

Leaded Brass—Exactly the same process is applicable to brasses, of which are included photographs of prints obtained from the following (Plate II): (i) Brasses, 60 : 40, containing 1·84 and 3·0% of lead; (ii) gun metal (6 specimens), 4·18% of lead; (iii) brass, 60 : 40, with no lead.

It will be noted with regard to the brass prints, as contrasted with those from steel, that, whereas the spots are sharper, the apparent amount of lead is relatively much less; this is probably due to the much lower solubility of the brass in the acid medium.

Demonstration of Segregation—The initial failure with gelatin paper was probably due to the difficulty of washing it free from the acetic acid. The *p*H requirements of the dithizone test are somewhat precise, and the small amount of cyanide in the developing liquid was probably insufficient to neutralise the residual liquid. In addition to this, it is desirable to form the lead chromate in, or behind, the gelatin layer, otherwise the dithizone ppt. may be washed off the surface.

Process—Prepare the specimen as for detection (*vide supra*). Soak a piece of "fixed" photographic paper in acetic acid soln. (1 : 1) until the curve of the paper is reversed; remove the excess of liquid with filter-paper and place on the specimen, remove air bubbles as usual and press down as firmly as the slippery nature of the acetic-gelatin surface will allow; leave for 5 min. Cover with a piece of dry filter-paper and drop on to this a 10% soln. of chromic acid, beginning in the middle and working outwards until the soln. has spread all over (the paper should not be too wet). Ensure contact of the filter-paper with the underlying gelatin paper, press down with dry filter-paper and allow to stand for 5 min. Strip off the gelatin paper, remove and discard the filter-paper and immerse the gelatin paper in acetic acid soln. (10%). Leave for 10 min., with occasional agitation. Wash the paper in running water for about 5 min., transfer to 10% potassium cyanide soln. and leave for 10 min. with occasional agitation. Rinse in water and transfer to a mixture in the proportion of 100 ml of 10% potassium cyanide soln. to 10 ml of a 0·1% soln. of dithizone in chloroform, shaking the mixture before the addition of the print.

Leave for ½ to 1 hour (or longer) according to the speed of development, with occasional gentle swirling. Finally, wash the paper in running water until the orange colour is almost removed from the back of the print, remove and dry (Plate III).

Notes—(*a*) The soln. of chromium trioxide and acetic acid does not keep and should be made up fresh daily.

(*b*) The dithizone-cyanide mixture must be fresh and should not be used, at most, more than three times before replacement. The chromic soln., the cyanide soln. and the dithizone soln. seem reasonably stable.

(*c*) Alloy steels may not show lead by the above treatment, and the following modification is necessary. After preparation of the specimen, as above, treat the surface with a 20% soln. of sodium hydroxide saturated with potassium persulphate and sodium phosphate. Leave for 30 min., rinse first with water and then with acetone, and proceed as usual.

(*d*) If, as is usual, the finished prints are covered with an orange stain, this can be removed by soaking in 50% pyridine soln. and re-washing.

(*e*) In using gelatin paper it has been found advantageous to cover it with a weighted glass plate during both the acetic acid and chromic acid attacks to prevent a tendency of the paper to detach itself from the specimen.

Thanks are due to Mr. D. G. Higgs for carrying out the work on the non-leaded samples and to the Director General of Scientific Research and Development for permission to publish this paper.

October, 1944

The Use of *p*-Dimethylaminobenzylidene Rhodanine as an Indicator for the Volumetric Determination of Cyanides

By J. A. RYAN and G. W. CULSHAW

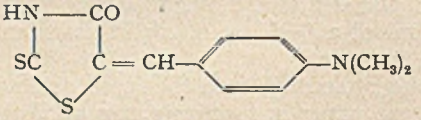
The standard method of determining cyanides volumetrically is by titration against standard silver nitrate soln., using potassium iodide or diphenylcarbazide as indicators. With the first indicator the end-point is shown by the appearance of precipitated silver iodide, and with the second by the pink colour becoming very pale violet on the colloidal precipitate before the opalescence is visible.[1]

With both indicators, when working in dilute solutions, *i.e.*, 0·2 *N* or less, the exact end-point is difficult to determine with accuracy. For instance, Jacobs[2] states that when the method is used to determine hydrocyanic acid in air, with potassium iodide as indicator, by means of 0·3% silver nitrate soln. (approx. 0·018 *N*) the end-point is somewhat difficult to see unless the illumination is favourable. Daylight is more satisfactory than artificial light. The end-point is quite sharp when the titration is carried out in semi-darkness, with a condensed beam of light passing through the solution in a generally horizontal direction. Satisfactory results may be obtained by the use of a focusing flashlight or the more powerful beam of a microscope illuminator. With such illumination a slight excess of silver nitrate produces a distinct Tyndall effect.

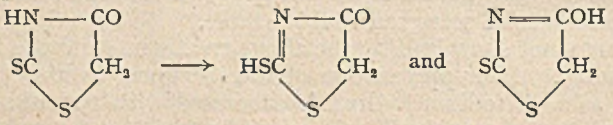
Recently, a determination of Prussian blue in spent oxide was required, and the usual method was employed, potassium ferrocyanide being separated and decomposed with boiling dil. sulphuric acid and the liberated hydrogen cyanide being absorbed in sodium hydroxide soln. The resulting sodium cyanide was then titrated against standard silver nitrate soln.

In the determination in question the silver nitrate used was 0·02 *N*, and with both potassium iodide and diphenylcarbazide it was difficult to observe the end-point. To overcome this, an indicator was sought which would give a sharp colour change at the first formation of silver argentocyanide.

It was thought that *p*-dimethylaminobenzylidene rhodanine[3]



might be of use for this purpose. In acetone solution this reagent is yellow, whilst the silver salt is red-violet and is formed from slightly acidified silver salt solns. on adding a solution of the reagent. This specific reaction of the *p*-dimethylaminobenzylidene rhodanine occurs only in acid solution. In alkaline solution, owing to the tautomeric change of the rhodanine component,



it forms –OH and –SH groups in such quantities that the formation of a colour or ppt. with nearly all heavy metals is possible.[4] However, this difficulty ought not to be encountered, provided that silver nitrate and alkali cyanide are the only solutions used in the final determination. Thus, for example, mercuric cyanide could be determined by the method of Rose,[5] the final filtrate being acidified and the hydrogen cyanide distilled off into sodium hydroxide soln.

The reactions involved in cyanide titration with silver nitrate are as follows. First $Ag^{\cdot} + 2CN' \rightleftharpoons [Ag(CN)_2]'$. When this reaction is complete further addition of silver nitrate yields insol. silver argentocyanide $Ag^{\cdot} + [Ag(CN)_2]' \rightarrow Ag[Ag(CN)_2]$.

The instability constant[6] of the complex ion $[Ag(CN)_2]'$ is $1{\cdot}0 \times 10^{-21}$, *i.e.*,

$$\frac{[Ag][CN]^2}{[Ag(CN)_2]} = 1{\cdot}0 \times 10^{-21}.$$

Thus, although dissociation does take place, the amount of silver ions in solution is too small

to affect the indicator. Furthermore, by the Law of Mass Action, addition of potassium hydroxide to the cyanide soln. should decrease the dissociation:

$$K[Ag(CN)_2] \rightleftharpoons K^{\bullet} + [Ag(CN)_2]' \rightarrow Ag^{\bullet} + 2CN'.$$

However, as soon as silver argento-cyanide is formed, which is comparatively strongly ionised, silver ions appear in the solution in sufficient quantity to be detectable by the indicator.

EXPERIMENTAL—To determine whether the use of *p*-dimethylaminobenzylidene rhodanine was satisfactory in practice, standard solns. of silver nitrate were titrated against solns. of sodium cyanide in alkaline solns. with the use of potassium iodide as well as the reagent as indicators.

Reagents—(i) Silver nitrate standard solutions: 0·1000 *N*; 0·0200 *N*; 0·0100 *N* prepared from A.R. silver nitrate. (ii) Sodium cyanide solutions of approx. strengths 0·2; 0·04; 0·02 *N*. (iii) Ammonium hydroxide: 6 *N*. (iv) Sodium hydroxide: 10% soln. B.D.H.; Laboratory Reagent. (v) Potassium iodide soln.: 10%. (vi) *p*-Dimethylaminobenzylidene rhodanine: 0·02% soln. of B.D.H. reagent in acetone.

Titrations—(1) The cyanide soln. (25 ml) was pipetted into a conical flask and 10 ml of sodium hydroxide soln. were added together with 3 drops of the indicator *p*-dimethylaminobenzylidene rhodanine, and the whole was titrated against silver nitrate soln. to the first appearance of a red colour.

(2) Twenty-five ml of the cyanide soln. were titrated against the silver nitrate soln. in presence of 2 ml of 10% potassium iodide soln. and 5 to 6 drops of 6 *N* ammonium hydroxide, until the first appearance of an opalescence against a black background in daylight.

TABLE I

Amount of 0·2 *N* NaCN taken for titration ml	0·1000 *N* $AgNO_3$ required in presence of KI ml	0·1000 *N* $AgNO_3$ required in presence of "rhodanine" ml
25	24·60	24·60
25	24·58	24·60
25	24·61	24·60

TABLE II

Amount of 0·04 *N* NaCN taken for titration ml	0·0200 *N* $AgNO_3$ required in presence of KI ml	0·0200 *N* $AgNO_3$ required in presence of "rhodanine" ml
25	24·84	24·80
25	24·78	24·80
25	24·99	24·81
25	24·96	24·79

TABLE III

Amount of 0·02 *N* NaCN taken for titration ml	0·0100 *N* $AgNO_3$ required in presence of KI ml	0·0100 *N* $AgNO_3$ required in presence of "rhodanine" ml
25	24·40	24·38
25	24·60	24·36
25	24·53	24·37
25	24·48	24·36
25	24·57	24·36

Notes—In making these titrations it was found that with 0·04 *N* and 0·02 *N* solns. and when potassium iodide was used as indicator the first appearance of a turbidity could only be discerned with difficulty, particularly with 0·02 *N* solns., whereas the *p*-dimethylaminobenzylidene rhodanine gave a sharp colour change in every instance from pale yellow to red-violet, provided that the amount of the reagent added was not such that a strong yellow colour tended to obscure the first appearance of the red-violet end-point.

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"SANDFIELD," SANDY LANE
LYDIATE, LANCS.

Some Experiences of Microbiological Assays of Riboflavin, Nicotinic Acid and other Nutrient Factors

By D. W. KENT-JONES, Ph.D., B.Sc., F.R.I.C., and M. MEIKLEJOHN

Discussion

following the paper read at the Meeting on November 1, *see* Analyst, 1944, 69, 330

Dr. E. Barton-Wright congratulated Dr. Kent-Jones on his clear and interesting rendering of a difficult subject. He thought that it was safe to say at the present time that microbiological methods of assay of the vitamin B-complex had come to stay. With the exception of aneurine (vitamin B_1) all the remaining members of the B-complex were difficult to estimate by chemical methods. All the chemical methods devised up to the present time were tiresome, tedious and of difficult manipulation, and it was impossible to assay more than one or two samples at a time. Moreover, the apparatus required was expensive. On the other hand, microbiological methods were reasonably expeditious and accurate; they required no special or expensive apparatus, and a relatively large number of samples could be assayed together. The assay of nicotinic acid, using the organism *Lactobacillus arabinosus*, required no special comment because the method was perfectly straightforward. This organism was less exacting in its vitamin requirements than *Lactobacillus helveticus* (*casei* ε), and he suggested that the eventual assay of pantothenic acid and biotin would be by means of this organism rather than *L. helveticus*. The assay of riboflavin with *L. helveticus* was rather more difficult, and in the assay of certain materials, notably cooked foods, *e.g.*, bread, special precautions had to be taken in the extraction process. He thought it would be better in the construction of the standard curve for riboflavin if Dr. Kent-Jones took more and lower levels of riboflavin and that the rather high blanks obtained for the nicotinic acid standard curve could be obviated by washing the inoculum once with sterile saline solution, as this high blank was entirely due to "carry over" of nicotinic acid from the inoculum.

Mr. A. L. Bacharach said that, in spite of—or because of—many years' experience in carrying out biological assays with whole animals, he welcomed the advent of microbiological testing with enthusiasm. Its advantages, which the authors of the paper had clearly pointed out, undoubtedly constituted it one of the most important of recent advances in methods for determining the nutrient analyses of foods. It was, however, strange that the treatment of results obtained by this method was along lines that have now been largely abandoned by those carrying out animal assays, in favour of relatively simple statistical procedures, which permitted extraction from the data of much more information than could be got by simply averaging interpolated values; notably these procedures made it possible to determine with accuracy the degree of inaccuracy of the assay. They made it possible, further, to discover whether the response curve, which related the size of dose to its effect, was the same for test substance and standard; if they were not the same, the assay was invalid, and probably indicated that the same active substance was not responsible for both sets of effects. As Dr. Kent-Jones had pointed out, it was necessary, by literature search or exploratory test, to know for assay purposes approximately the potency of the test substance. This being already known, it should be possible in an assay, without increasing the total number of observations, to reduce the number of points determined on the curve, but to make more replicate observations. These would furnish information about the mean variance (the square of the standard deviation) of a single observation, and about the extent to which the points on the "curve" departed from linearity. The use of these statistical methods was quite possible without any knowledge of the theoretical mathematics on which they were founded, and he was satisfied that their application to microbiological assay would prove as valuable as it had for tests with animals.

