THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society of Public Analysts was held on Wednesday, May 6th, 1936, at the Chemical Society's Rooms, Burlington House, the President, Dr. G. Roche Lynch, being in the chair.

Certificates were read in favour of:—John Glover, Arthur St. George Huggett, D.Sc., Ph.D., M.B., B.S., M.R.C.S., L.R.C.P., Frank Ernest Alban Leibbrandt, M.A., John Horsford Seager, M.Sc., and Alfred Pattinson Telford.

The following were elected members of the Society:—Ir. Willem Jan Pieter Pelle, George Hugh Walker, Ph.D., B.Sc., F.I.C., Herbert Wood Watson, M.Sc., Harold Frank Philip Webber, B.Sc., A.I.C.

The following papers were read and discussed:—"The Effect of some Impurities in Anaesthetic Ether: Peroxides," by J. H. Coste, F.I.C., F.Inst.P., and D. C. Garratt, B.Sc., Ph.D., F.I.C.; "The Determination of Bromides in Presence of other Halides," by F. W. Edwards, F.I.C., H. R. Nanji, Ph.D., A.I.C., and E. B. Parkes, M.Sc., A.I.C.; "A New Photographic Filter Cell," by C. Ainsworth Mitchell, M.A., D.Sc., F.I.C., and T. J. Ward; and "A Micro-Zeisel Apparatus for Determining Methoxyl and Ethoxyl Groups," by J. J. Chinoy, M.Sc., Ph.D.

The Determination of Moisture-Content by Distillation with Liquids Immiscible with Water

BY F. G. H. TATE, F.I.C., AND L. A. WARREN, PH.D., B.SC., A.I.C.

(*Read at the Meeting, April* 1, 1936)

PART I. METHODS AND APPARATUS

THE numerous papers describing methods for the determination of the moisture-content of a substance by entrainment distillation with an organic liquid immiscible with water afford evidence that the process is increasing in use and importance. It finds particular application in the rapid determination of moisture in materials not conveniently examined by other methods, but the process has been developed to provide ease of manipulation rather than great accuracy. Many forms of apparatus are described in the literature with no accompanying data whereby

the accuracy of the instrument may be determined, and where such data are quoted they are often in the form of comparison between results obtained with the instrument and results from other standard drying processes, such as the use of an oven at 100° C., which are notably open to criticism.

ENTRAINERS HEAVIER THAN WATER.—The most noteworthy methods are those due to Tausz and Rumm,[1] to Pritzker and Jungkunz,[3] and to Friedrichs.[5] The first consists in gently boiling the material to be examined with a suitable liquid in a round-bottom flask connected with a tall upright air condenser partly packed with glass beads. The upper end of this air condenser turns downward and leads to a Liebig condenser, which in turn is connected with a eudiometer adapted to collect a liquid. After its contents have been boiled for a sufficient length of time to remove all water into the cooled condenser, the flask is heated strongly to drive over sufficient liquid to wash the interior of the condenser free from water-drops. The water which has been collected as a layer on top of the entrainer in the eudiometer is transferred to a special calibrated capillary measuring-tube, designed to measure 2 ml. of water to 0·01 ml. Tausz and Rumm quoted data showing that an accuracy of 99·5 per cent. on 2 ml. could be obtained, but Thielepape and Fulde[7] found a variation of 2·5 per cent. on 2 ml. of water, and criticised the method for inconvenience in manipulation, since the apparatus required careful watching during distillation.

The type of apparatus due to Pritzker and Jungkunz[3] was an improved form of that devised by van der Werth.[2] The main feature was the collection of the water in a tube placed directly below a condenser with an internal cooling surface. The entrainer fell through the collected water and automatically returned to the flask. Fairbrother and Wood[4] criticised the form of condenser and preferred the Liebig type. They claimed an accuracy of 99·5 per cent., but gave no data in confirmation. The necessity for having a wide collection-tube to ensure a free return of the entrainer to the flask prevented accuracy of measurement. For their own instrument Pritzker and Jungkunz did not claim great accuracy, whereas Schimon[6] quoted results showing an error of 3 per cent. on 3 ml. of collected water, and Thielepape and Fulde[7] obtained an accuracy of about 98 per cent. The tendency for water-drops to become trapped in the condenser was emphasised by all the above workers, and by Lepper[8] and Lundin.[9] Lundin pointed out that any type of condenser which condensed an ascending stream of vapour must tend to trap water, and therefore designed an apparatus embodying the condensation of a downward stream of vapour, but as his apparatus was planned to collect 35 ml. water, it was useless for the accurate determination of small volumes. No results were quoted.

The method due to Friedrichs[5] appears to possess many good features. In this apparatus the water and vaporised entrainer are condensed in a downward stream, and the water is collected in a small tube, which projects above the main tube carrying the entrainer back to the distillation flask. There is no flow of liquid through the collecting tube, which can consequently be made of capillary dimensions, with correspondingly high accuracy of measurement. Insufficient data for estimation of the capabilities of the instrument were provided by Friedrichs, but both Schimon[6] and Thielepape and Fulde[7] gave good reports.

ENTRAINERS LIGHTER THAN WATER.—The original method of Marcusson[10] was greatly improved by Dean and Stark,[11] whose apparatus resembled that of Pritzker and Jungkunz in its essentials, but possessed the great advantage that the water was collected in a tube away from the main stream of returning liquid. The collecting tube could therefore be made of any dimensions in accordance with the accuracy of measurement required. The method has always been open to the criticism that a ring of water-drops tends to collect in the condenser-tube above the vapour, as Dean and Stark themselves pointed out. Many attempts have been made to eliminate this cause of error by modifications in the design (Normann,[12] Pritzker and Jungkunz,[13] Schaefer,[14] Boller,[15] Lundin and Lundin[16]), but in no instance where data are quoted does the accuracy of estimation seem to be very great. Jones and McLachlan,[17] in a critical survey of Dean and Stark's apparatus, recommended the removal of water droplets from the condenser by means of a copper-wire spiral, but although comparison of distillation and several other methods was made on many materials, there was no absolute determination of its accuracy when using pure water.

The Dean and Stark apparatus has been approved by the Association of Official Agricultural Chemists[18] and by the Institution of Petroleum Technologists for use in the tentative method for determining moisture in cattle feeds, following a report upon the apparatus by Bidwell and Sterling,[19] whose sole innovation consisted in using a collecting tube of narrower diameter, thereby gaining greater accuracy of measurement. Toluene and xylene were employed as entraining liquids, and the collecting tube was calibrated by distilling into it known volumes of water. The difficulty arising from water-drops sticking in the condenser was emphasised. These drops were removed into the tube by washing down the condenser with the liquid, brushing with a tube brush and using a small rubber squeegee. A comparison of the results by this method and the standard oven-drying methods for numerous food products gave good general agreement, with a tendency for the results by distillation to be higher than those by oven-drying. Of especial interest were the distillations of copper sulphate pentahydrate and sodium sulphate decahydrate, from which four and three molecules of water, respectively, were removed.

PRESENT INVESTIGATION.—From the above survey, the most satisfactory types of apparatus appeared to be those due to Friedrichs, and to Bidwell and Sterling.

Trials with Friedrichs' apparatus showed that in practice this type was far from satisfactory. The apparatus was tested by distilling known amounts of water and entrainer from the flask, and observing the amount collected in the previously-calibrated collection tube. Over a series of experiments, in which 0·5 to 1·0 ml. was used, the quantity of water collected varied from 84 to 97 per cent. of that introduced. The loss was traced to droplets of water clinging to the long narrow capillary tube, which could not be prevented by thorough cleaning. Further losses appeared to occur at the pressure-equalising hole, and by evaporation through bubbling in the collection tube. The losses were not constant, and little improvement was effected by first "saturating" the apparatus by a preliminary distillation of entrainer and water. Although perchloroethylene is generally

assumed to be the best heavy liquid available, it is open to the objections that water is appreciably soluble therein, and that some hydrolysis occurs on storing the moist liquid for long periods.

Preliminary trials with Bidwell and Sterling's apparatus indicated that the accuracy to be obtained was at least as great as with that of Friedrichs. The method of testing was the same as with the latter. The cork connections were found to be unsatisfactory and trouble was experienced with the method of removing water-drops from the condenser into the collecting tube. The introduction of a copper wire, as recommended by Jones and McLachlan,[17] did not effect complete removal. An attempt was made to reduce the surface tension between water and the glass surface by coating the latter with a substance such as sodium silicate. This, however, was found ineffective, and its solution in the water distilled introduced an uncertainty as to the purity of the water. For the same reason the introduction of acetone or alcohol into the distillation liquid was condemned as theoretically unsound, and in practice was found not to assist the removal of water.

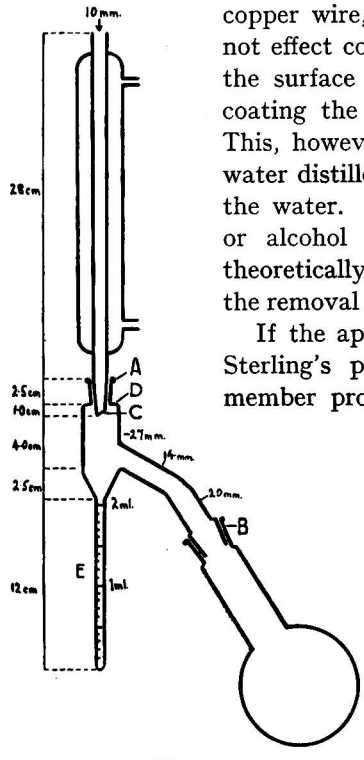


Fig. 1

If the apparatus is assembled as illustrated in Bidwell and Sterling's paper, two traps are formed where the internal member projects at each joint. Drops of water condense in these traps, and can be removed only with difficulty. Experience resulted in modifications which enable the apparatus to measure 2·00 ml. of water to within 0·01 ml., *i.e.* with an accuracy of 99·5 per cent. Moreover, the method, as now developed, is simple in operation, the apparatus is easy to construct and to clean and may be used for prolonged distillation, while the water collected can readily be removed for examination.

All these features were desirable in the work for which the apparatus was designed.

CONSTRUCTION.—The apparatus finally adopted conforms to the dimensions of the sketch (Fig. 1), and is constructed throughout of Pyrex glass, which appears to permit better drainage of water than soda-glass. Features essential to the satisfactory working of the apparatus are the following:

1. Cork connections are replaced at A and B by ground-glass joints, which may be of standard size.

2. The condenser tube, C, projects only a short distance into the cell below, to avoid the formation of a trap where water droplets can lodge. The projection should not be entirely eliminated (by grinding the condenser tube flush with its seating), as the condensed water then tends to adhere to the walls of the cell instead of falling cleanly into the graduated tube. The shoulder, D, is brought out squarely from the condenser-seating, thus avoiding any constricted space in the cell.

3. The internal member of the ground joint, B, does not project into the distillation flask.

4. The internal diameter of the graduated collecting tube should be 5 mm. A reading correct to 0·01 ml. can easily be taken with a tube of this diameter, but if the tube is narrower, water-drops tend to cling to the upper part without falling to the bottom.

5. The uppermost calibration-mark on the collecting tube is as close as possible to the junction of this tube with the cell above, to minimise the glass surface down which the collected water must drain. It is desirable to arrange for the maximum quantity of water to be collected, by using the necessary amount of material. This provides the greatest possible accuracy of measurement, and at the same time reduces to a minimum possible error due to loss of water by formation of a film on the glass surface.

6. The condenser tube has a smooth internal surface to facilitate drainage of water.

MANIPULATION.—The whole apparatus is thoroughly cleaned with chromic-sulphuric acid and washed out with water followed by acetone. It is then dried in a stream of dry air. A suitable quantity of the material to be examined is placed in the distillation flask, upon a layer of dry sand if it tends to cake or to stick to the walls of the flask. The sample is covered with liquid, and a few silica beads are added, if necessary, to prevent bumping. The apparatus is assembled after both ground joints have been treated with a trace of vaseline; the collecting tube is then filled with liquid poured down the condenser. The whole apparatus is lagged, so that the heating-bath can be kept at the lowest possible temperature. Heating is best effected by an oil-bath or an electrically heated air-bath, since use of a free flame or sand or asbestos baths generally leads to local overheating and possible decomposition. Distillation is conducted gently for as long as may be necessary; the time varies with the substance under examination, and can only be determined by trial. At the finish the bulk of the water will be found in the graduated tube, but there are always some water-drops adhering to the inside of the condenser. These are readily removed by a spray of entraining liquid. A suitable spray is made by sealing the end of a piece of glass tubing, and piercing four small holes near to the seal. With this a horizontal spray can be projected with considerable force on to the drops. The condenser is then removed, and any water-drops adhering to the walls of the cell are swept down with a camel-hair brush moistened with the entraining liquid.

CHOICE OF ENTRAINERS.—Aromatic liquids are unsatisfactory, as their high mutual solubility with water causes the distillate to be extremely turbid and prevents clean separation of distilled water. All the paraffins tested produced clean distillates, and by using them as entrainers the distilled water was collected in the graduated tube with the least difficulty. Even light petroleum (b.p. 60° C.) removed water in the presence of an anti-bumping agent; the possibility of covering a wide range of boiling-points with a number of liquids is very useful, as can be seen in several of the examples quoted later.

Commercial heptane was found very suitable for general use. Like all paraffins, it enables water to be distilled cleanly and rapidly. In distilling 2 ml. of water in the presence of an anti-bumping agent at least 99·5 per cent. can be recovered. It provides a bath temperature of 99 to 100° C. when under reflux,

and is therefore suitable for comparison with standard oven-drying, which commonly employs the same temperature. Caution must be used in comparing results by these two methods, as it has been found that in some instances decomposition can take place under a liquid boiling at a temperature at which the substance is stable in air.

EXPERIMENTAL RESULTS WITH WATER

(1·96 ml. water used in each case)

Entrainer	b.p.	Anti-bumping agent	Water collected			Water collected		
			¼ hr.	1 hr.	2½ hrs.	¼ hr.	1 hr.	2½ hrs.
			ml.	ml.	ml.	Per Cent.	Per Cent.	Per Cent.
Light petroleum..	60° C.	None	0·13	0·47	1·00	7	24	51
,, ,, ..	60° C.	Sand	0·91	1·95	—	46	99·5	—
,, ,, ..	60° C.	,,	1·89	1·94	—	96	99	—
Benzene	80° C.	None	0·58	1·76	1·96	30	90	100
,, ...	80° C.	Sand	0·92	1·94	—	48	99	—
Light petroleum..	80° C.	,,	0·43	1·94	—	23	99	—
,, ,, ..	80° C.	,,	1·09	1·96	—	55	100	—
Heptane	100° C.	None	—	1·96	1·96	—	100	100
,, ...	100° C.	,,	1·08	1·66	1·93	54	85	98·5
,, ...	100° C.	,,	1·61	1·94	1·94	82	99	99
,, ...	100° C.	,,	1·84	1·95	—	94	99·5	—
,, ...	100° C.	Sand	1·92	1·94	—	98	99	—
,, ...	100° C.	,,	1·63	1·95	—	83	99·5	—
,, ...	100° C.	Silica	1·85	1·95	—	94	99·5	—
,, ...	100° C.	,,	1·89	1·96	—	96	100	—
,, ...	100° C.	,,	1·85	1·96	—	94	100	—
,, ...	100° C.	,,	1·37	1·95	—	72	99·5	—
Toluene	110° C.	None	—	1·96	—	—	100	—
,, ...	110° C.	,,	—	1·94	—	—	99	—
,, ...	110° C.	,,	—	1·94	—	—	99	—
,, ...	110° C.	,,	—	1·96	—	—	100	—
Light petroleum..	120° C.	,,	—	1·96	—	—	100	—

From the above table it appears that high-boiling entrainers remove water faster than those boiling at a lower temperature. In addition, it is shown that a sample of free water can be distilled within an hour in the presence of sand or silica beads.

PART II. APPLICATIONS OF THE METHOD

Various substances were examined by the method described in Part I. In general, there was good agreement with oven-drying at 100° C.

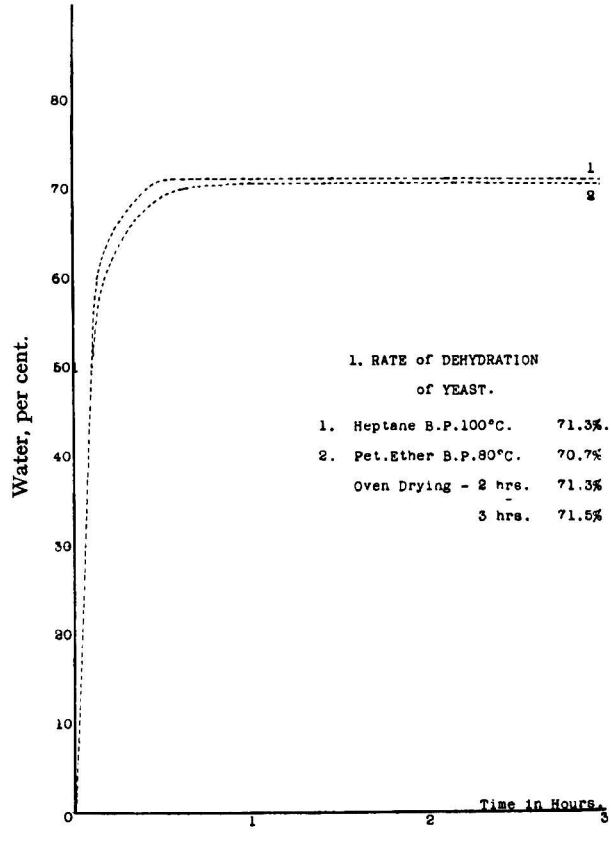
1. YEAST.—(*a*) *Oven-drying.*—The yeast sample (100 g.) was made up to 500 ml. with water and thoroughly mixed. Fifty ml. of this suspension were evaporated to dryness on the water-bath, and the residue was cooled, weighed, and heated at 75° C. in an oven to approximately constant weight. Decomposition caused the yeast to become brown.

	Per Cent.
Loss during drying on water-bath at 100° C. ..	69·8
,, in oven at 75° C. for 1 hr. ..	70·7
,, ,, 2 hrs. ..	71·3
,, ,, 3 hrs. ..	71·5

(*b*) *Distillation.*—(Graph 1.) Three g. of yeast were distilled with the solvent. The yeast did not become as brown during distillation as during oven-drying.

Entrainer	Water collected		
	½ hr. Per Cent.	1 hr. Per Cent.	4 hrs. Per Cent.
Heptane, b.p. 100° C. ..	71·3	71·3	71·3 ± 0·3
Light petroleum, b.p. 80° C...	69·0	70·0	70·7 ± 0·3

Agreement between the results from drying for 3 hours and from distilling for 3 hours with heptane was satisfactory.



Graph 1

2. ARTIFICIAL SILK YARN.—The moisture-content found by distillation with heptane agreed closely with oven-drying at 102–3° C. for 22 hours. The distillation method appears to be particularly useful for the examination of artificial silk yarn which has been lubricated with a preparation containing light mineral oil, as it enables the true water-content of the yarn to be determined, whereas the loss in the oven at 100° C. includes volatile oil.

	Distillation		Oven-drying	
Hours:	4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Viscose artificial silk yarn	9·9	10·1	9·7	10·0
Cuprammonium ,,	9·9	10·0	9·6	9·9
Lubricated viscose ,,	9·3	9·5	13·5	13·9

3. Cocoa Products.—Satisfactory agreement between the values for the moisture-content of cocoa-nib and cocoa-shell was obtained by distillation with heptane and by oven-drying at 100° C.

	Distillation		Oven-drying	
Hours:	4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Cocoa nib, unground ..	3·8	4·4	3·9	4·25
,, ground ..	4·2	4·4	4·1	4·3
Cocoa shell	10·8	11·5	10·8	11·3

4. Dried Milk Powder.—The value for moisture-content given by (*a*) distillation with heptane was compared with those by (*b*) direct drying at 100° C., and (*c*) drying at 100° C. after reconstitution by digestion with water in a closed pan for an hour at 100° C.

	1st sample			2nd sample		3rd sample	
Hours:	4	22	44	4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
(*a*)	3·0	4·1	4·6	4·1	4·1	4·6	4·9
(*b*)	2·4	2·9	2·9	3·7	4·2	3·9	4·5
(*c*)	—	4·5	5·1	—	5·2	—	5·6

The first sample was not placed on sand during distillation, consequently there was considerable decomposition after 22 hours. The results show close agreement between distillation for 4 hours and direct drying at 100° C. for 22 hours. In every instance drying after re-constitution resulted in much decomposition and browning.

The influence of the boiling-point of the entrainer upon the extent of dehydration of dried milk powder was very marked. In distillation with heptane only slight discoloration occurred after 22 hours ; but with light petroleum, b.p. 110° C., there was considerable decomposition. With light petroleum, b.p. 120° C., there was extensive decomposition even after 4 hours.

The advantages of using heptane as entrainer are well illustrated in this example:

Fourth sample

	Moisture	
	4 hours	22 hours
	Per Cent.	Per Cent.
Heptane, b.p. 100° C.	5·9	6·3
Light petroleum, b.p. 110° C. ..	6·2	7·4
,, b.p. 120° C. ..	7·7	15·0

There was no trace of undissolved material in the distilled water. To test for the possible presence of dissolved solids, the distillate from each sample was

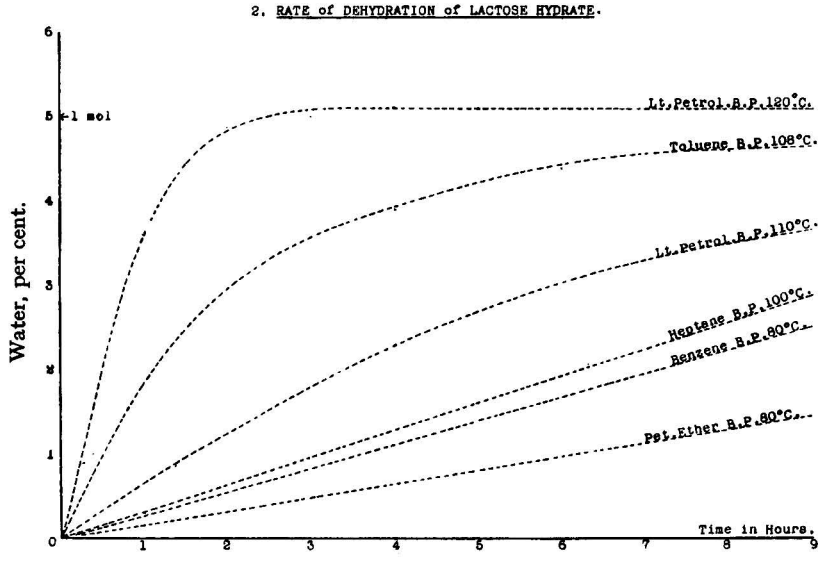
evaporated to dryness and the residue was weighed. The highest weight of residue was 0·001 g. (from 2·30 ml. of distillate).

5. LACTOSE HYDRATE ($C_{12}H_{22}O_{11}.H_2O$. H_2O = 5·0 per cent.).—The dehydration of lactose hydrate furnished another instance of the influence of the boiling-point of the entrainer upon rate of decomposition.

DEHYDRATION OF LACTOSE HYDRATE

	b.p. °C.	Water collected: 3 hours Per Cent.	Water collected: 22 hours Per Cent.
Light petroleum ..	80	0·5	3·6
Benzene	80	0·8	4·2
Heptane	100	0·9	4·5
Light petroleum ..	110	1·5	4·9
Toluene	110	3·6	4·8
Light petroleum ..	120	5·0	5·1

Dehydration with liquids boiling at or below 100° C. was slow and not complete in 22 hours. Light petroleum, b.p. 110° C., and toluene, b.p. 110° C., caused faster separation of water, although it was not complete in 22 hours. With light petroleum b.p. 120° C., rapid dehydration took place; one molecule of water was removed during the first three hours, after which the rate of collection of water diminished to zero.



Graph 2

The rates of dehydration form an interesting series of curves when plotted (Graph 2). In every instance the rate of collection of water was less than the rate of collection of a sample of free water when distilled from the same entrainer. This indicates that the water from the lactose hydrate was derived by decomposition,

since the removal of water from admixture with anhydrous lactose would be expected to occur at the same rate as from a mixture of free water and sand.

Lactose hydrate shows unusual behaviour when heated in air. If it is pure and dry no decomposition occurs (the specimen examined lost 0·1 per cent. during one hour and nothing during the next 17 hours). If, however, the hydrate is digested with water or a solvent such as alcohol, an equilibrium is established,

$$C_{12}H_{22}O_{11}.H_2O \rightleftharpoons C_{12}H_{22}O_{11} + H_2O,$$

and subsequent evaporation at 100° C. removes all water, leaving the anhydrous sugar. The sample examined lost 5·1 per cent. after treatment in this way with water or ethyl alcohol, and 5·3 per cent. with *n*-propyl alcohol. This loss of water of crystallisation also occurs under boiling paraffins, even at temperatures considerably below 100° C.

The possibility of loss of water during distillation at temperatures below those at which the substance is quite stable in air must therefore be considered when comparing results by oven-drying and distillation.

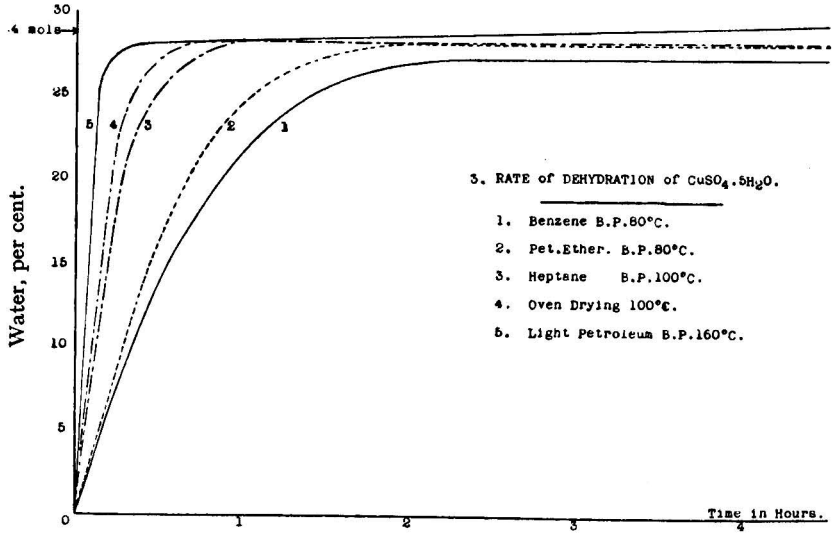
6. Copper Sulphate Pentahydrate.—Bidwell and Sterling[19] examined the dehydration of copper sulphate pentahydrate when distilled under toluene. They found that four mols. of water were lost, but they did not determine the rate of dehydration. In our experiments on the rate of dehydration a preliminary examination of the rate of loss in air at 100° C. was made (Graph 3, curve 4). The sample used was pure, finely-ground material which had effloresced slightly. A 1-g. sample lost 28·2 per cent. in 1 hour, then remained constant for 72 hours; a 5-g. sample lost 28·4 per cent. in 2½ hours, then remained constant for 72 hours (calc. for loss of $4H_2O$, 28·8 per cent.). The results with liquids are summarised below:

Dehydration of $CuSO_4.5H_2O$

Entrainer	b.p. °C.		Result—Graph 3
Benzene	80	Curve 1	Lost 27·0 per cent. in 2 hours. Constant at 27·4 per cent.
Light petroleum	80	,, 2	,, 28·1 per cent. in 2 hours. Constant at 28·1 per cent.
Heptane (1)	100	,, 3	,, 28·1 per cent. in 50 mins. Lost 28·6 per cent. in 4 hours. Well-marked break at 28 per cent.
(2)	100		,, 28·1 per cent. in 1 hour. Lost 28·3 per cent. in 4 hours. Well-marked break at 28 per cent.
(3)	100		,, 28·2 per cent. in 50 minutes. Lost 28·5 per cent. in 5 hours. Well-marked break at 28–29 per cent.
Light petroleum	160	,, 5	,, 28·3 per cent. in 1 hour. Lost 33·6 per cent. in 20 hours. Well-marked break at 28 per cent.

