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THE ANALYST

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COUNTY OF KENT

Appointment of County Analyst and Official Agricultural Analyst

Applications are invited for the above-mentioned appointment, which will become vacant on the 1st April, 1946. The basic salary will be at a rate not exceeding £1,250 a year, the remuneration to be fixed according to experience and qualifications. A war addition, at present at the rate of £59 19s. 3d. a year, and travelling and subsistence allowances are payable.

The appointment will be subject to the Local Government Superannuation Act, 1937, and the successful candidate will be required to pass a medical examination.

Applicants must possess the qualifications prescribed by or pursuant to the Food and Drugs Act, 1938, and the Fertilisers and Feeding Stuffs Act, 1926, and the appointment will require the approval of the Minister of Health and the Minister of Agriculture and Fisheries.

The person appointed will be desired also to accept appointment as Public Analyst by such of the autonomous Food and Drugs Authorities within the County as so wish and as the County Council may approve. He will be required to devote the whole of his time to the public offices to which he is appointed by or with the permission or approval of the County Council and to pay all moneys received into the County Fund.

Applications, stating age, qualifications and experience, accompanied by copies of not more than three recent testimonials, should be received by me not later than the 6th October, 1945.

Canvassing will disqualify.

W. L. PLATTS,
Clerk of the County Council.

COUNTY HALL, MAIDSTONE.
9th August, 1945.

CITY OF PORTSMOUTH

SECOND ASSISTANT ANALYST

Applications are invited for the post of Second Assistant Analyst in the Public Analyst's Department, the salary being Grade "C" of the Whitley Grading Scheme, namely, £390 per annum, rising to £435 per annum by increments of £15. The first increment of salary will be payable on the 1st April, 1947, and subsequent increments on the 1st April in each year. A cost of living bonus of £59 16s. 0d. is at present payable in addition to the salary.

Applicants must possess "The Associateship of the Royal Institute of Chemistry, Branch E (The Chemistry of Food and Drugs)."

The position is subject to the provisions of the Local Government Superannuation Act, 1937, and the Council's Sick Pay Scheme and other regulations.

The successful applicant will be required to pass a medical examination.

In accordance with the War-time practice of the Council, the post is a temporary one in the first instance and will be subject to termination by one month's notice on either side.

The person appointed will be required to undertake occasional Sunday duty in connection with examination for Diphtheria.

Applications, stating age, qualifications and experience, and accompanied by copies of not more than three recent testimonials, must be forwarded in an envelope endorsed "Application for Second Assistant Analyst," to reach the undersigned not later than the first post on Saturday, 8th September, 1945.

Canvassing, either directly or indirectly, will be a disqualification.

The Ministry of Labour and National Service have given permission under the Control of Engagement Order, 1945, for the advertisement of this vacancy.

FREDERICK STARKS,
Town Clerk.

THE ROYAL BEACH HOTEL,
SOUTHSEA, PORTSMOUTH.
10th August, 1945.

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Obituary

JAMES KEAR COLWELL

JAMES KEAR COLWELL, F.R.I.C., died in the Finchley Memorial Hospital after an operation on April 26th, 1945, in his 80th year. He spent his boyhood in Gloucester, going to the Crypt School, and had his first introduction to Food and Drugs Chemistry in the laboratory of George Embrey, who was Public Analyst for both the County and the City of Gloucester. Having won a scholarship at the Royal School of Mines, Colwell came to London in 1882. He worked for a time under Professor Percy Frankland, and afterwards entered his private laboratory, where he was engaged chiefly on water analysis. From there he went as assistant to Winter Blyth at Marylebone, where he stayed until he was appointed Public Analyst to the Clerkenwell Vestry, (which subsequently was fused into the Metropolitan Borough of Finsbury), of which he became Public Analyst 52 years ago. Very shortly afterwards he was appointed to a similar post with the Metropolitan Borough of Holborn. Later he became Public Analyst for the Borough of Bedford, the Metropolitan Borough of St. Pancras, the County of Bedford and the Borough of Luton in that order. He was at his desk until within a few days of his death.