Mr. E. C. Wood endorsed Mr. Bacharach's remarks, and suggested that a competent statistician, if consulted on the *design* of microbiological assays, could undoubtedly increase considerably the amount of information obtainable for a given expenditure of labour and materials. The greatly enhanced accuracy of microbiological assays, as compared with ordinary biological assays, using, *e.g.*, rats, was of course due to the fact that the quantity estimated in each test-tube was a measure of the aggregate growths of several million individuals. If several million rats could be used for an assay in each dosage-group, the accuracy of the result would doubtless be of the same order as that obtained by Dr. Kent-Jones.

Mr. H. C. H. Graves said that preliminary work on the lines proposed by Mr. Bacharach had already been done (Price and Graves, *Nature*, 1944, **153**, 461). In the course of the work it was found essential either to ensure uniformity of temperature at all parts of the incubator or, in the computation of results, to make proper allowances for previously determined non-uniformity of temperature. Mr. E. C. Fieller, the well-known statistician, had shown that in this way, with a total of as few as 8 tubes, 4 standard and 4 unknown, the standard of accuracy attained lay between 83 and 120% ($P = 0{\cdot}95$). A larger number of tubes, such as Dr. Kent-Jones recommended, would further increase the precision of the results. Dr. E. M. Nelson, of the United States Department of Agriculture (private communication), agreed that, using microbiological equipment standard in his Department, special precautions to ensure uniformity of temperature at all points within the incubator were essential. By using a mechanically stirred water-bath, as was now recommended in the riboflavin assay method published in a Supplement to the United States Pharmacopoeia XII, results agreed to within ±2%.

Mr. N. L. Allport said that a few months ago, being faced with the problem of determining riboflavin in certain food materials, he approached Dr. Kent-Jones for help. This was most generously accorded, and Dr. Kent-Jones and Miss Meiklejohn devoted a whole morning to giving detailed instructions for carrying out the procedure. He would like to state that, since then, he had conducted many determinations without experiencing any difficulty, and repeated trials in which known additional amounts of riboflavin had been first added to samples indicated eminently satisfactory recoveries. He had not indulged in any attempts

to modify the process beyond occasionally including two additional points on the graph and sometimes working in triplicate; in so doing he had apparently implemented, in part at least, the suggestions of Dr. Barton-Wright and Mr. Bacharach. However, there seemed to be no doubt that the method, when conducted precisely as described by Dr. Kent-Jones and Miss Meiklejohn, was quite satisfactory.

Mr. G. TAYLOR said that this method was based on the growth of micro-organisms, as determined by titration of their metabolic acidity. Dr. Kent-Jones, early in his account of the method, referred to turbidity as being also an indication of the amount of growth. Was there any practical alternative to the determination of acidity for the purpose of determining growth of the micro-organisms?

Dr. J. H. HAMENCE asked what were the smallest quantities of riboflavin and nicotinic acid which could be detected in foodstuffs by the methods described by the authors. Also, had any extraction methods been devised whereby riboflavin and nicotinic acid could be extracted from a very large quantity of material and so increase the sensitivity of the method?

Dr. A. J. AMOS said that this paper represented a most valuable contribution to a field which was already of importance, and would rapidly become more important, to the Public Analyst and the consulting chemist. The vast number of vitamin preparations and vitamin food adjuncts that had appeared on the market in recent years had led a few chemists to become experienced in the determination of vitamins, but it had remained a matter of personal choice whether any chemist had fitted himself to undertake such work. The position had, however, now changed, since the Regulations imposed by the recent Food Labelling Order required that in future the determination of vitamins would lie within the scope of each Public Analyst. Personally, he felt certain that the accepted methods of the future, certainly as far as the B_2 vitamins were concerned, would be microbiological. He would, however, sound a note of warning. Analysts should review these methods in their true perspective and should realise that because their reliability had been established for a range of foodstuffs it did not follow that they could, without modification, be applied with equal success to any type of material. A product of unknown composition might contain interfering factors, of either a stimulative or an inhibitory nature, and thus require a variation of the technique. The presence of such interfering factors was usually revealed by a drift in the readings obtained at different levels of the material, but even so, whenever a substance not previously tested was being examined, an analyst should adopt the only safe course and include in the assay a recovery test on the pure vitamin. An unsatisfactory recovery would be an indication that interfering factors were present, and the technique of the assay, or the method of preparing the sample, would then need to be modified so as to eliminate the effect of these.

Lieut. J. BERKELEY, M.B., put forward the hypothesis that bacteria suffer from deficiency of vitamins. It had long been known that an individual person could suffer from a food deficiency disease. In the *British Medical Journal* (August, 1944) there was a description of riboflavin deficiency, and it might be inferred that the *individual cell* suffered from "deficiency disease." It was now known that *Lactobacillus helveticus* required riboflavin for its multiplication. Who knew what enzymes or vitamins the numberless pathogenic bacteria required for successful reproduction? Further, might not the beneficial therapeutic effect of ultra-violet light in certain diseases be due to its destroying vitamins B, C, etc., in or near the surface of the skin, and so depriving pathogenic bacteria of necessary vitamins, thereby inhibiting their growth?

Corporal S. PARKIN, R.A.F. (Nutrition Section), School of Agriculture, Cambridge, referring to the variability of titre caused by the positioning of tubes in the incubator, suggested the advisability of increasing the incubation period. The standard adopted in the R.A.F. laboratory was now 4 days for *Lactohelveticus casei* instead of 72 hr. as recommended. The respiration of the organisms continued for approx. 6 days, and an increased incubation had been found to reduce deviations in the titratable acid produced from identically treated tubes of nutrients, plus standard inoculum, when subjected to variations in the temperature of the incubator.

Dr. KENT-JONES, replying to the discussion, said that Miss Meiklejohn and he would certainly give consideration to the point raised by Dr. Barton-Wright for improving the technique. With respect to the remarks of Mr. Bacharach, Mr. Graves and Mr. Wood on statistical analysis of results, he appreciated their observations but felt that it could be claimed that a high degree of accuracy was obtained, seeing that there was close agreement with duplicate tubes at three levels, that satisfactory checks with other workers had been obtained and, above all, that the recovery when known amounts of vitamin were added was excellent. He was grateful for the observations of Dr. Amos who had been associated with him in this work and he especially commended them to the meeting. In reply to Mr. Taylor and Dr. Hamence, he said that the literature strongly recommended using the determination of acidity rather than the determination of turbidity and he had experience only of the former procedure; it was possible to determine very small quantities of the vitamins, since in the assay tubes one required only 0·05–0·20μg of the vitamins and 5 ml of the food extract could be used to contain this small amount—in fact, as a rule, the food extracts had to be diluted to fit into the tube range. With respect to time of incubation they had not encountered erratic results when using 72 hours in their own incubator, but the longer time might lead to increased accuracy, although one had to bear in mind the importance of not complicating this test and also of keeping it as rapid as possible.

Erratum—October issue, p. 302. (MINERAL CONTENTS OF FOODS)—The two lines above Table I should read "All the results are expressed as percentages."

Notes

PRECIPITATION OF IRON WITH AMMONIA IN MIXTURES OF ETHYL ALCOHOL AND WATER

As is well known, the determination of iron by pptn. as ferric hydroxide with ammonia is tedious, for, owing to the nature of the ppt., filtration is slow and washing is difficult. If, however, the iron is pptd. as ferric hydroxide from a boiling mixture of alcohol and water (1 : 1), the ppt. is granular, filtration is rapid, and washing (with boiling 50% alcohol containing a little ammonia in solution) is easy. A long-stemmed funnel may be used, and the ppt. may be ignited while still moist. The results are very accurate; no salts insol. in 50% alcohol should be present.

The method of preventing shrinkage of the ppt. on the filter during washing is the same as with aqueous solns.[1] A hot 1% soln. of ammonium chloride in 50% alcohol is used as the washing liquid. The ppt. should not be allowed to stand on the filter until washing is complete, otherwise channels are formed and some of the ppt. escapes being washed. When very exact results are required, the ppt. is treated as prescribed for ppts. from aqueous solns.,[2] being dissolved in hot hydrochloric acid in 50% alcohol and re-pptd. with ammonia. As a rule, this procedure is unnecessary.

This method of pptng. iron may also be found suitable for other elements, *e.g.*, for the qualitative separation of certain cations, especially in semi-micro technique. In particular, it should find an application for the filtration of very voluminous ppts.[3] which can be made granular by pptn. from a mixture of alcohol and water.

It is possible that other solvents miscible with water, *e.g.*, methyl alcohol or acetone, may be found suitable in certain instances.

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October, 1944

A MACRO AND MICRO METHOD FOR THE ESTIMATION OF PYROGALLOL TANNINS AND CATECHOL TANNINS IN PRESENCE OF EACH OTHER*

CLASSIFICATION of the tannins into iron-blueing (pyrogallol) and iron-greening (catechol) tannins was suggested as long ago as 1772, but so far no analytical method has been described for the separate estimation of the tannins of these two groups when both are present in the same plant; this was pointed out by Mielke,[2] who described a number of abortive experiments that he had made. However, Naumann's investigations[3] of the distribution of the tannins in fungi seemed to us to offer a solution of this vexed problem.

Naumann found that fungi growing on wood contained either pyrogallol tannin or catechol tannin, even if wood such as oak, which is known to contain both tannins,[4] were used as a medium. Thus he found, for example, that *Polyporus* extracts from oak wood the pyrogallol tannin, whereas *Collybia* takes up the catechol tannin. From this we concluded (i) that *Polyporus* and *Collybia* assimilate their respective tannins, and (ii) that these two fungi produce either pyrogallase or catecholase, respectively, which thus destroy either the pyrogallol tannin or the catechol tannin. Our results confirm these deductions in every respect.

Nicholson, Nierenstein, Pool and Price[5] produced pyrogallase with the aid of *Aspergillus niger*. Using *Collybia crassipas* Schaeff, we obtained the identical enzyme, whereas with *Polyporus velutinus* Pers. we prepared catecholase. In our experience the production of these two enzymes by fungi is far superior to that by moulds, as much larger yields are obtained.

The fungi were grown on the following medium, which is based on the analytical data of Zellner[6]: calcium carbonate, 270 g; potassium carbonate, 20 g; magnesium phosphate, 20 g; sodium chloride, 5 g; ferric ammonium oxalate, 5 g; glucose, 100 g, dissolved in tap water. To this medium we added 50 g of pyrogallol or 40 g of catechol also dissolved in tap water. At the suggestion of Professor Zellner we used, in preference to the spores, the mycelia of these fungi, which were kindly supplied to us by Professor Zellner or the late Dr. O. V. Darbishire, Professor of Botany in this University. The fungi were grown in an incubator at 37° C. in large vessels made from Winchesters from which the tops had been cut off, and, in order to avoid the accumulation of carbon dioxide, sticks of sodium hydroxide, slightly moistened, were kept in the incubator. It took approximately 3 months for the solns. to become colourless; on adding a few drops of dil. ferric ammonium oxalate soln., however, they became either green or blue, thus showing that an excess of catechol or pyrogallol remained. The enzymes were prepared according to Nicholson, Nierenstein, Pool and Price,[5] who showed that they are a mixture consisting of a large number of enzymes, including tannase. The estimations were made in the apparatus described by Nicholson and Rhind,[7] fitted with either a blue or a green bulb.†

For our preliminary expts. we used acertannin,[8] a typical pyrogallol tannin, and paullinia tannin,[9] a typical catechol tannin. Both acertannin and paullinia tannin crystallise well and their constitution has been fully established. Acertannin, $C_{20}H_{20}O_{13}$, crystallises in two forms, *i.e.*, with two or four mols. of

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* I began this investigation more than 30 years ago, when I worked for some time at Kew. I take this opportunity of expressing my great appreciation of all the kindness shown me by the late Sir Arthur Hill, the Director of Kew Gardens, and the late Mr. Boodle, the Head of the Jodrell Laboratory.—M. N.