Examination of the graphs shows that for entrainers boiling at or above 100° C. there is only one well-marked change in the rate of collection of water, and this occurs at the composition $CuSO_4.H_2O$. The rate of collection during

the first hour approximates closely to the rate of collection for pure water. This indicates that the pentahydrate decomposes rapidly with liberation of four mols. of water, which distil over at the rate of free water. The rate then abruptly diminishes, with the formation of a break at the composition $CuSO_4.H_2O$. The rates of collection of water when $CuSO_4.5H_2O$ is distilled under benzene or light petroleum b.p. 80° C., do not show such well-marked breaks; this appears to be due to the slower rate of decomposition of the hydrate, but distillation of water ceases after removal of four mols., again indicating the formation of $CuSO_4.H_2O$. It is noteworthy that the rates of dehydration under benzene and light petroleum, b.p. 80° C., are very similar, indicating that the nature of the entrainer does not influence the rate of decomposition; also the extent and rate of decomposition of $CuSO_4.5H_2O$ under heptane at 100° C. and in the oven at 100° C. are very similar.



Graph 3

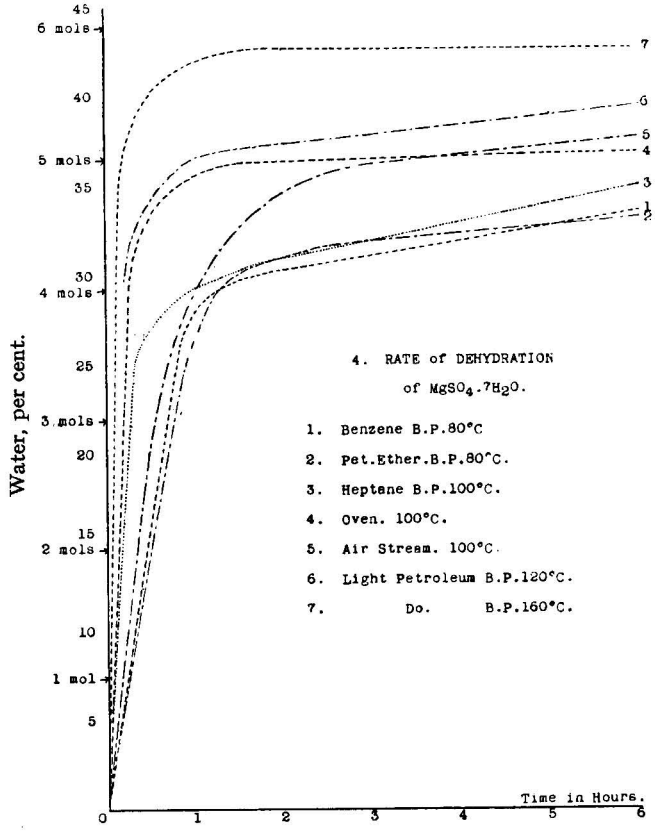
7. MAGNESIUM SULPHATE HEPTAHYDRATE.—Magnesium sulphate heptahydrate was examined in the same manner as copper sulphate pentahydrate. The possible stages of dehydration are indicated below.

LOSS OF WATER FROM $MgSO_4.7H_2O$

Loss of	1	mol.	water	from	$MgSO_4.7H_2O$	=	7·3	per cent.,	leaving	$MgSO_4.6H_2O$
,,	,, 2	,,	,,	,,	,,	=	14·6	,, ,,	,,	$MgSO_4.5H_2O$
,,	,, 3	,,	,,	,,	,,	=	21·9	,, ,,	,,	$MgSO_4.4H_2O$
,,	,, 4	,,	,,	,,	,,	=	29·2	,, ,,	,,	$MgSO_4.3H_2O$
,,	,, 5	,,	,,	,,	,,	=	36·5	,, ,,	,,	$MgSO_4.2H_2O$
,,	,, 6	,,	,,	,,	,,	=	43·8	,, ,,	,,	$MgSO_4.H_2O$
,,	,, 7	,,	,,	,,	,,	=	51·1	,, ,,	,,	$MgSO_4$

Dehydration in an oven at 100° C. caused rapid loss of five mols. of water (Graph 4, curve 4), leaving the dihydrate, which then decomposed very slowly,

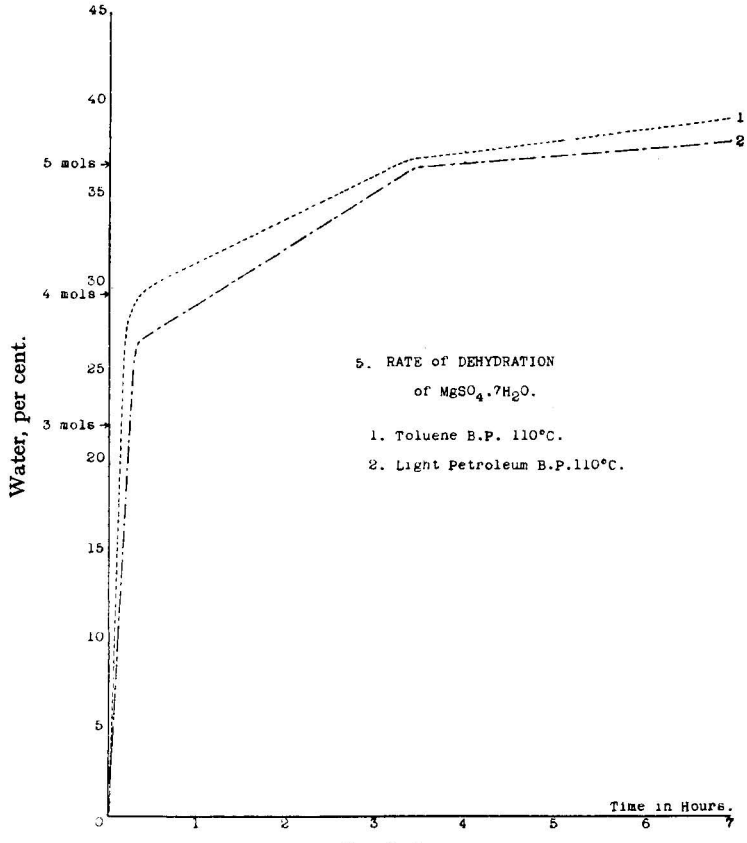
leaving a residue approximating to the monohydrate after 111 hours. Dehydration in a stream of dry air gave similar results (Graph 4, curve 5), a change in rate of loss occurring approximately at the composition $MgSO_4.2H_2O$. This result does not confirm the observations of Hannay,[20] who examined the rates of dehydration of various salt hydrates, and found a change of rate occurring at the composition $MgSO_4.3H_2O$. (Hannay's graph does not correspond with his published figures.)



Graph 4

The dehydration curves for $MgSO_4.7H_2O$ under benzene (Graph 4, curve 1), light petroleum, b.p. 80° C. (Graph 4, curve 2), heptane, b.p. 100° C. (Graph 4, curve 3), light petroleum, b.p. 110° C. (Graph 5, curve 2), and toluene, b.p. 110° C. (Graph 5, curve 1), show well-defined breaks at the loss of four mols. of water, when the residue corresponds with $MgSO_4.3H_2O$. Moreover, with light petroleum, b.p. 110° C., and toluene, equally well-defined breaks occur at loss of five mols. of water (residue $MgSO_4.2H_2O$). With light petroleum, b.p. 120° C. (Graph 4, curve 6), the break at loss of four mols. disappears, a break occurring at loss of five mols. water, while with light petroleum, b.p. 160° C. (Graph 4, curve 7), the only break occurs at loss of six mols. of water. The existence of the break after the distillation of four mols. of water is most readily explained by the formation of the hydrate $MgSO_4.3H_2O$ during the decomposition of the heptahydrate.

Except Hannay's data for the rate of dehydration of $MgSO_4.7H_2O$ in an air-stream, which have not been confirmed, this provides the only evidence available for the



Graph 5

existence of the trihydrate. That the break in the rate of distillation of the water after loss of four mols. of water is due to the formation of this hydrate is indicated by the following facts:

(1) Exactly similar breaks occur at the compositions $MgSO_4.2H_2O$, $MgSO_4.H_2O$, $CuSO_4.H_2O$, and $C_{12}H_{22}O_{11}$, during the distillation of magnesium sulphate, copper sulphate, and lactose hydrates, respectively.

(2) The position of the break is independent of the state of sub-division of the heptahydrate crystals.

(3) No breaks during the distillation of $MgSO_4.7H_2O$ occur at points not represented by molecular formulae ($MgSO_4.xH_2O$ where x is a whole number). Similarly, with copper sulphate and lactose, no well-defined breaks other than at $CuSO_4.H_2O$ and $C_{12}H_{22}O_{11}$ were observed.

The existence of magnesium sulphate trihydrate may therefore be inferred from the above evidence. It is remarkable that the trihydrate does not appear to be formed during the dehydration of the heptahydrate in air; the part played by the liquid is obscure.

The above results indicate that the process of dehydration under boiling liquids is of value in the study of the decomposition of salt hydrates. Further applications of the method are being undertaken.

SUMMARY.—1. The determination of moisture-content by distillation offers the following advantages over other methods:

(*a*) The water removed from the sample is collected, and its purity can be confirmed if necessary.

(*b*) Gases and vapours evolved by decomposition of the sample do not affect the volume of water collected, while volatile organic materials are often soluble in the liquid employed; hence these sources of error inherent in a weighing process are eliminated.

(*c*) Oxidation and skin-formation are avoided.

(*d*) The rate of dehydration can be readily determined on a single sample, without removing the sample from the apparatus, as is necessary in oven-drying.

(*e*) Observation of the rate of collection of the water can, in some instances, provide evidence that the water evolved is produced by decomposition. An extension of this permits the detection of lower hydrates during dehydration.

(*f*) A constant temperature of dehydration can be maintained indefinitely and without trouble.

(*g*) The method is of wide application, and can be applied to materials not easily examined by oven-drying methods.

2. Improved apparatus and technique enable an accuracy of 99·5 per cent. to be obtained on a distilled volume of 2 ml. of water.

3. Aromatic hydrocarbons are unsatisfactory for use as distilling media. Paraffins are suitable, and of these, commercial heptane is most useful for general purposes.

4. Comparison of the results by distillation and by oven-drying has been made for several substances.

5. Evidence was obtained for the formation of $CuSO_4.H_2O$; $MgSO_4.3H_2O$; $MgSO_4.2H_2O$; and $MgSO_4.H_2O$ during dehydration under boiling organic liquids. This is the only satisfactory indication of the existence of magnesium sulphate trihydrate.

We wish to thank Mr. A. H. Rheinlander, M.Sc., F.I.C., for his valued advice, and the Government Chemist, Dr. J. J. Fox, O.B.E., F.I.C., for permission to publish this work.

GOVERNMENT LABORATORY
April, 1936

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DISCUSSION

Mr. A. L. BACHARACH said that, contrary to frequent practice, the authors had made too few claims for their apparatus. He had mentioned five uses, but Mr. Bacharach thought that a further advantage was that one could measure the rate of loss of water with considerable accuracy on a single sample, which was difficult to do by oven-drying.

Mr. F. W. F. ARNAUD remarked that the authors of the paper had apparently used the apparatus and process only for the determination of moisture in solid materials. The process was applicable to the determination of moisture in tar oil and mineral oil emulsions, etc., and if these materials were distilled with paraffin (kerosene), the moisture determination so obtained was, he believed, in close proximity to the actual amount present. The process was, therefore, applicable to the determination of moisture in materials for which possibly no other process could be used with any great degree of accuracy.

Mr. G. GRINLING said that he had done a large amount of work with a simple apparatus of this type, as described by Tucker and Burke (ANALYST, 1936, 663). Working on marzipan for factory-control purposes he took 50 g. of material and 250 ml. of tetrachloroethane, and the results came out about 0·2 per cent. below those obtained by oven-drying; this was due to incomplete separation in the two burettes he had used. If, however, he re-distilled the turbid liquid, he obtained in each instance approximately 0·1 ml. of water, and by adding this to the original distillate the results were similar to those obtained with oven-drying. Oven-drying took about five hours, whereas by using tetrachloroethane it was possible to give the factory the results in about twelve minutes. This saving of time more than compensated for the slight difference in results between oven-drying and tetrachloroethane distillation.

Mr. T. MCLACHLAN drew particular attention to the advisability of using Pyrex glass. He thought it probable that many of the earlier workers had had trouble with water-rings because of the use of soda glass. With the ordinary Liebig condenser made of soda glass he too had invariably had this trouble. He asked whether the authors had had any experience with jam, honey or malt extracts.

Dr. E. B. HUGHES said that this process could be utilised in the opposite direction, as for example, for the determination of the essential oil-content of citrus products, water being added to the finely minced preparation and the mixture distilled in the usual way, the essential oil being collected in the calibrated receiver.

Mr. E. HINKS asked whether the authors had had any difficulty with dried milk owing to priming of solid matter. The Society's Milk Products Sub-Committee had tried distillation methods for the determination of water in dried milk; one of the reasons for not adopting such a method was that they had encountered experimental difficulties, of which priming was one.

Mr. TATE, replying to Mr. Hawkins, said that their apparatus had been made in the Government Laboratory, and he therefore had no information as to cost. Should any member desire to have the apparatus made, he would be glad to give full particulars to any maker.

Dr. L. A. WARREN was glad that the simplicity of the apparatus had been emphasised. The use of xylene did not appeal to them on account of the mutual solubility of xylene and water, while the high boiling-point rendered it very liable to decompose the substance under examination. In reply to Mr. Davis, he said that it appeared possible that the method would prove satisfactory for water in glycerin.* The use of tetrachloroethane, like that of xylene, was undesirable on account of the mutual solubility with water and the comparatively high boiling-point. The difficulties experienced with Friedrichs' apparatus had led to non-reproducibility of results. The authors' apparatus had behaved satisfactorily with tar oils, but, as there seemed to be no other method for the determination of water in these oils, it had not been possible to effect any comparison of results. For this reason the experiments with tar oils had not been mentioned in the course of the paper. Pyrex glass appeared to make the drainage of water more complete. The ring of water-drops always formed in the condenser, however, even when it was made of Pyrex glass, and it was essential to remove them by some means. It was for this reason that the small sprayer had been introduced. Materials such as honey or jam had not been examined, but he thought that the method might prove successful with such products.

* It has since been found that glycerin distils slowly when boiled with heptane. The method therefore cannot give accurate results.

The Calculation of Added Water from the Freezing-point of Watered Milks

BY G. D. ELSDON, B.Sc., F.I.C., AND J. R. STUBBS, M.Sc., F.I.C.

(*Communicated to the North of England Section, December* 14, 1935)

MOST of those who have considered the calculation of the amount of added water from the freezing-points of mixtures of milk and water have adopted the method of simple proportion, as represented by the formula of the American Association of Official Agricultural Chemists

$$W = \frac{100(T - T')}{T} \text{ per cent.}$$

where W is the amount of added water, T is the freezing-point depression of the original milk (or an average figure in cases where a comparison sample is not available) and T′ the freezing-point depression of the sample.

There do not appear to be many recorded instances where a milk has been diluted, the freezing-point depression observed, and the amount of added water calculated from the freezing-point depression compared with the amount known to be present. Writers who have given some consideration to the matter (*e.g.*

Monier-Williams[1]) have thought that the above simple formula, whilst not being exactly correct, was yet sufficiently near the truth for practical purposes.

Hortvet[2] published figures obtained from the examination of mixtures of milk with water, containing from six to sixteen per cent. of added water. He found that the amount of added water approximated closely to that present when calculated by the formula $W = \frac{100(T - T')}{T}$. Similar results were obtained by Denis-Nathan.[3]

As far as we are aware, the amount of added water in mixtures of milk and water is, in this country, expressed as grams of added water in 100 g. of the mixture, *i.e.* percentage by weight.

From a reading of *Methods of Analysis of the American A.O.A.C.* (3rd Edition, p. 223), there would appear to be some ambiguity as to the method of expression used by the Association. It is, perhaps, natural to assume that the formula specified is intended to give percentages by weight, as it is similar in form to the corresponding formula $\frac{8{\cdot}5 - \text{s.n.f.}}{8{\cdot}5} \times 100$ used for calculation of added water from the solids-not-fat which, according to the instructions of the A.O.A.C., are determined by weight. There is, however, an alternative method indicated for the calculation of added water, by means of Winter's table (Table XXIII in the Appendix), which gives the percentage *by volume.*

We have been unable to trace any statements by any of the authors quoted as to whether their percentages are by volume or by weight, with the exception of Henderson and Meston,[4] who recommend Winter's formula, and Hortvet,[5] who instructed his collaborators to make up their dilutions of milk by volume. Some of the discrepancies noticed may be due to the varying methods used. When the added water is calculated by volume in place of by weight the amount indicated will be higher. Twenty per cent. by weight is equivalent to 20·5 per cent. by volume. Our own figures are all given in grams of added water in 100 g. of the mixture.

Walder[6] found that the calculated amount of water was usually greater than that actually present, but Plücker and Steinruck[7] found that it was usually less (*cf.* also Buogo[8]).

Henderson and Meston[4] give a revised formula, based on those of Winter[9] and Bonnema,[10] but it is not fully worked out, and no results of the examination of known mixtures are recorded in their paper.

We have had in mind for some years this particular phase of the cryoscopy of milk, and recently attention was drawn to the subject at a meeting of the North of England Section, by Mr. H. M. Mason, who referred to the possibility that any water attached to the proteins might not be available as a solvent for the substances causing the freezing-point depression.

A preliminary experiment with a mixture of equal weights of water and of milk [F.P.D. (Hortvet) of milk 0·539° C.] gave a freezing-point depression of 0·258° C. Calculating the amount of added water by the A.O.A.C. formula, we obtain 52·2 per cent. or 2·2 per cent. above that actually present. In consequence

of the result of this experiment, we carried out a series of determinations of freezing-point depressions of various mixtures of milk and water with the following results:

FREEZING-POINT DEPRESSIONS OF MIXTURES OF MILK AND WATER

Actual added water Per Cent. by weight	Total solids of mixture Per Cent. by weight	F.P.D. found (Hortvet)	F.P.D. of original milk (Hortvet)	Water by A.O.A.C. formula Per Cent.	Water by suggested formula (*infra*) Per Cent. by weight
5	12·5	0·522	0·552	5·4	4·8
	12·1	0·516	0·547	5·7	5·0
10	11·4	0·484	0·547	11·5	10·2
		0·484	0·547	11·5	10·2
12½	10·7	0·470	0·546	13·9	12·5
	11·2	0·452	0·527	14·2	12·6
15	10·9	0·453	0·547	17·2	15·4
	10·6	0·452	0·546	17·2	15·4
	10·6	0·452	0·547	17·4	15·6
20	10·2	0·425	0·547	22·3	20·0
		0·424	0·547	22·5	20·2
25	9·6	0·399	0·547	27·1	24·5
	9·2	0·399	0·546	26·9	24·4
50	6·0	0·258	0·539	52·2	49·0
	6·4	0·261	0·547	52·3	48·9
75	3·2	0·133	0·547	75·7	73·3
90	1·3	0·060	0·547	89·1	87·9
		0·061	0·547	89·0	87·7

In order to attempt an explanation of these results and to endeavour to find a formula which will give results agreeing with the known composition of the mixtures, it is necessary to recall the laws governing the depression of the freezing-points of dilute aqueous solutions.

For small differences in concentration of dilute solutions of non-ionised substances the freezing-point depressions may be taken as proportional to the weight of substance dissolved in 100 g. of the solvent. In the case of mixtures of milk and water it is necessary to take into account several other factors, apart from this simple relationship. Thus it is necessary to consider:

(1) The effect of the weight of total milk solids on the weight of solvent present.

(2) The dissociation of the electrolytes.

(3) Any departure from a straight line of the graph connecting concentration with freezing-point depression in the case of lactose, as is the case with cane sugar according to Raoult's formula,

$$\frac{18{\cdot}72 \times P}{342 - (0{\cdot}99 \times P)}$$

where P is the weight in g. of solute in 100 g. of solvent.

(4) The water of hydration of the lactose and any denaturisation of the proteins (due to the splitting-off of water) during the drying of the total solids, which will increase the apparent amount of "free" water present.

(5) The effect of any substances dissolved in the water used for the dilution of the milk, on the freezing-point of the mixture, which will usually not exceed 1 per cent. of the water added.

In the above formula the concentration of solutions is expressed as weight of solute in 100 g. of solvent. The A.O.A.C. formula assumes that the same amount of solvent is present in the same quantity of all milks and mixtures of milk and water. This assumption is not correct and entails a considerable error. In milk of average quality, containing, say, 12·5 per cent. by weight of total solids, the active ingredients in 100 g. of milk are dissolved in 100 — 12·5 = 87·5 g. of water, whereas the A.O.A.C. formula assumes the solution to be in 100 g. (or ml.) of water. In the last column of the table we have placed results corrected for the fact that the added water should be calculated by using weights of solvent and not of solution (that is, referring always to 100 g. of water); we have used a revised formula, *viz.*:

$$\text{Added water} = \frac{T - T'}{T} \times (100\text{—T.S.}) \text{ per cent. by weight,}$$

where T is the freezing-point depression (Hortvet) of the original milk, T′ the freezing-point depression (Hortvet) of the mixture, and T.S. is the percentage of total solids in the mixture.

In the above experiments T was determined. In practice T will not generally be known. Where comparison is being made with an appeal-to-cow sample the freezing-point depression of this will be used, but where no appeal-to-cow sample is available an average figure can be substituted.

The revised formula gives the amount of added water* correctly (within the limits of experimental error) when this does not materially exceed 20 per cent. When the amount of added water is of the order of 25 per cent. it is under-estimated by about 0·5 per cent. The under-estimation increases with the quantity of added water present, and becomes about 2 per cent. when the added water is as high as 90 per cent. These results show a considerable improvement on the original formula, which invariably over-estimates added water where the amount does not exceed about 80 per cent. It is suggested that, in practice, the results given by the new formula can always be used, as any under-estimation is not material in any instances which are likely to be met with in practice.

Of the five points mentioned above as possibly having some influence on the calculation of the amount of added water, the first, *viz.* the influence of the solids of the milk, is greatest when the amount of added water is least. It appears from the experimental results that the effect of the increase in dissociation of the electrolytes on dilution of the milk does not become evident until such dilution is at least 25 per cent. There is, however, the possibility—in fact, the likelihood—that some of the factors may work in opposite directions; if so, they will tend to cancel each other and thus permit of the use of a comparatively simple formula.

The third point mentioned above, that is, the possible association of the lactose, is not likely to be of serious moment. A 5 per cent. solution of sucrose, *i.e.*

* As explained on p. 383, all our own figures for added water are expressed in percentages by weight.

5 g. of sugar in 100 g. of water, has, according to Raoult's formula, a freezing-point depression of 0·2777° C., whilst a 3 per cent. solution has a freezing-point depression of 0·1656° C.; if the freezing-point depression of the 3 per cent. solution were calculated from that of the 5 per cent. by simple proportion, the figure would be 0·1666° C.—a difference of only 0·001° C. from the actual figure. Lactose is not unlikely to have a somewhat similar range.

The expression 100—T.S. will not only include the "free" water in the milk (*i.e.* the water acting as a solvent for the crystalloids), but will also include any water of hydration which is attached to the lactose when in solution and which is expelled on drying, together with any water of combination of the proteins which is lost at the same time, *i.e.* more water than is present as solvent. The expression 100—T.S. will, therefore, tend to be too high, and this will tend to give too high a figure for the amount of added water. This tendency may, however, be reduced (in high dilutions even reversed) by the effect due to dissociation of the electrolytes, and this is what appears from the results recorded in this paper.

SUMMARY.—The factors influencing the freezing-point depression of mixtures of milk and water have been considered. It has been shown, experimentally, that the expression

$$\text{Added water} = \frac{T - T'}{T} \times 100 \quad \text{per cent.}$$

gives results which are too high. A revised formula

$$\text{Added water} = \frac{T - T'}{T} \times (100\text{—T.S.}) \text{ per cent. by weight,}$$

is suggested, which has been shown to give accurate results up to about 25 per cent. of added water, and slightly low results (possibly due to dissociation) above this figure.

We derived this formula from first principles, assuming that the freezing-point depressions of dilute solutions are proportional to the number of grams of any one substance dissolved in 100 g. of solvent, and assuming that differences in concentration do not affect the association, dissociation or hydration of the dissolved substances. Several alternative methods of derivation have been suggested to us, notably by Dr. G. W. Monier-Williams, Mr. Andrew More and Mr. A. N. Leather, to whom we are also indebted for reading the paper in typescript and for suggestions.

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The Determination of Casein by Formol Titration after Precipitation with Acid

By F. H. McDOWALL AND A. K. R. McDOWELL

The determination of proteins in milk by formol titration was first suggested by Steinegger[1] and Richmond.[2] In 1914, Walker[3] investigated the use of the method for the estimation of casein in milk (which had already been suggested by Steinegger), and, as a result of a large number of comparative analyses, he arrived at a factor to be used for conversion of the formol titre into the percentage of casein in the milk. The Walker method has since been widely used as a rapid method of determining casein in milk, and recently its use has been advocated as a means of determining the cheese-yielding capacity of milk supplied to cheese factories (McDowall[4]).

In the Walker method, the acidity produced by the action of formaldehyde on the neutralised milk is derived from both casein and albumin and from some of the non-protein nitrogenous constituents. The validity of the Walker factor for individual milks depends therefore on the uniformity of the proportion of casein to other substances reacting with formaldehyde (Report in the press, McDowall and Dolby). It cannot be used without modification for dried milks (Harrall[5]).

The details of the Walker method have recently been studied by Pyne,[6] and modifications in the technique have been suggested to increase the accuracy of titration. In the titration of milk to phenolphthalein with alkali, however, the end-point is somewhat indefinite, on account of the high buffering capacity of the milk. When casein is precipitated with acid, and separated by filtration, the greater proportion of the buffering substances, such as acetates and phosphates, remains in the filtrate.

It seemed possible, therefore, that these difficulties might be eliminated if casein could be titrated directly, after separation by precipitation with acetic acid and sodium acetate. If the method were satisfactory, there should be a constant relationship between the casein-content of the milk as determined by acetic acid precipitation and the Kjeldahl method, and the volume of alkali required for any fixed quantity of milk. In other words, there should be a factor for converting the formol titre of the precipitated casein into percentage of casein in the milk.

Experimental

Precipitation and Separation of Casein.—The milk (20 ml.) was diluted, and the casein was precipitated according to the procedure recommended by Moir.[7] One hour after the addition of sodium acetate, the liquid in which the precipitate was suspended was removed from the beaker by suction through an asbestos mat on a filter disc in a 2·5-cm. funnel. Distilled water (90 ml.) was added to the beaker and, after five minutes, was removed as before. This washing was repeated twice. It was not found possible to remove all the aqueous portion from the beaker in each washing, as it was necessary to avoid the formation of an impervious

layer of casein on the filter-mat. The asbestos mat and filter-disc were blown back into the beaker used for the precipitation.

SOLUTION AND TITRATION OF THE CASEIN PRECIPITATE.—(*a*) Addition of *N*/10 sodium hydroxide solution in the cold to the exact end-point to phenolphthalein.

Solution of casein was slow, even with continuous stirring, and the end-point to phenolphthalein was not any more distinct than for the ordinary Walker titration. When the neutral point had been reached 4 ml. of formalin were added, and the titration was repeated to the same end-point. Quite uniform results could be obtained for the factor:

$$\frac{\text{Per cent. of casein in milk}}{\text{ml. } N/10 \text{ NaOH required for formol titration of precipitated casein from 20 ml. of milk.}}$$

For example, for two milks the factors obtained were:

1·12, 1·10, 1·06 } mean, 1·09 1·09, 1·08, 1·09 } mean, 1·09.

(*b*) Addition of excess of *N*/10 sodium hydroxide, in the cold. The difficulty due to slow solution of the casein was partly eliminated by addition of excess of *N*/10 alkali (11 ml.) followed by vigorous stirring for two minutes. Sulphuric acid *N*/5 was then added until the correct shade of pink had been reached. (A colour standard, consisting of 20 ml. of milk and 6 to 8 drops of 0·01 per cent. aqueous rosaniline acetate solution made to the same volume as the titration liquid was used.) With this technique the following "factors" were obtained for eight milks:—0·99, 1·02, 1·01, 1·04, 1·04, 1·09, 1·06, 1·05. Average, 1·04. The figures are the averages of triplicate determinations for each milk. Some of the triplicates for the individual milks agreed quite well, whilst others showed appreciable discrepancies.

(*c*) Addition of excess of *N*/10 sodium hydroxide solution at boiling water-bath temperatures.

An experiment in duplicate with one sample of milk and various times of heating in the water-bath gave the following results (Table I). The casein-content of the milk was 2·79 per cent.