Colwell was a man of the highest integrity with a thoroughly genial disposition, always ready with sound advice and a helping hand for his friends in time of trouble. Not the least of his good qualities was the extraordinary cheerfulness with which he suffered the complete deafness that afflicted him during the last two years of his life. He will be mourned by a wide circle of friends.

A. LICKORISH

The Precipitation of Titanium by Tannin from Chloride Solution

BY W. R. SCHOELLER AND H. HOLNESS

IN the first paper of this series one of us (S.) has shown that zirconium is pptd. by tannin from chloride solns. containing free hydrochloric acid,[1] and that the reaction can be used for its quantitative separation from a number of elements, including iron and vanadium. Cupferron has the disadvantage of pptng. these two metals; hence they must be eliminated prior to the determination of zirconium by that reagent. As a preliminary step in the study of the application of tannin in zirconium analysis we have investigated its action upon titanic chloride solns. and the results are recorded below.

In the paper cited above a serial order of precipitability by tannin from chloride soln.—"probably" Sn-Zr-Ti-Th-V-M^{III}—was indicated, and it was stated that the zirconium-tannin ppt. "includes any titania present." If the co-pptn. of titanium is quantitative, this may not be a disadvantage of the method, because it would give the sum of the two oxides in one operation, the subsequent determination of the titania in the mixed oxides being a routine matter. Our experimental evidence proves that the serial order here reproduced is substantially correct; the statement referring to the co-pptn. of titanium, however, should be qualified, the titanium complex being so much more sensitive to hydrochloric acid than the zirconium compound that complete pptn. of the titanium requires reduction of the acidity to below 0·02 *N*. If this condition is realised, tannin pptn. gives the sum of $ZrO_2 + TiO_2$.

At 0·4 *N* acidity, *ca.* 90% of the zirconia is pptd., while the pptn. of titanium from pure chloride soln. is inhibited. This behaviour persists when the two elements are in association, the cream-coloured TP^1 yielding more or less pure zirconia, whilst the orange to brown TP^2 recovered after suitable neutralisation contains nearly all the titania. We attach no quantitative value to this differentiation. The pptn. interval between titanium and thorium in chloride soln. is too small for quantitative work, and Schoeller's rule for tannin pptns. from oxalate solns.[2] appears to hold good also for chloride solns., *viz.*, "two neighbours in the

series cannot be separated from each other by the tannin process, at least not without elaborate fractionation." This rule applies to vanadium, iron and aluminium, which we succeeded in separating from titanium in chloride soln. in spite of the very low acidity required for complete titanium pptn. The same procedure (described below) gave a clean separation of iron, aluminium and vanadium from zirconium and a subordinate amount of titanium in solutions simulating that from a titaniferous zirconium mineral.

Theoretical

Before describing our experimental work we wish briefly to discuss the tannin pptn. of titanium and zirconium in two different media, *viz.*, oxalate and chloride solns. (*a*) In feebly acid oxalate soln. half-saturated with ammonium chloride there is pronounced differentiation,[3] ascribed to "titanyloxalates being less stable than zirconyloxalates": the titanium is pptd. first, and the separation is quantitative. The position of titanium and zirconium in the serial tannin pptn. from oxalate soln. is Ta-Ti-Nb-V-Fe^{III}-Zr-Th. (*b*) In slightly acid chloride soln. there is well-marked differentiation, and it is the zirconium that is pptd. first: Zr-Ti-Th-V-Fe^{III}.

The inversion of the relative positions of titanium and zirconium in series (*a*) and (*b*) may be explained in terms of the current theory of tannin pptns., according to which the negatively charged tannin particles neutralise the positive charge of suspensions of metallic hydroxides or other compounds, yielding adsorption complexes by reciprocal flocculation. In other words, tannin does not ppt. true molecular solns.; and if a metal is not (or only partly) pptd., we infer that the acidity of the soln. prevents (or impairs) hydrolytic decomposition of the dissolved metallic compound. Hence acidity control is the vital factor in all tannin work. Thus we must assume that in oxalate soln. titanyloxalic acid is completely hydrolysed before zirconyloxalic acid is affected by progressive neutralisation.