† We have to thank the Osram Company for the trouble they took to obtain a perfect colour match and for kindly presenting us with several lamps.

water, and becomes anhydrous at 160° C. Tannase hydrolysis yields 2 mols. of gallic acid and 1 mol. of aceritol. Under the same conditions paullinia tannin, $C_{38}H_{36}O_{20}$, yields 2 mols. of catechin carboxylic acid, $C_{15}H_{14}O_6$.COOH, and 1 mol. of glucose. Paullinia tannin was originally prepared from guarana paste made from the extract of the crushed seeds of *Paullinia cupana* H.B. et K., but the yield is poor. We find that the seedless pods of *Paullinia cupana var. oppositofolia*, Filat. give as much as 43% of well-crystallising paullinia tannin. During this investigation we examined, in collaboration with Mr. S. N. Barr, a large number of various kinds of *Paullinia* pods, and our results will be published elsewhere.

In addition to these two naturally occurring tannins, we also used "Syntans,"[10] prepared from either pyrogallol or catechol by condensation with formaldehyde in presence of hydrochloric acid, as described by Nierenstein and Webster.[11] These two Syntans crystallise well, and their constitution is that of the Neradols, which are generally known in industry as A, B, etc. Neradols. Following this system, we suggest the names α- and β-Neradols for Syntan from pyrogallol and catechol, respectively. Mixtures of paullinia tannin and acertannin by themselves or with either α-Neradol, β-Neradol, or mixtures of the two Neradols by themselves, gave satisfactory results, the errors being ±0·3%.

Errors of the same magnitude were also obtained with the following pyrogallol tannins: 3 samples of commercial gallotannin obtained from three different sources, Mitchell's glucose-free gallotannin,[12] 2 samples purified according to Geake and Nierenstein,[13] 2 samples of gallotannin freed from free glucose according to Nierenstein, Spiers and Hadley,[14] and the synthetic tannins of Armstrong, Barton and Nierenstein.[15]

So far, paullinia tannin is the only known crystallising catechol tannin. The tanning materials were therefore extracted with a mixture of carbon tetrachloride and chloroform (1 : 1), followed by extraction with ether, so as to remove the catechins present, and then exhaustively percolated with cold water under aseptic conditions. The tannin content was estimated, first by the method of Trotman and Hackford,[16] and then by Nierenstein's method.[17] We obtained errors of about the same magnitude with the following substances: Assam and China tea, oakwood (and also firwood), chestnut bark, oak bark, birch bark, gambier cutch, acacia cutch. Some of the cutches investigated were of commercial origin and others were made by us in the laboratory.

Micro-analysis—For this we used tall Nessler tubes of 10 ml capacity and had no difficulty in estimating 0·1 mg of the tannins mentioned. Our method gave results comparing favourably with those obtained by the method recently described by Nierenstein.[17]

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THE PHOTOMETRIC ANALYSIS OF COPPER-BASE ALLOYS: I. THE DETERMINATION OF IRON AND MANGANESE

The original methods devised by Vaughan[1] for the determination of manganese and iron in complex brasses have been applied to copper-base alloys in general without restriction as to iron or manganese content. "Standard blanks" (made from alloy samples of known composition) are used in conjunction with a suitable neutral filter.[2] Sample weights and aliquots must be such that the final concentrations are: *Iron*—not greater than 0·65 mg/110 ml—preferably between 0·5 and 0·6 mg/110 ml. *Manganese*—not greater than 1·6 mg/100 ml—preferably between 1·0 and 1·4 mg/100 ml.

Method—Dissolve 0·5 g of alloy in 15–20 ml of acid mixture (450 ml of sulphuric acid (sp.gr. 1·84), 200 ml of nitric acid (sp.gr. 1·42) and 1400 ml of water); when solution is complete, boil for a few min., cool and make up to 100 ml with water. "Fuming" to remove nitric acid is unnecessary.

Iron determination—Take a suitable aliquot in a volumetric flask graduated at 100 and 110 ml (*cf.* Stross[3]), and add either 20 ml of 15% nitric acid or 20 ml of 10% hydrochloric acid and 10 ml of 0·5% ammonium persulphate soln. Make up to 100 ml with water and, prior to taking a reading, make up to

the 110-ml mark with 20% sodium thiocyanate soln. Measure the absorption with the Spekker Absorptiometer, using 2-cm glass cells and Ilford Spectrum Blue-Green filters 603 or Wratten No. 75. Set the Absorptiometer to read 0·265 with a standard blank of a final concn. of 0·540 mg of iron per 110 ml, controlling the setting by means of a neutral filter—Ilford 602 or Hilger grey H.508.[3]

Manganese determination—Oxidise an aliquot by boiling with 0·05 g of potassium periodate[4] and 10 ml of 30% phosphoric acid, bringing the copper concn. to 0·25 g where necessary. Cool, transfer to a 100-ml volumetric flask and make up to the mark with water. Measure the absorption of this soln., using Wratten 72 filters, 2-cm glass cells and a neutral filter (Hilger H.508), and a setting at 0·15.[2]

The instrument is calibrated with standard solns. of the two elements without making a supplementary copper graph.

This composite method has proved useful for over a year on a wide range of copper-base alloys and is quick and accurate. Table I gives typical results.

TABLE I

Sample No.	1	2	3	4	5	6	7	8	9
Iron, photometric, % ..	5·28	10·50	5·17	1·04	1·05	1·03	0·96	2·13	2·20
,, gravimetric, % ..	5·26	10·48	5·27	1·04	1·01	1·00	0·96	2·10	2·15
Manganese, photometric, %	0·46	0·41	0·43	0·20	0·15	0·17	1·01	1·89	2·06
,, volumetric, %	0·44	0·42	0·43	0·19	0·15	0·18	0·99	1·91	2·10

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

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THE DETERMINATION OF UNSAPONIFIABLE MATTER IN PACIFIC DOGFISH LIVER OIL BY THE S.P.A. METHOD

I HAVE used the S.P.A. method for the determination of unsaponifiable matter in an oil[1] for several years with very good results. There is rarely difficulty due to emulsification, and duplicate analyses usually agree closely.

Kirsten[2] reported that the method affords a more complete extraction than does the modified Kerr-Sorber method.[3] B. E. Bailey, formerly of this Station, and I confirmed this conclusion by determination of the unsap. matter in the liver oil of two Pacific dogfish (*Squalus suckleyi*) by both methods, with the following results.

Unsap. matter in	Modified Kerr–Sorber method %	S.P.A. method %
Dogfish liver oil, (A)	21·4	28·1, 28·1
,, ,, (B)	10·5	14·6, 14·9

However, with Pacific dogfish liver oils high in unsap. matter, the recommended three extractions are insufficient to remove completely the unsapon. matter. In one expt. the procedure was carried out with two oils as prescribed, except that five instead of three extractions were made. At another time two samples of dogfish liver oil[3] were saponified and extracted, one four times and the other three times. In both expts. each separate extract was washed three times with alkali and water and then with water to neutrality. The weights of oil saponified were between 2·3 and 2·4 g, less than the limit of 2·5 g given in the method, a limit that was emphasised in a later paper.[4] The results follow.

TOTAL EXTRACT AS % OF OIL

No. of extractions	Oil 1	Oil 2	Oil 3	
			i	ii
1	13·75	14·57	17·45	17·09
2	19·14	19·27	24·79	24·84
3	20·44	20·57	26·41	26·23
4	20·82	20·98		26·60
5	20·94	21·15		

With oil 2 the fourth extract gave a slight reaction for cholesterol by the Liebermann-Burchard test, and even the fifth showed faintly the fluorescence characteristic of vitamin A in ultra-violet light. The extracts of the two samples of oil 3 were combined, dissolved in ethanol and titrated with alcoholic sodium hydroxide soln. Each required 0·3 ml of 0·04 *N* alkali, which is equivalent to the limit specified by the method.

In another expt., simulating more closely the actual procedure, four 2·3-g samples of dogfish liver oil 3 were saponified. Two were extracted three times and yielded 26·4 and 26·1% of unsap. matter respectively. The other two were extracted four times and yielded 26·8 and 26·8% of unsap. matter. Each extraction was made with vigorous shaking for 1 min.

It would therefore seem that four extractions give a more complete removal of unsaponifiable matter from Pacific dogfish liver oil than three. It should be emphasised that the only oils with which this examination has so far been undertaken were from the livers of Pacific dogfish.

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October, 1944

Ministry of Food

STATUTORY RULES AND ORDERS*

1944—No. 1156. General Licence, dated October 6, 1944, under the Fish (Supplies to Catering Establishments) Order, 1943. Price 1d.

This General Licence replaces an earlier General Licence and specifies the fish that catering establishments may obtain without limit as to quantity.

"Cured (pickled) herrings" means herrings (gutted or ungutted), hand packed in tiers with salt in barrels, but does not include roused or sprinkled herrings.

— **No. 1165. The Fish (Maximum Prices) (No. 2) Order, 1944. Dated October 11, 1944.** Price 4d.

This Order replaces the Fish (Maximum Prices) Order, 1944, No. 511, as amended by S.R. & O., 1944, No. 875.

"Fillet" *now* means fish from which all guts, bones, head, tail and fins and, in the case of fresh and smoked skinned fish, flaps, have been removed, and in the case of haddock and whiting includes a block fillet not exceeding 3/4 lb. in weight, provided that for the purposes of this definition—(*a*) the small bones in the flaps need not be removed; (*b*) flaps sold separately shall not be regarded as fillets.

— **No. 1199. Order, dated October 21, 1944, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1944.** Price 1d.

This Order alters the composition of National Baby Chick Feed and National Chick Feed by including a minimum of 20% of maize in the former and altering the proportion of cut wheat and maize grits in the latter.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Cholesterol to Estimate the Egg Solids Content of Noodles. R. C. Koehn and F. A. Collatz (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 451–455)—In 1941 the cholesterol method (*J. Assoc. Off. Agr. Chem.*, 1942, **25**, 63, 69) for estimation of the egg solids content of noodles, etc., was adopted officially in place of the lipoid phosphorus method. The cholesterol method, unlike the older procedure, requires no correction for losses in manufacture or long storage. The sample is saponified, the unsaponifiable portion is treated with bromine to form insol. cholesterol dibromide, and this is collected by filtration, dissolved in ether and alcohol and hydrolysed by alkali. The resultant aq. soln. of potassium bromide is oxidised with hypochlorite and the bromate is determined iodimetrically. Two lots of durum patent flour and 3 lots of commercially frozen egg yolks were examined by the method of Haenni (*J. Assoc. Off. Agr. Chem.*, 1941, **24**, 119; ANALYST, 1941, **66**, 245). The unsap. matter of egg yolks was found to be somewhat higher than the average result reported by Haenni, but the sterol content was the same, and a value of 2·88% (dry basis) may be taken as an accurate estimate of the cholesterol content of commercial frozen egg. The value found with both samples of durum flour (0·028%) varied more widely from the values previously reported (aver. 0·024%), the difference being probably due to seasonal variation, milling practice, geographical origin or variety of wheat. Three samples of noodles of known composition were examined. Duplicate determinations agreed satisfactorily, and when the factor 0·028% of cholesterol in durum flour was used, the results agreed closely with the known amount of egg present. With the factor 0·024%, agreement, although still satisfactory, was not so close, but this was to be expected, since the flour was that used for the determination of the factor. Results by the lipoid phosphorus method were consistently low. After 5–6 months' storage in paper cartons, the cholesterol content of the noodles had not altered, whereas the lipoid phosphorus content had decreased. Commercial noodles made to contain 5·6% of egg solids (dry basis) were examined by the method and, by use of the factor 0·028% for the cholesterol content of flour, satisfactory results were obtained. A. O. J.

Action of Copper Sulphate on Phenylosazones of the Sugars. Phenyl-*d*-glucosotriazole. R. M. Hann and C. S. Hudson (*J. Amer. Chem. Soc.*, 1944, **66**, 735–738)—Heating a suspension of phenyl-*d*-glucosazone with an aqueous soln. of copper sulphate leads to the formation of aniline

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

and a very stable substituted triazole, now named phenyl-*d*-glucosotriazole (m.p. 195–196° C.). The triazole is only slightly sol. in water at ordinary temps., crystallises easily and has characteristic m.p., rotation and crystalline appearance; it is suggested as a reagent for confirming the identity of phenylglucosazone, especially with small samples. The following test is recommended. Heat under reflux for 1 hr. a suspension of 0·2 g of powdered osazone in 18 ml of water, 1 ml of 0·5 *N* sulphuric acid, 0·6 g of copper sulphate pentahydrate and 12 ml of *iso*propyl alcohol, using porous porcelain fragments to ensure even boiling. The osazone dissolves within 15 min., the soln. then being deep red; during the next 30 min. the colour fades to orange and finally yellowish-green. Concentrate the soln. on the steam-bath by an air current to about 5 ml; the osotriazole crystallises during evaporation and on further keeping the soln. in the refrigerator for 3 hr. Filter the tan-coloured ppt., wash with water, boil with 30 ml of water and 0·1 g of decolorising carbon, and filter hot. Long needles of pure osotriazole separate on standing in the cold overnight; yield 20–40 mg. In 25 ml of pyridine soln. 18·3 mg gave a rotation α_D −0·23° in a 4-dm tube, the calculated value being −0·24. Methyl alcohol may be substituted for *iso*propyl alcohol if the refluxing time is increased to 2 hr.; yield 56 mg, m.p. 195–196° C., observed rotation for 50 mg in 25 ml of pyridine soln. in 4-dm tube, −0·64°, calculated −0·65°. The test would be applicable to phenyl-*l*-glucosazone, the osotriazole of which would be dextrorotatory; the positive detection in nature of the *l*-forms of glucose, mannose or fructose, is of biological interest. This reaction appears to be general with all sugar phenylosazones, and the properties of several of the derivatives are given. E. M. P.