TABLE I

Effect of time of heating with excess N/10 NaOH on the formol titre of precipitated Casein

Time of heating (minutes)	0	5	10	20	60
Formol titration NaOH, ml. *N*/10	2·55, 2·65 } 2·60	2·64, 2·67 } 2·66	2·61, 2·64 } 2·63	2·54, 2·58 } 2·56	2·61, 2·55 } 2·58
Casein per cent. / Formol titre, ml.	1·07	1·05	1·06	1·09	1·08

It was found that the casein dissolved readily after heating for 5 to 10 minutes in the boiling water-bath, although the results show that there is a considerable

margin of safety since the amount of hydrolysis was negligible, even after heating for 1 hour.

The casein being now in solution, and the fat emulsified, the end-point was much more definite and there was very little fading.

An attempt was made to reduce the final volume of solution for titration by the use of 1 ml. of 1·0 *N* sodium hydroxide solution instead of 11 ml. of *N*/10 solution, followed by the 5 to 10 minutes' heating in the water-bath and titration with *N*/5 acid and *N*/10 alkali. The results indicated an appreciable amount of hydrolysis. (Table II.)

TABLE II

Effect of using strong Alkali for solution of the Casein

No. of milk	1		2		3		4	
Formol titre	3·07		3·59		3·88		4·18	
Sodium hydroxide, *N*/10 ml.	3·07	3·06	3·55	3·57	3·91	3·90	4·34	4·26
	3·03							
Casein per cent. / Formol titre ..	0·96		0·73		0·75		0·74	

The "factor" had been thus reduced from 1·05 to 0·96 and 0·74. These results are in agreement with the findings of Tague,[7] who was able to prepare disodium caseinate (the salt neutral to phenolphthalein) by adding excess of *N*/10 alkali and heating in the water-bath, but who found that stronger alkali on prolonged heating caused a slow decomposition.

The following procedure was therefore finally adopted:—The milk (20 ml.) was diluted with 100 ml. of water at 42° C., and the casein was precipitated according to the directions of Moir. The casein was separated and washed three times as indicated above, leaving about 20 to 30 ml. of liquid in the beaker at the final washing. Sodium hydroxide solution (11 ml. of *N*/10) was added, and the beaker was placed in the boiling water-bath for 5 minutes, with occasional stirring. The solution was then neutralised back to the standard colour with *N*/5 acid; 4 ml. of formalin were added, and a titration with *N*/10 sodium hydroxide solution was carried to the same end-point.

The following results, arranged in order of increasing "factor," were obtained with 21 samples of milk (Table III).

The average factor, 1·051, agrees well with the average factor 1·04, obtained for the formol titration in the cold, of casein precipitated from 8 milks (see p. 388).

The results indicate that the method could be used, with reasonable accuracy, for the determination of casein in milk. The casein-content of the milk (g. of casein per 100 g. of milk) would be given by the formula:

Ml. of *N*/10 NaOH for formol titration of casein from 20 ml. of milk × 1·05.

The manipulation involved, however, is not so simple as may appear, since the filtration under suction requires a considerable amount of attention. The method in its present form is not considered suitable for use in dairy factories. Experiments are in hand with the object of eliminating the filtration by the use of a centrifuge.

TABLE III

Formol titration of Casein precipitated from individual Milks

No.	Casein in milk Per Cent.	Formol titre ml.	Mean ml.	Factor = Casein per cent. / Formol titre
1	3·16	3·15, 3·17, 3·18	3·17	1·00
2	2·92	2·90, 2·81, 2·87	2·86	1·02
3	2·68	2·64, 2·66, 2·55	2·62	1·02
4	2·84	2·76, 2·78	2·77	1·03
5	2·94	2·91, 2·81	2·86	1·03
6	3·08	3·01, 2·98	3·00	1·03
7	2·70	2·55, 2·55, 2·66	2·59	1·04
8	3·10	3·00, 2·97	2·99	1·04
9	2·94	2·86, 2·77	2·82	1·04
10	2·79	2·64, 2·67	2·66	1·05
11	3·22	3·05, 3·06	3·06	1·05
12	2·95	2·82, 2·77	2·80	1·05
13	2·58	2·49, 2·43	2·46	1·05
14	2·63	2·45, 2·50	2·48	1·06
15	2·59	2·45, 2·38, 2·39	2·41	1·07
16	2·70	2·52, 2·54	2·53	1·07
17	3·16	2·98, 2·91	2·95	1·07
18	2·57	2·38	2·38	1·08
19	3·25	3·03, 2·97	3·00	1·08
20	3·00	2·84, 2·73	2·79	1·08
21	2·79	2·52, 2·48	2·50	1·12

SUMMARY.—The casein-content of milk can be determined with reasonable accuracy by formol titration to phenolphthalein of the curd obtained on precipitating 20 ml. of milk with acetic acid and sodium acetate. The formol titre × 1·05 gives the casein-content in grams per 100 g. of milk.

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The Use of 2:4-Dinitrophenylhydrazine as a Reagent for Carbonyl Compounds

By N. R. CAMPBELL, A.I.C.

While employing 2:4-dinitrophenylhydrazine for the characterisation and identification of aldehydes and ketones, it was observed that there were a considerable number of discrepancies in the recorded melting-points. Analysts referring to older tables will also find discrepancies between these and more recent papers. It was considered that a revision of many of these melting-points and a tabulated comparison of the recorded figures might clear the way to a more extended use of this valuable reagent. This has been done and certain hitherto unrecorded derivatives have been prepared and examined.

The 2:4-dinitrophenylhydrazine was prepared by the method of Brady,[5] *iso*propyl alcohol being used in place of the more costly pure ethyl alcohol. The product was pure and did not require recrystallisation.

The dinitrophenylhydrazones were prepared as suggested by Brady,[5] and certain experimental details may be of interest. It was found that the addition of dilute sulphuric acid was unnecessary, but that even when separation did not occur on cooling, boiling gently under reflux for from five to thirty minutes sufficed for condensation. Purified industrial methylated spirit was used as a substitute for ethyl alcohol, and in no instance did this cause complication. Recrystallisation was effected, whenever possible, from alcohol, but acetic acid was frequently found necessary, and when this solvent failed, xylene usually gave good results.

Two compounds not prepared by Brady's method were the dinitrophenylhydrazones of phenanthraquinone and chrysoquinone. These were obtained by boiling one mol. of the quinone with two mols. of dinitrophenylhydrazine in glacial acetic acid solution for thirty minutes. The crude substances did not require re-crystallisation.

Attention is drawn to the variations in the m.p. of the dinitrophenylhydrazone of acetaldehyde as recorded by various authors. These have been attributed by Bryant[8] and others to the existence of a meta-stable form melting at 147° C. and a stable form melting at 168° C. It is true that the *crude* product obtained by Brady's method has m.p. 147° C., but I have been unable to confirm Bryant's statement that a meta-stable modification melting at this temperature is produced when the liquid phase is allowed to solidify. Indeed, no signs of such a meta-stable modification could be detected when the liquid was undercooled far below its m.p., in a narrow tube, and then inoculated with a crystal of the crude substance having m.p. 147° C. The so-called meta-stable form is undoubtedly merely impure substance, the impurity being probably crotonic aldehyde dinitrophenylhydrazone, derived from some crotonic aldehyde produced from the acetaldehyde by the sulphuric acid used in Brady's method. Confirmation of this was obtained by the addition of excess of freshly distilled acetaldehyde to a cold pyridine solution of dinitrophenylhydrazine. Under these conditions the formation of crotonic

TABLE I

2:4-DINITROPHENYLHYDRAZINE DERIVATIVES OF ALDEHYDES

Aldehyde	Solvent	Colour	Melting-point °C.	Recorded melting-points
Formaldehyde	Alcohol	Yellow	166°	(1) 155°, (4) 155°, (6) 167°
Acetaldehyde	Alcohol	Orange-yellow	168°	(1) 147°, (4) 147°, (6) 167°
Propionaldehyde	Alcohol	Orange	154°	(3) 155°, (4) 155°, (6) 156°
n-Heptylaldehyde (Oenanthol)	Alcohol	Yellow	108°	(3) 106°, (4) 106°
Phenylacetaldehyde	Alcohol	Yellow	121°	(5) 110°
Cinnamic aldehyde	Acetic acid	Red	255° (decomp.)	(5) 248°
Furfuraldehyde	Xylene	Red	229°	(1) 202°
Benzaldehyde	Acetic acid	Orange	237°	(1) 203°, (2) 235°
Salicylaldehyde	Acetic acid	Light red	252° (decomp.)	(1) 237°, (2) 248°
m-Hydroxybenzaldehyde ..	Alcohol	Red	260° (decomp.)	(5) 259°
p-Hydroxybenzaldehyde ..	Acetic acid	Purple-red	280° (decomp.)	(1) 157°
o-Nitrobenzaldehyde	Xylene	Yellow	250° (decomp.)	(1) 192°
m-Nitrobenzaldehyde.. ..	Acetic acid	Yellow	292–293° (decomp.)	(1) 268°
o-Chlorobenzaldehyde ..	Acetic acid	Orange	206–207°	
Anisaldehyde	Acetic acid	Orange-red	253–254° (decomp.)	(5) 250°
Vanillin	Acetic acid	Red	271° (decomp.)	
Piperonal	Acetic acid	Red	266° (decomp.)	(5) 265°
Cumic aldehyde	Acetic acid	Red	243°	(5) 241°
Citral	Alcohol	Orange	116°	(4) 108–110°α, 96°β
Citronellal	Alcohol	Yellow	77°	(4) 78°
n-Butyraldehyde*		Yellow		(3) 122°, (4) 122°, (6) 123°
*iso*Butyraldehyde		Yellow		(3) 182°, (4) 182°, (6) 187°
n-Valeraldehyde		Yellow		(4) 98°
*iso*Valeraldehyde		Yellow		(3) 123°, (4) 123°
Trimethylacetaldehyde ..		Yellow		(4) 210°
n-Caproic aldehyde		Yellow		(3) 104°, (4) 104°
n-Octyl aldehyde		Yellow		(4) 106°
n-Nonyl aldehyde		Yellow		(3) 96°, (4) 96°
n-Decyl aldehyde		Yellow		(4) 104°
n-Undecyl aldehyde		Yellow		(4) 104°
n-Duodecyl aldehyde ..		Yellow		(4) 106°
Acrolein		Orange-red		(4) 165°
α-Methyl-β-ethyl acrolein ..		Crimson		(4) 159°
p-Nitrobenzaldehyde		Orange		(5) 320°
2:4-Dinitrobenzaldehyde ..		Yellow		(7) 258°
2:4:6-Trinitrobenzaldehyde ..		Orange		(7) 208°

* The solvents, colours and melting-points of this and the following derivatives tabulated are recorded from the literature for convenience of reference.

TABLE II

2:4-DINITROPHENYLHYDRAZINE DERIVATIVES OF KETONES

Ketone	Solvent	Colour	Melting-point °C.	Recorded melting-points °C.
Methyl ethyl ketone	Alcohol	Orange	110–111°	(3) 115°
Acetone	Alcohol	Yellow	126°	(1) 118°, (2) 128°
Methyl propyl ketone.. ..	Alcohol	Orange-yellow	143–144°	(4) 141°
*cyclo*Pentanone	Alcohol	Yellow	146–147°	(4) 142°
*cyclo*Hexanone	Alcohol	Yellow	162°	(4) 160°
Diacetone alcohol	Alcohol	Light red	202–203°	
Benzylidene acetone	Acetic acid	Red	227°	(4) 223°
Dibenzylidene acetone ..	Acetic acid	Red	180°	
Cinnamylidene acetone ..	Acetic acid	Purple-red	222–223°	
Dicinnamylidene acetone ..	Acetic acid	Red	208°	
Pyruvic acid	Alcohol	Yellow	218°	(4) 213°
Acetoacetic ester	Alcohol	Yellow	93–94°	(1) 95°, (2) 96°
Benzoylacetoacetic ester ..	Acetic acid	Orange	222–223°	
Mesityl oxide	Acetic acid	Red	203°	(4) 200°
Acetylacetone	Alcohol	Yellow	209°	
Diacetyl	Anisole	Orange	charred above 300°	
Acetophenone	Acetic acid	Orange-red	249–250°	(4) 237°
Benzylidene acetophenone ..	Acetic acid	Orange-red	244° (decomp.)	(4) 208
Cinnamylidene acetophenone	Acetic acid	Red	218–219° (decomp.)	
β-Acetylnaphthalene	Acetic acid	Red	262° (decomp.)	
l-Carvone	Acetic acid	Red	193°	(4) 189°
d-Carvone	Acetic acid	Red	190°	
Menthone	Alcohol	Orange	146°	(4) 145°
Pulegone	Alcohol	Red	147°	(5) 142°
Piperitone	Alcohol	Red	119°	
α-Ionone	Alcohol	Orange	150°	(5) coml. 125–128°
d-Camphor	Alcohol	Deep yellow	177°	(5) 175°
Benzophenone	Acetic acid	Orange	238–239°	(1) 229°
β-Benzoylnaphthalene ..	Acetic acid and alcohol	Orange-yellow	257–258°	
p-Benzoyldiphenyl	Acetic acid	Orange	214°	
Benzoin	Alcohol	Yellow	245°	(4) 234°
Furoin	Alcohol	Orange-red	216–217°	
Benzil	Alcohol	Yellow	189°	(1) 183–184°, (4) 185°
Phenanthraquinone		Dark red	312–313° (decomp.)	
Chrysoquinone		Red-brown	308–309° (decomp.)	

TABLE II—*continued*

Ketone	Solvent	Colour	Melting-point °C.	Recorded melting-points °C.
Methyl *n*-butyl ketone* ..		Orange-red		(4) 106°
Methyl *n*-amyl ketone ..		Orange-yellow		(4) 89°
Methyl *n*-hexyl ketone ..		Orange		(4) 58°
Methyl *n*-nonyl ketone ..		Yellow-orange		(4) 63°
Methyl *n*-undecyl ketone ..		Yellow-orange		(4) 69°
Methyl *iso*propyl ketone ..		Yellow-orange		(4) 117°
Methyl *iso*butyl ketone ..		Orange-red		(4) 95°
Methyl *iso*amyl ketone ..		Orange		(4) 95°
Methyl *iso*hexyl ketone ..		Orange-yellow		(4) 77°
Methyl *cyclo*hexyl ketone ..		Orange		(4) 140°
Di-ethyl ketone		Pale orange		(4) 156°
Ethyl *n*-propyl ketone ..		Orange-yellow		(4) 130°
Ethyl *iso*butyl ketone ..		Orange-yellow		(4) 75°
Di-*n*-propyl ketone		Orange-yellow		(4) 75°
*cyclo*Heptanone	Alcohol	Orange-yellow		(5) 148°
*cyclo*Octanone	Alcohol	Orange-yellow		(5) 163°
*cyclo*Pentadecanone	Alcohol	Yellow		(5) 105°
Benzoyl acetone	Alcohol	Pale yellow		(5) 151°
Allyl acetone		Orange		(4) 104°
Ethyl oxomalonate		Lemon		(4) 128°
Levulinic acid		Yellow		(4) 92°
Methyl benzoyl formate ..		Orange-yellow		(4) 171°
Fenchone	Alcohol	Orange-yellow		(5) 140°
Pinacolone		Orange-yellow		(4) 125°
Methyl heptenone		Orange-red		(4) 81°
α-Indanone		Orange-red		(4) 258°
n-Butyroin		Yellow		(4) 99°
*pseudo*Ionone		Deep red		(4) 143°

aldehyde is very improbable, and the crude product obtained by pouring the solution into water and then adding dilute hydrochloric acid had m.p. 164° C.

There is no difficulty in purifying the acetaldehyde dinitrophenylhydrazone prepared by Brady's method, provided that alcohol is used as a solvent, a single recrystallisation from this usually being sufficient. Benzene, xylene and similar solvents are less suitable, and, if used, often necessitate several re-crystallisations. The possibility of impurities being produced by the aldol condensation is, however, one which should be carefully borne in mind by analysts.

* The solvents, colours and melting-points of this and the following derivatives tabulated are recorded from the literature for convenience of reference.

SUMMARY.—(1) *iso*Propyl alcohol can be conveniently used in place of the more costly duty-paid pure ethyl alcohol in the preparation of 2:4-dinitrophenylhydrazine.

(2) The melting-points of many dinitrophenylhydrazones recorded in the literature are erroneous. Tables of corrected melting-points are given, and these tables include all melting-points given in the literature and also the melting-points of several hitherto undescribed dinitrophenylhydrazones.

(3) The so-called meta-stable modification of acetaldehyde dinitrophenylhydrazone is shown to be merely impure material.

(4) Attention is drawn to the possibility of an impure derivative being obtained, owing to the aldol condensation taking place when Brady's method of preparation is used.

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Anthranilic Acid and its Use in the Determination of Zinc, Cadmium, Cobalt, Nickel and Copper

BY R. J. SHENNAN, B.Sc., A.I.C., J. H. F. SMITH, B.Sc., A.I.C., AND A. M. WARD, D.Sc., F.I.C.

FUNK and Ditt[1] have described gravimetric methods for the precipitation of zinc, cadmium, cobalt, nickel, and copper from neutral solutions by means of sodium anthranilate. They dealt also with a volumetric procedure[2] in which the anthranilic acid was dibrominated, whereas in an earlier paper, Day and Taggart[3] had determined anthranilic acid by tribromination. Gotô[4] has studied the effect of varying the *p*H of buffered acetate solutions on the precipitation of anthranilates. It is with two aspects of the subject, namely, the conditions of the volumetric determination, and the solubility effects of anthranilates in acetate solutions, that the present paper is mainly concerned.

VOLUMETRIC DETERMINATION OF ANTHRANILIC ACID.—*Dibromination.*—The effects of varying the concentration of acid, the excess of brominating solution, and the time were studied. A solution containing 10 g. of anthranilic acid per litre in 4 *N* hydrochloric acid was prepared. For each titration 10 ml. of this solution [≡0·1000 g. of $C_6H_4(NH_2)CO_2H$] were diluted to about 100 ml. in a stoppered bottle, and treated first with hydrochloric acid to produce the required acidity (see below), and then with a known volume of *N*/10 potassium bromate-bromide solution. After the times shown 5 ml. of *N*/5 potassium iodide were introduced, the solution was diluted, and the liberated iodine was titrated by *N*/10 sodium thiosulphate in presence of starch. In the first two experiments, the concentration of hydrochloric acid was approximately *N*, and in the others, it was greater than 1·6 *N*.

N/10 Potassium bromate ml.	*N*/10 Sodium thiosulphate ml.	Time Minutes	Calc. $C_6H_4(NH_2)CO_2H$ g.
32·00	0·70	15	0·1073
32·00	3·20	0	0·0987
32·00	1·90	0	0·1031
32·00	2·00	0	0·1028
32·00	1·80	0	0·1035
32·00	2·20	0	0·1021
34·00	0·95	15	0·1132
37·00	5·45	15	0·1081
50·00	16·10	0	0·1162
50·00	8·10	15	0·1436

It appears, as stated by Funk and Ditt,[2] that the stage of complete dibromination is not reached immediately if the concentration of acid is less than 1·6 *N*. Observing the conditions specified by Funk and Ditt, we obtained results indicating a tendency to tribromination, and this became more marked the larger the excess of bromate added.

Tribromination.—The results given below, each on 10 ml. of solution, lead us to prefer the method of tribromination, described by Day and Taggart.[3] The concentration of acid exceeded 1·6 *N*, and the period of bromination was 30 minutes at room temperature.

N/10 Potassium bromate ml.	*N*/10 Sodium thiosulphate ml.	$C_6H_4(NH_2)CO_2H$ g.
50·00	7·50	0·0971
55·00	10·90	0·1007
60·00	15·90	0·1007
65·00	21·00	0·1005
70·00	26·10	0·1003

The method involving tribromination was also tested on zinc anthranilate precipitates, and exact results were obtained.

The end-points were sharp and permanent; no difficulties, such as are sometimes met with in the titration of 8-hydroxyquinoline,[5] were experienced.

Precipitations by means of Anthranilic Acid

Zinc.—Zinc (8·000 g.) was dissolved in hydrochloric acid, and the solution was made just alkaline with sodium hydroxide, and then faintly acid with acetic acid, and diluted to 2 l. The zinc in 25 ml. of solution was determined gravimetrically by means of 8-hydroxyquinoline (found: zinc, 0·09944 g.).

Determinations by means of anthranilic acid were carried out on the above solution (25 ml.) by Funk and Ditt's gravimetric procedure, and the effects of adding ammonium acetate (5 g.) or sodium tartrate (5 g.) were also observed. The results were as follows [(An) signifies $C_6H_4(NH_2)CO_2$] :

	$(An)_2Zn$ g.	Zinc g.
Without buffer	0·5158	0·09991
	0·5161	0·09997
	0·5159	0·09993
With ammonium acetate (5 g.)	0·5068	0·09817
	0·5068	0·09817
With sodium tartrate (5 g.) ..	0·5114	0·09906
	0·5120	0·09917

Cadmium.—Cadmium sulphate (39·03 g.) was dissolved in water, the solution was made just alkaline and then just acid with acetic acid, and diluted to 2 l. The cadmium-content of the solution (15 ml.) was determined by precipitation as cadmium sulphide, followed by conversion into anhydrous cadmium sulphate for weighing (found: cadmium, 0·1292 g. per 15 ml.).

In each precipitation by means of sodium anthranilate, the solution (15 ml.) was diluted to 100 ml. with water and boiled, and the metal was precipitated by addition of the reagent (30 ml.). Filtration, washing and drying were as used for zinc. Results on unbuffered solutions and on solutions containing sodium acetate (5 g.), ammonium acetate (5 g.) or sodium tartrate (5 g.) are shown below.

	$(An)_2Cd$ g.	Cadmium g.
Without buffer	0·4429	0·1295
	0·4427	0·1294
	0·4419	0·1292
	0·4419	0·1292
With ammonium acetate ..	0·4295	0·1255
	0·4325	0·1264
With sodium acetate.. ..	0·4345	0·1270
	0·4342	0·1269
With sodium tartrate ..	0·4207	0·1229
	0·4226	0·1235

The following results show the effect of varying acidity on the extent of precipitation from solutions (140 ml.) containing ammonium acetate (5 g.):

N acetic acid, ml.	0,	5,	15,	30
Weight $(An)_2Cd$, g.	0·4295,	0·4305,	0·4240,	0·4065
Cadmium g.	0·1254,	0·1258,	0·1240,	0·1188

COBALT.—Cobaltous chloride (32·40 g.) was dissolved in water, and diluted to 2 l. Cobalt was determined in an aliquot portion (100 ml.) by electrolysis, and traces of cobalt remaining in solution were separated as sulphide, ignited and weighed as Co_3O_4 (found: cobalt, 0·0967 g. per 25 ml.). Precipitations as anthranilates were made from boiling solutions, and the precipitates were allowed to stand overnight in contact with the solutions. The results were:

	$(An)_2Co$ g.	Cobalt g.
Without buffer	0·5432	0·09669
	0·5425	0·09657
With ammonium acetate ..	0·5300	0·09434
	0·5314	0·09458
With sodium acetate.. ..	0·5347	0·09517
	0·5332	0·09491
With sodium tartrate ..	0·5390	0·09594
	0·5397	0·09607

NICKEL.—A solution of 38·28 g. of nickel sulphate in 2 l. was used. Analyses were made by electrolysis and determinations of traces remaining in solution were effected by means of dimethylglyoxime (found: nickel, 0·0773 g. per 25 ml.); nickel was also determined as the dimethylglyoxime complex (found: nickel, 0·0770 g. per 25 ml.). The determinations of nickel by means of anthranilic acid were carried out on 25 ml. of solution exactly as with cobalt.

	$(An)_2Ni$ g.	Nickel g.
Without buffer	0·4452	0·07898
	0·4452	0·07898
With ammonium acetate ..	0·4345	0·07709
,, sodium acetate ..	0·4320	0·07665
,, ,, tartrate ..	0·4378	0·07767

The determination of nickel from unbuffered and from tartrate solutions gave somewhat high results. This was confirmed by carrying out estimations on a solution of AnalaR nickel chloride, containing 32·40 g. in two litres (found, by electrolysis and dimethylglyoxime: nickel, 0·09888 g. per 25 ml.).

	$(An)_2Ni$ g.	Nickel g.
Without buffer	0·5679	0·1008
With ammonium acetate (5 g.)	0·5569	0·09881
,, sodium acetate (5 g.)	0·5512	0·09781
,, ,, tartrate (5 g.)	0·5582	0·09904

COPPER.—A solution containing AnalaR copper sulphate (12·923 g. per l.; 25 ml. ≡ 0·08227 g. Cu) was used, and 25 ml. were taken for each determination, precipitation being effected from the boiling solution.

	$(An)_2Cu$ g.	Copper g.
Without buffer	0·4342	0·08224
	0·4341	0·08222
With ammonium acetate ..	0·4335	0·08212
	0·4338	0·08217
,, sodium acetate ..	0·4337	0·08215
	0·4337	0·08215

The solubility of copper anthranilate in acetate solutions, unlike that of the anthranilates of zinc, cadmium, cobalt and nickel, is therefore negligible, and a study of the effect of varying *p*H on the extent of precipitation was accordingly made. In the following series of experiments there were taken 25 ml. of the standard copper solution and 20 ml. of 25 per cent. w/v ammonium acetate solution; sodium hydroxide or acetic acid was added, and the total volume was made up to 145 ml. with water. The copper salt was precipitated from boiling solution by addition of sodium anthranilate reagent.

*p*H	Weight of ppt. g.	*p*H	Weight of ppt. g.	*p*H	Weight of ppt. g.
9·63*	Nil	6·05	0·4344	4·39	0·4334
8·76*	0·0552	5·75	0·4333	4·04	0·4340
8·71*	0·2711	5·25	0·4343	3·04*	0·4126
8·31*	0·3923	4·96	0·4342	2·60*	0·3102
7·35*	0·4324	4·64	0·4342	2·39*	0·1780

The *p*H values marked with an asterisk were measured by means of a quinhydrone-calomel-glass electrode system, and the remainder by means of hydrogen and calomel electrodes. Variable results were obtained when using the hydrogen electrode in the alkaline copper solutions, but no difficulties were experienced with the glass electrode. When plotted, the above points fall on a curve similar to those obtained for 8-hydroxyquinoline.[6] Complete precipitation takes place over the range *p*H 7·3 to 3·3.

The following results relate to solutions, not containing ammonium acetate, to which *N* hydrochloric acid or glacial acetic acid was added, the total volume, prior to addition of reagent, being 125 ml. All measurements of *p*H were made with the glass electrode.

N HCl ml.	*p*H	$(An)_2Cu$ g.	Acetic acid ml.	*p*H	$(An)_2Cu$ g.
5	2·00	0·4017	20	2·64	0·4130
10	1·72	0·2597	40	2·30	0·3938
15	1·57	Trace	60	2·00	0·3142
20	1·34	Nil	100	1·58	0·2860

We conclude, therefore, that the method can be extended but little beyond the conditions specified by Funk and Ditt, who, in each instance, carried out precipitations from neutral unbuffered solutions. They stated that the precipitates are partly soluble in ammonium acetate solutions, but the figures which they gave indicated only very slight solubility effects. Gotô does not appear to have detected the solubility of the precipitates in presence of sodium acetate, which he used as buffer.

All the precipitates are beautifully crystalline and very easy to handle, and, except in the case of nickel, we find the determinations under the conditions specified to be highly accurate; the range of separations which can be effected is, however, very limited.

PRECIPITATIONS WITH 3-AMINO-2-NAPHTHOIC ACID.—Other amino-carboxylic acids may give similar types of precipitates to the anthranilates, but may not be

subject to solubility effects in presence of acetate buffers. Some experiments were, therefore, made with 3-amino-2-naphthoic acid 

, m.p. 214° C. A 3 per cent. solution of the reagent was prepared by dissolving 3 g. of the acid in 15·9 ml. of *N* sodium hydroxide diluted to 100 ml. with water.

Samples (10 ml.) of copper sulphate solution (Cu, 0·03291 g. per 10 ml.) were diluted to 100 ml. with water, and boiled, and 15 ml. of reagent were added; a precipitate formed at once and coagulated after a few minutes. After the solution had cooled, the precipitate was filtered off on a sintered glass crucible (G.4), and washed first with a 0·01 per cent. solution of sodium amino-naphthoate and then with alcohol. It was dried at 130° C. for half-an-hour, cooled and weighed.