Zirconium chloride solns. have been investigated by Britton,[4] who found that they are considerably hydrolysed even if free acid is present, and he inferred that they contain a highly dispersed basic chloride; no evidence for the existence in the solns. of $(ZrO)^{++}$ ions or of zirconyl chloride could be obtained. This we consider to be confirmed by the fact that zirconyl chloride is obtained only upon addition of a large amount of strong hydrochloric acid to the chloride soln., when a crystalline ppt., $ZrOCl_2.8H_2O$, is obtained. When dissolved in water, this salt probably forms the dihydroxydichloride, $Zr(OH)_2Cl_2$, which then undergoes hydrolysis. It is this highly disperse, positively charged phase which accounts for the ready pptn. of zirconium by tannin from a chloride soln. The fact that zirconium is not pptd. by tannin from slightly acid sulphate soln. is explained by the hydrolysis of zirconium sulphate into sulphuric acid and complex zirconylsulphuric acids, in which the metal forms part of the anion.[5]

Titanium chloride solns. contain hexachlorotitanic acid, H_2TiCl_6, in which the metal remains in the anion as long as the acidity is sufficient to prevent the reaction:

$$H_2TiCl_6 + (2 + x)H_2O \rightarrow TiO_2.xH_2O + 6HCl.$$

When this sets in, the titania forms a slightly disperse phase which adsorbs tannin, yielding a denser ppt.

Actually there is a decided difference in the appearance of the two ppts.; Schoeller[1] has already remarked that the zirconium ppt. "flocculates well, but hardly adheres to the glass at all," which we take as an indication of reciprocal flocculation. The titanium complex, on the other hand, always gives a dense red ring round the beaker and glass rod at the edge of the hot liquid, where "metatitanic" acid must have formed by hydrolysis. At 0·2 *N* acidity, *ca.* 70% of the titanium is pptd.; the recovery curve then drops steeply to *ca.* 10% in 0·3 *N* acid (see F).

Experimental

A. Gravimetric Determination of Titanium—Two solns. of hexachlorotitanic acid (prepared from titania purified by fractional tannin pptn. from oxalate soln.) were standardised by pptn. with ammonia and ignition of the ppt. to TiO_2; 20-ml portions gave 0·0459 and 0·0508 g respectively.

The tannin pptn. was carried out as described for zirconium,[1] with this difference, that (as in all the tests here recorded) much closer attention had to be given to the adjustment of the acidity, which was effected with *N* ammonia. Twenty ml of stock soln. were diluted, treated with 10 ml of saturated ammonium chloride soln. and 1 g of tannin dissolved in 15 ml of hot water, and 18 ml of *N* ammonia were added to the boiling-hot liquid. The final

volume was 112 ml, and the acidity was reduced to 0·018 *N*. The dark red ppt. was collected on No. 541 Whatman paper, washed with 2% ammonium chloride soln., and ignited. Found, 0·0459 and 0·0507 g respectively (Exp. 1). If only 16 ml of *N* ammonia were added, giving 0·036 *N* acidity, the recovery from the first stock soln. was 0·0451 g (Exp. 2).

B. SEPARATIONS—The separation of titanium by itself from aluminium, iron, vanadium, etc., by tannin pptn. from chloride soln. is not of great practical importance because recourse can always be had to the tannin separation from oxalate soln., an accurate method much used in practice.[6] With zirconium it is different, since this element cannot be separated from the others by the same method. We have studied the separations here discussed in order to satisfy ourselves that we can recover any titanium present in a chloride soln. along with the zirconium, and to work out the conditions under which this can be done. We experimented with a pure titanium soln. (D) and mixed zirconium-titanium solns. (E). In view of its application to mixed solns., the following procedure prescribes pptn. in two fractions, the major TP^1 at a higher acidity than the minor TP^2, since this technique had been found necessary with zirconium to ensure a clean separation.