Hexabromide Method for Detection of Small Quantities of Linolenic Acid in Animal Fats. Detection of Horse Meat in Admixture with Pork or Beef. G. K. Crowell (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 448–451)—The method is a modification of that of Paschke (*Z. Unters. Lebensm.*, 1938, **76**, 476; ANALYST, 1939, **64**, 47) taking into account the solubility of the pptd. hexabromide and preventing pptn. of fatty acids other than hexabromide fatty acids during bromination. Pass the meat sample twice through a meat grinder, warm it to *ca.* 60° C., cover it with light petroleum and when boiling has ceased add more light petroleum to cover the sample and leave it overnight at room temp. Filter the extract through a dry filter, remove the solvent on the steam-bath in a current of nitrogen or carbon dioxide and store the fat in a refrigerator. Boil 10 g of the fat under reflux with 100 ml of 0·5 *N* alcoholic potassium hydroxide for 30 min. Remove *ca.* 80 ml of alcohol by distillation and to the warm residue in a separating funnel add 250 ml of water, 15 ml of 5 *N* sulphuric acid, 250 ml of sat. salt soln. and 50 ml of dry ether. After shaking vigorously withdraw the ethereal layer, wash it with three 15-ml portions of sat. salt soln., filter, cool to 5°–10° C. and make up to 50 ml at this temp. with dry ether. To 10 ml (2 g) of the ethereal soln. in a tared centrifuge tube (6 in × 1 in.), add 15 ml of anhydrous ether saturated with hexabrominated fatty acid prepared from horse fat (Bailey and Baldsiefen, *J. Ind. Eng. Chem.*, 1920, **12**, 1189; *cf.* ANALYST, 1921, **46**, 104) and 2 ml of glacial acetic acid. Cool the tube for 15 min. in ice and salt (−5° to −10° C.) and add from a burette, drop by drop, 2 ml of brominating soln. (2 ml of bromine and 8 ml of glacial acetic acid), keeping the tube in the ice-bath during bromination and for 5 min. after. Place the stoppered tube in the refrigerator overnight. Cool the tube in the ice-bath for 15 min., remove the stopper and centrifuge at 900–1000 r.p.m. for 2–4 min. Decant all the ether from the tube, add 10 ml of washing ether (prepared by adding 2 ml of glacial acetic acid to 25 ml of the ether saturated with hexabrominated fatty acid) to the residue, stir vigorously, cool the tube for 15 min. and centrifuge as before. Repeat the washing process until all occluded bromine has been removed, but avoid excessive washing. Dry the tube at 105°–110° C. for 30 min. and weigh the brominated fatty acid. The m.p. of the brominated acid from horse fat agrees with that given in the literature for linolenic acid hexabromide: 179·5°–180° C. (corr.). The following results were obtained for the hexabromide values (mg per g of fat)—beef fat, 2·0; pork fat, 7·5; horse fat, 56·8. Known mixtures of horse fat with beef or pork fat gave results agreeing with the calculated figures. The method does not lend itself to the quantitative determination of horse meat in admixture with beef or pork, since horse meat contains less fat than is usually present in beef and pork. The fats used in the investigation were extracted from lean meat containing the average amount of fatty tissue. The use of fatty tissue was avoided, since its composition varies with its position in the animal. A. O. J.

Detection of Adulteration of Olive Oil used in Packing Maine Sardines. M. D. Voth (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 455–458)—In the course of an investigation it was noted that the Hanus iodine value of the surplus oil drained from canned fish was not materially different from that of the original oil used for packing, and expts. were made with fish canned in olive, peanut, maize, cottonseed and soya oils. It was evident that, when plain or smoked Maine sardines are packed in oil, very little change takes place in the Hanus iodine value or in the n_D^{25} of the packing oil during and after processing. Expts. with other fish (herring, kipper, bloater, smoked mackerel and smoked salmon) showed that the amount of oil in the fish had a direct bearing upon the extent of change that takes place in the iodine value of the oil, but that it is only in very oily fish, such as smoked mackerel, that the change is considerable. In these expts. only the drained oil was examined. It is possible that the oil retained in the fish may have a somewhat different iodine value from that of the oil exuded in the heat process. Adulteration of the olive oil used in packing Maine sardines can thus be detected by determining the Hanus iodine value of the drained oil. The n_D^{25} also serves as a rapid sorting test. A. O. J.

Adsorption of Fatty Acid by the Linear Component of Maize [Corn] Starch. T. J. Schoch and C. B. Williams (*J. Amer. Chem. Soc.*, 1944, **66**, 1232–1233)—The presence of small amounts of fatty acid in maize starch markedly represses iodine adsorption. Thus, the iodine adsorptions (determined potentiometrically) of commercial maize starch containing 0·65% of fatty material were 4·1–4·4 and 5·3% for the original starch and the defatted starch respectively (*cf.* Wilson, Schoch and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1380, 1421; ANALYST, 1943, **68**, 192). Experimental evidence indicates that this effect is due to preferential adsorption of fatty acid by the

presumed linear-chain component, which is selectively precipitated by butanol (*cf.* Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2957; ANALYST, 1943, **68**, 119) and is responsible for the blue iodine adsorption complex. The higher fatty acids (*e.g.*, oleic acid) also act as selective precipitants for the linear chain component. Hence, before potentiometric determination of iodine adsorption, cereal starches must be completely defatted. The proportion of linear chain component can be calculated approximately as the ratio of iodine adsorption of defatted maize starch (5·3%) to that of the repeatedly recrystallised butanol-precipitated fraction (19·0%), *i.e.*, 28%. While the result is higher than that obtained previously from pptn. with butanol, it agrees with values given by improved methods of pptn. with certain amyl alcohols. These results indicate that the so-called α-amylose of this starch is part of the linear chain component, made polar through adsorption of maize starch fatty acid.

E. B. D.

Biochemical

Micro-determination of Volatile Fatty Acids in Blood. J. F. McClendon (*J. Biol. Chem.*, 1944, **154**, 357–360)—Add the blood to 5 times its vol. of 2% $ZnSO_4.7H_2O$ soln., mix and add 12 ml of the mixture to 2 ml of 0·5 *N* sodium hydroxide. Centrifuge or filter and put 7 ml of the filtrate (equiv. to 1 ml of blood) into the still of the apparatus (Fig. 1) by means of a pipette introduced

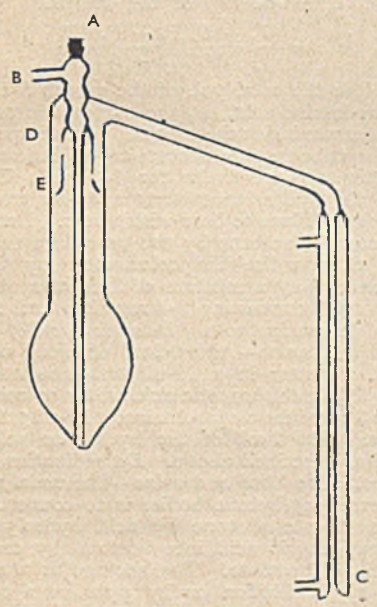

Fig. 1

at A. Add 0·5 ml of syrupy phosphoric acid (through which steam has been blown while it is hot) at A, circulate cooling water through the condenser, and place a 50-ml conical flask, graduated at 30 ml, under C. Immerse the still in a glycerin bath and heat this, together with the steam generator, by means of micro-burners. When the bath temp. reaches 120° C., insert the stopper at A, so that steam enters from B and bubbles through the filtrate in the still. Regulate the burners so that the temp. of the bath is kept at 120° C. and the water-seal at D is maintained. Collect 30 ml of distillate, add 3 drops of 0·04% bromothymol blue soln. and bubble air, purified by passage through sodium hydroxide soln. and wet Permutit, through the distillate in a fine stream for 10 min. and during the titration. Titrate with 0·01 *N* sodium hydroxide (CO_2-free) until the colour is identical with that of a mixture of 30 ml of CO_2-free water, 3 drops of indicator and 3 mg of sodium acetate. Observe the end-point with the aid of a "daylight" fluorescent lamp and an opal glass diffuser. Make a blank expt., using Ringer's soln. in place of blood, and subtract the titre from the value found in the determination. Under the conditions specified above, all the caproic acid, butyric acid and acetic acid are recovered in the distillate, but probably not all the formic acid. Negligible amounts of pyruvic acid, β-hydroxybutyric acid or lactic acid collect in the distillate. F. A. R.

Determination of Oxalic Acid in Urine. H. H. Powers and P. Levatin (*J. Biol. Chem.*, 1944, **154**, 207–214)—The method depends on the extraction of the oxalic acid from the urine with ether, precipitation as calcium salt from an acidified 60% alcoholic soln., and titration of the oxalate with permanganate by the iodimetric technique. The extraction apparatus consists of a 25-ml test-tube, about 15 cm long, into which dips a funnel-shaped tube, 20 cm long, made from half of a 25-ml pipette. The stem end of this tube is sealed and four small holes are made near the sealed end. A 3-mm hole is drilled near the top of the test-tube, and the test-tube and funnel are suspended in a larger test-tube, 30 × 2·5 cm, by means of a nichrome wire hooked through this hole and passing over the edge of the outer tube. A small "cold-finger" condenser is inserted in the outer tube, so that the condensate will collect in the funnel and so flow through the holes at the bottom into the small test-tube. The extraction is carried out on an electric hot-plate covered with an asbestos sheet, in which a small hole is cut to take the bottom of the tube. *Method*—The urine may be collected with a few ml of formaldehyde as preservative. Fill a 25-ml graduated flask to the mark with the urine, add 1 ml of conc. hydrochloric acid, mix and immerse in a boiling water-bath for 30 min. to hydrolyse the oxaluric acid. Cool, filter and transfer 10 ml to the inner tube of the extraction apparatus. Put 3 or 4 glass beads, 2 ml of water and about 25 ml of ether into the outer tube, and adjust the hot-plate so that ether drips from the condenser tube at about 100 drops per min. After 6 hr. extraction remove the inner tube and rinse it down with 2 ml of 95% alcohol. Add 1 ml of 2% acetic acid to the contents of the outer tube with gentle agitation, drive off the ether by warming to about 70° C., and transfer the aqueous soln. to a 15-ml conical centrifuge tube, the tip of which is drawn to an inside diam. of 1 mm. The transfer is best effected by siphoning with the aid of gentle suction. Rinse the sides of the extraction tube with 2 ml of 95% alcohol and transfer the washings to the centrifuge tube. Repeat the washing. Add 0·5 ml of 10% calcium chloride soln. to the centrifuge tube and stir with air blown through a fine capillary. Add 2 ml of a mixture of 60 ml of 95% alcohol, 10 ml of 2% acetic acid and 20 ml of water so that it forms a layer on the surface of the liquid in the tube. Leave overnight, centrifuge for 30 min., decant and invert to drain for a few min. Rinse the sides of the tube with 2 ml of the acid-alcohol soln. and break up the ppt. with a fine glass stirring-rod. Remove the rod, wash with 3 ml of

the acid-alcohol soln., again centrifuge for 30 min., decant, drain and heat on the water-bath to remove alcohol. To the ppt. add 1 ml of 20% sulphuric acid and 0·5 ml of 4% manganese sulphate soln., mix with a fine glass rod and add exactly 3 ml of 0·01 *N* potassium permanganate. Stir with a current of air, leave for 8–10 mins. and then add 0·5 ml of 10% potassium iodide soln. and mix. Add 4 drops of 1% starch soln. and 2 drops of saturated barium hydroxide soln. (the white precipitate of barium sulphate renders the end-point easier to see). Titrate the excess permanganate with 0·01 *N* sodium thiosulphate from a microburette. Subtract from the titre the reagent blank and multiply the result by 0·45 to convert to mg of oxalic acid. Apply a volume correction of 1·04 for the hydrochloric acid added. Recoveries of oxalic acid averaging 90% were obtained, even with concentrations as low as 0·75 mg per 100 ml.