Found: 0·2257 g. $[C_{10}H_6(NH_2)CO_2]_2Cu$, corresponding with 0·03294 g. of copper
0·2250 ,, ,, ,, 0·03283 g. ,,
0·2254 ,, ,, ,, 0·03289 g. ,,

Similar precipitates were obtained with nickel and cobalt solutions, but these precipitates, like that of the copper compound, were very finely divided, and filtration was slow; each filtration and washing in the above copper determinations occupied about one-and-a-half hours. On this account, the matter was not further investigated.

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Notes

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

ULTRA-VIOLET LIGHT AS AN AID IN THE FLUORESCENCE TEST FOR BROMINE

THE fluorescent nature of both fluorescein and eosin suggests that ultra-violet light might prove of assistance in sensitising the Baubigny test for bromine, in which the former compound is converted into the latter by the action of this gas (cf. *Compt. rend.*, 1897, **125**, 654; Abst., ANALYST, 1898, **23**, 23). Preliminary experiments on these lines were in progress when the paper by Seaber dealing with this reaction appeared (ANALYST, 1936, 14), and it was realised that the procedure therein described offered one of the best methods hitherto available for carrying it out. Mr. Seaber was kind enough to lend me the various standard and other stains obtained by his method, and these were examined in the filtered ultra-violet light from the usual form of mercury-vapour lamp.

Although the stains were examined in the dry state, and the fluorescence consequently was not so characteristic as with those obtained with solutions of fluorescein and eosin, it was quite apparent that inspection in ultra-violet light heightened the contrast between the eosin stain and the surrounding paper impregnated with fluorescein, the former appearing as a deep brown against the vivid golden background of the latter. This not only facilitated matching, but also increased the sensitiveness of the method, the very faint stains being rendered more pronounced. Thus, the appearance in ultra-violet light of the stain produced by 0·0000017 g. of bromine was equivalent to that produced by 0·000007 g. of bromine when viewed in ordinary light. When a little carbon (derived from the air) was present, the contrast was heightened still more. In a few instances (*e.g.* blanks and "negative" results) a faint stain was visible in ultra-violet light, although nothing was apparent in ordinary light.

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SODIUM DIETHYLDITHIOCARBAMATE AS A REAGENT FOR CERTAIN MICRO-CRYSTAL REACTIONS

THE colour reactions of sodium diethyldithiocarbamate with metals are well known, but, so far as we are aware, the use of this compound as a reagent for producing characteristic micro-crystals has not been recorded. The reagent is prepared by shaking a little of the solid salt with water in a test-tube, and filtering the liquid after a short interval; it is advisable not to use a solution more than 2 weeks old. About 0·3 ml. is placed in a test-tube (length 6 cm., diam. 6 mm.) fitted with a solid ground-glass stopper, and 0·3 ml. of a neutral solution of the substance to be tested is added, together with 0·2 ml. of a suitable organic solvent (usually benzene). The mixture is shaken, and the layer of solvent is transferred in a capillary pipette to a depression in a microscope slide, care being taken that no water is carried over with it. The solvent is then allowed to evaporate at room temperature, and the residue is examined under a low power of the microscope; sometimes it is an advantage first to add a drop of alcohol to the residue on the slide and to allow this also to evaporate before making the observation.

The results obtained are as follows:

Cadmium.—This provides the best example of the use of the method. Large isolated hexagonal crystals with well-defined facets are formed, the sensitiveness being 0·01 mg. of Cd (1:20,000).

Mercuric salts crystallise (but with greater difficulty) in groups of brown plates. The sensitiveness is 0·1 mg. $Hg^{\cdot\cdot}$ (1:2,000), and it is not greatly affected by the presence of an equal quantity of cadmium.

Antimony and *bismuth* give unsatisfactory results, as they do not easily crystallise, but form oily drops. This is an important point, because the presence of such drops may hinder the crystallisation of the complexes of other metals which may be present.

Manganous salts form elongated hexagonal crystals, the sensitiveness of the reaction being 0·01 mg. (1:20,000). The method cannot be used satisfactorily in the presence of ferric iron or copper salts, as these form crystals which are isomorphous with those of the manganous complex.

Lead, *zinc* and *strontium* produce isomorphous elongated rectangular plates, which frequently occur in groups of radiating crystals. There is reason to believe, however, that these represent only an intermediate stage in the formation of a more stable and less characteristic crystalline form, and it is therefore doubtful whether the reaction has much practical significance in such cases. The sensitiveness with lead is of the same order as with mercuric salts, and it is unaffected by the presence of an equal quantity of mercuric salts.

Cobalt forms almost rectangular brown-green plates. The principal value of this reaction, however, is the fact that the corresponding nickel complex is entirely different; it is produced only with difficulty, and is then formed as tiny green hexagons. The reactions may therefore be used to detect cobalt in the presence of nickel, although it is unreliable as a test for the latter, either in the pure state or in a mixture.

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A TEST FOR POROSITY IN THE COATING OF TIN PLATE

THE various methods recommended either require considerable time to carry out or do not produce a permanent pictorial record of the porosity of the tin coating. A count of pinholes is usually made, and the number per sq.cm. calculated. Such calculations often fail to reveal the true nature of the defects in the tin coating, in that they do not clearly portray isolated porous areas or streaks of exposed base metal. By the method here described, which is essentially a modification of the use of ferricyanide, a permanent record of the quality of the tin coating is obtained.

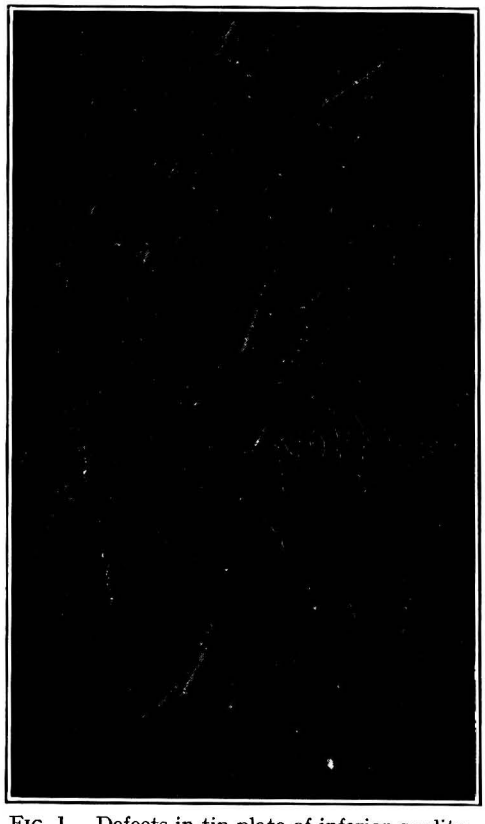

FIG. 1. Defects in tin plate of inferior quality.

METHOD.—To 750 ml. of a 7 per cent. solution of potassium ferricyanide in distilled water are added 10 drops of concentrated sulphuric acid (sp.gr. 1·840). In this solution suitable sheets of plain "Cellophane" paper (No. 300) are soaked for at least fifteen minutes until thoroughly permeated by the solution. The sheets are then withdrawn, drained for a minute to remove superfluous solution, and laid flat on the area of the tin plate to be tested. The surface of the tin plate should first be thoroughly cleaned by washing with carbon tetrachloride, followed by anhydrous acetone. Care should be taken to bring the total area of the prepared "Cellophane" paper into intimate contact with the tin plate. This is conveniently achieved by means of a photographic squeegee. The sheets are allowed to "develop" for 45 to 60 minutes, during which time they are covered with filter paper damped with the ferricyanide solution to prevent evaporation and lifting. The sheets are then carefully removed from the plate, washed for a few seconds, and dried, when it will be found that imperfections in the tin coating are indicated by the formation of deep blue stains. The prints thus obtained furnish a permanent pictorial record of the defects in the tin coating.

The "Cellophane" prints may, if desired, be used as photographic negatives and contact prints, as illustrated in Fig. 1, can be obtained.

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SOME ANALYTICAL CHARACTERISTICS OF DATE-STONE OIL

WILLIAMS* has recently drawn attention to the fact that date stones contain about 8 per cent. of a pungent oil, whose properties require investigation.

DATE STONES.—The following analyses of ground date stones, together with those of their extracted oils, may be of interest:

	Mixed Per Cent.	Deglet Nour variety Per Cent.	Iraq Per Cent.
Moisture	7·96	9·82	6·46
Ash	0·89	0·86	1·12
Protein	5·25	5·30	5·22
Carbohydrates (by diff.)	65·53	58·53	62·51
Fibre	13·60	18·10	16·20
Oil	6·77	7·39	8·49

Ground date stones have been used as a cattle food.

DATE-STONE OIL.—The stones were ground in a high-speed laboratory disintegrator, and the oil was extracted by percolation in the cold with petroleum spirit (b.p. 40°–60° C.). A preliminary grading by sieving through a standard 30-mesh sieve increased the proportion of oil in the first (mixed) sample to 9·27 per cent., while the fibre was decreased to 9·0 per cent., a large portion of the cellulose material being rejected by the sieve.

The oil thus obtained was a pale yellowish-green liquid with a pleasant (not pungent) odour. The following values were obtained:

	Mixed	Deglet Nour variety	Iraq
Sp.gr. at 15·5° C.	0·9201	0·9203	0·9207
Refr. index, n_D^{40}	1·4580	1·4574	1·4580
Saponification value	206·1	212·6	208·3
Iodine value	54·5	50·2	53·4
Unsaponifiable matter, per cent. ..	1·98	0·51	0·40
Free acids (as oleic), per cent. ..	0·5	0·2	0·3
Reichert–Meissl value	1·0	0·9	1·1
Polenske value	3·0	2·7	2·9
Insoluble fatty acids, per cent. ..	88·7	91·5	88·9
Insoluble bromides	Nil	Nil	Nil
Halphen test	Negative	Negative	Negative
Bieber test	,,	,,	,,

The separated fatty acids were solid and of a yellow or yellowish-green colour. They gave the following values:

	Mixed	Deglet Nour variety	Iraq
M.p.	22·0° C.	22·0° C.	22·0° C.
Solid. pt.	17·5° C.	17·5° C.	17·5° C.
Refr. index, n_D^{40}	1·4467	1·4465	1·4465
Iodine value	56·6	56·1	56·4

Exposed to screened filtered ultra-violet light in quartz tubes all the oils showed a bluish-purple fluorescence, whilst the separated acids fluoresced greenish-blue.

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* Report of Chemical Laboratory, Rustamia, Baghdad; *Food Manufacture*, Oct. 1935, p. 352.

Report of the Analytical Methods Committee

THE REICHERT–POLENSKE–KIRSCHNER PROCESS

THE Society has been requested to supply a description of the method for the determination of the Reichert–Wollny or Reichert–Meissl, Polenske and Kirschner values of butter-fat, and the Analytical Methods Committee reports as follows:

The process in general use is based on the method described by Polenske,[1] with Kirschner's[2] extension which came into use after the work of Revis and Bolton.[3] Slight variations in the dimensions of the apparatus, however, have appeared in the literature, and chemists also use methods differing in certain details.

The dimensions and form of the apparatus described below agree with those prescribed by Polenske, and the tolerances in dimensions are in accordance with the apparatus in common use. These have been approved by the Scientific Glassware Committee of the British Standards Institution.

Particular care has been taken to avoid modification of the method in any way that would invalidate data already accumulated, but several details, likely to cause slight differences in the results, such as the amount of water to be added to the saponified fat, and the size of the particles of pumice added to ensure regular boiling, have been specified. Some chemists add the water measured cold, and others add the same volume measured hot; again, pumice has been used varying from fragments of the size of a pea, as recommended in the now redundant Reichert–Wollny method agreed between the Government Laboratory and this Society for determining the proportion of butter-fat in margarine,[4] to very finely divided powder.

As the original Reichert process, using 2·5 g. of fat, and as the Reichert–Meissl process, using 5 g., have been obsolete since Wollny[5] modified the Meissl process nearly 50 years ago, and as the name Reichert is common to the different forms, it may now be used alone in place of the indiscriminate use of the hyphenated forms, Reichert–Meissl, Reichert–Meissl–Wollny, Reichert–Wollny and Reichert–Polenske, when applied to the soluble volatile acid value.

VOLATILE FATTY ACIDS OF BUTTER-FAT

REICHERT–POLENSKE–KIRSCHNER PROCESS

This process does not determine the *total* quantities of volatile fatty acids, soluble and insoluble in water, present in combination in the fat. The amounts of these acids actually determined by the process are dependent on strict adherence to the dimensions of the apparatus and the details of procedure.

PREPARATION OF THE FAT FOR ANALYSIS.—Heat a portion of the sample of butter in a beaker until the fat separates from the water and curd. To facilitate separation and filtration, it is advisable that the temperature should not be above 50° C. Filter the fat layer through a dry paper into a dry vessel, and, if necessary, re-filter the filtrate until it is clear and free from water. Liquefy the fat completely before taking samples for analysis. Exposure of the warm fat to the air should be as short as possible.

REAGENTS.

Glycerol.

Concentrated sodium hydroxide solution (50 per cent. by weight).—Sodium hydroxide is dissolved in an equal weight of water and the solution is stored in a bottle protected from carbon dioxide. The clear portion free from deposit is used.

Dilute sulphuric acid solution.—Approximately 25 ml. of concentrated sulphuric acid are diluted to 1 l., and adjusted until 40 ml. neutralise 2 ml. of the sodium hydroxide (50 per cent.) solution.

Pumice powder.—Pumice, ground, passing through a sieve B.S. No. 50,* and remaining on a sieve B.S. No. 90.

Phenolphthalein solution.—0·5 g. of phenolphthalein dissolved in 100 ml. of industrial methylated spirit.

Alcohol.—Industrial methylated spirit neutralised to phenolphthalein immediately before use.

Sodium hydroxide solution.—Approximately $N/10$ solution of sodium hydroxide, of accurately determined strength.

Barium hydroxide solution.—Approximately $N/10$ solution of barium hydroxide, of accurately determined strength.

Silver sulphate, powdered.

All reagents must be of the quality required for quantitative chemical analysis.

APPARATUS.—100-ml. graduated cylinder; Class B., B.S.S. No. 604.
Fifty-ml. pipette complying with the N.P.L. Class B regulations.

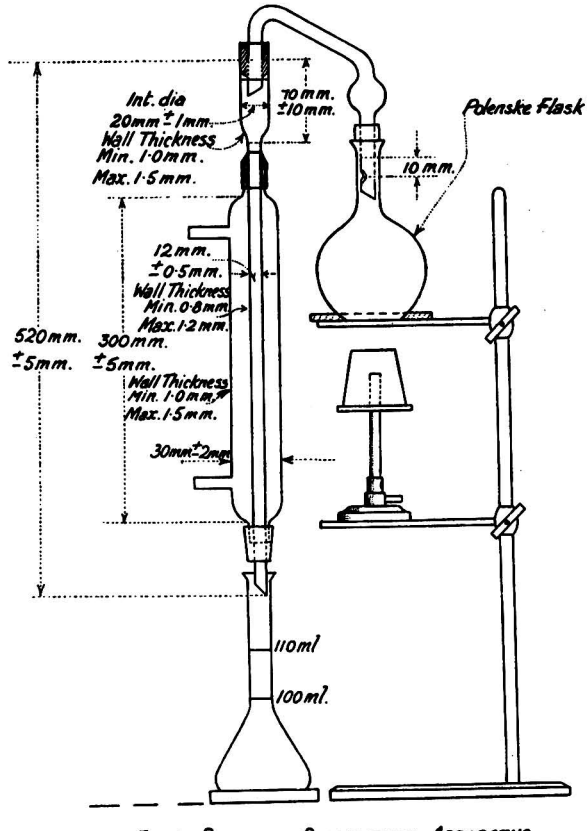

FIG. 1 POLENSKE DISTILLATION APPARATUS

* See British Standard Specification for Test Sieves, No. 410, 1931.

The assembly of the apparatus for the distillation is shown in Fig. 1, and details of the constituent parts are given below:

Flat-bottom boiling flask (Polenske).*—The flask shall be made of resistance glass and shall conform with the following details:

Volume contained to bottom of neck	310 ml.	±	10 ml.
Length of neck	75 mm.	±	5 mm.
Internal diameter of neck	21 ,,	±	1·0 ,,
Overall height	160 ,,	±	5 ,,
Diameter of base	45 ,,	±	5 ,,

Still-head.†—The still-head shall be made of glass and shall conform with the dimensions given in Fig. 2.

Condenser.—The condenser shall be made of glass and shall conform with the dimensions given in Fig. 1.

Receiver.—The receiver shall be a flask with two graduation marks on the neck, one at 100 ml. and the other at 110 ml., conforming with the British Standards Specification for Sugar Flasks, No. 675.

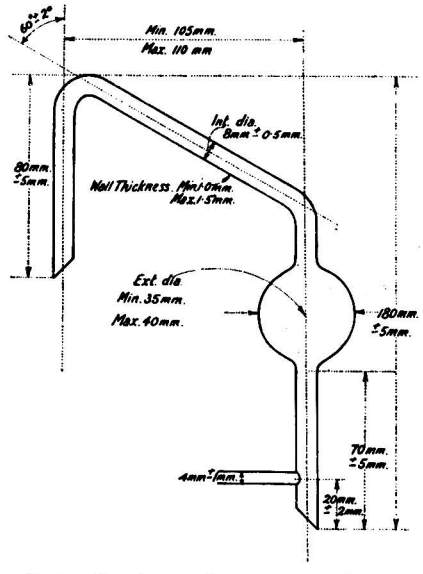

FIG. 2. STILL HEAD FOR POLENSKE DISTILLATION APPARATUS.

*Asbestos board.**—An asbestos board, 120 mm. diameter, 6 mm. in thickness, with a circular hole about 65 mm. diameter.

PROCEDURE.—Weigh 5 g. (tolerance not exceeding 0·01 g.) of the fat into a Polenske flask. Add 20 g. of glycerol and 2 ml. of concentrated sodium hydroxide solution. (The burette containing the soda solution must be protected from carbon dioxide, and, before withdrawal of the solution for tests, the nozzle must be wiped clean from carbonate, and the first few drops of solution rejected.) Heat over a naked flame, with continuous mixing, until the fat, including drops adhering to the upper parts of the flask, is saponified, and the liquid becomes perfectly clear. Cover the mouth of the flask with a watch-glass.

[Make a *blank* test without fat, but using the same quantities of reagents and following the same procedure, and avoiding over-heating during the heating with soda (indicated by darkening of the solution).]

Measure 93 ml.‡ of boiling distilled water, which has been vigorously boiled for 15 minutes, into a 100-ml. graduated measuring cylinder. When the soap is sufficiently cool to permit addition of the water without loss, but before the soap has solidified, add the water to the flask, draining the cylinder for 5 seconds, and dissolve the soap.

* During distillation the Polenske flask must fit snugly into the hole in the asbestos board so as to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos board may be conveniently prepared by bevelling the edge of the hole, soaking in water, moulding the edge with a flask and drying.

† The position of the side hole of the still-head relative to the stopper of the Polenske flask should also be as shown in the diagram.

‡ The temperature of the water in a cylinder rinsed out and refilled with boiling water is between 85 and 90° C.; 90 ml. of cold water correspond with 92·7 ml. at this temperature, or, allowing 0·3 ml. for water retained on draining, 93 ml. altogether. Addition of the water hot avoids loss of time in dissolving the soap.

If the solution is not clear (indicating incomplete saponification), or is darker than light yellow (indicating over-heating), the saponification must be repeated on a fresh sample of the fat.

Add 0·1 g. of powdered pumice, followed by 50 ml. of the dilute sulphuric acid solution, and connect the flask at once with the distilling apparatus, shown in Fig. 1. Heat the flask, without boiling, until the insoluble acids are completely melted, then increase the flame and distil 110 ml. in 19 to 21 minutes. The flow of water in the condenser must be sufficient to keep the temperature of the issuing distillate between 18° and 23° C. When the distillate reaches the 110-ml. mark, remove the flame and replace the 110-ml. flask by a cylinder of about 25-ml. capacity to catch drainings. Close the 110-ml. flask with a stopper, and, without mixing, place it in water at 15° C. for 10 minutes so as to immerse the 110-ml. mark. Remove the flask from the water, dry the outside, and invert the flask carefully, avoiding wetting of the stopper with the insoluble acids. Mix the distillate by four or five double inversions without violent shaking. Filter through a dry 9-cm. No. 4 Whatman filter paper, which fits snugly into the funnel. Reject the first runnings and collect 100 ml. in a dry flask; cork the flask, and retain the filtrate for titration as at *R* below. The filtrate must be free from insoluble fatty acids.

Detach the still-head and wash the condenser with three successive 15-ml. portions of cold distilled water, passing each washing separately through the cylinder, the 110-ml. flask and the filter, nearly filling the paper each time, and draining each washing before filtering the next. Discard the washings.

Dissolve the insoluble acids by three similar washings of the condenser, the cylinder and the filter with 15 ml. of neutralised alcohol, collecting the solution in the 110-ml. flask and draining the alcohol after each washing. Cork the flask and retain the solution for titration as at *P* below.

(*R*)—Reichert* (or Soluble Volatile Acid) Value

Pour 100-ml. of the filtrate containing the soluble volatile acids into a titration flask, add 0·1 ml. of solution of phenolphthalein, and titrate with *N*/10 barium hydroxide solution† until the liquid becomes pink, rinsing the 100-ml. flask with the nearly neutralised liquid towards the end of the titration.

[If the Kirschner value (see below) is to be obtained, the titration flask must be dry before use; note the actual volume of barium hydroxide solution used; drain the 100-ml. flask into the titration flask, close with a cork, and continue as at *K* below.]

If the amounts of barium hydroxide solution used for the titration of the sample and the blank are equivalent to x ml. and x_b ml. *N*/10, respectively, the

$$\text{Reichert value} = (x - x_b) \times \frac{110}{100}$$

(*P*)—Polenske (or Insoluble Volatile Acid) Value

Titrate the alcoholic solution of the insoluble volatile acids after addition of 0·25 ml. of phenolphthalein solution with *N*/10 barium (or sodium) hydroxide solution, until the solution becomes pink.

If the amounts of barium (or sodium) hydroxide used for the titration of the sample and the blank are equivalent to y and y_b ml. *N*/10, respectively, the Polenske value $= P = (y - y_b)$.

* It is considered that the name "Reichert" is preferable to the cumbrous and sometimes inaccurate names associated with one or more of those who have modified Reichert's original process, such as Meissl, Wollny and Polenske.

† *N*/10 sodium hydroxide solution may be used for the titration if the Kirschner value be not required.

(*K*)—Kirschner Value

Add 0·5 g. of finely powdered silver sulphate to the neutralised solution from (*R*), above. Allow the flask to stand in the dark for one hour, with occasional shaking, and filter the contents through a dry filter. Transfer 100 ml. of the filtrate to a dry Polenske flask, add 35 ml. of cold distilled water (recently boiled for 15 minutes), 10 ml. of dilute sulphuric acid solution and a loosely-wound 5 mm. coil of 30 cm. of aluminium wire (about 1 mm. thick or S.W.G. about 18 to 20), or 0·1 g. of pumice powder. Connect the flask with the standard apparatus and repeat the process as described above, *i.e.* the distillation of 110 ml. in 19 to 21 minutes, the mixing (but without cooling for 10 minutes), the filtration, and the titration of 100 ml. of the filtrate with *N*/10 barium hydroxide solution.

If the amounts of barium hydroxide solution used for the titration of the fat and the blank are equivalent to z ml. and z_b ml. of *N*/10, respectively,

$$(K),\ \text{the Kirschner value} = (z - z_b) \times \frac{(100 + a) \times 121}{10000}$$

where a represents the actual volume in ml. of barium hydroxide solution used in the titration for determination of the Reichert value (see *R* above).

Note.—Polenske values, and, to a much slighter extent, Reichert values, have been found to be low when determined at low barometric pressures, such as may occur at high altitudes. The following factors may be applied to values determined at a barometric pressure of p mm. of mercury, to convert them to the values determined under normal pressure (Kirkham).[6]

Corrected Reichert value

$$= \left(\frac{(\text{Observed value} - 10)\ \log\ 760}{\log p}\right) + 10$$

Corrected Polenske value

$$= \text{Observed value} \times \left(\frac{760 - 45}{p - 45}\right)$$

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6. V. H. Kirkham, Analyst, 1920, **44**, 293.

Official Appointments

ANDREW MORE, as Deputy Government Chemist (April 17th).

HAROLD EDWARD MONK, as Public Analyst for the County of Worcestershire, in place of C. C. Duncan (retired), May 2nd.

GEORGE HUGH WALKER, as Public Analyst for the County Borough of Salford, in place of H. E. Monk, appointed to Worcestershire (May 2nd).

HAROLD EDWARD MONK, as Public Analyst for the County Borough of Worcester, in place of C. C. Duncan (retired), May 19th.

ERNEST ROBERT ANDREWS, as Deputy Agricultural Analyst for the County of London, in place of E. T. Shelbourn (appointed Agricultural Analyst), May 19th.

HAROLD EDWARD MONK, as Agricultural Analyst for the County of Worcestershire and the County Borough of Worcester, in place of C. C. Duncan (retired), May 19th.

EDWARD THOMAS SHELBOURN, as Agricultural Analyst for the County of London, in place of J. H. Coste (retired), May 19th.

GEORGE HUGH WALTER, as Agricultural Analyst for the County Borough of Salford, in place of H. E. Monk (appointed to Worcestershire), May 19th.

Notes from the Reports of Public Analysts

The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.

STATES OF JERSEY

REPORT OF THE OFFICIAL ANALYST FOR THE YEAR 1935

THE total number of samples analysed during the year was 4778, including 766 samples of foods, drugs and waters examined for the Public Health Committee, and 3223 of soils and fertilisers for the Agricultural Committee.

BORIC ACID ON IMPORTED FOWLS.—Ten fowls, sent by post from the Irish Free State, were submitted for examination by the States Veterinary Surgeon; on eight of them boric preservative was found, and they were condemned.

FERTILISER INGREDIENT FROM SEAWEED.—Two samples, from a private source, of a white powder obtained by dissolving, filtering and evaporating the ash of "Colley" seaweed, were found to contain 35·7 and 46·6 per cent. of potash (K_2O) respectively.

LEAD IN JERSEY WATER.—The action of Jersey well waters on metals continues to be a serious matter, especially in respect of the action on lead pipes. In 35 supplies lead was found in the water, and in one case in which the consumer was ill, a sample contained the very high proportion of 1 grain of lead per gallon. Other samples received also indicated that certain rain waters here exert some attack on lead.

The only certain remedy is to avoid the use of lead (or "compo") pipes and pumps, except for waterworks water. C. P. MONEY

Legal Notes

Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.

NON-ALCOHOLIC MEAT AND MALT WINE

ON April 17th the Scissett Industrial Co-operative Society was summoned at Barnsley West Riding Police Court for selling as non-alcoholic meat and malt wine, a preparation containing less than 4 per cent. of meat extract and less than 25 per cent. of malt extract.

Mr. C. Phythian, prosecuting, stated that the wine was described on the label as one which strengthened and built up the whole system. On a separate leaflet, which was with the bottle, was a statement that the preparation nourished, strengthened, and built up the blood, brain and nervous system; that its basis was the juice of the finest grapes along with all the active principles of meat and malt wine; and that it contained a proportion of glycerophosphates.

Mr. F. W. Richardson, F.I.C., West Riding County Council Analyst, said that the sample contained 24 per cent. of carbohydrates, consisting of maltose, dextrose, laevulose and maltodextrins (which were of no more nutritive value than ordinary sugar), and not more than 1 per cent. of meat extract; the nitrogen and phosphorus pentoxide contents were 0·129 per cent., and 0·117 per cent., respectively; diastase, grape juice, and glycerophosphates were absent. In his opinion a meat and malt wine should contain not less than 4 per cent. of meat extract and 25 per cent. of malt extract. The preparation was sold at 4s. 6d. per bottle, and with his suggested proportions of meat extract and malt extract it would cost 4d. per pint. So far as his experience went, all non-alcoholic meat and malt wines were worthless.

Mr. C. J. H. Stock, B.Sc., F.I.C., Durham County Analyst, said that, like Mr. Richardson's, his suggested standard for such a non-alcoholic preparation would be 4 per cent. of meat extract and 25 per cent. of malt extract. His reason for this opinion was that a true meat and malt wine contained about 16 or 17 per cent. of alcohol, and a non-alcoholic meat and malt wine should, therefore, contain an equivalent proportion of malt.