C. PROCEDURE—Adjust the volume of the chloride soln. (0·05 to 0·1 g of $(Zr,Ti)O_2$), containing at least 5 g of ammonium chloride and a known amount of hydrochloric acid, to 200 ml, and its acidity to *ca.* 0·1 *N* for titanium, or 0·25 *N* for zirconium and titanium, or 0·5 *N* for zirconium with vanadium, neutralising any excess acidity with *N* ammonia. Boil, stir, and add a freshly-made soln. of 1 g of tannin in 15 ml of hot water; allow to boil gently for 1 or 2 min., set aside to cool, and collect after 2 hr. on an 11-cm No. 541 filter containing creamed filter-pulp and moistened with the acid wash liquor. Wash with an accurately measured amount of a cold 2% soln. of ammonium chloride in dil. hydrochloric acid (0·1 *N* in absence, 0·5 *N* in presence, of zirconium). Ignite the wet TP^1 in a tared porcelain crucible.

Minor fraction—To the hot filtrate and washings, of known bulk and acidity, gradually add a calculated volume of *N* ammonia to reduce the final acidity to 0·005–0·01 *N*; it is best to use a burette for the final addition. Iron is usually present, and its tannin complex is so sensitive to mineral acid that it acts as an indicator; hence the darkening produced by addition of ammonia disappears when the boiling liquid is stirred. Stop short of permanent discoloration (at this point, a spot test on blue litmus paper should give a pronounced red colour). Boil for 1 or 2 min., set aside in the cold and proceed as for TP^1. Wash with 0·1 *N* acid 2% ammonium chloride soln. and ignite TP^2 separately, for a purity test if desired.

A simple test for complete titanium pptn. (zirconium is completely pptd. in TP^2) consists in treating the hot filtrate from TP^2 with dil. ammonia until a very slight flocculent ppt. is obtained. It should not be red, and should readily dissolve on addition of a few drops of *N* acid.

D. PURE TITANIUM SOLNS.—The effect of varying the acidity in the pptn. of TP^1 was studied (see Table I). The weight of metallic chloride added is calculated to oxide. TiO_2 taken: 0·0459 g.

TABLE I

			TP^1		TP^2		TiO_2	
Exp.	Added, g		Acidity as *N*	g	Acidity as *N*	g	Found, g	Error, g
3	Al_2O_3	0·378	0·045	0·0438	0·006	0·0019	0·0457	−0·0002
4	do.	do.	0·093	0·0401	0·006	0·0055	0·0456	−0·0003
5	do.	do.	0·21	0·0123	0·006	0·0328	0·0451	−0·0008
6	Fe_2O_3	0·250	0·045	0·0407	0·006	0·0047	0·0454	−0·0005
7	do.	do.	0·093	0·0348	0·006	0·0108	0·0456	−0·0003
8	do.	do.	0·21	0·0230	0·006	0·0226	0·0456	−0·0003
9	V_2O_4	0·250	0·039	0·0538	0·006	0·0024	0·0562	+0·0103
10	do.	do.	0·082	0·0425	0·006	0·0037	0·0462	+0·0003
11	ThO_2	0·200	0·05	0·1017				
12	do.	do.	0·10	0·0676				

Except in Exp. 9 (in which the initial acidity was too low for a clean separation, vanadium requiring a higher acidity than do aluminium and iron), the weighed titania ppt. in Exps. 3 to 10 were found free from the metal added. In Exp. 5, with a high initial acidity, TP^2 became the major fraction, yet it proved free from aluminium after pptn. at a final acidity as low as 0·006 *N*. This must be due to its having formed gradually as the soln. containing the tannin ppt. was being neutralised with *N* ammonia. Hence the separation of titanium

from aluminium and iron appears feasible in a single pptn. In Exp. 1 of the earlier paper,[1] on the other hand, the zirconium complex was heavily contaminated with alumina after pptn. at 0·1 *N* acidity, which may be explained by its sudden formation at an acidity intended for complete pptn.

We interrupted the unpromising Exps. 11 and 12 for the time being, as we intend to make a fuller investigation of the application of tannin in thorium and rare-earth analysis.