F. A. R.

Direct Determination of 5-Keto-*d*-Gluconic Acid. W. E. Militzer (*J. Biol. Chem.*, 1944, **154**, 325–330)—5-Keto-*d*-gluconate can be estimated by measuring the time necessary to reduce cold Benedict's reagent. Sugars such as glucose, galactose, mannose and fructose, and 2-keto-*d*-gluconate only interfere when present in high concns., but *l*-ascorbic acid and its oxidation products, dehydroascorbic acid and 2,3-diketo-*l*-gulonic acid, and related compounds interfere and must be removed. Put 5 ml of Benedict's reagent (dissolve $CuSO_4.5H_2O$, 18 g; sodium carbonate, 150 g; potassium citrate, 200 g; potassium thiocyanate, 125 g in water, add 5 ml of 5% potassium ferricyanide soln. and dilute to 1 litre) into test tubes, 5 in. × 1 in., coated on the outside with white enamel to a height of 2 in. from the bottom. Immerse the tubes in a constant temperature bath at 25° C. and add from a pipette 5 ml of the soln. to be tested or of substandards made from a standard soln. prepared by dissolving exactly 2·5 g of calcium 5-keto-*d*-gluconate and 0·75 g of oxalic acid dihydrate in 20 to 30 ml of warm water, filtering off the calcium oxalate and diluting the filtrate to 50 ml. Shake during the addition of the gluconate soln. and note with a stop-watch the time taken for the complete disappearance of the blue colour, counting from the first moment of adding the gluconate soln. For maximum accuracy, the time taken to add the soln. to the reagent should be 15 to 20 sec. Plot the concns. of 5-keto-gluconate in the standard solns. against the times required for complete reduction and from these curves calculate the concn. of 5-keto-gluconate in the unknown soln. Generally, the reduction times can be duplicated within 5 sec.

F. A. R.

Micro Method for the Determination of Acetone and Ketone Bodies. L. A. Greenberg and D. Lester (*J. Biol. Chem.*, 1944, **154**, 177–190)—The method was developed primarily to measure the concentration of acetone vapour in air, but it can also be used to measure the amount of ketone bodies in blood and urine. It is ten times more sensitive than previous methods, and with 0·2 ml of blood, concns. as low as 0·1 mg of acetone or 0·2–0·3 mg of total ketones per 100 ml of blood can be estimated with errors of ± 0·05 and ± 0·12 mg % respectively. In determining acetone in air the absolute sensitivity is 0·1–0·2μg with an error of ± 0·06μg. The acetone is converted to its 2 : 4-dinitrophenylhydrazone, which is extracted with carbon tetrachloride and then determined colorimetrically. Interference from keto-acids can be prevented by extracting the carbon tetrachloride soln. of the mixed hydrazones with alkali. This also removes the dinitrophenylhydrazones of formaldehyde and (unless large quantities are present) of acetaldehyde.

Measurement of colour intensity—Put 2 ml of a 0·1% soln. of 2 : 4-dinitrophenylhydrazine in 2 *N* hydrochloric acid into a 10-ml glass-stoppered graduated cylinder, add the acetone soln. to be analysed and dilute to 5 ml. Add exactly 2 ml of carbon tetrachloride, stopper the cylinder and shake for 10 min. Run off the supernatant soln., wash the carbon tetrachloride layer twice with water and then shake for 3 min. with 3 ml of 0·5 *N* sodium hydroxide soln. Measure the colour intensity of the carbon tetrachloride soln. in a Klett micro-colorimeter tube against a similar tube containing carbon tetrachloride, using a filter transmitting at 420mμ. From the reading, subtract the blank obtained by using distilled water in place of the soln. to be analysed. Small losses occur during the extraction procedure, but these are approximately compensated for by the enhanced colour due to the presence of a little dinitrophenylhydrazine and of alkali, so that reproducible and constant results are obtained, provided the volumes specified above are strictly adhered to. *Determination of acetone in air*—Put 3 ml of the 2 : 4-dinitrophenylhydrazine soln. into the tube or flask in which the air sample is to be collected, evacuate with a water-pump and measure the negative pressure. The vol. (*V*) of the sample

$$= \frac{(V_1 - S)(P_1 - P_2)273}{760(273 + t)}$$

where V_1 is the total vol. of the vessel, S the vol. of the hydrazine soln., P_1 the barometric pressure, P_2 the pressure inside the tube and t the temperature. Open the tube in the atmosphere to be examined and, after closing, shake for 1–2 min. Transfer 2 ml of the hydrazine soln. to a 40-ml graduated cylinder and dilute to 5 ml with water. Determine the amount of acetone by the procedure described above. Standardise the colorimeter by treating in the same way a mixture of 3 ml of a standard acetone soln. containing 2–4 mg/litre and 2 ml of the acid hydrazine soln. *Determination of acetone in blood and urine*—Add 0·2 ml of blood or urine to 1 ml of water in a 10-ml test-tube and then 3 ml of 5% trichloroacetic acid; filter or centrifuge and put 3 ml of the clear soln. into a 10-ml graduated cylinder. Add 2 ml of the hydrazine soln. and proceed as described above. *Determination of total ketone bodies in blood and urine*—The usual method for determining ketone bodies is to convert acetoacetic acid into acetone by acid hydrolysis and β-hydroxybutyric acid into acetone by oxidation with acid dichromate. The acetone is then separated by distillation or by pptn. as a complex mercury salt. In the present method, distillation is avoided, and the hydrolysis and oxidation are carried out simultaneously in a Pyrex glass test-tube which is surrounded by a water-jacket and into which is inserted a "cold-finger" condenser to within 3 cm of the bottom. First deproteinise the blood or urine by adding a 0·2-ml sample to 2·3 ml of water, allowing the blood to lake and then adding 1 ml of 0·15 *N* barium hydroxide followed by 1 ml of 2·5% $ZnSO_4.7H_2O$ soln. The baryta soln. should be so adjusted that 5 ml will produce a permanent pink colour when 5 ml of the zinc soln. are titrated, with phenophthalein as indicator. Mix, centrifuge for 2 or 3 min., and transfer 3 ml of

the supernatant liquid to the refluxing tube containing several glass beads. Add 0·6 ml of a 0·46% soln. of potassium dichromate in 15·6 *N* sulphuric acid and insert the "cold-finger." Heat under reflux by means of a micro-burner for 10 min., allow to cool somewhat and then add 0·5 ml of 10% potassium dichromate soln. Close the tube and continue heating for a further 10 min. Cool the apparatus and transfer 3 ml of the soln. to a 10-ml graduated cylinder. Add 0·4 ml of 15% sodium sulphite soln. to destroy the dichromate, and proceed as described above. From the reading subtract a blank obtained by adding 0·6 ml of acid dichromate soln. and 0·5 ml of 10% dichromate soln. to 3 ml of water and carrying through the procedure subsequent to the refluxing. Standard solns. are similarly prepared, using 3 ml of standard acetone soln. instead of water. F. A. R.

Agricultural

Orange-coloured Pigment of Cottonseed. C. H. Boatner, M. Caravella and C. S. Samuels (*J. Amer. Chem. Soc.*, 1944, **66**, 838–839)—An orange-coloured pigment is isolated from cottonseed as follows. Re-extract the ethereal extract of the sample with dil. sodium hydroxide soln. containing sodium dithionite ($Na_2S_2O_4$); neutralise with conc. hydrochloric acid and withdraw the yellow ethereal layer which separates. After separation of gossypol "acetate" from this layer by addition of glacial acetic acid, an orange-coloured solid is slowly pptd. from the filtrate on standing. Extract this product with acetone, and then crystallise it from hot acetone and finally from hot chloroform-ether mixture. The pigment is less sol. than gossypol in most organic solvents, insol. in alkali, and melts at 212° C. (corr.) to form a solid of deeper colour, m.p. 238–239° C. (decomp.). It does not give a ppt. with aniline, reduce Fehling soln. or react in the fuchsin-aldehyde test. The products obtained with hydroxylamine and with dinitrophenylhydrazine differ in m.p. from the corresponding gossypol derivatives. In conc. sulphuric acid the pigment forms a brilliant yellow solution which slowly changes colour to the scarlet characteristic of gossypol in sulphuric acid. Conc. hydrochloric acid also converts the pigment into gossypol. Prior treatment with conc. hydrochloric acid therefore alters the spectral absorption of the colour produced by the antimony chloride test. Occurrence of high concns. of this pigment—which is also distinguished spectroscopically from gossypol—in some cottonseed meals is regarded as being partly the reason for the lack of correlation between the gossypol content and toxicity of these meals when gossypol is determined as the dianiline compound. E. B. D.

Organic

Identification of Monochloroacetic Acid as Barium Monochloroacetate. J. B. Wilson and G. L. Keenan (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 446–448)—When considerable proportions of monochloroacetic acid are present and when other acids soluble in ether are not present in excessive amounts, it is possible to identify this acid as its barium salt. To 100 ml of the sample, containing 50 mg or more of monochloroacetic acid, add 3 ml of sulphuric acid and extract the mixture with an equal vol. of ether, or, if emulsions form, in a continuous extractor for 1–2 hr. To the ethereal extract in a separating funnel add phenolphthalein indicator and 5 ml of 0·1 *N* barium hydroxide and shake for 30 sec. If the aq. layer is pink, filter it into a small beaker, add 0·05 *N* acetic acid until the colour is discharged, evaporate to 1 or 2 ml on the steam-bath and allow the residual liquid to evaporate spontaneously in the air and finally in a desiccator. If 5 ml of 0·1 *N* barium hydroxide does not give a pink aq. layer, add 5 ml more and shake before separating. Repeat the extraction with barium hydroxide soln. several times or until a pink soln. is obtained, evaporating each extract in a separate beaker. Examine the crystals under a polarising microscope. Barium monochloroacetate crystallises from water in hexagonal plates with sharply pointed ends and with two opposite sides only *ca.* 1/3rd the length of the remaining equal sides. In the overlapping layers usually formed, the sharply pointed ends are visible even in finely powdered material. In parallel polarised light (crossed nicols) the extinction is parallel and the elongation negative in the more elongated plates. The plates invariably extinguish sharply with crossed nicols and, therefore, interference figures are not observed in convergent polarised light. Since the plates persistently lie in one orientation, the significant refractive indices were determined by the statistical method, and the lowest and highest, arbitrarily designated n_α (1·582 ± 0·002) and n_γ (1·611 ± 0·002), were measured on plates showing the maximum amount of double refraction. The procedure was successfully applied to identify 10 mg of monochloroacetic in 100 ml of water and 30 mg per 100 ml in creme soda and orange soda beverages. From other samples containing substances that interfered with crystallisation no identifiable crystals were obtained. Solns. containing dichloroacetic acid yield a vitreous hygroscopic barium salt. Crystalline barium trichloroacetate is formed under the conditions of the method, but it has not yet been found suitable for microscopical study, although its crystalline habit is distinctly different from that of the monochloroacetate. A. O. J.

Isolation and Properties of some Naturally-occurring Octadecenoic [Oleic] Acids. R. C. Millican and J. B. Brown (*J. Biol. Chem.*, 1944, **154**, 437–450)—Theoretically, 16 isomeric octadecenoic acids can exist, each differing in the position of the double bond, but only two or three of these, in addition to oleic acid, have been found in nature. Petroselenic (6 : 7-octadecenoic) acid has been found in parsley seed and ivy seed oil, vaccenic (11 :12-octadecenoic) acid was found in whale oil and beef fat, and has been reported in butter fat, mutton fat and lard, and the presence of 10 : 11-octadecenoic acid has been suggested in pork liver lipids. The preparation of pure oleic acid was formerly extremely difficult, but improved methods, in particular low-temperature crystallisation, have simplified it. A method, due to Foreman, has now been employed for the isolation of the oleic acid fraction from several animal and seed fats and oils. The method comprises careful fractionation of the methyl esters of the oil through an electrically-heated and packed column (which gives a product containing only C_{18} esters), removal of methyl stearate by crystallisation from acetone or methyl alcohol at −25 to −30° C., and removal of methyl linolate by crystallisation of the filtrate at −60° C. The resulting methyl oleate is saponified with alcoholic potassium hydroxide soln., the alcohol is removed under reduced pressure, and the acid is liberated and purified by distillation.

Oleic acid prepared in this way from olive oil had a purity of 99·8%, and was taken as a standard in the present work. The following analytical data were determined for each of the oleic acids obtained by this procedure from other oils: n_D^{20}, m.p., iodine value and thiocyanogen value. These were compared with the corresponding values for pure oleic acid, *viz.*, 1·4600, 13·3–13·5° C., 89·98, 89·90, respectively. Several of the fractions were also oxidised to the corresponding dihydroxy acids, and these were compared with dihydroxystearic acid, m.p. 131–132° C., prepared from oleic acid. The seed fats of olive, cottonseed, maize and linseed oil appeared to contain oleic acid and no other isomer, whilst the oleic acid fractions obtained from soya bean and rape-seed oil, lard, beef tallow, adrenal phosphatides, pork liver lipids and human fat appeared to be mixtures of octadecenoic acids, of which oleic acid was the principal component. These results support Bertram's conclusion that beef fat and lard contain vaccenic acid (*Biochem. Z.*, 1928, **197**, 433). F. A. R.