Dr. Wilfred Vining, M.D., F.R.C.P., of Leeds University, said that the meat extract present had no food value, and that 2 oz. of the best beef steak was equivalent in protein to 2½ gallons of the wine.

Dr. J. A. Fraser, Assistant County Medical Officer of Health, said that the wine would certainly produce fat by reason of the carbohydrates present, but possessed no flesh-forming qualities.

Mr. G. Raymond Hinchcliffe, defending, submitted that the bottle of wine was no more and no less than a bottle of non-alcoholic meat and malt wine, properly labelled. The magistrates were not concerned with the value of the wine. Hundreds of thousands of bottles had been sold throughout the length and breadth of Great Britain during the last five-and-a-half years, and, so far as was known, the West Riding County Analyst was the only person to take exception to its contents. The statements contained in the leaflet, though rather of a puffing nature, were literally true. As there was no legal standard for meat and malt wine, it rested with the Bench to fix its own standard, and he submitted that the wine in question was above what the minimum standard for such a wine should be. The Bradford Stipendiary Magistrate had dismissed a summons in which the same wine was concerned.

Mr. C. H. Manley, M.A., F.I.C., Leeds City Analyst, stated that his standard for such a non-alcoholic preparation would be 1 per cent. of meat extract and 4 per cent. of malt extract, and that, accepting Mr. Richardson's maximum figure

for meat extract present, the present preparation contained 1 per cent. of meat extract and 6·8 per cent. of malt extract; he quoted the B.P. Codex, 1934, to show that less importance was now attached to the digestive action of diastase than formerly; there was present, moreover, in the carbohydrates, in addition to approximately 2 per cent. of dextrin and 4 per cent. of maltose, 18 per cent. of glucose (including laevulose) in the grape juice (customarily employed to impart the wine flavour), the superior energising value of which over ordinary sugar was well recognised. Further, as no mineral phosphates were present, the phosphorus pentoxide in excess of that equivalent to the nitrogen found might well be due to the presence of a proportion of glycerophosphate.

Dr. W. Macadam, Professor of Clinical Medicine, and Lecturer in Dietetics at Leeds University, said that he did not attach overmuch importance to the actual amount of meat extract present; quite small quantities of this, in his opinion, were sufficient to exert on the gastric juices that stimulating effect which indirectly made possible the effective digestion of the food which followed. He considered the wine of beneficial value in certain affections and in various stages of convalescence. He quoted Pavlov's experiments on dogs as proof that a combination of sugars with meat extract did not diminish, but rather increased, the stimulating action of the latter.

The Chairman (Mr. W. Humphries), in giving the judgment of the Bench, said: "It is a very important case, and we are prepared to accept the standard put up by the defence and dismiss the case."

Costs were not allowed, as it was considered that the County Council had done the proper thing in bringing the case.

MERCURY OINTMENT

ON April 17th three appeals were heard at the London Sessions against convictions and fines for selling mercury ointment not of the nature, substance and quality demanded.

In dismissing the two appeals against the penalties, with £5 5s. additional costs in each case, the Chairman (Sir Percival Clarke) said that he thought that when the chemist was asked to supply a particular article, and when precautions were taken to write its name down, it was of great importance that he should supply that article. He thought that the magistrate was right in inflicting the penalties.

In the third appeal Mr. Christmas Humphreys, appearing for the respondents (the Bethnal Green Borough Council), said that the facts were the same in substance as in the other two cases, except that the chemist supplied golden ointment which contained such a small percentage of oxide of mercury that it would be useless for the treatment of a complaint for which mercury ointment was required.

An agent for the inspector of the Bethnal Green Borough Council said that she asked for a box of mercury ointment, and also handed over a slip on which "mercury ointment" was written. She received three yellow tubes, for which she paid 4½d. each. She denied that a shop assistant had told her to take golden eye ointment and to bring it back if it was not what was required.

Two assistants gave evidence that they had not had a request for mercury ointment, and had supplied golden eye ointment, requesting the purchaser to bring it back if it was not what was wanted.

Dr. W. O'Donovan, physician at the Skin Hospital, said that he considered it inadvisable to put mercury ointment in the hands of the public, because it was liable to produce a rash if used without medical advice. He had never known a demand in the East End of London for mercury ointment by the public for self-treatment.

The Chairman said that the Court accepted the evidence of the shop assistants, and allowed the appeal, with £10 10s. costs against the respondents.

LINIMENT OF TURPENTINE

ON April 30th a druggist was summoned at the Thames Police Court for selling liniment of turpentine not of the nature, substance and quality demanded.

Mr. E. Fail, for the Stepney Borough Council, said that a sample of liniment of turpentine purchased at the defendant's shop was found to contain no camphor and only 25 per cent. of turpentine. The assistant who supplied the liniment said that he frequently sold liniment containing turpentine, but was rarely asked for liniment that conformed to the B.P. standard. The demand for it was so rare that it was not even kept in the shop, although he made it up if required. He sold a great deal of white liniment, which was made up according to the formula published by the National Insurance Committee. When the sampling officer's assistant asked for liniment of turpentine, he asked him whether he required white liniment, and was told that he did. Accordingly he filled the bottle with the formula liniment and charged 2s. The price would have been the same if he had supplied B.P. liniment. Had he thought that B.P. liniment was required he would have supplied it.

The Magistrate (Mr. Everard Dickson) dismissed the summons.

Ministry of Health

THE MILK (SPECIAL DESIGNATIONS) ORDER, 1936*

THE Minister of Health, after consideration of the representations made to him and after consultation with the Minister of Agriculture, has now made the new Milk (Special Designations) Order which came into operation on the 1st June, 1936.

PRINCIPAL OBJECTS OF THE ORDER.—The new Order has two main objects—to transfer from the Minister to local authorities the duty of granting licences to producers of certain graded milks, and to improve and simplify the special designations of milk.

LOCAL AUTHORITIES TO GRANT LICENCES.—The Minister of Health undertook the work of granting licences to producers of Certified and Grade A (Tuberculin Tested) milks only while the graded milk scheme was in its early stages. This work is appropriate to local authorities who already grant all other licences under the scheme, and the number of licences has now increased to an extent which makes devolution necessary.

THE NEW DESIGNATIONS.—An alteration in the designations of the grades of milk is also much needed. The present designations are "Certified," "Grade A (Tuberculin Tested)," "Grade A," and "Pasteurised." The existence of so many grades creates confusion, and some of the designations are not such as to give to consumers a clear indication of the nature of the milk purchased. Accordingly it is proposed to reduce the number of grades to three—"Tuberculin Tested," "Accredited" and "Pasteurised."

"TUBERCULIN-TESTED" MILK.—"Tuberculin Tested" will replace the existing designations "Certified" and "Grade A (Tuberculin Tested)."

The chief characteristic of "Certified" and "Grade A (Tuberculin Tested)" milks is that they both come from herds which have been subjected to a stringent test for the absence of tubercle. The new designation "Tuberculin Tested" which, when the Order comes into force, will be the only designation for raw milk from tuberculin-tested cows, will indicate this characteristic more clearly than the existing designations.

* H.M. Stationery Office, Kingsway, London, W.C.

"Tuberculin-tested" milk may, if desired, be pasteurised subject to the conditions of the Order, and where this is done, it must be sold as "Tuberculin-Tested Milk (Pasteurised)." This milk will have the double security of tuberculin testing (as a safeguard against bovine tuberculosis) and pasteurisation (as a safeguard against all milk-borne diseases).

"Tuberculin-tested" milk will be subject to bacteriological tests which are of great importance for the purpose of ensuring the cleanliness and good keeping quality of milk. But these tests are not tests for disease-causing organisms, and the Minister of Health, having regard to the need for simplification, has not felt it necessary to retain the specially stringent bacteriological test which is at present applied to "Certified" milk. The only other material characteristic of "Certified" milk is that it is required to be bottled on the farm. Accordingly, when the new grade of "Tuberculin-tested" milk is bottled on the farm the Order will permit it to be described as "Tuberculin-tested Milk (Certified)." This will be of advantage to producers who now hold licences for the production of "Certified" milk and desire to retain in some form the use of this designation.

"ACCREDITED" MILK.—"Accredited" milk will replace the present "Grade A" milk and be subject, broadly, to the same conditions, *i.e.* it will be raw milk from cows which are regularly inspected by a veterinary surgeon, but are not tuberculin-tested. It will be subject to the same bacteriological tests as "Tuberculin-tested" milk. The designation "Accredited" will carry a clearer meaning than "Grade A," as it will indicate that milk sold as "Accredited" comes from the herds of producers enrolled under the Accredited Producers' Scheme initiated by the Milk Marketing Board. This Scheme has already met with considerable success, and there are now nearly 16,000 producers enrolled under the Scheme. Where "Accredited" milk is bottled on the farm the Order will permit the words "Farm Bottled" to be added to the description.

"PASTEURISED" MILK.—"Pasteurised" milk will, as at present, be milk which has been held at a temperature of 145°–150° F. for 30 minutes. There has been no need to alter this designation, as it indicates clearly that milk so described gives the protection which pasteurisation affords against all forms of milk-borne disease.

BACTERIOLOGICAL TESTS.—After the 31st December next the present method of prescribing the bacterial standard by a "plate-count" test of 200,000 bacteria per ml. will be superseded in relation to raw "Tuberculin-tested" and "Accredited" milks by a colour test, as recommended in the recent report of the Medical Research Council (Special Report Series, No. 206). The test for *coliform bacillus* will, however, be retained as at present.

PERMISSION TO USE UP STOCKS OF BOTTLE-CAPS, ETC.—In order that existing stocks may be used up and unnecessary loss to producers and distributors avoided, bottle-caps and labels complying with the existing Order will be allowed until the 31st December next, but apart from this it will not be permissible after the 31st May to sell milk under any of the designations which the new Order abolishes.

OTHER PROVISIONS OF THE ORDER.—The new Order contains a number of other provisions, including a power to licensing authorities to charge less than the prescribed maximum fees for licences, if they wish to do so, or to forego fees altogether.

CIRCULAR TO LOCAL AUTHORITIES.—A circular explaining the new Order is being sent to local authorities. This deals with a number of administrative points, including inspection and sampling. Amongst other matters, the attention of local authorities is drawn to a new chemical test (the phosphatase test) for determining whether or not milk has been properly pasteurised.

Medical Research Council

THE BACTERIOLOGICAL GRADING OF MILK*

THIS Report is divided into two parts: Part I is a critical study of the bacteriological technique used in the grading of milk: the plate count, the coliform count, the methylene blue test and miscellaneous tests, and Part II is a critical study of the interpretation of these tests by comparison with hygienic or unhygienic methods of production, together with a discussion of the general principles of bacteriological grading of milk and general conclusions and recommendations.

PART I. THE TECHNIQUE OF THE TESTS

SECTION A. THE PLATE COUNT.—The sub-sections include: difficulties and pitfalls of sampling; time and temperature for holding samples; types of pipettes and methods of delivery; diluents and methods of dilution; nutrient value of various extracts and peptones; choice of medium; effect of *p*H; amount of medium per plate; methods of mixing and pouring; incubation for 2 and 5 days at 22°, 30°, and 37° C.; methods of counting and magnitude of error. These and other points are studied in detail, investigated practically, and their experimental errors worked out. Each sub-section has its summary and conclusions, and the final sub-section gives the method recommended by the author for the performance of the plate count. One notes that when the best conditions are observed the experimental error in the milk agar 2-day count at 37° C., when one plate only is used, is assessed at ±75 per cent. or three times the standard error of ±25 per cent. That is to say, a count of 100,000 per ml. would represent a number somewhere between 25,000 and 175,000.

SECTION B. THE COLIFORM COUNT.—The sub-sections include:—(1) Qualitative examination of Raw and Pasteurised Milk, Cow Dung and Foodstuffs for Coliform Organisms and the Differentiation of these Organisms into *Coli*, *Aërogenes*, *Cloacae*, Intermediate and Irregular Types. (2) Observations on the Quantitative Estimation of Coliform Organisms by the plating and dilution methods, including the relative rate of growth of *B. coli* and *B. aërogenes* in milk at 37° and 22° C. (3) Quantitative Estimation of *Coli aërogenes* bacilli in milk, for which four methods are described of which Method III is regarded as "peculiarly suitable for a rapid estimation of the faecal coli count," this method consisting in the incubation of a double set of MacConkey tubes at 37° C. and 44° C., all tubes showing acid and gas at 37° C. being sub-cultured into Koser's citrate to see whether citrate positive organisms are present, the latter being *aërogenes-cloacae*-intermediate types, and those growing at 44° C. faecal types. It is pointed out that the dilution method gives a very high experimental error, the count being from 70 per cent. below to 260 per cent. above the true value when 5 tubes to each dilution are used.

SECTION C. THE METHYLENE BLUE TEST.—After an historical introduction a modified method for the performance of this test is described, the distinctive features of which are:—(1) Strict control of temperature, (2) the maintenance of a homogeneous suspension of fat globules and bacteria, and (3) performance of the test in the dark. Sub-section 2 deals with the electrometric method of estimating reduction potential in milk and the apparatus used for this measurement is described. An account is given of various investigations which show that, apart from micro-organisms, there is a reducing system in milk, evident only under anaerobic conditions, due to some enzyme destroyed by pasteurisation; this enzyme is absorbed on the fat-globules, so that the reducing capacity is lost on

* Special Report Series. No. 206, by G. S. Wilson, M.D., F.R.C.P., D.P.H., assisted by R. S. Twigg, R. C. Wright, C. B. Hendry, M. P. Cowell, and I. Maier. H.M. Stationery Office, 1935. Price 7s. 6d. net.

the removal of the fat and restored to separated or pasteurised milk by the addition of raw cream or of the water-soluble fraction of raw cream after shaking out with ether and separating. Reasons are adduced for believing that the natural reducing system in raw milk plays a part, though not the principal part, in the reduction of methylene blue by active bacterial growth. It is shown that under aerobic conditions the leucocyte-content has little effect, if any, on the reducing activity, and the same applies to dead micro-organisms. The reducing activity of micro-organisms in general, and of *B. aërogenes*, *B. coli*, *Staphylococcus aureus*, a large micrococcus and of other micro-organisms comparatively is investigated. Sub-section 11 records observations on the bacterial flora present at the time of reduction and the number of organisms present. Sub-section 14 deals with practical considerations, including a comparison of the results obtained by the new method and the old, and Sub-section 17 assesses the total error of the new method, giving a comparison of the results obtained by four separate workers, showing a mean coefficient of variation of 1·12 as compared with C.V. of 21·54 and 15·49 for raw and pasteurised milk plate counts. It is claimed that the new test gives a better defined end-point, and more concordant results, than the old.

SECTION D. MISCELLANEOUS TESTS.—The following are considered:—The sediment test and the leucocyte content; the Breed smear method; estimation of acidity; determination of *p*H; the bromthymol blue test; the keeping quality test; the rate of increase of plate count on incubation; laboratory pasteurisation test; the Frost little-plate method; and the Bury smear-culture method.

PART II. INTERPRETATION OF THE TESTS USED

SECTION E. COMPARISON OF THE TESTS WITH THE HYGIENIC CONDITIONS OF PRODUCTION.—Sub-section 1 gives the scheme adopted for marking by farm inspection. It is to be noted that the method of collecting and transmitting samples involves the admixture of morning and evening milk at 8·0 a.m. and holding at atmospheric temperature until 12.0 noon, when the samples are cooled in brine and sent in a brine-cooled chamber to the laboratory. Comparison with farm conditions is therefore very considerably complicated by this period of incubation. An analysis is given of the results obtained in the three periods—July, October–November and April–May—in the form of correlation coefficients between the various tests and marks for farm inspection, and between the various tests with one another. From Table CXXXVIII the following are selected:

Correlation between	July	Oct.–Nov.	April–May
Farm inspection and plate count 37° C.	−0·330	−0·511	−0·102
,, ,, ,, presumptive coliform count	−0·356	−0·380	−0·120
,, ,, ,, reduction time 37° C.	+0·301	+0·320	+0·120
and from Table CXXXIX for the July period alone			
Presumptive coliform count and general cleanliness		..	−0·454
Reduction time and general cleanliness		..	+0·447

The correlation of the farm marks with the plate and coliform count is of course negative and with the reduction time positive, and in the instances quoted in approximately the same degree. The author, however, regards the presumptive coliform count as more poorly correlated with farm inspection. Sub-section E.2 records an investigation of the rate of increase of plate count on incubation of raw milk, and it is demonstrated that milk produced under good conditions may show such lag in multiplication that there is little change in 6 hours; on the other hand, it may fail to show this lag and show multiplication up to 8 to 9 generations. Four generations appear to be the average from Table CXL; sub-section E.5 records an investigation of the relation of plate count to reduction time. An interesting synoptic table is given on p. 345, showing the percentage of samples reducing within a given time with plate counts at 37° C. of 0 to 30,000, 30,000 to 200,000,

200,000 to 1,000,000, and over 1,000,000. From this it would appear that as many as 34 per cent. with plate counts of 200,000 to 1,000,000 would pass the methylene blue reduction test.

SECTION F. DISCUSSION OF THE BACTERIOLOGICAL GRADING OF MILK.—The sources of bacteria in milk are considered: the udder, the barn and milking utensils, the human personnel (of particular importance from a health point of view), imperfect cooling, the complicity of cleanliness as applied to milk. The author enumerates 5 general principles for the grading of milk, which may be briefly given as follows:—(i) The unwisdom of paying too much attention to any one examination. (ii) The unfairness of penalising a producer on the basis of one sample. (iii) The necessity for a different standard for summer and winter. (iv) The limitation of grading to four broad classes only—clean, moderately clean, moderately dirty and dirty. (v) The milk not only of the best producers, but of all producers for human consumption should be graded. "If these principles," the author says, "be accepted it follows that what is required for bacteriological grading of milk is a simple inexpensive test with a small experimental error, which can be used on a large scale by relatively unskilled workers." He then proceeds to consider the available tests, with a view to deciding which best meets the case, and in succession rules out all but the modified methylene blue test, which he recommends as fulfilling most of the requirements demanded. D. R. W.

(See Review, p. 446.)

Ceylon

REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

IN his Annual Report the Government Analyst (Mr. J. V. Collins, M.Sc., F.I.C.) states that 3256 articles were examined for various Government Departments; these included 441 samples of milk, 101 samples of water, and 2015 articles in connection with criminal cases.

CEYLON MILK SUPPLY.—The percentage of genuine samples of milk was only 2 per cent. better than in 1934, 272 of the 441 samples being adulterated. It is deplorable that so much of the milk sold to the public is adulterated. As a case in point the situation in Tangalla calls for special mention. No samples were received from this town from 1929 to 1934. During 1935, 37 samples were received, of which only 6 were genuine. On one day alone 17 samples from different vendors were received, of which only one was genuine. This appalling state of affairs is an indication as to what is probably occurring in many outstation towns where sampling is infrequent and where the fines inflicted are out of proportion to the profits which are made from this form of adulteration.

Of the 272 adulterated samples, 131 showed under 25 per cent. of added water, 115 gave between 25 and 50 per cent., 16 showed between 50 and 60 per cent., and 10 contained over 60 per cent. The highest percentage of added water recorded during the year was 77 per cent. Diluted sweetened condensed milk was again detected as an adulterant of fresh milk.

IDENTIFICATION OF OLD BLOOD STAINS.—In a case of murder from the Police Court of Rakwana a dagger sheath made of palm leaf was sent for examination for blood. The murder took place in October, 1907—over 28 years ago, and the exhibit was kept in the Police Court since that date until the accused surrendered to the court. The stains were dark brown in colour and readily gave the leuco-malachite green and benzidine tests. Haemin crystals were obtained by the method of Takayama after the stain had stood for 8 hours. Haemin crystals were also obtained by the sodium iodide method.

MEDINAL POISONING.—In a case of suicidal poisoning by medinal the analytical figures, when calculated back to the original medinal, gave the following results:—223 g. of the stomach yielded 1·69 grains of medinal, 711 g. consisting of one kidney and part of liver 5·07 grains; 671 g. of brain substance 2·1 grains; 290 ml. of urine 4·75 grains. The amount recovered from the urine is of particular interest as the patient lived for approximately 48 hours. The doctor withdrew the urine after 24 hours, and this specimen, which theoretically should have contained the bulk of the drug, was unfortunately not preserved for examination. The specimen examined by us was that obtained at the post-mortem and represented the urine secreted from 24 hours after ingestion of the poison until death. The amount taken by the deceased could not be definitely determined, but, judging by the empty containers found, the dose was probably in the region of 150 grains.

NUX VOMICA WITH DATURA.—In a case in which a mixture of powdered nux vomica bark, datura seeds, and ganja seeds was given to a man in his coffee, the police report stated that he "fell off to sleep and was robbed." The suppression of the typical strychnine symptoms by the presence of datura and ganja is of interest.

CASTS OF HOUSE-BREAKING IMPLEMENTS.—In eleven cases of house-breaking and theft house-breaking implements, clay and sand were produced for examination. Successful casts of the sharp point of the breaking implement were taken from a mud wall by means of Wood's metal (a fusible alloy usually containing 50 per cent. of bismuth, about 25 per cent. of lead, 12 to 13 per cent. of tin, and about 12 to 13 per cent. of cadmium).

British Standards Institution

ABSTRACT OF DRAFT BRITISH STANDARD SPECIFICATION FOR DENSITY BOTTLES (CD (C) 9870)*

THE foreword to the specification indicates how well adapted to the determination of density are bottles calibrated to contain a definite volume of liquid.

The specification itself is divided into three parts.

PART I comprises the specification for density bottles and deals with

- Range of sizes:—Four sizes are specified, *viz.* 10 ml., 25 ml., 50 ml., and 100 ml.
- Definition of capacity.
- Material and construction.
- Tolerances.
- Inscriptions.
- Arrangements for testing.

PART II deals with the determination of density by means of British Standard Density Bottles and gives simple tables for facilitating computations.

PART III relates to the use of density bottles in conjunction with the measurement of liquid in bulk.

* Copies of the draft specification will be sent to members of the Society of Public Analysts and Other Analytical Chemists, for the purpose of technical criticism and comment, on application to the British Standards Institution, 28, Victoria Street, London, S.W.1.

The Sale of Poisons

REGULATIONS FOR COLOURING INSECTICIDES AND WEED KILLERS

THE Secretary of State for Home Affairs, acting under Section 23 of the Pharmacy and Poisons Act, 1933, has made the following Rules:

(1) The Poisons (Colouring) Rules, 1936 (S.R. and O., 1936, No. 363).*

(2) The Poisons (Amendment) Rules, 1936 (Provisional).*

(1) The Poisons (Colouring) Rules, 1936, require the addition of a dye to certain arsenical substances sold for use in agriculture and horticulture, such as sheep dips, fruit sprays, and vermin- and weed-killers, and replaces the colouring provisions of the Arsenic Act, 1851, which lapsed on May 1st, 1936. The poisons scheduled in the Rules are: arsenates, arsenites, copper acetoarsenites, halides of arsenic, organic compounds of arsenic, sodium thioarsenates and sulphides of arsenic. The Rule does not apply to (*a*) lead arsenate paste or lead arsenate powder; or (*b*) poisons which are of themselves of a distinctive colour; or (*c*) sheep dips which are already of a distinctive colour; or (*d*) articles to be exported to purchasers outside the United Kingdom.

(2) The Poisons (Amendment) Rules, 1936, make additions to the articles in the Third Schedule to the Poisons Rules, 1935, which are exempted from the provisions of the Act. The poisons affected are ammonia, dinitrophenol and potassium hydroxide (caustic potash). Appendix I of the recently issued Home Office Memorandum on the provisions of the Pharmacy and Poisons Act affecting shops other than chemists' shops (Poisons, No. 1 (Shopkeepers)) includes these exemptions.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Ammonia-content as an Indication of the Quality of Milk. H. Kluge. (*Z. Unters. Lebensm.*, 1936, **71**, 232–245.)—Tillmans and his co-workers (*Z. Unters. Nahr. Genussm.*, 1914, **27**, 59; Abst., ANALYST, 1914, **39**, 173) described two methods for determining the ammonia-content of milk, but these are somewhat cumbersome for routine purposes. The method of Folin and Bell (*J. Biol. Chem.*, 1917, **29**, 529) for the determination of ammonia in urine as modified by Kolb (*Chem.-Ztg.*, 1924, **48**, 557; Abst., ANALYST, 1924, **49**, 488) was applied by Burstein and Frum (*Z. Unters. Lebensm.*, 1935, **69**, 421; Abst., ANALYST, 1935, **60**, 699) to its determination in milk. It gives satisfactory results when the ammonia-content is relatively high, but with fresh milk of low ammonia-content the final solution gives a greenish colour with Nessler's reagent, which makes accurate comparison difficult. The method has therefore been modified as follows:— Twenty-five or 50 ml. of milk are treated with an equal volume of 20 per cent. trichloroacetic acid, added drop by drop with shaking. The serum is filtered and, if still turbid, is shaken with kieselguhr and animal charcoal and re-filtered. To 25 ml. of the clear serum 10 per cent. sodium hydroxide solution is added until

* Copies of the new Rules (price 1d. each net, post free 1½d.), and the Home Office Memorandum (price 3d. net, post free 4d.), may be purchased directly from H.M. Stationery Office at London, Edinburgh, Manchester, Cardiff, or through any bookseller.

it is only slightly acid (25 ml. usually require about 5·5 ml. of sodium hydroxide solution). The liquid is then transferred with 3 water washings to a 100-ml. flask containing 2 g. of sodium-permutit which has been purified by washing first with 20 ml. of water and 2 ml. of 10 per cent. sodium hydroxide solution, then successively with water twice, with 20 ml. of 2 per cent. acetic acid and again with water twice. The serum is shaken with the permutit for about 3 minutes, after which it is poured off and the permutit is washed with water by decantation three times. Twenty ml. of water and 2 ml. of 10 per cent. sodium hydroxide solution are introduced into the flask, and after the liquid has been diluted to about 80 ml., 2 ml. of Nessler's reagent are added, the flask is filled to the mark with water and vigorously shaken. After 5 to 10 minutes the colour is compared with standard ammonia solutions in the usual way. The stock standard solution, which is stable for a month, contains 3·8792 g. of ammonium sulphate per l. This is diluted tenfold as required, 1 ml. being then equivalent to 0·1 mg. of ammonia. Blank determinations should give a final solution yielding no colour with Nessler's reagent. The accuracy of the method was tested by determining varying amounts of ammonia of the order 0·1 to 1·0 mg. per 100 ml. which had been added to milk. The errors in these determinations varied from −0·04 to +0·03 mg. per 100 ml. The ammonia-content of fresh milk was found to average about 0·12 mg. per 100 ml. Ten samples of milk from cows varying in the time since calving from 9 days to 11 months contained from 0·14 to 0·17 mg. per 100 ml.; they showed no dependence upon the stage of lactation. Two cows infected with *B. abortus*, Bang gave a somewhat high value, *viz.* 0·24; on the other hand, a cow having a mild form of mastitis gave the normal value of 0·14. Raw milk containing 0·20 mg. of ammonia per 100 ml. gave the following values after being heated under various conditions:—0·26 (half-an-hour at 65° C.), 0·20 (momentary heating at 71° C.), 0·23 (momentary heating at 85° C.), 0·27 (after boiling), and 1·18 (after sterilising four times at 120° C.). The effect of contamination with dirt was investigated by adding cow dung to a portion of a sample of pure milk. The clean portion gave ammonia values of 0·08, 0·12 and 0·23, respectively, after 1, 6 and 20 hours, whilst the contaminated portion gave a value of 0·10 immediately after adding the dung, and values of 0·30, 0·80 and 1·00, respectively, 1, 6, and 20 hours later, the acidity showing only a slight increase. With milk allowed to become sour spontaneously the following values were obtained:—0·12 (1 hour), 0·14 (6 hours), 0·23 (20 hours), the corresponding figures for another sample being 0·20, 0·26 and 0·30. The acidity of these samples increased only slightly. In the earlier stages of souring without great increase in acidity a continuous increase in the ammonia-content occurs. Milk with an original acidity of 6·6 (Soxhlet–Henkel scale) and an ammonia-content of 0·28, kept at room temperature for longer periods, gave the following values:—0·36 (6 hours), 0·50 (24 hours), 0·66 (29 hours), 0·44 (32 and 34 hours), 4·00 (53 hours), the acidity having risen uniformly to 30·8. When stored at 6 to 8° C. the values were:—0·42 (24 hours), 0·40 (29, 32 and 34 hours), 0·32 (53 hours), the acidity rising only slightly. These figures indicate the advisability of keeping milk at low temperatures. The milk kept at room temperature showed a decrease of ammonia-content at one stage with rising acidity, and only on further souring an increase in ammonia-content. To confirm

this, fully sterilised milk was inoculated with pure cultures of *Streptococcus lacticus* and of *B. coli* and incubated for definite periods up to 72 hours at 37° C. The occurrence of this decrease in ammonia-content at some stages of the souring was confirmed. In these experiments the trichloroacetic acid serum was turbid and required clarification with kieselguhr and charcoal, and further experiments showed that, although it was possible that the bacteria consumed ammonia, absorption phenomena also played a part in its disappearance. Fully sterilised milk was incubated for 8 hours with pathogenic bacteria. The following caused a distinct rise in the ammonia content:—*B. proteus* (which gave the highest increase), *B. prodigiosus*, *B. coli*, *B. typhus*, *B. paratyphus*, *Staphylococcus*, *B. Ruhr*, *B. pseudodiphtheriae*. *Streptococcus lactis* caused no change, and *B. diphtheriae* caused a decrease. The addition of formaldehyde to milk inhibits the formation of ammonia. Examples of the application of the determination of ammonia-content to the practical control of milk supply are given. A. O. J.