E. MIXED ZIRCONIUM-TITANIUM SOLNS.—Two solns., prepared by addition of known amounts of titanium stock soln. to solns. of zirconyl chloride in 0·1 *N* hydrochloric acid, were standardised as before; 25-ml portions gave:

Soln. 1: 0·0057 g of TiO_2 + 0·0497 g of ZrO_2 = 0·0554 g of (Zr, Ti)O_2.
„ 2: 0·0057 „ „ + 0·0581 „ „ = 0·0638 „ „

Exps. 15 to 20 (Table II) prove that the sum of the dioxides was satisfactorily determined in presence of substantial amounts of the added metals, a higher acidity being again required for vanadium, as in Schoeller's Exps. 10 and 11.[1]

TABLE II

			TP^1		TP^2		(Zr, Ti)O_2	
Exp.	Added, g	(Zr,Ti)O_2 Taken, g	Acidity as *N*	g	Acidity as *N*	g	Found, g	Error, g
15	Al_2O_3 0·378	0·0554	0·266	0·0462	0·006	0·0088	0·0550	−0·0004
16	do. do.	0·0638	0·157	0·0603	0·004	0·0038	0·0641	+0·0003
17	Fe_2O_3 0·250	0·0554	0·266	0·0484	0·006	0·0070	0·0554	0·0000
18	do. do.	0·0638	0·157	0·0585	0·004	0·0050	0·0635	−0·0003
19	V_2O_4 0·250	0·0638	0·25	0·0638	0·004	0·0047	0·0685	+0·0047
20	do. do.	0·0638	0·48	0·0499	0·003	0·0138	0·0637	−0·0001

F. DIFFERENTIATION OF ZIRCONIUM AND TITANIUM—The major ppts. in Exps. 17 and 18 were chocolate-brown, due to a coloration ascribed to cyclogallipharic acid[7] present in commercial tannin; the ignited ppts. were pure white. In Exps. 15 and 16 the difference in colour of TP^1 and TP^2 made it quite evident that TP^1, pptd. at higher acidity, contained much less titanium than TP^2. Exps. 21 to 23 (Table III) show that there is pronounced differentiation in a common chloride soln.

TABLE III

	Taken, g		TP^1			
Exp.	ZrO_2	TiO_2	Acidity as *N*	g	Colour	TiO_2 in TP^1, g
21	0·0298	0·0225	0·25	0·0353	orange	0·0066
22	0·0298	0·0225	0·51	0·0187	pale buff	0·0012
23	0·0497	0·0057	0·51	0·0337	cream	0·0008

Finally, we conducted two series of tests, with pure zirconium and titanium chloride solns. respectively, on the lines of and including Exps. 1 and 2 (A), with the acid concn. as the only variable. The weights of the ignited *TP* (which to save space we have not reproduced here) gave the % recovery of each oxide at the acidity given in Table IV. It is intended to reproduce these results in graph form in a forthcoming paper, as this investigation is being extended to certain other metals.

TABLE IV

ZrO_2	Acidity, as *N*	0·10	0·20	0·30	0·40	0·50
	% Recovered	100	98·3	94·2	88·2	79·8
	Acidity, as *N*	0·60	0·70	0·80	0·90	1·0
	% Recovered	66·6	49·6	31·9	13·1	3·5
TiO_2	Acidity, as *N*	0·018	0·036	0·043	0·087	0·13
	% Recovered	100	98·3	97·2	95·2	88·7
	Acidity, as *N*	0·174	0·21	0·274	0·33	0·4
	% Recovered	78·0	69·7	15·8	6·7	nil

SUMMARY—Titanium is quantitatively pptd. by tannin from chloride solns. containing free hydrochloric acid below 0·02 *N* concentration; it can thus be separated from aluminium, iron and vanadium, but not from zirconium and thorium. Titanium and zirconium can be recovered together by tannin pptn. from chloride solns. containing aluminium, iron and vanadium under controlled acidity conditions. The separation procedure is described. In

chloride soln., tannin ppts. zirconium at a higher acidity than it does titanium, whilst in oxalate soln. the reverse occurs; the theoretical aspect of this inversion in the order of precipitability is discussed.

We wish to thank the Governors, Principal and Head of the Science Department of the S.W. Essex Technical College for permission to carry out this investigation.