Analysis of Chlorinated Xylenol Mixtures with Special Reference to the Unchlorinated Phenols Content. W. H. Ritchie and W. E. Hamer (*J. Soc. Chem. Ind.*, 1944, **63**, 247–249)—Commercial chlorinated xylenol mixtures contain unchlorinated phenols (xylenols, ethylphenols and higher homologues) as well as mono- and higher chlorinated compounds. Efficient fractionation (at reduced pressure) removes the unchlorinated and some monochlorinated material, and determination of the chlorine content provides data for calculating the free phenols content of the distillate and, hence, of the original mixture. The apparatus consists of a 130-ml flask fitted into an asbestos box containing a circular nichrome wire heater rated at about 200 watts, the source of heat being the low-voltage secondary 20–50 volts of a transformer, controlled by a variable resistance. A reflux rate measurer is attached to the flask by ground-glass joints, and above this is a 33 theoretical plate, spiral screen column of 50-mesh gauze stainless steel, the spiral being pitched 6 turns to the inch on a central holding rod and fitting on to a Pyrex glass tube; the packed length is 2 ft. and internal diam. 0·6 in. (Lecky and Ewell, *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 544). The number of theoretical plates was determined with a test mixture of benzene and ethylene chloride (Bragg, *Ind. Eng. Chem.*, 1942, **34**, 1083). The column is surrounded by a vacuum jacket which, for high-boiling liquids of the phenol type, is preferably heated by fitting a nickel sheath to slide tightly over the jacket, covering this with 2 or 3 layers of asbestos paper, winding over this insulation a nichrome wire to dissipate 200 watts max. at the voltage of the transformer secondary, and covering the nichrome winding with asbestos paper or string; the heat developed in the winding is controlled by a variable resistance. The top of the column carries a 6-in. water condenser and a simple capillary tap take-off, which ensures a very small retention. The distillation is carried out at a reduced pressure of 50–70 mm of mercury; below this the amount passing through the column is very small and flooding easily occurs. The distillate is removed intermittently, 1 ml every 12 min.

Charge the flask with 80–100 g of the mixture to be evaluated and, when the correct pressure is reached and kept constant by a manostat, apply heat to the column jacket and control by the variable resistance so that the wattage dissipated by the heater is 70–75. Apply heat to the flask, boiling the contents so that flooding occurs, this "wetting-out" of the packing being necessary to give max. efficiency; allow the flooding to subside and repeat flooding until the flood liquid is boiling and vapours rise to the condenser. Allow to subside and adjust the heating until the distillation rate is just below the flood-point. After equilibrium is reached, as shown by the temperature at the top of the column becoming steady, collect the distillate in a roughly calibrated receiver made by sealing a side-arm on to a test-tube, changing the receiver after each portion of 5 ml has been collected. Weigh each fraction accurately and determine the sp.gr. The sp.gr. falls for several fractions and then suddenly rises; stop the distillation at this stage, which represents the cutting-point between the "free" xylenols plus monochloro-compounds and the mono- plus polychloro-compounds. For routine testing, collect the distillate in one fraction; at the cutting-point the distillation almost stops for a time and then drops of distillate are more viscous and obviously of higher sp.gr. Unite the distillate fractions up to the cutting-point and determine the chlorine content. From the results calculate the percent. of unchlorinated xylenols. Retention by the column and presence of traces of dichloro-compounds in the distillate cause the results to be slightly low, but with an efficient column the latter effect can be ignored; the max. error due to column retention is of the order of 1%. The small percent. of higher homologues of phenol present will make the results up to *ca.* 1% too high. E. M. P.

Determination of Primary plus Secondary Amines. J. Mitchell, Jr., W. Hawkins and D. M. Smith (*J. Amer. Chem. Soc.*, 1944, **66**, 782–784)—Primary plus secondary amines can be estimated by acetylation, based on the reactions

$$RNH_2+(CH_3CO)_2O=CH_3CONHR+CH_3COOH$$
$$RNHR'+(CH_3CO)_2O=CH_3CONRR'+CH_3COOH$$
$$(CH_3CO)_2O+H_2O=2CH_3COOH.$$

Excess of acetic anhydride after acetylation is determined by hydrolysis followed by titration of the excess of water with Karl Fischer reagent under conditions previously reported (Smith, Bryant and Mitchell, *J. Amer. Chem. Soc.*, 1941, **63**, 1700). The procedure is as follows. Prepare the acetylating reagent by mixing 1·5 g-mol. (142 ml) of pure acetic anhydride in sufficient dry pyridine to make 1 litre of soln. The hydrolysis reagent is 100 g of (J. T. Baker C.P. or Merck A.R.) dry sodium iodide and 22 ml of water in 1 litre of pyridine soln. The preparation of the Karl Fischer reagent has been described by Smith, Bryant and Mitchell (*J. Amer. Chem. Soc.*, 1939, **61**, 2407). In absence of hydroxyl, transfer the sample, containing up to 10 mg-equiv. of primary plus secondary amine to a 250-ml glass-stoppered graduated flask, weighing the sample directly or adding it volumetrically in pyridine soln. Add exactly 20 ml of the acetylating reagent. Stopper the flask (also a flask containing a blank), shake, and leave for 30 min. at room temp. (In presence of primary hydroxyl, limit the sample size to 10 mg-equiv. of amine plus hydroxyl and allow an extra 30 min. at 60 ± 1° C. to effect complete acetylation.) Add 25 ml of the hydrolysis reagent, using a calibrated pipette. Place the flask in a water-bath at 60° ± 1° C., momentarily raise the stopper to allow for expansion of the included air, firmly stopper and maintain at 60° ± 1° C. for 30 min. Allow to cool spontaneously to room temp. and titrate for water with Karl Fischer reagent. Titrate 10 ml (using a calibrated

pipette) of the hydrolysis reagent for water. Determine free water in the sample by titrating a portion of the sample acidified with glacial acetic acid. Total anhydride added is equiv. to the water used during hydrolysis of the blank, which is determined by subtraction of the water found from the total water added before hydrolysis. Excess of anhydride after acetylation is determined by subtracting water found from total water added to the sample, including free water originally present. The net anhydride is equiv. to the primary plus secondary amine in the sample. Under the modified conditions primary alcohols react quantitatively, but not secondary alcohols; tertiary hydroxyl reacts only slightly. No interference is encountered from amides, urethans, nitriles or tertiary amines. Acetamide, urea and acetanilide do not react. Acetonitrile and adiponitrile reacted to the extent of 0·7% and 0·4%, respectively; dimethylaniline to the extent of 1·3%, possibly owing to secondary amine impurity. The method can be used for the indirect determination of tertiary amines by correcting the total base titre for primary plus secondary amine. E. M. P.

Determination of Amino Alcohols by means of Karl Fischer Reagent. D. M. Smith, J. Mitchell, Jr., and W. Hawkins (*J. Amer. Chem. Soc.*, 1944, **66**, 715–716)—The esterification method for the determination of alcoholic hydroxyl (Bryant, Mitchell and Smith, *J. Amer. Chem. Soc.*, 1940, **62**, 1), depending on the reaction ROH + $CH_3COOH = CH_3COOR + H_2O$, using boron trifluoride as catalyst, has been extended to include hydroxylamines. The water liberated, which is equiv. to the hydroxyl esterified, is determined by titration with Karl Fischer reagent. The procedure is as follows. Prepare the esterification reagent by dissolving about 200 g of boron trifluoride gas in sufficient (Merck C.P.) glacial acetic acid to make one litre of soln. Weigh the sample, containing up to 0·1 g-equiv. of hydroxyl, into a 50-ml glass-stoppered graduated flask about one-third filled with glacial acetic acid, preferably chilling the flask before adding the sample. Make the soln. up to volume with acetic acid. Using a calibrated pipette, transfer 5 ml to a 250-ml glass-stoppered graduated flask and add 20 ml of the esterification reagent. (Where convenient, weigh the sample, containing up to 10 mg-equiv. of hydroxyl, directly into the 250-ml flask, adding 5 ml of glacial acetic acid to the cooled flask containing the sample before adding the reagent.) Stopper the flask tightly and place it, and a control containing 20 ml of reagent and 5 ml of acetic acid, in a water-bath or oven at 70 ± 2° C. for 2 hr. Allow the flasks to cool spontaneously to room temp., add 10 ml of dry pyridine (dry Baker C.P. or Eastman Kodak No. 214–H pyridine used without further purification) to each flask, and titrate with Karl Fischer reagent prepared as previously described (Smith, Bryant and Mitchell, *J. Amer. Chem. Soc.*, 1939, **61**, 2407). After correction for the water in acetic acid, esterification reagent and pyridine, the mg-mol. of water present after reaction, less the water originally present, are equal to the mg-equiv. of free hydroxyl in the sample. The water originally present may be found by titrating 5 ml of the original acetic acid soln. with Karl Fischer reagent. A correction for the water in the acetic acid is obtained by titrating a 5-ml portion and correcting to the vol. of acid present in the sample soln. The procedure is applicable in presence of ammonia or amines, but is affected by aldehydes, ketones, acetals and ketals (Bryant, Mitchell and Smith, *J. Amer. Chem. Soc.*, 1940, **62**, 1). Ortho-esters react quantitatively with the acetic acid in the esterification reagent. E. M. P.

Identification of 2-Methyl-1,4-naphthoquinone. A. Novelli and J. S. Conticello (*J. Amer. Chem. Soc.*, 1944, **66**, 842)—In Novelli's test for 2-methyl-1,4-naphthoquinone (menadione) by means of 2,4-dinitrophenylhydrazine (*Science*, 1941, **93**, 358) the coloured product is insol. in water and must be extracted with amyl alcohol. For very small amounts of menadione, the yellow colour of 2,4-dinitrophenylhydrazine interferes with the green of the product. To avoid these drawbacks, *p*-carboxy- or *p*-sulphonphenylhydrazine, in 1% alcoholic or in cold saturated aq. soln. respectively, may be used, as follows. Add 1 ml of either reagent to 0·1–1 ml of menadione soln., heat for 10 min. at 70–80° C., cool and add 1 ml of 1% sodium hydroxide soln.; the stable reddish-violet colour formed is proportional in intensity to the concn. of quinone present. One μg is detectable. E. B. D.

Potentiometric Determination of Acidity in Highly Coloured Materials. Application to New and Used Petroleum Lubricants containing Additives. L. Lykken, P. Porter, H. D. Ruliffson and F. D. Tuemmler (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 219–234)—The development is described of potentiometric methods for the titration of acidic components of materials soluble only in non-aqueous solvents; the methods are especially applicable to highly coloured or opaque solns. in which coloured indicators are useless. It is claimed that determinations may be made of free and combined acids and bases in many kinds of lubricant, animal and vegetable oils and fats, asphalt, resins, polymers, emulsions and other substances before or after saponification; that in a single titration it is possible to determine two or more components of a mixture of acids or bases which are not distinguishable in aqueous titrations; that estimates may be made of the dissociation constants of acids and bases which are sparingly sol. in water. No useful relation between acid values determined by these methods and those obtained with the use of coloured indicators has been found. The requirements of the method are discussed and the objections to various electrode systems are given. A glass and calomel electrode system is adopted and used in a 1 to 1 benzene and *iso*propyl alcohol mixture containing about 0·5% of water. The behaviour of about 40 different combinations of solvents and electrodes is detailed. Solutions of hydrochloric acid or potassium hydroxide in *iso*propyl alcohol are generally used as reagents. Inflection points in the titration curve or fixed cell potentials are taken as end-points. For particulars of the apparatus used (some items of which are critical), procedure, precautions, interpretation and details of the behaviour of specified materials, the original should be consulted. L. A. D.