Effect of Homogenisation on some of the Characteristics of Milk Fat. I. A. Gould and G. M. Trout. (*J. Agric. Res.*, 1936, **52**, 49–57.)—The rapid development of rancidity in homogenised raw milk and the absence of a similar development in milk pasteurised before homogenisation have been noted by several investigators. The view of Dormer and Widmer (*Lait*, 1931, **11**, 545), who attributed the rancidity to fatty acids produced by lipase, is generally accepted. These authors and also Halloran and Trout (Abst., *Proc. Ann. Meeting Amer. Dairy Sci. Assoc.*, 1932, **27**, 17) observed an increase in the titratable acidity of homogenised raw milk, the increase appearing immediately after homogenisation and varying directly with the pressure used. Pasteurisation before homogenisation prevented the occurrence of this increase. Doan and Minster (*Penn. Agric. Exp. Stat. Bull.*, 287, 1933) obtained similar results by *p*H measurement. The decrease in surface tension of homogenised raw milk noted by Halloran and Trout (*loc. cit.*) was ascribed by Doan (*Milk Dealer*, 1933, **23**, 40) to the liberation of lower fatty acids and their concentration at the surface. Measurements of these changes have usually been made indirectly upon the milk rather than directly upon the fat. In this work the effect of homogenisation upon the constants of the fat itself is investigated. The fat obtained by churning the separated cream was purified by washing, melting and filtering. Homogenisation of raw and pasteurised milk at 1500 lb. per sq. in. pressure caused only negligible changes in the Reichert–Meissl and Polenske values and in the refractive index of the fat. If only lower fatty acids are liberated by homogenisation no changes in the Reichert–Meissl and Polenske values would be expected, since these are liberated in the determination of the constants. Although butyric acid, having a lower refractive index than milk fat, should by its presence reduce the refractive index, this lowering is compensated by the slightly higher refractive index of the glycerol simultaneously liberated. The acid value of the fat is materially changed. The average acid value of fat separated from raw homogenised milk immediately after processing was about 4 times that of pasteurised unhomogenised milk, and, after storage of the raw homogenised milk for 24 hours the acid increased to 18 times that of the pasteurised milk. It is known that the homogenisation process increases the

surface area of the fat 4 to 6 times. It appears, therefore, that the entire immediate increase in acid value is explained by the increased surface exposed to enzyme action. The greatest change in acid value occurs during the first 24 hours of storage, the average increase in this period being 1652 per cent. The value increased on an average by 533 per cent. during the first few minutes. The acid value of milk pasteurised before homogenisation showed little change. With raw homogenised milk at the end of 5 days' storage about 48 per cent. of the butyric acid has been liberated as the free acid. The titratable acidity and the *p*H value of raw homogenised milk stored at 35 to 40° F. were determined daily in comparison with a control sample, which was pasteurised but not homogenised. The values indicate a marked increase in titratable acidity and decrease in *p*H value during the 5-day storage period. The average results were as follows:—Acidity 0·161 (control), 0·172 ($\frac{1}{4}$ to $\frac{1}{2}$ hour), 0·208 (1 day), 0·223 (2 days), 0·227 (3 days), 0·229 (4 days), 0·237 (5 days), the corresponding *p*H values being 6·43 (control), 6·40, 6·28; 6·28, 6·26, 6·21, and 6·19. The titratable acidity followed increases in the free acids in the fat somewhat more closely than did the *p*H values. In view of the small number of experiments the author hesitates to assume that this will always occur, but it appears safe to say that determinations of rancidity development may be made as satisfactorily by titration as by potentiometric methods. The measurement of free fatty acids by titration of the separated fat appears to be a more accurate and more sensitive means of determining the rate of fat decomposition than determinations made upon the milk itself. A. O. J.

Extract of Orris Rhizome added to Wine. H. Mohler. (*Z. Unters. Lebensm.*, 1936, **71**, 266–268.)—In Tuscany orris rhizome is said to be used for imparting aroma to wines of the Chianti type. To what extent the practice occurs is not known; it is not legally permissible in Italy or in Switzerland. A quantity of a brown liquid containing a sediment, and labelled "Alcoolato Giaggiolo" ("Giaggiolo" = orris), was admitted to have been used for imparting aroma to certain artificial wines. The liquid was identified as an extract of orris rhizome. Microscopical examination of the sediment revealed the presence of oval starch grains with a radiate hilum, prismatic calcium oxalate crystals, a few scalariform vessels, and cellular debris. Sclerenchymatous elements were absent. The liquid had a violet-like odour and a taste bitter and harsh at first, but aromatic afterwards. When decanted from the sediment it contained 42 per cent. by volume of alcohol, traces of acids, esters and higher alcohols. Tannin was present and the liquid reduced Fehling's solution. Extraction with ether separated a hard yellowish solid. Ethereal extraction of the distillate yielded a strongly aromatic substance. From the semi-solid residue fatty acids and an unsaponifiable oil of unpleasant odour were separated. Steam-distillation of the sediment yielded a residue containing traces of diacetyl and a little furfural, and a distillate containing fatty acids—mainly lauric and myristic, with traces of caproic, caprylic and capric acids. The brown liquid was unsuitable for spectrophotometric examination and the distillate was therefore used. By comparison of the absorption spectrum curves of the sample with those of an extract of orris rhizome the identity of the sample was confirmed. A. O. J.

Component Glycerides of Cacao Butter. T. P. Hilditch and W. J. Stainsby. (*J. Soc. Chem. Ind.*, 1936, **55**, 95–101T.)—In view of the recent work on seed-fats the authors have returned to the study of cacao butter glycerides, particularly since the first conclusions (ANALYST, 1929, **54**, 242) were based on the yield of fully saturated glycerides obtained after removal of unsaturated mixed glycerides by oxidation with permanganate in acetone, and supported by examination of the mono-azelao-disaturated glycerides produced in this operation. The 1927 specimen, and also a fresh sample of cacao butter, have been subjected to a series of analyses, including component analyses of the whole fats and a study of the fully saturated glycerides present after complete hydrogenation and after hydrogenation to intermediate stages of saturation. The original fats have also been partly separated by crystallisation from acetone, and each separated fraction examined by the same method as applied to the fats as a whole. The following is now suggested as the composition of the glycerides:—oleopalmitostearin, 52; oleodistearin, 19; stearodiolein, 12; palmitodiolein, 9; oleodipalmitin, 6; and palmitostearins, saturated, 2 per cent. by weight. β-Palmito-oleo-stearin must comprise a large part of the trebly mixed glycerides, and β-oleodipalmitin and β-oleodistearin are probably the isomerides mainly present, whilst both α- and β-stearodiolein may occur. The molar composition is a good illustration of the tendency in seed-fats for the component fatty acids to be distributed as evenly as possible among the glycerol molecules, since in every 100 mols. of the mixed fatty acids there are 26 palmitic, 34 stearic, and 40 oleic (and linolic) mols. There are more triglyceride molecules containing only stearic and oleic acids than only palmitic and oleic acids, just as there is more stearic than palmitic acid in the total acids of the fat. Of the total mono-oleo-glycerides, two-thirds are oleopalmitostearins, the remaining third alone consisting of either oleodistearins or oleodipalmitins, both the oleodipalmitins and the oleodistearins occurring in much smaller quantities than might be expected. Various numerical relations are brought out, but at present their precise significance remains obscure. D. G. H.

Contribution to the Study of the Adulterants of Maté. T. J. Rumi. (*Industria y Quimica*, 1935, **1**, 69–72.)—Maté (Bot. *Ilex paraguariensis* St. Hill), Voadeira (mixture of *Prunus subcoriacea*, *Villaresia congonha* Miers var., and *Ilex dumosa* Reiss (Congonilla)), Sapopema (Botanical species not known), Pecegueiro bravo (*Prunus Sub.* (Chodat and Hassl.) Koehne), De Anta (species of the genera *Rudgea* or *Faramea*, family Rubiaceae), Cauna (species of the genus *Rapanea*, possibly *Rapanea martensis* Mes, family *Mirsinaceae*), Orelha de Mico (*Villaresia con.* Miers var.), and Congonha (*Ilex dumosa* Reiss (Congonilla)) were studied. Photographs are given of Pecegueiro bravo, Cauna, Orelha de Mico, and Congonha, with a photomicrograph of *Ilex paraguariensis*. The table below shows the results of chemical analyses. Determinations of moisture, total ash, ash soluble in hydrochloric acid (1 + 9), and caffeine were carried out by the methods of the Oficínas Quimicas Nacionales (Leyes, *Decretos y Resoluciones*, 1933, **1**, 80–82). The method for caffeine is that of Grandval and Lajoux, modified by Keller and Beittner and by Katz (*cf.* L. Gugliamelli and L. P. J. Palet, *An. Soc. Quím. Arg.*, 1915, **3**, 371), the chloroform solution being washed with 1 per cent.

potassium hydroxide solution, as recommended by Power and Chestnut (A.O.A.C., *Official and Tentative Methods of Analysis*, 1930, 151). For the determination of aqueous extract 2 g. of sample were extracted in a 150-ml. Soxhlet extractor. The following results were obtained:

	Ilex paraguariensis		Voadeira		Sapopema		Pecegueiro bravo	
	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.
Moisture	10·71	9·13	8·33	8·86	9·15	8·76	7·39	7·55
Total ash	4·97	5·43	3·77	4·39	4·47	3·98	1·63	4·30
Insoluble ash ..	0·14	0·34	0·35	0·27	0·06	0·74	0·03	0·11
Caffeine	0·73	1·25	—	—	None	None	—	None
Aqueous extract ..	31·90	34·95	23·40	46·50	22·45	35·95	20·35	41·25
Chloroform extract	—	—	—	8·02	—	—	1·93	10·72
Extract in chloroform +5 ml. of ammonia	4·55	13·36	4·18	—	1·81	6·11	—	9·03

	Anta		Cauna		Orelha de Mico		Congonha	
	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.
Moisture	10·25	9·67	7·38	8·00	—	7·90	—	—
Total ash	7·67	10·20	2·97	4·42	3·55	5·65	2·77	3·31
Insoluble ash ..	1·45	2·48	0·065	0·035	0·025	0·11	0·04	0·085
Caffeine	None	None	None	None	—	None	—	None
Aqueous extract ..	20·55	35·45	21·22	38·05	26·18	37·80	21·74	36·65
Chloroform extract	—	5·93	—	8·64	—	7·69	—	9·84
Extract in chloroform +5 ml. of ammonia	2·08	5·65	3·78	8·54	3·25	8·14	5·02	9·88

The leaves of Pecegueiro bravo contained 702 mg. of hydrocyanic acid per kg. of dry leaf; the method used for the determination was that of Liebig–Kohn–Abrest (U. Hordh., *An. Asoc. Quím. Arg.*, 1935, **23**, 85–86). The presence of Pecegueiro bravo in maté can be detected by applying Guignard's hydrocyanic acid reaction: maté containing 5 per cent. of Pecegueiro bravo gave a negative (yellow) reaction; with 10 per cent. a slight (light orange) reaction; and with 15 per cent. a positive (orange) reaction.

One sample was hairy, suggesting an adulterant, but had a high caffeine content; it proved to be *Ilex paraguariensis* (maté), containing young leaves. Adulteration of maté by stems of the plant can be detected by a determination of the fibre-content, the stems containing roughly twice as much fibre as the leaves.

E. M. P.

Reaction of Opium Alkaloids with certain Oxidising Agents. M. Pesez. (*Bull. Soc. Chim.*, 1936, **3**, 675–676.)—A few mg. of alkaloid (free or in the form of salt) are dissolved in 0·5 ml. of alcohol in a test-tube, treated with 1·5 ml. of strong sulphuric acid, and heated for 3 minutes in a water-bath. The tube is cooled, and 5 ml. of water are added. The colourless liquid is divided into three parts: one is tested with a drop of hypochlorite, the second with a drop of bromine water, the last with a drop of 1 per cent. sodium nitrite solution. With each

a fine orange-red colour is produced. A pink tinge is thus obtained after a few seconds with 0·05 mg. of morphine or codeine. Brucine and adrenaline give a positive (yellowish-brown), strychnine a negative, reaction.

For the detection of the above oxidising agents, 1 ml. of 5 per cent. alcoholic codeine solution is cooled and treated with 2 ml. of strong sulphuric acid, and the mixture is placed in a water-bath for three minutes, when it assumes a pale violet colour. It is again cooled, and slowly diluted with 20 ml. of distilled water. The reagent becomes colourless and keeps well. For the detection of bromine, 5 ml. of the solution are treated with 1 ml. of the reagent. After a few minutes a red colour is obtained; in very dilute solution, the brownish-pink tinge develops within 5 minutes. Nitrous acid gives a positive reaction at a concentration of 0·05 mg. in 5 ml. Chlorine and hypochlorite react at the same concentration. Nitric, chloric and persulphuric acids and their salts, and peroxides of hydrogen or of metals give no reaction. W. R. S.

Investigation of Endrine. H. J. Van Giffen. (*Pharm. Weekblad*, 1936, 73, 526–528.)—According to the label on the package the percentage composition of endrine supplied by a London firm was:—ephedrine, 0·75; menthol, 0·5; camphor, 0·5; eucalyptol, 0·5; liquid paraffin was the solvent. Ephedrine is determined by shaking 10 g. (accurately weighed) for 2 minutes with a mixture of 1·25 ml. of hydrochloric acid and 3·75 ml. of water, the water layer being removed and filtered, and the operation repeated 3 times. The combined filtrates are evaporated in a vacuum until almost dry, and are then made strongly alkaline with sodium hydroxide solution and extracted with 70 ml. of chloroform, the extract being dried with fresh sodium sulphate and filtered; this operation is repeated thrice, 10 ml. of chloroform being used each time. The combined filtrates are evaporated to 2 ml., 5 ml. of 0·1 *N* sulphuric acid being added from a micro-burette, and the last traces of chloroform are removed on the water-bath, a gentle stream of air being used to assist this operation. A drop of a mixture of 62·5 mg. of methylene blue and 133 mg. of methyl red in 150 ml. of strong alcohol is then added, and the liquid is back-titrated with 0·1 *N* sodium hydroxide solution, the end-point being a change from violet to green (1 ml. acid ≡ 20·15 mg. of ephedrine hydrochloride). Volatile compounds are determined by shaking 5 g. of anhydrous sodium sulphate with the oily layer remaining from the original separation, and filtering; a weighed quantity of filtrate is heated at 50° C. until no further loss in weight occurs, the volatile compounds being thus expelled. If the solution remaining after the titration is evaporated in a vacuum to 3 ml., identification tests for ephedrine may be applied as follows:—(1) One ml. of the residue is made alkaline with sodium hydroxide solution and extracted with ether. The filtered extract is evaporated, and the residue is dissolved in a mixture of 2 drops of alcohol and 0·5 ml. of dilute hydrochloric acid. The ephedrine hydrochloride is then precipitated by addition of an excess of ether and collected in the centrifuge, after which it may be washed with ether and dried, and the m.p. (216° C.) determined. (2) A mixture of another ml. of the same residue and 1 drop each of copper sulphate and sodium hydroxide solutions produces a violet colour, which may be extracted by shaking with an equal volume of ether. (3) A mixture of the last ml. of residue and 0·5 ml. each of

sodium hydroxide and fresh 1 per cent. potassium ferricyanide solutions evolves an odour of benzaldehyde when warmed. Other tests carried out on the original endrine are:—(1) One ml. is warmed with a solution of 100 mg. of vanillin in 10 ml. of hydrochloric acid, when a green-blue colour results. (2) If a drop of endrine is distributed over the walls of a test-tube and exposed to bromine vapour, brick-red crystals form. Results obtained for a sample prepared by the author according to the above formula, and for a purchased sample, were, respectively:—ephedrine hydrochloride, 0·7455, 0·66 per cent.; volatile compounds, 1·46, 1·68 per cent.; the identification tests were positive with both samples. J. G.

Biochemical

Distribution of Lead in Human Bones. S. L. Tompsett. (*Biochem. J.*, 1936, **30**, 345–346.)—The lead-content of tibiae, femora, ribs and vertebrae from the human body was determined by a method previously described (Tompsett and Anderson, *Biochem. J.*, 1935, **29**, 1851; Abst., ANALYST, 1935, **60**, 772). Femora and tibiae contained much higher concentrations of lead than ribs and vertebrae. In a series of nineteen cases, the amounts of lead found were, rib 4·0 to 17·5, vertebra 3·4 to 16·5, femur 18·2 to 108·3, and tibia 15·3 to 96·5 mg. of lead per kg. of fresh bone. S. G. S.

Photometric Determination of Titanium in Animal Tissues. L. Maillard and J. Ettori. (*Compt. rend.*, 1936, **202**, 594–596.)—Minute amounts of titanium occurring naturally in muscle and blood were determined by a microchemical adaptation of the cupferron precipitation, followed by the hydrogen peroxide colorimetric method. The ash of a 100-g. sample is dissolved by heating with conc. sulphuric acid; the solution is diluted to give a concentration of about 5 per cent. of sulphuric acid, a little tartaric acid is added, together with a few mg. of iron in the form of a solution of ferric sulphate to act as collecting agent. The titanium, etc., is precipitated with cupferron. The precipitate is ashed, and the residue is dissolved in sulphuric acid. The diluted solution, to which tartaric acid is added, is neutralised, rendered slightly acid, saturated with hydrogen sulphide and made ammoniacal, and the precipitate of iron sulphide with traces of copper sulphide is filtered off and rejected. The filtrate containing the titanium is acidified and boiled to remove hydrogen sulphide. The titanium is again precipitated with cupferron, this time with the addition of a few mg. of zirconium (as sulphate) as collecting agent. The precipitate is filtered off, ashed, and dissolved in a few drops of sulphuric acid. The solution is diluted, with the addition of 0·3 ml. of perhydrol, to 10-ml. volume, transferred to a 40-cm. colorimeter tube of narrow cross-section, and the depth of colour is determined by a colorimeter of the Pulfrich type which has been calibrated with standard colours prepared with known amounts of titanium. The sensitiveness of the method is stated to be one ten-thousandth of a mg. (0·1γ). Human and other mammalian muscle was found to contain 8γ of titanium in 100 g.; blood contained 3γ in 100 g. S. G. C.

New Colour Reaction of Hexoses and their Polymers and its Application to the Colorimetric Determination of Glucose in Blood. J. A. Sanchez. (*J. Pharm. Chim.*, 1936, **23**, 377–387.)—If 15 ml. of pure sulphuric acid (sp.gr. 1·84)

are added to 5 ml. of a 1 : 5000 solution of dextrose, and the mixture is shaken so as to accelerate the rise in temperature, a red colour is produced and becomes more intense after a few minutes; the specified concentration of dextrose should not be exceeded. The relationship of colour-intensity to quantity of dextrose is linear for the range 0·0001 to 0·0003 g., and the colour is stable for several days. The colour is produced by sucrose, lactose, maltose, raffinose, glycogen, starch and other polysaccharides, and in a different shade by fructose, galactose and mannose, but not by pentoses. From analogy with the production of furfuraldehyde by the dehydration of pentoses by sulphuric acid, it is considered that the colour is due to the formation of hydroxymethylfurfuraldehyde, and this would also account for the selectivity for hexoses. The absorption spectra of the colours from galactose and mannose show single bands ranging from 556$m\mu$ to about 480$m\mu$, whilst the other hexoses mentioned above show a wider band (566$m\mu$ to the end of the spectrum) with 3 zones of intensity (maxima at 530, 492 and 552$m\mu$, in order of increasing intensity). For the examination of blood a mixture of 1 ml. of serum obtained by decantation or centrifugal separation, 1 ml. of 20 per cent. trichloroacetic acid solution and 2 ml. of water is stirred well with a glass rod and filtered, 2 ml. of filtrate being diluted with 3 ml. of water, and the liquid added to 15 ml. of the sulphuric acid reagent. The mixture is then heated in the water-bath for 5 minutes and compared with the standards when cool. It is important that red corpuscles should be absent as haemolysis alters the shade to a grey colour, but even when this precaution is taken a greyish haze is apparent in the sample, which is attributed to the action of the reagent on minute particles of nitrogenous matter not removed by the trichloroacetic acid. It is overcome by adding some dealbuminated serum, free from dextrose, to the standards, and this is prepared by incubating non-haemolysed blood for 24 hours at 37° C., the serum then being removed by decantation and ground in a mortar with an equal volume of the trichloroacetic acid reagent. The serum obtained by filtration is stable, and under the conditions of the reaction produces a greyish haze, but no red colour. A range of standards may then be prepared by adding to each of five 5 ml. graduated tubes 1 ml. of the above serum and 0·1 to 0·5 ml. of a 1 : 1000 solution of dextrose, each mixture being then made up to a total of 5 ml. with distilled water, and treated exactly as described above in order to produce the red colour. It is important that the quantities mentioned should be used so far as possible, but if the colour of the sample is too intense to be matched, the reaction should be repeated on 1 ml. of filtrate (*i.e.* 0·25 ml. of serum). Destruction of dextrose in blood by enzymes may be followed in this way, and it has been shown that 1 g. of dextrose or less is destroyed after 24 hours at 37° C. by the action of total coagulated blood, although it is unaffected by the dealbuminated serum prepared as described above. The enzyme probably responsible for this is destroyed at 54° C. J. G.

Determination of Starch in Plant Tissue, with Particular Reference to the Apple Fruit. C. S. Hanes (*Biochem. J.*, 1936, **30**, 168–175.)—The tissue is extracted with 70 to 80 per cent. alcohol, and the residue is boiled with dilute alcoholic hydrochloric acid solution to convert the starch into soluble starch, which is completely extracted by hot water. The soluble starch in the extract is next

selectively hydrolysed by β-malt-amylase prepared from ungerminated barley. This enzyme is preferred because of its specificity as a starch "reagent," because maltose is produced almost exclusively, and because a definite hydrolysis limit exists. It was found that throughout a season the apple starch was hydrolysed to the same extent by this enzyme. The preparation of the enzyme and the starch are described in appendixes. S. G. S.

Observations on the Excretion of Vitamin *C* in Human Urine. B. Ahmad. (*Biochem. J.*, 1936, **30**, 11–15.)—The reducing capacity of human urine varies with different dietary conditions. If the vitamin *C* content of the food is kept constant, the reducing action increases with an increase in the intake of meat. The evidence is generally in favour of this increased reducing action being due almost entirely to ascorbic acid, and it therefore appears that high meat diets cause the excretion of vitamin *C* in the urine. S. G. S.

Critical Remarks on the Determination of Ascorbic Acid. M. van Eekelen and A. Emmerie. (*Biochem. J.*, 1936, **30**, 25–27.)—Attention is drawn to precautions which must be observed in removing interfering substances by the authors' method of precipitation with mercuric acetate (*Biochem. J.*, 1934, **28**, 268, 1153) in the determination of ascorbic acid by titration with 2 : 6-dichlorophenolindophenol. The solution or extract to which mercuric acetate is added must be slightly acid (*p*H about 5·0). Trichloroacetic acid is preferred to acetic acid, because the precipitation of small quantities of cysteine and ergothionine is more complete in the presence of this acid. Excess of mercuric acetate must be avoided, and for this reason the amount of reagent necessary must be determined on a separate sample. In order to avoid irreversible oxidation, the precipitate should be centrifuged off and hydrogen sulphide passed into the solution not more than 10 minutes after the mercuric acetate is added. When urine contains little ascorbic acid 10 ml. may be taken and mixed with 20 ml. of 20 per cent. mercuric acetate solution. When these precautions were observed, a recovery of 95 to 100 per cent. was obtained. The use of lead acetate as a precipitant (Dewjatnin and Doroschenko, *Biochem. Z.*, 1935, **280**, 118) is criticised, on the ground that cysteine is not removed and that, since hydrogen sulphide is not used, reversibly oxidised ascorbic acid is not determined. The authors have also found (*Acta Brev. Neerl.*, 1934, **4**, 141) that, although pure ascorbic acid solutions are not affected by lead acetate in slightly acid or neutral medium, the addition of ascorbic acid to urine, and subsequent treatment with lead acetate, involves serious losses. The reduction of silver nitrate by tissues and extracts as a criterion for their ascorbic acid content is also criticised, because substances such as cysteine and glutathione can inhibit the reduction. The quantitive determination of ascorbic acid by the tungstic acid method of Fujita *et al.* (*Biochem. Z.*, 1935, **277**, 298) cannot be used in the presence of adrenaline (suprarenal extracts), because adrenaline inhibits the reaction and the values obtained are too low. S. G. S.

Comparison of Titrimetric and Colorimetric Determinations of Ascorbic Acid. K. Wachholder and H. H. Podestà. (*Hoppe Seyler's Z. physiol. Chem.*, 1936, **239**, 149–161).—The vitamin *C* content of human urine and

of various organs of rabbits and cats has been determined by several titrimetric methods and by a colorimetric method. The highest values were obtained with the method of Tillmans (titration with 2 : 6-dichlorophenolindophenol solution), but this is rejected, together with that of Fujita (*Biochem. Z.*, **1935**, **277**, 296) and the use of Bezssonoff's reagent (*Bull. Soc. Chim. biol.*, **1934**, **16**, 1160), as not being specific. Titration with methylene blue solution, as suggested by Martini and Bonsignore (*Biochem. Z.*, **1934**, **273**, 170), and the colorimetric method, using Folin's phosphotungstic acid reagent, are preferred as being more nearly specific, although giving lower values. S. G. S.

Colorimetric Determination of Phosphoric and Arsenic Acids with Ascorbic Acid. R. Ammon and K. Hinsberg. (*Hoppe Seyler's Z. physiol. Chem.*, 1936, **239**, 207–216.)—Ascorbic acid may be used as the reducing agent in the colorimetric determination of phosphoric or arsenic acid by the molybdate method. The solution of the phosphoric or arsenic acid (which should contain not more than 3 mg.) is placed in a 25-ml. flask, and 5 ml. of a 20 per cent. solution of trichloroacetic acid, followed by 1 ml. of a 2·5 per cent. solution of ammonium molybdate in 5 *N* sulphuric acid, are added. Five mg. of ascorbic acid are introduced, the contents of the flask are diluted to 25 ml. with water, and the flask is placed in a water-bath at 37° C. for 20 minutes. The extinction coefficient of the solution is then determined in a colorimeter. When phosphoric and arsenic acids are present together, the extinction coefficient is determined in the same manner. Another determination is then made with the water-bath at 70° C., and with the addition of sodium bisulphite to the reaction mixture. This prevents colour formation due to the arsenic acid, and therefore allows the amount of phosphoric acid to be determined. The colorimeter should be calibrated by making the determination with known amounts of material. S. G. S.