REFERENCES

1. Schoeller, W. R., ANALYST, 1944, **69**, 259.
2. —— "*The Analytical Chemistry of Tantalum and Niobium*," London, 1937, p. 148.
3. Powell, A. R., and Schoeller, W. R., ANALYST, 1930, **55**, 608.
4. Britton, H. T. S., "*Hydrogen Ions*," London, 1942, Vol. II, p. 57; *J. Chem. Soc.*, 1925, **127**, 2134.
5. Mellor, J. W., "*A Comprehensive Treatise on Inorganic and Theoretical Chemistry*," Vol. VII, London, 1941, p. 154.
6. Schoeller, W. R., and Powell, A. R., "*The Analysis of Minerals and Ores of the Rarer Elements*," London, 1940, p. 91, 97.
7. Nierenstein, M., ANALYST, 1944, **69**, 260, footnote.

SOUTH-WEST ESSEX TECHNICAL COLLEGE
WALTHAMSTOW, LONDON, E.17

April, 1945

Determination of the Original Freezing-point of Sour Milk

BY F. J. MACDONALD

(*Read at the Meeting, April* 4, 1945)

THE determination of the freezing-point is now generally accepted as offering the best means of deciding, irrespective of the amount and composition of the solids-not-fat, whether a milk is genuine, and of estimating the extent of adulteration when water has been added. For the result to be of any practical value, however, it is essential that the milk shall have undergone no serious decomposition, since any such changes, involving for the most part the splitting of the lactose molecules into a larger number of smaller molecules, cause a substantial increase in the depression. When only slight souring has taken place it is possible to apply a suitable correction to the observed freezing-point, but beyond this stage some indirect method must be employed to arrive at the original freezing-point.

It is helpful at this juncture to consider what individual contributions to the total depression of the freezing-point are made by the various constituents of a normal milk. It is generally agreed that the lactose and the alkali chlorides are together responsible for *ca.* 70% of the depression, but the factors governing the remaining 30% do not appear to be definitely established. Table I shows Coste and Shelbourne's[1] conception of the partial depression caused by certain milk constituents, and Table II that of Porcher and Chevallier.[2]

TABLE I

Constituent	%	Δ° C.
Alkali chlorides	0·1	0·110
Lactose	4·7	0·250
Other salts and ions	—	0·200
Total ..		0·560

TABLE II

Constituent	%	Δ° C.	Constituent	%	Δ° C.
Sodium and potassium chlorides ..	0·180	0·111	Lactose	5·00	0·293
Potassium phosphates	0·210	0·067	Non-protein N ..	0·060	0·021
Potassium, calcium and magnesium citrates	0·315	0·027	Colloidal complex	—	0·025
Potassium sulphate	0·015	0·004	Total		0·552
Sodium bicarbonate	0·025	0·004			

Various attempts[3,4,5] have been made on the basis of the lactose/chloride relationship to derive a formula which would enable the presence of added water to be detected, but all suffer from the defect that it seems impossible to fix a standard sufficiently accurately for genuine milk. Thus, although the original proportions of lactose and chloride may be estimated in the sour milk, this method will not serve as a means of obtaining the desired

information. H. J. Evans[6] has evolved a method, based largely on Coste and Shelbourne's[1] figures, for arriving at the original freezing-point of a sour milk. Satisfactory results were obtained, but the process is very lengthy.

RELATIONSHIP OF LACTOSE AND SOLUBLE ASH DEPRESSIONS—In view of the inadequacy of the lactose/chloride ratio as a reliable index of added water, it was decided to investigate the mutual relationship of the depressions due to the lactose and soluble ash and to see whether this was sufficiently constant to serve as a basis for the calculation of the freezing-point of milk. By so doing the effect of factors other than the alkali chlorides would be included, although in itself the soluble ash could not be considered as offering a complete measure of the mineral matter originally in true solution in the milk. It was thought that the direct determination of the freezing-point of a solution of the water-soluble ash would yield more information in a far shorter time than any chemical determination of the individual constituents.