Inorganic

Determination of Bismuth. I. Gravimetric Analysis with Phenylarsonic Acid. A. K. Majumdar (*J. Indian Chem. Soc.*, 1944, **21**, 119–124)—A method for the precipitation of bismuth as phenylarsonate (suggested formula $C_6H_5AsO_3.Bi(OH)_2$, which contains 49·06% of

bismuth) from an acetic acid soln. buffered with sodium or ammonium acetate (*p*H 5·1–5·33) is described. By this method bismuth can be separated from thallous, alkali, alkaline earth, cobalt, silver, mercuric, cadmium, copper, nickel, zirconium, thorium, tin and lead salts. Iron, aluminium, beryllium, uranium, titanium, chromium, zinc, manganese, antimony, mercury(ous), citrate, tartrate, oxalate, phosphate, arsenate, fluoride and chloride must be removed. *Reagents*—Phenylarsonic acid, 1% soln. in water; sodium acetate, 10%; ammonium acetate, 10%; acetic acid, 2 *N*. *Method I*—Add an excess of reagent to a faintly acid soln. of bismuth nitrate (0·02 to 0·2 g of bismuth in *ca.* 250 ml) and then sufficient sodium acetate soln. to neutralise the mineral acid. Boil and stir until the gelatinous ppt. becomes crystalline. Add a little more sodium acetate if the ppt. remains gelatinous, but avoid a large excess. Leave to settle, filter hot on a Gooch crucible, wash with hot water and dry at 110–120° C. *Method II*—Add excess of reagent to a bismuth soln. acidified with nitric acid (if sulphate ions are present bismuth sulphate may precipitate at this stage; add a little more nitric acid and warm to effect solution before continuing). Neutralise with 2 *N* ammonia, acidify with acetic acid and add 2 ml in excess and 20 ml of ammonium acetate soln. Dilute to 250 ml, boil, and stir until the ppt. becomes crystalline. *Separation from copper, silver, cadmium, cobalt and nickel*—Add excess of reagent to a nitric acid soln. containing bismuth and any of the above, neutralise with ammonia and add sufficient potassium cyanide to keep the other cations in soln., acidify with acetic acid and proceed as in Method II. *Separation from zirconium and tin*—Add excess of reagent to a 5% hydrochloric acid soln. to ppt. the zirconium or tin and filter. Wash the zirconium ppt. with 1% hydrochloric acid and the tin ppt. with 4% nitric acid. Evaporate the filtrate and washings to dryness and "fume" with the min. amount of sulphuric acid. Dissolve the residue in a few drops of nitric acid, to prevent hydrolysis, and dilute with water. Proceed as in Method II. *Separation from thorium*—Precipitate the thorium from a nitrate soln. (400 ml), containing 75 ml of acetic acid and 5 g of ammonium acetate, by dropwise addition of the reagent. Filter off the ppt., wash, dissolve in hydrochloric acid (1 : 1), re-ppt. and filter. Evaporate the combined filtrates, "fume" with the min. quantity of sulphuric acid and proceed as in Method II. A volumetric method is under investigation. C. F. P.

Colorimetric Determination of Chromium in Steel. L. Singer and W. A. Chambers (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 507–509)—A rapid method is described not involving any separation, based on the fact that the colourless ferric perchlorate intensifies the colour of the chromic acid. Take 1 g of steel for chromium up to 0·1%, and 0·5 g for higher percentages. Dissolve in 10 ml of nitric acid (1 : 1) and 20 (15) ml of perchloric acid in a 125-ml beaker. Evaporate until dense fumes are evolved and boil gently for 5 min. to peroxidise the chromium. Cool in running water, dissolve the solids in 20 ml of water, transfer the soln. to a glass-stoppered 50-ml flask and adjust the volume. Transfer an aliquot to the absorption tube of a Klett-Summerson photoelectric colorimeter, reduce the soln. with a small crystal of ferrous ammonium sulphate (0·01–0·02 g), and adjust the instrument to zero. Insert another aliquot and take a second reading, which gives the colour due to chromium. Unless appreciable amounts of other highly coloured ions are present, make subsequent measurements by taking a reading with the oxidised soln. first, then reducing with ferrous salt prior to the second reading, and take the difference between the two. An absorption tube of 12·5 mm internal diameter and a colour-filter transmitting at 410–480 $m\mu$ were used. The results are read on a graph constructed with samples of known composition similar to that of the test sample. W. R. S.

Polarographic Reduction of Rhodium Compounds. J. B. Willis (*J. Amer. Chem. Soc.*, 1944, **66**, 1067–1069)—Tests made with chloride solns. after treatment with excess of various complex-forming compounds proved that polarographic steps could be observed, indicating the possibility of analytical application. Reduction of a number of complexes took place through the rhodous stage; direct reduction to metallic rhodium was not observed. A pyridine-potassium chloride soln. gave a well-defined step at the rhodous stage, and it is thought to be the most suitable medium for analytical work. W. R. S.

Analysis of Anhydrous Hydrofluoric Acid. W. B. Sherry, C. F. Swinehart, R. A. Dunphy, and S. C. Ogburn (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 483–486)—The detailed description of the method of sampling, the constructional details of the Saran weighing tube (made by The Harshaw Chemical Co., Cleveland, Ohio) and the general precautions required in these operations should be read in the original. The anhydrous acid is received in the tared weighing tube containing ice, and the determinations are made on aliquots of aqueous acid taken by weight. *Sulphur dioxide*—Determine this first to minimise risk of loss. Into a tared waxed 250-ml beaker containing 50 ml of water, 10 ml of 0·1 *N* iodide-iodate soln. and a Bakelite rod, pour 50 g of an aliquot portion and weigh to ±0·5 g. Titrate the excess of liberated iodine, using starch and 0·1 *N* thiosulphate. If no colour appears upon addition of the starch, titrate with iodide-iodate to a blue end-point. *Total acidity*—Submerge a platinum weighing-bottle containing the weighed aq. acid (7–8 ml) in a 200-ml platinum dish containing 25 ml of water, 1 ml of 0·1% phenolphthalein soln. and 75 ml of *N* sodium hydroxide; loosen the cover, stir, and titrate with more alkali to the first appearance of permanent pink. Heat to boiling and continue the titration if the colour fades. *Hexafluorosilicic acid*—Weigh 50 g (±0·5 g) of aq. acid in a platinum beaker (dull finish). Stir in 0·2 g of sodium chloride and evaporate to dryness on a steam-bath. Dissolve in 25 ml of water, stir in 2 g of potassium chloride, and add 1 ml of phenolphthalein soln. Cool in ice for at least 15 min., titrate cold with silica-free *N* sodium hydroxide to near the end-point, and finish with 0·1 *N* sodium hydroxide to the first pink persisting for at least 15 sec. The vol. of alkali used is not required. Heat the soln. to boiling, titrate with 0·1 *N* alkali to a pink end-point, reheat to boiling and finish to a pink persisting for 45 sec. 1 ml of alkali consumed in the hot titration ≡ 0·0036 g of H_2SiF_6. A correction factor should be applied for iron, if present. For 0·02% of Fe, add 0·007% of H_2SiF_6; for 0·04%, 0·017; for 0·06%, 0·031; for 0·10%, 0·049%. *Sulphuric and fluosulphonic acids*—Evaporate a 50-g aliquot in a platinum dish to dryness on a steam-bath, add 10 ml of water and again evaporate. Repeat

until no odour of hydrofluoric acid can be detected while the liquid is hot, then evaporate once more. Add 25 ml of water free from carbon dioxide, 1 g of neutral sodium fluoride free from silica to form ferric and aluminium complexes, and potassium oxalate if any copper, nickel, or lead is present. Titrate with 0·1 *N* alkali (phenolphthalein as indicator), and calculate to sulphuric acid equivalent. *Water* is found by difference. W. R. S.

Microchemical

Microscopical Identification of Strontium, Ammonium, Copper and Zinc by means of their Crystalline Picrolonates. W. V. Eisenberg and G. L. Keenan (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 458–462)—The method previously described for the identification of sodium and potassium (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 177; ANALYST, 1944, **69**, 225) is applicable to the detection of strontium, ammonium, copper and zinc. To a fragment of the unknown substance, or to a neutral or very slightly acid drop of soln., add a drop of a 0·5% soln. of picrolonic acid in 50% alcohol. Warming is not usually necessary. Each cation is identifiable by its characteristic crystal, which forms immediately and is visible at 200×. When the crystalline form has been observed, allow the ppt. on the slide to dry at room temp. and examine it by the immersion method with the polarising microscope. The tabular prisms (with "envelope" and "roof-top" modifications) of strontium picrolonate are formed in presence of relatively large concns. of barium and calcium ions, and the bifurcated yellow rods of ammonium picrolonate are formed slowly even in presence of relatively large concns. of sodium and potassium ions. For the detection of copper the test is very sensitive. Copper, zinc, sodium and potassium ions form yellow needles and rods, and their identification requires optical-crystallographic determinations on several crystals. The minimum refractive indices (n_α) are —strontium, 1·448; ammonium, 1·644; zinc, 1·650; copper, 1·734. The ammonium and zinc compounds have an intermediate index (n_i) of 1·734, and the maximum index ($n\gamma$) for all four compounds, as well as for the potassium and sodium salts, is >1·734. The strontium salt shows inclined extinction, the ammonium, zinc and copper salts parallel extinction. The strontium salt shows negative elongation, and the ammonium, zinc and copper salts positive elongation. Strontium and ammonium picrolonates are thus identifiable by their crystalline form. With the picrolonates of the other four elements, needles exhibiting inclined extinction will indicate potassium, and those showing parallel extinction will indicate copper, sodium or zinc. The elongation is negative for sodium and positive for copper and zinc. Copper and zinc crystals are distinguished by their refractive indices. The procedure described is most effective with salts of these cations present in dry physical mixtures (*e.g.*, in food and drug products), from which the unknown material may be isolated as minute fragments under a low power, wide-field binocular microscope. A. O. J.

Colorimetric Micro-determination of Sulphate. B. Klein (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 536–537)—The sulphate is pptd. with benzidine salt, and the ppt. is diazotised and coupled with N-(1-naphthyl) ethylenediamine dihydrochloride (Bratton and Marshall's reagent, *J. Biol. Chem.*, 1939, **128**, 537). Introduce the sample soln. (0·015–0·15 mg of sulphate) into a 15-ml centrifuge tube, with narrowed conical bottom, containing 1 ml of glacial acetic acid and 1 ml of benzidine hydrochloride soln. (4 g of the salt in 250 ml of 0·2 *N* hydrochloric acid). Mix, add 2 ml of acetone-alcohol (1 : 1), close the tube and roll it between the hands. Set aside for 30 min. in ice, then centrifuge for 10 min. (2500 r.p.m.). Pour off the clear liquid in one quick motion and touch the end of the inverted tube with a clean filter pad; allow the tube to drain on the pad for 5 min., rinse down with 2 ml of acetone-alcohol without disturbing the ppt., centrifuge for 5 min., pour off and drain as before. Repeat the washing once more. Dissolve the washed ppt. in 2 ml of 0·2 *N* hydrochloric acid, place the tube in ice, add 1·0 ml of freshly-prepared 0·1% sodium nitrite soln., mix by shaking, and set aside for 3 min. Add 1 ml of 0·5% ammonium sulphamate soln., mix and set aside for 2 min. Add 1·0 ml of a 0·1% soln. of the reagent, mix and set aside for 20 min. Transfer the violet soln. to a 50-ml graduated flask, adjust the volume, and examine an aliquot portion in a Klett-Summerson photoelectric colorimeter, using a green filter (Klett No. 54). Make a blank assay on 2 ml of 0·2 *N* hydrochloric acid to obtain the zero reading. Standardise on either a benzidine soln. (which need only be diazotised and coupled) or a potassium sulphate soln. The former is made from 0·4014 g of pure hydrochloride dissolved in 100 ml of 0·2 *N* hydrochloric acid, and 1 ml of this stock soln. is diluted to 100 ml with the dil. acid; 1 ml ≡ 0·015 mg of sulphate. Phosphates should not be present in the material to be analysed; chloride can be tolerated below a weight ratio of Cl:S = 30. Above this, the benzidine sulphate pptn. is incomplete. W. R. S.

Physical Methods, Apparatus, etc.

Spectrographic Determination of Lead in Pectinous Materials. C. R. Jeppesen, E. J. Eastmond and H. G. Logan (*J. Opt. Soc. Amer.*, 1944, **34**, 313–318)—Lead in solns. of apple pomace extract, about 1 part in 10^7 or 10^8, and in solid pectin, about 1 part in 10^6, may be determined spectrographically on dry-ashed samples. Samples in solution contain 1·5% to 2·5% of total solids and the solid samples have ash contents 0·3% to 0·8%. Dry-ashing avoids contamination from chemical reagents, gives a sample which will burn satisfactorily on the arc and concentrates the lead. Use of an added internal standard and a spectroscopic buffer avoids difficulties associated with variability of ash composition. Maximum stability of the arc may be obtained by using specially-shaped graphite electrodes; these are ¼ in. diam. and each has a centre post, ¼ in. diam., ⅛ in. long, turned on the end. In addition, the positive (lower) electrode has its centre post surrounded by a moat, 1/32 in. wide, 3/32 in. deep, in which the sample is placed. With an arcing current of 15 amp. the arc strikes between the two posts and wanders less than with the usual centre-cup electrodes. *Procedure*—Mix some of the dry-ashed sample in a mortar with an equal quantity of a synthetic ash (5% of basic magnesium carbonate, 25% of calcium carbonate, 25% of sodium carbonate, 30% of silicic acid, 5% of ferrous sulphate and 10% of chromium oxide) and three times its weight of lithium carbonate containing 0·233% of bismuth. Also prepare standard samples by adding known amounts of lead to some of the synthetic ash and mixing the same additional quantities of the

synthetic ash and standard buffer as for the unknown samples. Arc about 15 mg of the sample on the positive electrode of a D.C. pure graphite arc and prepare the spectrogram in the normal manner. Calibrate by using the standard samples to obtain a relation between lead content and ratio of the intensities of the lines Pb2833A and Bi2898A. Concns. of lead as small as 0·000001% in the original sample and 0·005% in the ash have been detected and measured. With triplicate exposures, the results of repeated determinations on a single sample gave a standard deviation of 3·7% and, with separately ashed samples, 7%. B. S. C.