Agricultural

Rapid Determination of Barium Silicofluoride in Insecticides. J. Vinas and J. Save. (*Ann. Falsif.*, 1936, **29**, 152–154.)—Barium silicofluoride is largely used, either alone or mixed with such substances as talc, chalk, rice, starch, etc., as an insecticide. To determine the amount present, 0·5 g. of the silicofluoride (or a corresponding amount of the preparation) is suspended in 200 ml. of boiling water, and titrated boiling with *N* sodium hydroxide solution in the presence of phenol red. Titration is slow, owing to the small solubility of barium silicofluoride. The solution turns yellow, but the pink colour must be maintained to prevent decomposition of the barium fluosilicate when boiling. Titration is stopped as soon as the pink is permanent. Alkaline salts and sulphates decompose the silicofluoride, but when these are present the quantity of active silicofluoride should be determined, since decomposition, which is rapid at the boiling point, goes on slowly in the powder in the presence of moisture. The relatively high solubility of sodium silicofluoride (0·6 per cent.) enables it to be determined in the presence of barium silicofluoride. It may be present as an impurity, and is important owing to its scorching effect on foliage. Ten g. of the

silicofluoride (or the equivalent of the preparation) are suspended in 100 ml. of water, mixed 3 or 4 times in 2 hours and filtered, and 20 ml. of the filtrate are titrated with $N/10$ sodium hydroxide solution. One ml. = 0·0047 g. Na_2SiF_6, and 0·2 g. (representing the dissolved barium silicofluoride) is subtracted from the quantity thus found. The barium may also be determined as sulphate by treating 0·5 g. of barium silicofluoride (or the equivalent of powder) with 20 ml. of hydrochloric acid and 50 ml. of water, boiling for 30 minutes, cooling, making up to 500 ml., filtering and determining the barium as sulphate in 200 ml. D. G. H.

Organic

New Kjeldahl Method for the Determination of Nitrogen in Foods, Feeding Stuffs, Leather, etc. A. E. Beet and D. G. Furzey. (*J. Soc. Chem. Ind.*, 1936, **55**, 108–109T.)—The catalyst mixture used in the present series of experiments consisted of 2 lbs. of potassium sulphate, 5 ozs. of mercuric sulphate (preferable to oxide which is liable to contain traces of nitrate), and 1 oz. of selenium, finely powdered and well mixed. One g. of finely ground material, 10 g. of catalyst mixture, and 20 ml. of conc. sulphuric acid are shaken in a 300-ml. flask, boiled briskly until the liquid is a pale lemon-yellow colour, and then for a further 10 minutes. The ammonia formed is then determined by distillation. This method was compared both for speed and accuracy with that recommended in the Fertilisers and Feeding Stuffs Regulations, 1932, in which anhydrous sodium sulphate and copper sulphate are used. It was found that, if the "after-boil" is omitted, results are about 2 per cent. (on the nitrogen-content) too low in the copper sulphate method, and 0·5 per cent. with the new catalyst. Ten minutes' "after-boil" is found to be ample, and with this time any loss of nitrogen liable to occur with too long digestion is prevented. Nitrogen was determined in a large number of feeding stuffs by both methods, and the results agreed closely. The new method was found to reduce by half to two-thirds the time of digestion needed by the copper sulphate method. D. G. H.

Phosphotungstic and Silicotungstic Acids as Reagents for Organic Bases. E. and M. Kahane. (*Bull. Soc. Chim.*, 1936, **3**, 621–625.)—The authors have prepared salts of these acids with primary, secondary, and tertiary amines, quaternary ammonium bases, pyridine, quinoline, and urea and guanidine derivatives. A 10 per cent. solution of either reagent is added to the strongly acid solution of the base. Precipitation is more complete in the cold, but it is sometimes necessary to boil the solution so as to produce a precipitate which settles well and can be washed by decantation. The washing is done with water or dilute hydrochloric acid, after which the precipitate is air-dried. The portion to be analysed is dried at 100° C. to constant weight, and is then calcined at a dull red heat without special precautions. The residue is weighed as $12WO_3.HPO_3$ or $12WO_3.SiO_2$. The results proved that the precipitates dried at 100° C. are definite anhydrous tribasic phosphotungstates and tetrabasic silicotungstates. They can be utilised as criteria for the purity of a base or, if they are sufficiently insoluble, for its quantitative determination, or again, for the indirect determination of two

bases in admixture. Certain compounds, such as glycocoll and adrenaline, gave very soluble salts, whilst others containing several nitrogen atoms in the molecule gave ill-defined salts. The authors consider that, of the latter class of compounds, only those containing a single basic nitrogen atom are capable of giving well-defined phospho- and silicotungstates. W. R. S.

Detection of Oxalic Acid. A. S. Komarowsky and W. A. Nasarenko. (*Z. anal. Chem.*, 1936, **104**, 413–416.)—The procedure of Tananaeff and Budkewitsch (ANALYST, 1936, 135) is criticised for its lack of specificity. The authors show that other organic acids (including tartaric, citric, lactic, and salicylic) induce decolorisation of indigo by dichromate. Arsenious acid has the same effect; ferrous salts, nitrites, and complex-formers (*e.g.* molybdates, zirconium salts) also interfere. Feigl and Frehden's diphenylamine test (*Mikrochemie*, 1935, **18**, 272) is recommended for the detection of oxalic acid. W. R. S.

Determination of Small Quantities of Benzoic Acid. E. B. Johnson (*J. Soc. Chem. Ind.*, 1936, **55**, 109–110T.)—The colorimetric method adopted is as follows:—A known volume of the solution containing the benzoic acid is acidified with a few drops of conc. sulphuric acid, and shaken three times with ether, and the combined extracts are evaporated. A small quantity of potassium nitrate and conc. sulphuric acid are added to the residue, and the mixture is heated in boiling water for 1 hour, diluted, cooled and made up to a known volume. A small quantity of zinc is added to an aliquot portion of the solution, which is again heated for 1 hour, and the remaining zinc is filtered off and washed. It is not necessary to precipitate the zinc if no great excess of nitrating solution has been used. Ten ml. of the aminobenzoic acid solution are then diazotised with 30 ml. of saturated sodium nitrite solution, and, after 5 minutes, 3 ml. are added to 1 ml. of alkaline β-naphthol solution (1 g. of β-naphthol in 100 ml. of 10 per cent. sodium hydroxide solution) in a 50-ml. Nessler tube, and made up to a height of 5 cm. Into a companion tube containing 5 ml. of water the dye solution (0·25 g. of azogeranine B, British Dye Stuffs Corporation; Colour Index No. 31, in 100 ml. of water, not filtered), is run until a match is obtained on looking down the tubes side by side on a white surface. The volume added is noted, and the amount of aminobenzoic acid is read off from a curve. Outside the limits 5·7 ml. and 17·8 ml. of dye solution, corresponding with 100 to 300 p.p.m. of aminobenzoic acid, results are misleading. For α-naphthol the procedure is the same, but the dye used is Neolan Pink B (Clayton Aniline Co.), and the test is unreliable outside the limits 50 and 200 p.p.m. The graphs were obtained by examining solutions of known concentrations by this method. D. G. H.

Determination of Lignin in Woods. K. F. Bamford and W. G. Campbell. (*Biochem. J.*, 1936, **30**, 419–427.)—The methods for the determination of lignin in wood by means of 72 per cent. sulphuric acid are criticised, and stress is laid on the lack of uniformity in these methods. It is shown that a preliminary hydrolysis with dilute sulphuric acid does not prevent the formation of carbohydrate condensation products during the isolation of lignin, unless precautions are taken to remove the products of hydrolysis as soon as possible after they are

formed. The following procedure is suggested: After a preliminary extraction with alcohol-benzene (1 : 2) a 2-g. sample of air-dried wood, having a moisture content of about 10 per cent., is digested with 25 ml. of 72 per cent. sulphuric acid at 10° ± 0·5° C. for 5 hours with hard woods and for 6 hours with soft woods. The acid is diluted with water until a concentration of 3 per cent. is obtained, and the mixture is boiled under a reflux condenser for 2 hours. The lignin residue is then collected in an alundum crucible of porosity R.A. 360, washed free from acid and dried at 105° C. When xylosė, fructose and sucrose were treated with sulphuric acid under these conditions only negligible amounts of insoluble residues were obtained. S. G. S.

Differentiation of Casein and Blood Albumin Glues in Plywood by Means of the Microscope. B. J. Rendle and G. L. Franklin. (*J. Soc. Chem. Ind.*, 1936, **55**, 105–106T.)—Adhesives used in plywood manufacture are usually derived from either casein or blood albumin, but synthetic resin cements, mostly of the phenol-formaldehyde type, are being increasingly used, and animal glues and so-called vegetable glues, made principally from cassava starch, find a limited use. It appears, from the limited amount of material examined, that casein and blood albumin may be distinguished by their natural colour, microscopical structure and optical properties. The casein glue layers are colourless or nearly so, with a fine granular structure, giving a slightly anisotropic appearance under crossed nicols, with a sparkling effect against a dark background. Blood albumin glue layers appeared distinctly green under the microscope, with an opaque glassy structure, and showing extinction under crossed nicols. A satisfactory stain and mounting medium consists of a 2 per cent. aqueous solution of methyl blue mixed with a 2 per cent. solution of eosin in 50 per cent. alcohol, in the proportion of 3 to 1, the mixed solution being added to liquefied glycerin jelly until the colour is that of blue-black writing ink. The section is covered with one drop of the mixture, and the slide is gently heated until bubbles appear, thereby intensifying the stain. Casein glue is stained purplish pink, intermediate between "amaranth pink" and "pale amaranth pink" in Ridgway's "Colour Nomenclature 1912," and blood albumin wine red or "vinaceous purple" (Ridgway). The wood itself is stained pale mauve. D. G. H.

Inorganic

Some Metallic Combinations of Thiosemicarbazide and the Thiosemicarbazones. V. Harlay. (*J. Pharm. Chim.*, 1936, **23**, 392–403.)—It is shown that thiosemicarbazide and the thiosemicarbazones produce a series of crystalline silver and copper compounds containing the corresponding acid radical of the metallic salt used. These are as follows:—*Thiosemicarbazide.*—With silver nitrate:—(1) An equimolecular compound (nitrate of silver thiosemicarbazide) forming small compact white crystals, which turn brown on exposure to light and are insoluble in 5 to 20 per cent. nitric acid and slightly soluble in water. The compound is precipitated in the amorphous state by simple mixture of solutions of the two constituents in the presence of dilute nitric acid and a slight excess of silver nitrate, and when heated on the water-bath the crystals form. Fine, long colourless

needles are obtained if the salt is re-crystallised from hot water. The reaction may be used to determine free and combined thiosemicarbazide. (2) An amorphous precipitate, forming white crystals and containing 2 molecules of silver nitrate and 3 of thiosemicarbazide is formed when the quantity of the former is equal to or less than two-thirds of the quantity required in reaction (1); if the quantity of silver nitrate exceeds two-thirds, a mixture of both compounds results. With silver sulphate:—(1) The reaction is analogous to that obtained with the nitrate (*supra*); the precipitate is amorphous, unless produced in the presence of boiling alcohol, from which it crystallises, after filtration, in tufts of colourless elongated prisms. (2) Oily drops obtained during the early stages of precipitation in the above reaction, if separated and washed with alcohol and ether, form a colourless transparent varnish, which becomes brown on exposure to light in contact with water, and dissolves in the latter to the extent of 0·15 per cent. It can be crystallised slowly from water in the dark, the crystals containing 1 mol. of silver sulphate and 4 mols. of thiosemicarbazide.

Cupric salts (hydrochloride, nitrate or sulphate) produce a blue-violet colour, and subsequently, a precipitate (brown, brownish-purple and deep blue in colour, respectively) containing 1 atom of copper linked by its double valency-bond to 2 mols. of thiosemicarbazide, and substituting 2 sulphydryl hydrogen atoms; the solubility in water is greatest for the hydrochloride and least for the sulphate.

Acetone Thiosemicarbazone.—With 0·1 *N* silver nitrate solution:—(1) The precipitate produced by adding an appropriate quantity of this reagent to a solution of the thiosemicarbazone in alcohol dissolves in excess, and subsequently deposits fine white needles consisting of 1 mol. of the former and 2 of the latter. (2) By adjustment of the proportion of the reactants a crystalline compound, containing 2 and 3 mols., respectively, may be similarly obtained. With cupric salts several crystalline complexes were obtained, of which the following were identified:—(1) Addition of copper nitrate solution to a solution of thiosemicarbazone in a mixture of water and acetone, made alkaline with ammonia, yielded small black crystals when the ratio of copper to thiosemicarbazone was 1:2; they were insoluble in water, alcohol or ether, but dissolved slightly in these solvents in the presence of a mineral acid. The original green solution developed a yellow shade on standing. (2) and (3) The crystalline hydrochloride or sulphate of this complex was obtained by dissolving the base in a solvent acidified with the appropriate acid, or by rapidly adding appropriate volumes of the corresponding cupric salt to a solution of acetone thiosemicarbazone in acetone. The hydrochloride forms large yellow prisms, slightly soluble in cold water, soluble in warm water, and insoluble in ether. The sulphate forms yellow prisms containing 1 mol. of acetone of crystallisation; they are decomposed by water, liberating the acetone and producing fine, pale grey needles of the sulphate.

Benzaldehyde Thiosemicarbazone.—Two crystalline compounds, analogous to those obtained from acetone thiosemicarbazone (*supra*), were produced by the action of silver nitrate with acetone or methyl alcohol as solvent. A third stable and crystalline compound was also obtained as a result of the union of the two others, and this accounts for the difficulty experienced in producing one of these.

J. G.

Spot Tests for Gold. R. N. Costeanu. (*Z. anal. Chem.*, 1936, **104**, 351–355.)—A drop of the test solution is placed on filter-paper which has been impregnated with a reducing agent and dried at a temperature not exceeding 40° C. The agent may be stannous chloride, benzidine in alcohol, pyrogallol, hydroquinone, hydrogen peroxide or formaldehyde in alkaline solution, hydrazine hydrate, hydroxylamine hydrochloride, or mercurous nitrate. By comparison of the spots with those produced by solutions of known gold-content, it is possible to effect an approximate quantitative determination (ANALYST, 1935, **60**, 779).

W. R. S.

Qualitative Reactions of Rhenium. L. C. Hurd. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 11–15.)—Existing data on qualitative reactions of rhenium are critically reviewed. Rhenium compounds, when heated to 900° C. in hydrogen, are reduced to metal, which, when re-heated in oxygen, gives the volatile heptoxide. In the presence of fixed bases, loss of rhenium when ignited in air is negligible or slight. Prolonged digestion of rhenium minerals with nitric or sulphuric acid should be avoided by reason of the danger of volatilising per-rhenic acid. Oxidising materials of the type of pyrolusite may apparently be dissolved in hydrochloric acid without danger of losing rhenium. Evaporation of hydrochloric acid solutions of potassium per-rhenate on a water-bath caused loss of some rhenium, but addition of potassium chloride prevented loss. Alkaline fusion of minerals is satisfactory. Whilst rhenium heptasulphide can be quantitatively precipitated by hydrogen sulphide from solutions containing as much as 33 per cent. of hydrochloric acid (presumably of conc. hydrochloric acid) by weight, the precipitation takes place slowly, and may be incomplete when the hydrochloric acid concentration is below 4 per cent. Owing to the very minute amounts of rhenium present in the known minerals, direct spectroscopic examination is not recommended, and preliminary concentration is necessary. Only four lines, which occur in the visible spectrum, *viz.* the 346$m\mu$ triplet (345·18, 346·05 and 346·37$m\mu$) and the strong 488·91$m\mu$ line, are of value to the analyst for spectroscopic purposes. Spark and emission spectra have been studied. Microscopic precipitation of rubidium and caesium per-rhenates (sensitiveness 0·1γ per 35 cu.mm.) is probably the best for identifying per-rhenic acid, but care is necessary to avoid confusion with chlorostannates and chloroplatinates. Organic compounds found to yield characteristic, but not specific, products with per-rhenic acid, were nitron, methylene blue, acriflavine, brucine and strychnine. In Kronmann and Bibikowa's reaction, which serves to distinguish between nitron nitrate and nitron per-rhenate, nitron acetate and sodium sulphide are allowed to react with a soluble per-rhenate in 10 per cent. gelatin solution; a drop of titanium trichloride solution is added to the mass after setting, when a brownish-yellow colour forms around nitron per-rhenate crystals (sensitiveness, 10γ of rhenium). With dimethyl glyoxime, a yellow complex is formed. Rhenium gives a blue-green flame in the oxidising region, but the colour is easily masked by that of other elements. Borax or phosphate bead-tests yield, in a reducing flame, a grey colour due to dispersed metallic rhenium. The Geilmann test is the most convenient for the detection of heptavalent rhenium in the absence of molybdenum. To the hydrochloric acid

solution, stannous chloride and ammonium or potassium thiocyanate are added, yielding a yellow-brown rhenium thiocyanate, which is soluble in ether, butyl alcohol, or *cyclo*hexanol, but insoluble in carbon disulphide or carbon tetrachloride. Rhenium does not form a compound with ethyl xanthate analogous to the violet-red compound given by molybdenum; in presence of molybdenum, therefore, xanthate may be added, the red compound extracted with chloroform, and tests for rhenium applied to the aqueous portion by the use of the Geilmann reaction. In the Prescott and Johnson system of qualitative analysis rhenium concentrates with arsenic, whilst in the Noyes and Bray system it is found in the tellurium-copper group, and appears in the rhodium-iridium filtrate. S. G. C.

Colorimetric Determination of Iron with Thiocyanate. K. Steinhäuser and H. Ginsberg. (*Z. anal. Chem.*, 1936, **104**, 385–390.)—The instability of the red complex is counteracted by the use of ether containing sulphur dioxide. The sulphate solution (50 ml.), containing not more than 0·01 to 0·25 mg. of ferric oxide, is treated with 5 ml. of hydrochloric acid, 10 ml. of 50 per cent. potassium thiocyanate solution and 25 ml. of alcohol, and shaken with successive portions of ether (15, 10, 10, and 10 ml.) containing 10 per cent. of ether saturated with sulphur dioxide. The combined extracts are made up to 50 ml. with ether, and matched in a Pulfrich photometer against standards treated in the same manner. The colour is perfectly stable. The vessels used should be cleaned by treatment with the reagents used in the determination, and it is pointed out that ether containing sulphur dioxide attacks the skin. Phosphates, fluorides, silver and mercury salts, and salts of organic acids interfere with the method. W. R. S.

Detection and Rapid Determination of Zirconium in Minerals. N. A. Tananaeff and A. W. Tananajewa. (*Z. anal. Chem.*, 1936, **104**, 346–351.)—The method is based on the precipitation of the phosphate in strongly mineral-acid solution, which is a specific reaction for zirconium. The powdered mineral (1 g.) is cautiously fused with 4 g. of sodium hydroxide in an iron or nickel crucible, the heat being increased after 15 minutes. The crucible is cooled, 1 g. of sodium peroxide is added, and the fusion is resumed and finished over a blast-burner. The fluid melt is poured on to a nickel sheet, cooled, transferred to a beaker, and treated with 25 to 30 ml. of hydrochloric acid. The crucible is cleaned with dilute acid, which is added to the bulk, and the solution is evaporated to dryness on a steam-bath. The residue is digested, hot, for 10 minutes with 25 ml. of 15 per cent. sulphuric acid, and the cloudy liquid is treated with a hot solution of 0·10 g. gelatin, which facilitates filtration by coagulating colloidal silica. The solution is filtered after 10 minutes, the filter is washed with 60 ml. of 15 per cent. acid; the filtrate is boiled and treated with 2·5 g. of sodium phosphate dissolved in 5 ml. of 15 per cent. acid, and stirred briskly from time to time. A precipitate or opalescence, appearing within 2 hours, proves the presence of zirconia. Traces require 24 hours for deposition.

For a quantitative determination, the precipitate is collected, washed 3 to 4 times with 15 per cent. acid, and then with hot 5 per cent. ammonium nitrate solution until free from acid, and then ignited and weighed. The weight divided

by 2·15 (or by 2 for minute quantities) gives the quantity of zirconia. The method is claimed to be accurate within 5 per cent. for quantities up to 1 per cent., and within 10 per cent. for higher percentages. The determination can be made in ten hours. W. R. S.

Rapid Volumetric Determination of Titanium. H. B. Hope, R. F. Moran and A. O. Ploetz. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 48–49.)—The method was designed for application to titanium oxide pigments. Reduction by zinc amalgam and titration with iron alum is employed, the apparatus (Fig. 1) being used to maintain a non-oxidising atmosphere. A 0·1 to 0·2-g. sample of the oxide is dissolved by heating with 20 ml. of conc. sulphuric acid and 15 g. of ammonium sulphate. The apparatus is filled with boiled-out 1 per cent. sulphuric acid up to the level of the stop-cock, and both stop-cock and pinch-clip are closed. Fifteen ml. of zinc amalgam [prepared by heating 15 g. of powdered zinc with 300 g. of mercury and 5 ml. of dilute sulphuric acid (1 : 4) for 1 hour on a water-bath, and separating from any solid residue, which is discarded], together with the cooled sample solution and 75 ml. of water, are placed in the tap-funnel of the apparatus; two 5-grain tablets of sodium bicarbonate are added, and the stopper C (with cork A removed) is inserted. When effervescence has subsided, two more bicarbonate tablets, broken into small fragments, are dropped in through tube B. As soon as gas evolution has ceased, cork A is inserted and the apparatus is vigorously shaken for 5 minutes. The stop-cock and pinch-clip are opened, the amalgam is allowed to drop into flask D, and they are closed again. Stopper C is withdrawn and rinsed into the funnel with water, and the solution is rapidly titrated with standard ferric alum solution (30 g. in 1000 ml. of water containing 10 ml. of sulphuric acid), after the addition of 5 ml. of saturated potassium thiocyanate solution. The stop-cock is opened, and the aqueous liquid in the stem of the funnel is caused to rise into the funnel by squeezing tube E. The titration is then completed. The ferric alum standard solution is standardised by reduction by the above method and titration with 0·1 *N* permanganate solution. Good test-results are cited. S. G. C.

Fig. 1

Volumetric Determination of Indium. H. B. Hope, M. Ross and J. F. Skelly. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 51–52.)—The method involves titration of indium acetate solution with potassium ferrocyanide, diphenylbenzidine being used as internal indicator. In the absence of metals of Groups I and II, the solution (containing 10 to 15 mg. of indium) is rendered faintly ammoniacal and boiled, and the indium hydroxide is filtered off and washed sparingly with hot water; the precipitate is dissolved in 15 ml. of hot glacial acetic acid by pouring the acid repeatedly through the filter, which is washed with 10 ml. of glacial acetic acid and then with three 5-ml. portions of hot water. If any iron is present, 5 ml. of 10 per cent. potassium fluoride solution are added in order to

prevent the formation of Prussian blue in the subsequent titration. The solution (which should contain 60 per cent. by volume of glacial acetic acid) is cooled; 2 drops of indicator (2 per cent. of diphenylbenzidine in conc. sulphuric acid) are added, and the solution is titrated with standard potassium ferrocyanide solution (2·5 g. of potassium ferrocyanide and 0·2 g. of potassium ferricyanide per l.). The colour-change at the end-point depends on whether iron is present or not. In the absence of iron the change is from slate-blue to pea-green, whilst if iron and fluoride are present, it is from dull green to bright blue. Both changes are sharp and should persist for 10 seconds. The end-point cannot be obtained in presence of chlorides. The potassium ferrocyanide solution should be standardised by titration of a solution of known indium-content under the same conditions. *Determination of indium in dental alloys.*—These alloys, which constitute one of the major applications of indium, may contain also gold, silver, platinum metals, copper and zinc. The following sulphide separation process is suggested:—The alloy is dissolved in *aqua regia*, 5 to 10 ml. of sulphuric acid are added, and the liquid is evaporated until fumes of sulphuric acid are given off; the solution is diluted, and enough hydrochloric acid is added to make it "about 0·1 *N* in total acidity" (to prevent precipitation of indium sulphide). The solution is heated to boiling, and hydrogen sulphide is passed into the hot liquid in a rapid stream for 30 minutes. The sulphides are filtered off without delay, the filtrate is boiled to expel hydrogen sulphide; indium hydroxide is precipitated with ammonia, and the process is continued as already described. S. G. C.

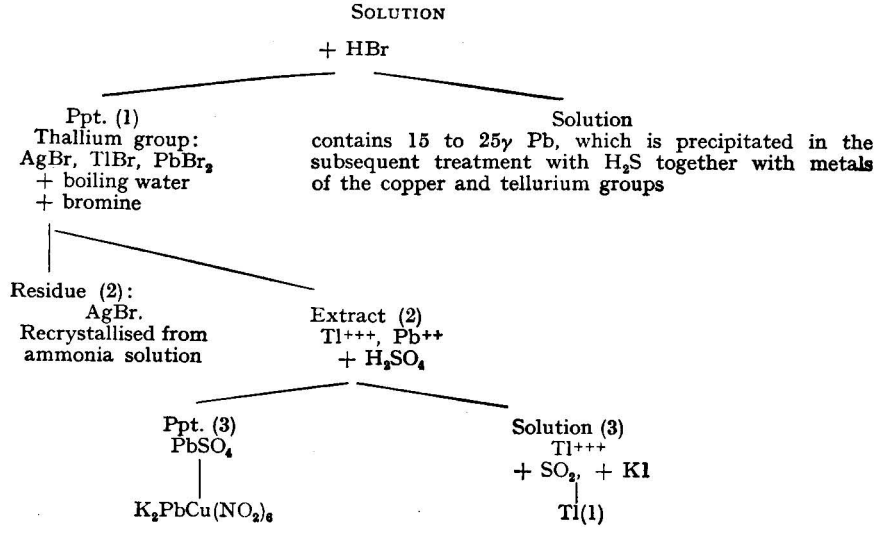
Lanthanum Acetate as a Reagent for Fluoride. J. Fischer. (*Z. anal. Chem.*, 1936, **104**, 344–346.)—The author did not succeed in obtaining satisfactory results with Meyer and Schulz's method for the quantitative determination of fluorine (ANALYST, 1925, **50**, 637). Attempts at modifying and improving it were unsuccessful. On the other hand, the adsorptive power of lanthanum fluoride, whilst vitiating the quantitative results, can be utilised in increasing the sensitiveness of the qualitative test for traces of fluorine. The solution to be tested (1 ml.) is treated with 0·5 ml. of saturated sodium acetate solution, 1 drop of 0·2 per cent. eosin solution, and 0·5 ml. of weakly acid 1 per cent. lanthanum acetate solution. The liquid is boiled, cooled, and centrifuged; a red precipitate is obtained if fluorine is present. The reaction can be applied on a micro-scale, 2γ of fluorine producing a visible red precipitate in a pointed Emich tube. The following do not interfere: sulphate, dichromate, silicate, chloride, nitrate, nitrite, chlorate, or perchlorate, but phosphate, oxalate, molybdate, or sulphate should not be present.

W. R. S.

Microchemical

Qualitative Separations on a Micro-scale. A. A. Benedetti-Pichler and W. F. Spikes. (*Mikrochem.*, 1936, **19**, 239–244.)—A method is described for the separation, identification and determination of the ions of the thallium group of Noyes and Bray (thallium, silver and lead) in a 1-mg. sample. The group is precipitated by adding hydrobromic acid to the filtrate of a preceding

treatment with formic acid at boiling temperature. The procedure is outlined in the following scheme:



In test analyses 0·01 ml. of the test solution are treated with 0·002 ml. of 2 *M* hydrobromic acid and centrifuged, the liquid is tested for completeness of precipitation, and the precipitate is washed with 0·005 ml. of *M* hydrobromic acid. It is then treated with 0·01 ml. of water, heated on the water-bath and centrifuged, and the warm supernatant liquid is removed by means of a capillary pipette of wide bore (0·5 to 0·6 mm.). If crystallisation of lead bromide occurs in the capillary, it is necessary to treat precipitate (1) with 0·01-ml. portions of water until all the lead is extracted. The aqueous extracts are combined. Precipitate (1) is treated with 0·01 ml. of saturated bromine water, the mixture is stirred and centrifuged, and the bromine-water extract is added to the aqueous extract (2). The bromine-water extract must contain excess of bromine, otherwise some thallium might be left behind with the silver. The residue (2) is washed again with 0·01 ml. of hot water, and the amount of silver present is determined by comparison with a standard precipitate of known silver-content. The pure precipitate dissolves readily in ammonia and will re-crystallise slowly on a watch-glass. Extract (2) is treated with 0·002 ml. of 3 *M* sulphuric acid, any suspended white lead sulphate is centrifuged to the bottom, and the deposit is estimated by comparison with a known amount of lead sulphate. The precipitate is washed with 0·001 ml. of *M* sulphuric acid, and a portion is tested by the triple nitrite reaction. The thallium is precipitated by saturating the solution with gaseous sulphur dioxide or solid sodium sulphite and adding 0·001 to 0·002 ml. of *M* potassium iodide solution. The thallium precipitate is estimated by comparison, and the presence of thallium is confirmed by determining the solubility of the precipitate or by the flame-test. The methods permit of the detection of 0·05 per cent. of silver, 0·2 per cent. of thallium, and 3 per cent. of lead in 1 mg. of a solid sample, with limiting proportions 1:1000. J. W. M.