METHOD—(1) *Depression due to Lactose*—A stock solution of lactose (Judex A.R.) was standardised polarimetrically. From this soln. a series was prepared to cover the range from 1% to 5·5% w/w, the strength being checked gravimetrically with Fehling's solution. The freezing-points of these solns. were determined on the Hortvet cryoscope, and the depressions were plotted against the concn. From the graph was derived the following relation between concentration and depression:

$$\Delta^\circ \text{C.} = 0{\cdot}063 \times \%\ \text{w/w of anhydrous lactose.}$$

(2) *Depressions due to Soluble Ash*—Fifty g of milk were ashed, with the usual precautions against loss of chloride, and the ash was boiled with 50 ml of water for 15 min. The mixture was cooled and its weight was adjusted to 50 g, less the weight of fat in this quantity of the milk. After mixing, the insoluble matter was allowed to settle and the soln. was decanted and its freezing-point determined on the Hortvet cryoscope. In every instance the freezing-point was determined within *ca.* 1 hr. of the soln. being made, since irregular results were obtained when the solns. were allowed to stand overnight or pre-cooled.

(3) *The Ratio of the Combined Depressions of Lactose and Soluble Ash to the Total Depression of the Milk*—Twenty samples of fresh milk, some watered, were taken and determinations were made of fat, S.N.F., lactose and freezing-point. The remaining portion of each sample was sealed and allowed to stand in the laboratory for 3 or 4 weeks. At the end of this time 50-g portions of the decomposed milk were weighed and ashed, and the freezing-points of their soluble ash solutions were determined as above.

The results obtained are shown in Table III, F being the ratio Δ milk/(Δ lactose + Δ soluble ash).

TABLE III

No.	Fat %	S.N.F. %	Δ° C. Milk	Lactose % w/w	Δ° C. Lactose	Δ° C. Sol.-ash	F	Δ° C. milk (calculated)	Difference °C.
1	2·70	8·65	0·536	4·60	0·290	0·135	1·26	0·548	+0·012
2	3·70	8·65	0·540	4·40	0·277	0·141	1·29	0·540	nil
3	4·00	9·29	0·548	4·83	0·304	0·117	1·30	0·543	−0·005
4	4·60	9·28	0·549	4·86	0·306	0·110	1·32	0·537	−0·012
5	3·80	8·50	0·540	4·58	0·289	0·139	1·26	0·552	+0·012
6	3·40	8·99	0·547	4·66	0·294	0·120	1·32	0·532	−0·015
7	4·30	9·15	0·547	4·69	0·295	0·116	1·33	0·530	−0·017
8	4·60	8·61	0·545	4·20	0·265	0·146	1·33	0·530	−0·015
9	3·70	8·81	0·546	4·39	0·277	0·148	1·28	0·548	+0·002
10	5·80	8·48	0·545	4·52	0·285	0·129	1·32	0·534	−0·011
11	0·50	9·50	0·558	4·88	0·307	0·126	1·29	0·558	nil
12	0·55	9·68	0·555	4·86	0·306	0·131	1·27	0·564	+0·009
13	4·50	8·21	0·568	3·38	0·213	0·224	1·30	0·564	−0·004
14	1·20	9·20	0·543	4·76	0·300	0·125	1·28	0·548	+0·005
15	2·60	8·33	0·544	4·01	0·253	0·180	1·26	0·559	+0·015
16	3·30	8·10	0·504	4·10	0·258	0·132	1·29	0·504	nil
17	3·20	8·13	0·495	4·09	0·258	0·129	1·28	0·499	+0·004
18	3·00	7·86	0·482	4·04	0·255	0·120	1·29	0·482	nil
19	3·85	8·11	0·542	3·94	0·248	0·175	1·28	0·546	+0·004
20	4·20	8·10	0·545	3·87	0·244	0·185	1·27	0·553	+0·008

From these results it was found that the combined depressions of lactose and soluble ash were responsible on the average for *ca.* 77·5% of the total depression of the milk, and that this ratio was of fair constancy whether the milk was genuine, watered or of abnormal composition. The average value for the ratio F is 1·29. The following formula, derived from

these results, enables the freezing-point of a milk to be calculated from the results for lactose and soluble ash depression.

$$\Delta^\circ \text{ C. milk} = 1{\cdot}29\ (\%\ \text{w/w of lactose} \times 0{\cdot}063 + \Delta^\circ \text{ C. soluble ash})$$

In the last two columns of Table III are shown the calculated freezing-points of the twenty samples, based on the above formula, together with the differences from the observed freezing-points. It will be noted that the margin of error ranges from 0·017° C. below the observed values to 0·015° C. above them, and that there is a clear distinction between genuine milks deficient in S.N.F. and those in which the deficiency is due to added water.