Spectrographic Method for Small Amounts of Calcium in Magnesium Metal. T. Whitehead and A. J. Boyle (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 455–456)—Calcium may be determined in amounts ranging from 0·0005 to 0·1% by the use of a high voltage spark between a sample of magnesium metal and a high purity graphite electrode. Since no satisfactory chemical method was found for the estimation of these small calcium concns., the calcium content of those samples selected to serve as standards for calibration was determined by a spectrographic procedure. Solns. of these samples, with and without known small additions of a calcium salt soln., were examined spectrographically by arcing or sparking impregnated graphite electrodes. Extrapolation of the curves obtained for these synthetic solns. gave the calcium contents of the selected metal samples. These then served as a basis for the construction of a final calibration curve for the routine method. B. S. C.

Photometric Method for the Determination of Hemicellulose. C. J. Barton and A. J. Prutton (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 429–430)—The first step in the production of viscose rayon is the steeping of the pulp in 18% sodium hydroxide soln. A rapid and reliable method for the determination of the alkali-soluble material, hemicellulose, is necessary where a close control of the concn. of the steeping soln. is required. *Procedure*—Take an amount of sample, according to the amount of hemicellulose present, as follows. Concn. range 0·00–0·12% required 25 ml of sample; concn. 0·12–0·30%, 10 ml; concn. 0·30–1·20%, 2·0 ml; concn. 1·2–2·4%, 1·0 ml. For samples less than 25 ml add sufficient pure sodium hydroxide soln. or distilled water to bring the vol. to 25 ml. The sodium hydroxide concn. should be approx. the same as that used in the hemicellulose solns. from which the calibration curve is prepared. Add 5 ml of *N* potassium dichromate and then, in a 250-ml Erlenmeyer flask, add cautiously 25 ml of conc. sulphuric acid. Boil the soln. for 30 sec. and cool. The final vol. is 49 ± 1 ml and is stable in colour. Measure the transmission of a suitable thickness (13 mm) at 600$m\mu$ with a spectrophotometer or absorptiometer and deduce the hemicellulose concn. from a calibration curve prepared from samples of known concn. Determine the hemicellulose in the calibration samples iodimetrically from the excess of dichromate. Beer's Law holds up to a hemicellulose concn. at which nearly all the chromic acid is reduced. The photometric method gives a precision under routine conditions of ±1·5% and determinations can be completed in 5 min. B. S. C.

Infra-red Analysis of Butadiene. L. J. Brady (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 422–424)—Refined butadiene contains varying amounts of impurities such as butene-1, butene-2, perhaps traces of *iso*butylene and acetylenes, together with small amounts of saturates, the latter usually amounting to less than 5% of the total impurities present. At wavelength 6·9μ, butadiene is relatively transparent, but the above impurities all absorb strongly. In particular, the absorptions of the major impurities, butene-1 and butene-2, at this wavelength are of the same order of magnitude. Thus, as long as the concns. of the total impurities remains constant, fluctuations in their relative amounts will have no significant effect on the optical densities of refined butadiene samples. Measurement of the transmission of the sample at 6·9μ enables the total impurities to be determined, the butadiene being determined by difference. A simplified form of infra-red spectrometer may be used as a plant control instrument. After standardisation, only 10 min. is required to make duplicate infra-red determinations of butadiene, whereas the maleic anhydride method requires 5 to 6 hr. B. S. C.

Chemical Analysis by Powder Diffraction. L. K. Frevel (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 209–218)—This paper illustrates by a number of examples the application of X-ray powder diffraction methods to chemical analysis and deals briefly with several of the difficulties, encountered in practice, which can give rise to incorrect interpretation of the patterns. The subjects discussed include absorption edges, fluorescence of the specimen, resolution (including suitable standards for calibrating cameras), absorption in the specimen, preparation of the specimen, the validity of published standard patterns (several examples are given of standards in which the compound concerned is wrongly described or obviously impure, and a few in which the diffraction data are incorrect), and the distribution of reference lines with respect to inter-planar spacing. There is already difficulty in locating desired standard patterns, and this will increase as the number of standards grows. A systematic classification is proposed which is based on knowledge of the elements present in the specimen whose pattern is sought, and is intended to be used in conjunction with preliminary spectrographic and chemical examination. Many tabulated data illustrating the points discussed are given. The tables comprise: (i) chemical, microscopical and diffraction analysis details of a boiler scale; (ii) diffraction data for the same boiler scale, showing the setting-out of the measurements and the comparison with standards; (iii) the preparation of powder specimens and difficulties and precautions associated with various methods; (iv) details of the examination of a sample of magnesium oxide, showing the setting-out of the data and the scope of the information which should be recorded; (v) ambiguities arising from solid solution or *iso*morphism; (vi) partial ambiguities due to *iso*morphism; (vii) ambiguities arising from structural similarities; (viii) defect structures; (ix) index to the patterns of aluminium-containing substances, which with (x) is a model of part of the proposed classification; (x) index to the patterns of iron-containing substances; (xi) powder diffraction data, exemplifying the use of the proposed tables. A bibliography of 63 items is given. L. A. D.

Physical Methods of Separating Fibres. R. Lassé (*Melland Textilber.*, 1943, **24**, 466–470)—For qualitative or quantitative analysis of mixtures of textile fibres, disintegrate 2 sq.cm. of the

conditioned and weighed sample and shake the fibres vigorously with a liquid of suitable sp.gr. (*vide infra*) in a 7-ml stoppered glass tube with a conical base. Then place the tube in water at 60° C. until separation by flotation is complete, pour off the floating fibres, with all but *ca.* 0·5 ml of the liquid, through a filter, and dry and weigh the resulting mat of fibres. Insert in the mouth of the tube a cork having a slit in its side and holding a narrow tube with a bent pointed end, and evaporate the residual liquid by drawing air through the tube; weigh the residual fibre. The method is rapid, requires very little sample, and may be applied to most textile mixtures; many examples are given. The microscope is an aid in ascertaining when separation is complete; the fibres should be stained selectively and mounted in medicinal paraffin. The range of the method may be extended by the use of selective solvents for certain fibres, or by altering the relative floatibility or sp.gr. of the constituents of fibre mixtures which are otherwise similar in these respects. Thus, wool is first de-fatted by ether, or colloidal metal (*e.g.*, silver, gold or copper) is deposited on the fibres; the last method has proved useful in separating ramie and matt viscose rayon. The principal liquids used alone or mixed for separation by flotation are: acetone, ether, ethyl alcohol, benzene, glacial acetic acid, chloroform, carbon tetrachloride and bromoform. Fibres which may be separated in this way (with their sp.gr.) are cuprammonium rayon, 1·52; viscose rayon, 1·42–1·53; linen, 1·46; cotton, 1·48–1·56; plant fibres, 1·50; natural silk, 1·37; acetate rayon (often sol. in the reagent), 1·25–1·33; sheep's wool, 1·26–1·34; "Tiolan," 1·305. J. G.

Determination of the Permeability to Aromas of Packaging Materials. F. Kiermeier (*Chem. Tech.*, 1943, **16**, 204–206)—Since it is not practicable to test packaging materials for permeability to all of the many components of odours and flavours, it is necessary to select a few typical substances, *e.g.*, ethyl acetate (representing fruit esters), trimethylamine (fish odours), naphthalene (representing solid compounds which sublime readily at normal temps.) and limonene (representative of solid compounds which are water-insol. and do not readily vaporise). Permeability to ethyl acetate is determined by using the sample to be tested to seal a metal cup containing a known vol. of a 0·5% aq. soln. of the reagent, and analysing the residual soln. by the potassium dichromate method after storage under appropriate conditions. A similar method is used for trimethylamine; the reagent is titrated with 0·1 *N* HCl. A correction for losses due to vaporisation into the air-space inside the cup is obtained from a blank test in which a metal sheet is substituted for the sample. In tests with naphthalene, a filter-paper is impregnated with the reagent and placed between 2 discs of the sample, the edges being sealed; the rate of loss of wt. of the assembly is measured, but a correction (obtained by omitting the impregnated paper) must be made to eliminate the effect of changes in the relative humidity of the laboratory. A vessel containing limonene is sealed with the sample and placed in a closed glass-vessel with a dish of conc. sulphuric acid. The vapour passing through the sample is absorbed in the acid, and may be determined by the potassium dichromate method. Data for 16 samples are tabulated. J. G.

Reviews

PLASTICS FOR PRODUCTION. By P. I. SMITH. Pp. viii + 180. London: Chapman & Hall, Ltd. 1944. Price 12s. 6d.

According to some very enthusiastic technologists we have reached the beginning of what future historians are to call the "Plastic Age," and many books are now being written about the universal materials of that age. The present work, however, is not concerned with "boosting" of that character; to quote from the preface, it is an attempt to "present a balanced review . . . a sober and, indeed, somewhat critical study" of the more important plastics. Repercussions on civilisation are not discussed, nor are the economics of the subject. In fact, it is refreshing to read a book on plastics in which not even the sources of raw materials are examined, but which is devoted solely to a description of the properties of these undoubtedly important products.

A warning is necessary, however, for the chemist will find but little chemistry in this account, while the analyst is not considered at all. Besides discussion in general terms and from many aspects, this book consists largely of numerical values for physical and mechanical characteristics, including thermal, optical and electrical data where relevant. The reader is advised of pitfalls in interpreting such data, and the value of actual service tests is pointed out; a difficulty is that "published results appertaining to one particular resin may not necessarily apply to a similar and apparently identical product manufactured at a different plant." Chemical information is nearly restricted to the behaviour of thermo-plastic resins towards solvents, etc., this being important for materials employed for tank linings and similar purposes. A few errors of a chemical nature occur; for example, on p. 69 phosphorus is mentioned instead of the pentoxide as a desiccant, while on p. 156 "H_2O_2 100% vol." is an unfamiliar expression. "Nylon" is described correctly on p. 20 as a super-polyamide, and it is unfortunate, and possibly misleading, that tables of its properties should be given (p. 169) without any textual matter, immediately following a description of poly*iso*butylene.

Two features of the book which detract from the pleasure of reading it are: (1) the constant use of "Deg." instead of the degree sign (31 times on one page!), and (2) the inclusion of references in the main body of the text, often in a very extended form. These defects, however, are of a secondary character, and the book will be found useful, particularly for reference to the many numerical data. G. H. WYATT

HACKH'S CHEMICAL DICTIONARY. 3rd Ed. Revised and Edited by JULIUS GRANT, M.Sc., Ph.D., F.R.I.C. Pp. ix + 925. London: J. & A. Churchill, Ltd. Price 60s.

It is about thirty years since Ingo Hackh conceived the idea of a chemical dictionary on novel lines. Besides giving definitions like those in ordinary chemical dictionaries, it was intended also to illustrate the evolution of chemistry and to include technical names as well as terms used in cognate sciences, such as physics, astronomy, geology and botany. His effort met with immediate success, and within a few years there was a demand for a second edition. In the preparation of this, Dr. Grant collaborated with Dr. Hackh, with the result that much more space was given to the work of British chemists than in the first edition.

Hackh died in 1938, and the third edition has been edited by Dr. Grant alone, who has made the dictionary still more representative of the work of both nations. The historical notices have been well distributed between European, American and British chemists, and a few new portraits have been added. Of the older portraits, some are still good, whilst others have begun to look like caricatures, owing to the blocks having become worn. Another minor point is that the wording of the notice on Jocelyn Field Thorpe tends to suggest that he was an originator as well as the later editor of *Thorpe's Dictionary of Applied Chemistry*.

The total number of definitions in the Dictionary is now more than 57,000, including a good selection of trade names, such as Diakon, Hemit, Luminogram, Freon, Gummou and so forth. Where necessary, there are good diagrams, *e.g.*, of apparatus, alchemical symbols, chemical structure, crystallograms, and periodic arrangements of the elements. As regards the definitions of chemical compounds, we have tested the Dictionary on a number, including some unusual ones, with which we happen to be familiar, and have not once found it wanting.

The work should continue its successful career as a handy book for general reference.

EDITOR

INAUGURAL MEETING OF THE PHYSICAL METHODS GROUP

THE inaugural meeting of the Physical Methods Group will take place on Wednesday, February 7th, 1945. Particulars of the meeting will be sent to members of the Group in due course. The Hon. Secretary will be pleased to receive the names of any further members of the Society who wish to become members of the Group.

Papers for Publication in THE ANALYST

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

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

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

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