Iodimetry. II. Micro-Iodine Determination by raising to a Higher Power. F. Rappaport and H. Engelberg. (*Mikrochem.*, 1934–35, **16**, 1–12.)—The principle of the method consists in the conversion of iodide into iodate, from which 6 atoms of iodine are liberated for every 1 atom in the original compound, and then the further conversion of the liberated iodine to iodate, and a repetition of the process to the 4th or 5th power, whereby if 1 ml. of *N*/100 thiosulphate were the original requirement, 7776 ml. would be the final requirement of the 5th power. The iodine should be originally present as iodide, but iodate or free iodine may be converted into iodide with alkaline sulphite solution. The test solution is acidified with dilute phosphoric acid, using methyl red as indicator. It is oxidised with bromoacetic acid, and the excess of bromine is removed *in vacuo* by gently heating in a stream of steam in the apparatus shown in Fig. 1. The steam is derived from the litre flask, A, and the connection with flask B is intended to lessen the intensity of the flow. The test solution is placed in the 250-ml. flask, E. The water in A is heated to boiling with the tap in position I, and then it is carefully turned to position II while the manometer shows a pressure of 20 mm. The contents of E should not be heated above 30° C. About 4 minutes after the brown colour has faded, the tap, *f*, is closed and tap *g* is turned to position III. The U-tube contains soda-lime to absorb any bromine vapour in the air. The vacuum is then released, the 250-ml. flask is lowered, and the tube, *d*, is rinsed with double-distilled water. After treatment with 2·5 ml. of potassium bisulphate the iodine liberated by cadmium iodide is distilled off in steam and collected in an alkaline sodium sulphite solution, the apparatus shown in Fig. 2 being used: this is similar to the micro-Kjeldahl distillation apparatus. The absorption flask, E, is interchangeable with E, and is used in its place in the apparatus in Fig. 1 when the process is being repeated. *Reagents.*—(i) *Absorption*

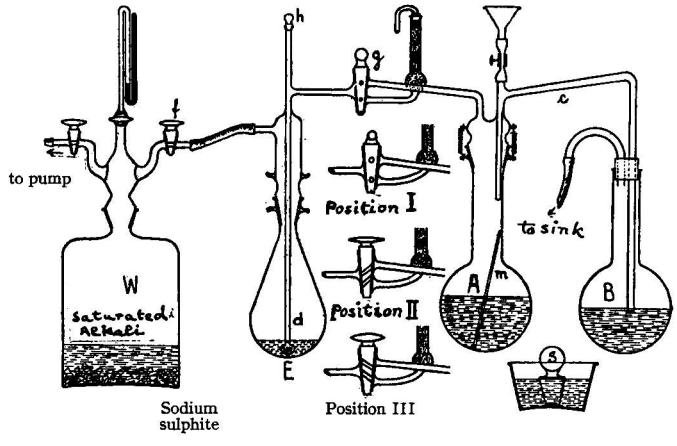


Fig. 1

liquids: (*a*) sodium hydroxide: 8 g. of pure sodium hydroxide dissolved in about 200 ml. of water; (*b*) sodium sulphite: 4 g. of anhydrous sodium sulphite (this keeps for a limited time) in 200 ml. of water. A mixture of 25 parts of (*a*) and 15 parts of (*b*) is made immediately before use, 2 ml. of this being sufficient. (ii)

Dilute phosphoric acid: 10 ml. of syrupy phosphoric acid diluted with water to 100 ml. (iii) *Indicator:* 15 mg. of methyl red dissolved in 10 ml. of alkali (i, *a*) and diluted with water to 1 l.; 2 drops are used. (iv) Bromoacetic acid: 25 g. of bromine dissolved in 100 ml. of pure glacial acetic acid by addition, drop by drop, until the brown colour is permanent. The bromination is best carried out in another room. (v) Five per cent. A.R. (iron-free) potassium bisulphate solution; 2·5 ml. are used. (vi) Cadmium iodide solution: 0·5 g. of cadmium iodide (this may be obtained from the firm Schuchardt, who pack this amount in small bottles, which should be opened in a vacuum desiccator) dissolved in 25 ml. of water; 10 ml. of this solution are dropped in from the funnel (Fig. 2), which is protected from dust and light. (vii) Double-distilled water (*Mikrochem.*, 1934, **15**, 302). (viii) Solid potassium iodide. (ix) Twenty per cent. sulphuric acid. (x) 0·01 or 0·005 *N* sodium thiosulphate. (xi) 0·25 per cent. starch solution. When the repetitions to a suitable power are complete, 1 to 2 ml. of sulphuric acid (ix) and a granule of potassium iodide are added, and the iodine is titrated with thiosulphate. It is essential that a blank test to the 4th or 5th power should give no positive iodine reaction. Good results were obtained on amounts of iodine from 1·2γ upwards. J. W. M.

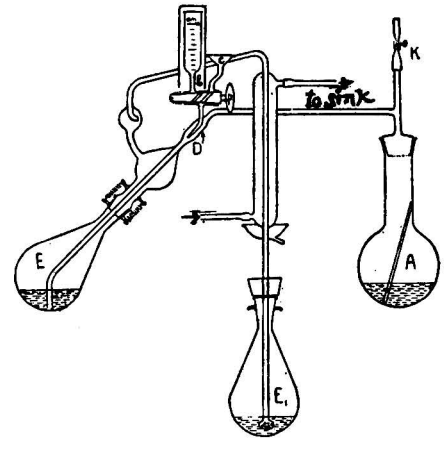
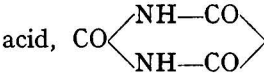


Fig. 2

Micro-determination of Lignin. A. J. Bailey. (*Mikrochem.*, 1936, **19**, 98–107.)—The Ross and Potter method (*Pulp and Paper Mag. Canada*, 1929, **27**, 541) is adapted to the micro-scale. The sample of powdered wood (3 mg.) is weighed into a dry 5-ml. micro-beaker, inside a weighing bottle. The sample is moistened with about 2 drops (40 mg.) of 40 per cent. formaldehyde and 1·64 times its weight of 72 per cent. sulphuric acid and allowed to stand, with frequent stirring, for 10 minutes. Then 95 per cent. sulphuric acid to the amount of 2·76 times the weight of formaldehyde is added, and the liquid is stirred until solution is complete. The final concentration of sulphuric acid should not exceed 72 per cent., correct weights being added by means of pipettes delivering calibrated drops. After the addition of 7 drops of chloroform and acetic acid (1 : 6) the solution is diluted with 4 ml. of water and stirred. The chloroform is expelled on a water-bath, and the lignin is collected on a tared (platinum) Munroe crucible, washed with 2 ml. of 5 per cent. hydrochloric acid, dried at 130° C., cooled, weighed in the weighing bottle, ignited, and weighed again. Analyses of the wood of fir, hemlock-spruce and spruce are compared with those given by the macro-method; it is claimed that the Ross and Potter method gives the best index of lignin-content, as compared with results obtained by the ligno-sulphonic acid method. A bibliography of 48 references is given. J. W. M.

Sensitive Test for Potassium. H. Fredholm. (*Z. anal. Chem.*, 1936, **104**, 400–405.)—5-Nitrobarbituric acid,  , yields a crystalline, sparingly soluble potassium salt. The corresponding salts of ammonium, rubidium, magnesium, and barium are likewise fairly insoluble, but the precipitates present a distinctive appearance under the microscope, which is illustrated by reproductions of photographs. The compounds are obtained by treatment of the slightly acid chloride solutions with a 0·1 *N* solution of the reagent in 40 per cent. alcohol. The potassium salt is readily distinguishable from that of rubidium. The quantitative determination of potassium and some other metals by means of nitrobarbituric acid is under investigation. W. R. S.

Microchemical References, 1935, Part I. (Appendix to *Mikrochem.*, 1936, **19**.)—The references are grouped under the following subjects:—I. *Pure microchemistry*.—(i) General and apparatus, 147 references, 6 books. (ii) Inorganic analysis, 155 references, 3 books. (iii) Organic analysis, 65 references, 1 book. (iv) Preparative chemistry, 2 references. (v) Physical chemistry, 105 references. II. *Applied microchemistry*.—(i) Biological chemistry, 140 references. (ii) Medical and pharmaceutical chemistry, 143 references, 4 books. (iii) Mineralogical chemistry, 53 references, 1 book (in Russian). (iv) Technical chemistry, 195 references, 2 books. Appendix, containing further references to publications which appeared in 1933 and 1934 under the same headings, to be added to those previously published, about 380 references. Under each heading the references are listed in alphabetical order of the authors' names. J. W. M.

Collected References. Cadmium. K. Hiller and F. Maetiek. (*Mikrochem.*, 1936, **19**, 147–161.)—Brief details, together with 85 references, are given of the methods of qualitative analysis of cadmium published since 1926. Quantitative methods include spectrographic, 14 references; polarographic, 4 references; gravimetric and volumetric, 9 references; and determination of cadmium in organic compounds, 3 references. J. W. M.

New Form of the Micro-Kjeldahl Flask. A. Solbys. (*Mikrochem.*, 1936, **19**, 304–305.)—The usual shape of Kjeldahl flask, with pear-shaped bulb and cylindrical tube, is slightly altered, so that one side of the flask is straight and the pear-shaped bulb is at the other side, giving a lop-sided effect. In this model of flask loss by spurting is prevented (model patented and obtainable from Haack, Vienna). J. W. M.

Physical Methods, Apparatus, etc.

Fluorescence Thermoscope. H. Eichler. (*Chem.-Ztg.*, 1936, **60**, 357.)—Solutions of Magdala red in certain organic compounds containing a hydroxy or carboxyl group are violet and non-fluorescent in the solid state, but have a strong yellow-red fluorescence when melted, and as the change from one to the other corresponds very sharply with the m.p., it may be used to indicate the temperature

at which this occurs. The apparatus is a thin-walled glass tube, weighted with shot so that it floats vertically if it is to be used in a liquid; the top portion contains the indicating mixture which is added in the molten state and allowed to set, the tube being then sealed. A container made from transparent foil may be used for measurements with solids. Suitable substances are *o*-phthalic acid, salicylic acid, benzoic acid, resorcinol, thymol, phenol, *o*-, *m*- or *p*-cresol, acetic acid and formic acid; for temperatures below 0° C., formaldehyde, methyl alcohol, ethyl alcohol, glycerol or acetone may be used. Solutions of certain of these substances in water containing Magdala red also show this property. It is advisable to calibrate the end-point against a thermometer rather than to rely on the accepted m.p. of the substance; if the colouring matter used in alcohol thermometers is replaced by Magdala red, the readings are more easily made. J. G.

Dielectric Constant of Mineral Powders. J. L. Rosenholtz and D. T. Smith. (*Amer. Mineralogist*, **1936**, **21**, No. 2, 1–11.)—This method of separation of powdered minerals, which is adapted from that proposed by H. S. Hatfield (*Bull. Inst. Min. and Met.*, **1924**, Nos. **233** and **234**), depends on the fact that if grains of powder are immersed in a liquid they will be attracted to electrodes also immersed in the liquid and connected with a suitable A.C. supply, when the liquid has a lower dielectric constant (ϵ) than that of the sample, and *vice-versa*. Current from a 110-volt, 60-cycle A.C. supply is converted to 220 volts by means of a small step-up transformer in series with a switch and a 2000 ohm resistance, the function of which is to avoid burning the needles by conducting grains of mineral. The current is carried to two insulated biological dissecting-needles mounted together and bent so that the points are 1 mm. apart. The mineral is powdered to pass a 250-mesh, but not a 300-mesh sieve, and if its dielectric constant is low it should also be dried at 110° C. to eliminate the effect of surface-moisture. A speck is added to a measured 3 to 4 ml. of carbon tetrachloride (ϵ 2·24 at 20° C., temperature coefficient −0·0014, see *Intl. Critical Tables*, **6**, 83), and the needles are immersed, whereupon there is a distinct attraction towards the points, which may be observed under a binocular microscope. Methyl alcohol (ϵ 33·7 ± 1 at 20° C., temperature coefficient −0·18, *loc. cit.*) is then added from another burette, and when the dielectric constant of the mixture equals that of the sample the grains will remain stationary between the points; an extra drop of the alcohol then causes repulsion. "Back-titration" gives erratic results, but except with calcite minerals, no trouble due to flocculation is experienced. A single separation takes 5 minutes. Mixtures of the above solvents may be used over the range 2·24 to 33·7, ϵ being a straight-line function of the amounts of the constituents, but allowance must be made for the temperature-coefficient of the methyl alcohol; for higher values triply-distilled water (ϵ 81 at 20° C.) must be used, although this is unnecessary for most minerals. No appreciable error occurs as a result of volatilisation, and carbon tetrachloride suppresses any tendency of the methyl alcohol to ignite as a result of sparking at the needles. Average values of ϵ at 20° C. are tabulated for 160 mineral powders, and are reproducible usually to within 5 per cent., and always to within 10 per cent. Two-thirds of the samples had values below 10, and perfect separations were then possible, even when the difference

in ϵ were only about 1; with other samples separation was accomplished for differences of about 2 in ϵ, the particles being allowed to drop into a minute glass spoon under the needles. Minerals having values over 81 (argentite, arsenopyrite, bornite, braunite, copper, corvellite, enargite, galena, gold, graphite, haematite, manganite, molybdenite, pyrolusite, pyrrhotite, silver and smaltite) were attracted strongly to the points, and usually arced between them. No exact results were obtainable for minerals having values between about 33·7 and 81 (anthracite, chrysotile, cobaltite, ilmenite, magnetite, marcasite, proustite, pyrargyrite, pyrite and zincite). The method may be used in conjunction with other methods as an aid to diagnosis, although it must be remembered that the values obtained will not necessarily agree with those in the literature, as they are dependent on the working conditions (*e.g.* the size of the particles and the frequencies used). Other sources of error are, the fact that a slight excess of methyl alcohol may affect ϵ considerably without producing a noticeable difference in attraction or repulsion, and the effects of changes in temperature and pressure. According to theory, mineral grains should orient themselves with respect to the needles in such a way that the maximum values of ϵ will be obtained for anisotropic minerals; in practice, however, this is questionable, especially with minerals which cleave into thin plates or flakes. J. G.

Reviews

THE THEORY OF EMULSIONS AND THEIR TECHNICAL TREATMENT. By WILLIAM CLAYTON, D.Sc., F.I.C. Third Edition. Pp. ix + 458, with 91 illustrations. London: J. & A. Churchill. 1935. Price 25s.

Once again Dr. Clayton has placed chemists and technologists under a great debt by the appearance of this timely and stimulating volume. To have covered a field so wide, and at the same time one which it must be particularly difficult to correlate and reduce to order, is a feat of which any man may well be proud. Dr. Clayton's reputation in the field of emulsion technology, and in the application of the concepts of colloid chemistry to edible materials in general, is such that it would be something of an impertinence merely to express approval of what he has accomplished. The real measure of the value of the labour, the immense labour, which must have gone to the compilation of a work of this kind, is the fact that in a relatively short space of time a third edition of the work has been called for. The scope and size of the various editions is, perhaps, the best index of the rapid progress which is being made in the chemistry and technology of emulsions. Starting with a slim volume of 160 pages in 1923, the second edition (1928) had already grown to 283 pages, and this in turn has been followed by a completely revised edition 458 pages in length. Clearly this work is the most authoritative one, in any language, and, that having been said, there is little need to say more. This book is indispensable alike to the academic and to the industrial chemist concerned with the behaviour of disperse systems. W. C. M. LEWIS

ESSENTIALS OF PHYSIOLOGICAL CHEMISTRY. By ARTHUR K. ANDERSON. Pp. 257 + v. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1935. Price 13s. 6d.

Probably many biochemists deplore the regular appearance of new text-books of biochemistry, especially elementary text-books, believing that there are already more than enough to satisfy the legitimate needs of the rising generation. Professor Anderson, however, must be exempted from this general censure, for he has two excellent excuses. In the first place, he is concerned not for the welfare of the young biochemist, nor yet for the more scientific upbringing of the medical student, both of whom are already well looked after. This book, as he explains in the preface, is written for the "student of human nutrition" and the dietician. In the second place, the rapidity with which discoveries in various branches of the subject are being made demands either the regular publication of new text-books or the regular revision of old ones. Even during the few months that have elapsed between the writing of this book and its appearance on this side of the Atlantic, further developments have made necessary the re-writing of many paragraphs in the chapters on hormones and vitamins.

There is nothing revolutionary about *Essentials of Physiological Chemistry*; it follows conventional lines, but the contents are specifically limited to the biochemical processes closely associated with nutrition. The first chapter briefly describes the physical chemistry of the functioning human organism, and is followed by accounts of the chemistry of the carbohydrates, lipids and proteins. After a short chapter on foods and one on enzymes, the seven chapters following are devoted to discussing the processes of digestion and of metabolism. Blood and urine form the subjects of the next two chapters, and the book terminates with accounts of the endocrine organs and the vitamins, which are quite up-to-date, subject, of course, to the reservation already made. The author's style is simple and straightforward. He states that he has avoided controversy as much as possible, but in so doing he has laid himself open to the charge of over-simplification, and even of partisanship. How many chemists would agree, for example, with the statement made on p. 230, that ergosterol may be converted into vitamin *D* by chemical means?

There are remarkably few typographical errors; such as there are appear to be confined to stray valency bonds in certain of the formulae. Thus the formula of haemin on p. 178 contains a tervalent carbon atom and a bivalent nitrogen atom, whilst on p. 210 adrenaline is given two quinquivalent carbon atoms, and on p. 145 acetone and acetoacetic acid are given the following curious constitutions: CH_3–C=O–CH_3 and CH_3–C=O–CH_2–COOH. One of the worst errors is the inclusion on p. 79 of an ancient formula for cholesterol, for which the responsibility is quite unfairly put on Windaus! This, in spite of the fact that the modern formula for ergosterol is given on a later page. Incidentally, could not this latter formula have been more elegantly represented? Another criticism which the reviewer feels to be legitimate concerns the table on p. 141, which purports to summarise the chemistry of muscle contraction. As it stands at present, however, this table is very difficult to follow, and the time spent in deciphering it might be better occupied with studying the two pages of text more closely. Finally,

sitosterol contains 29 carbon atoms, not 27, as stated, and surely some mention might have been made of the connection between the flavins and "vitamin *G*."

These criticisms are not intended to detract in any way from the merits of the book, but are made in the hope that the matters to which attention has been called will be amended in later editions, which without doubt will be needed in the not very distant future.

F. A. ROBINSON

DIE FERMENTE UND IHRE WIRKUNGEN. By Professor CARL OPPENHEIMER. Supplement. Pp. 320. The Hague: W. Junk. 1935. Price £1 8s. net.

The present publication forms the first part of the supplementary volume to the main work of the same title published in four large volumes during the period 1924–1929. The whole supplement will, it is estimated, occupy about 1600 pages, and will be complete by about 1937.

The supplement consists of records and critical discussions of recent work in the "special part" of the enzyme field, *i.e.* is supplementary to portions of Vols I and II of the original work, which covered general properties of enzymes and special treatment of individuals. Volume III of the original dealt with methods of preparation and measurement, and Vol IV with technical applications.

The new volume opens with a section on the esterases, which term is often confused with the term "lipases," the two having meanings which overlap. The author, quite logically, uses the term "esterase" to cover generally all enzymes which promote the hydrolysis of the ester type of compound, whether organic or inorganic acids, alcoholic or phenolic hydroxyl groups are involved. Sub-division of the whole group of esterases gives sulphatases and phosphatases which hydrolyse esters of sulphuric and phosphoric acids, lipases which act on esters and glycerides of fatty acids, whether fats or not, lecithases, tannase, etc.

The next part of the volume deals with carbohydrases, a term covering all enzymes which hydrolyse compounds built up partly or wholly of carbohydrates. The linkage broken is an oxygen bridge connecting what were two hydroxyl groups —one alcoholic and the other either alcoholic or phenolic. Oppenheimer classifies the carbohydrases into (1) oligases which split either hexosides (*e.g.* α- and β-glucosides, mannosides, fructosides, etc.), or hetero derivatives of sugars, such as digitalin, phenol glucoside, etc., which contain a non-sugar group; (2) polyases, such as amylase, inulase, pectinase, etc., which attack polysaccharides.

The system of classification is most useful, in view of the growing multiplicity of enzymes, and if orderly classification can keep pace with new discovery much will be achieved for which the biochemist should be thankful.

The section on the amylases (diastase)—(about 150 pages of this part have been issued so far)—reviews very thoroughly the numerous complicated, and in some ways conflicting, advances of the last five or six years. An interesting survey is provided of the nature of starch—both the chemical molecule and the micelle or aggregate of chemical molecules which gives rise to colloidal properties. This is followed by a consideration of the mechanism of the breakdown of starch by α- and β-amylases, a field in which a considerable revival of activity has been manifested during the past few years.

In all, 480 pages of the supplement fall within the scope of this review. Throughout this part, the most recent work in the various fields is described and critically reviewed at length. There are extensive citations from the literature on almost every page. The classified bibliography is to be expected at the end of the supplement. To the biochemist and others working in this field this volume is indispensable as a standard book of reference. R. H. HOPKINS

INCOMPATIBILITIES IN PRESCRIPTIONS. RUDDIMAN and NICHOLS. Sixth edition. Pp. 300 + Index, 37 pp. New York: John Wiley & Sons; London: Chapman & Hall. Price 13s. 6d. net.

Although the title suggests that this work is of chief importance to pharmacists and physicians, yet a perusal of its pages shows that it will be of service to analysts who are called upon to deal with chemicals and drugs when compounded in the form of medicines for human use.

The description of any incompatibility must of necessity beg the assumption that the mixing of two or more ingredients produces a result which was not intended or expected, and the trained chemist can hardly be expected to agree that mercuric chloride and potassium iodide are incompatible, unless the use for such a combination is known. The pharmacist mixes solutions of them daily in the dispensing of prescriptions intended to contain the double iodide. To analysts the resulting mixture of iodides is known as a precipitant of alkaloids, and consequently, it would seem futile to mix such ingredients with any medicine containing an alkaloid. In such a case the use of the word incompatible would be justified, and the physician should avoid such combinations; yet it is by no means unusual to find double iodides, Donovan's solution, to wit, prescribed with vegetable solutions containing alkaloids.

The authors have compiled a very comprehensive list of "incompatibles" which will be of service in the examination of pharmaceutical preparations, for the number of reactions possible or probable in a mixture of four of five ingredients selected from many hundreds provides limitless possibilities for an analyst.

The book is divided very usefully into two parts: (1) enumerating the incompatibilities of drugs and medicinal chemicals; (2) prescriptions with criticisms and explanations of what may happen to particular ingredients in over five hundred selected prescriptions, and how to avoid many of the decompositions which are possible in them. These are followed by a table of solubilities which will be of particular service to the users of such chemicals and compounds, for it includes many substances infrequently used in this country, although often employed in the United States of America.

One of the most useful pages in the book gives in tabular form the effect of mixing numerous solids together; to the uninitiated it will be surprising to find what a number of solids form fluids when rubbed together.

A study of the 434 criticisms of specified prescriptions will afford the opportunity for making many useful notes, and possibly for some arguments, but to the analyst, some of the recipes will suggest that prescription writing is not always preceded by an exact chemical knowledge, for if it were, many of the combinations could never have been ordered.

It is not always possible to consult a physician regarding his written prescription, and every pharmacist must, at times, encounter almost impossible problems, the results of which may or may not eventually come before the analyst for investigation. C. EDWARD SAGE

THE BACTERIOLOGICAL GRADING OF MILK. Medical Research Council. Special Report Series, No. 206. By G. S. WILSON, M.D., F.R.C.P., D.P.H. Assisted by R. S. TWIGG, R. C. WRIGHT, C. B. HENDRY, M. P. COWELL, and I. MAIER. Pp. 392. H.M. Stationery Office. 1935. Price 7s. 6d. net.

A summary of this Report is given elsewhere in this number (p. 414), and the following remarks are offered by way of general comment and criticism.

While one greatly admires this masterly work and the manner in which Professor Wilson and his able team have investigated practically every detail of all the methods of bacteriological examination extant for the grading of milk, and while one is bound to admit that most of his criticisms of present-day methods are justified, many bacteriologists experienced in milk examinations will find themselves unable wholly to accept his conclusions.

With regard to the coliform count, his indictment is based largely upon the fact that he and other workers find that roughly from 25 to 50 per cent. of the coliform bacilli isolated from raw milk belong to the *aerogenes-cloacae*, intermediate types, and from this it is argued that their presence does not necessarily represent faecal contamination, direct or indirect, but contamination from the dust of grains, meals, feeding cakes, hay, and so on. This argument is unsatisfactory, because it does not take into account the smallness of the numbers in which these types are present in such feeding stuffs when reasonably clean, nor the very small extent to which these feeding stuffs are used in many rural districts, particularly in the spring, summer and autumn. It cannot be accepted, therefore, that this source of contamination is sufficient to account for 25 to 50 per cent. of the coliform content of milk, and it would appear more probable that the presence of *B. coli* in milk originates indirectly from faecal contamination, the ratio of *aerogenes* to faecal types (normally 2 to 5/100) being modified by conditions, such as desiccation, which are more favourable to the former. This view is supported by experience of milk competitions, the results of campaigns for cleaner methods of production, and in particular the better cleansing of the udders and flanks of the cows. These have resulted in a marked fall of the coliform count in the last decade, so that, whereas formerly it was a common experience to find 1000 to 10,000 or more per ml., it is now very unusual, less than 10 to 100 being commonly found, and this has resulted without any special steps being taken to reduce contamination from feeding-stuffs. Moreover, one's experience is that a high coliform count almost invariably indicates some lack of cleanliness in production, which subsequent careful inspection reveals. One therefore joins issue very strongly with the author over his criticism of the coliform count.

Apart from the conditions of milk-supply in London (the bulk of which is pasteurised), and in the large cities, people expect to obtain their milk within three or four hours of the time of production. It is, therefore, much more desirable to ascertain the condition of the milk so delivered than to subject it to indefinite

conditions of storage for a number of hours and then test it. Professor Wilson's suggestion that samples of evening milk should be left for 18 hours, and those of morning milk for 12 hours, at atmospheric temperature before examination, invites criticism. Summer temperature (1st May to 31st October) may vary from 50° to 80° F., and by actual experiment the variation in the plate count with the same milk, after being held for 12 hours at these extremes of temperature, may be from 200 to 440,000 per ml. The author has not worked out the coefficient of variation due to possible changes of atmospheric temperature.

It is claimed that the modified methylene blue reduction test is suitable for the grading of good-quality milk such as certified, but does Table CLVII quite justify this claim? In this table, one finds it recorded that 67·8 per cent. of churn milk and 61·9 per cent. of rail-tank winter milk giving plate counts of 30,000 to 200,000 per ml. did not reduce methylene blue in 6 hours, and that 24·0 and 10·7 per cent., respectively, did not reduce it in 8 hours. Unless one is prepared to extend laboratory hours considerably, milk of better quality would apparently not come within the range of distinction of the modified methylene blue test. Moreover, one finds in Tables CLIV and CLV that the number of samples with plate counts of 200,000 to 1,000,000 not reducing methylene blue in 6 hours were 30, 16, 35, 38, and 28 per cent. in the five periods November–December, 1932, April–May, 1933 (Table CLIV), July, 1933, October–November, 1933, and April–May, 1934 (Tables CLV), so that a considerable number with very high plate counts would be passed by this test.

Notwithstanding these criticisms, Professor Wilson's report is one of the greatest value. There is much to be learnt from it, and one cannot read it without much profit in the improvement of one's technique and judgment in the problems of grading milk bacteriologically. The problem that Professor Wilson has attacked, namely, that of finding a simple inexpensive test for grading milk, is an extremely difficult one—one might almost say insolvable, in view of the many variables and contributing factors. The test that he suggests—the modified methylene blue reduction test—is certainly simple and inexpensive, and he claims that, as modified, it has a fairly well-defined end-point and small experimental error; it may be expected to give a fairly reliable, though rough, measure of non-specific bacterial growth in milk. While he does not claim that it gives a measure of faecal contamination, he does claim that it is well correlated with hygienic conditions of production, and that it compares very favourably, and even to advantage, with any of the tests in present-day use. What one greatly regrets, however, is that it is not sensitive enough to apply to milk without a preliminary period of incubation, for a new variable is thereby introduced far greater than any involved in the plate count and coliform count.

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