(4) *Application to sour milks of unknown original freezing-point*—Eighteen samples of fresh milk were taken, of which the original freezing-points remained unknown to me until the conclusion of the experiment. These samples were allowed to stand in the laboratory for 3 or 4 weeks before examination. The fat and S.N.F. were then determined by the method[7] previously published, which also gave the corrections for the volatile decomposition products of lactose. The total acidity and the residual lactose provided the remaining data for the original lactose, and the freezing-point of the soluble ash soln. was determined as above. The results obtained are shown in Table IV.

TABLE IV

No.	Fat %	S.N.F. %	Calculated original lactose % w/w	Δ° C. lactose	Δ° C. sol-ash	Δ° C. milk calculated	Δ° C. milk observed	Error °C.
1	3·70	8·81	4·72	0·297	0·128	0·548	0·543	+0·005
2	3·63	8·81	4·72	0·297	0·134	0·556	0·543	+0·013
3	3·70	8·93	4·60	0·290	0·131	0·541	0·541	nil
4	3·30	8·75	4·69	0·295	0·130	0·548	0·546	−0·002
5	3·15	8·23	4·50	0·284	0·125	0·528	0·523	+0·005
6	2·95	7·88	4·22	0·266	0·117	0·494	0·498	−0·004
7	3·17	7·93	3·96	0·249	0·110	0·463	0·478	−0·015
8	3·40	8·82	4·45	0·280	0·131	0·530	0·541	0·011
9	3·05	7·72	3·85	0·243	0·104	0·448	0·460	−0·012
10	2·80	8·35	4·15	0·261	0·162	0·546	0·550	−0·004
11	2·92	7·47	3·79	0·239	0·118	0·461	0·451	+0·010
12	3·05	7·77	3·94	0·248	0·119	0·472	0·472	nil
13	3·45	8·83	4·56	0·287	0·125	0·532	0·541	−0·009
14	2·60	7·03	3·55	0·224	0·108	0·428	0·435	−0·007
15	2·98	7·37	3·81	0·240	0·107	0·448	0·450	−0·002
16	3·70	8·83	4·45	0·280	0·132	0·531	0·539	−0·008
17	2·90	8·07	2·16	0·136	0·305	0·569	0·560	+0·009
18	4·26	8·17	3·78	0·238	0·187	0·548	0·544	+0·004

The error on these eighteen samples ranged from 0·015° C. below the original observed value for the depression to 0·013° C. above it, genuine and watered milks being easily distinguished. The above method thus provides a means of deciding whether a sour milk was genuine or watered, and of determining the extent of any adulteration, with an error not exceeding ±3%.

SUMMARY—A method is described by means of which the original freezing-point of sour milk may be estimated by determination of its initial lactose content and the freezing-point of a solution of its soluble ash.

I wish to thank the Governing Director of the Express Dairy Co., Ltd., for permission to publish this work, and Dr. J. G. Davis of the National Institute for Research in Dairying, Reading, for supplying Samples No. 11, 12, 13 and 14.

REFERENCES

1. Coste, J. H., and Shelbourne, E. T., ANALYST, 1919, **44**, 158.
2. Porcher, C., and Chevallier, G., *Le Lait*, 1923, **3**, 369.
3. Mathieu, L., and Ferré, L., *Ann. Falsif.*, 1914, **7**, 12.
4. Kopatschek, F., *Milchw. Zentralbl.*, 1922.
5. Post, P., *Z. Unters. Lebensm.*, 1926, **52**, 371.
6. Evans, H. J., ANALYST, 1936, **61**, 666.
7. Macdonald, F. J., *Id.*, 1944, **69**, 173.

CENTRAL LABORATORIES
EXPRESS DAIRY CO., LTD.,
CLAREMONT ROAD, CRICKLEWOOD, N.W.2

December, 1944

Discussion

Dr. J. R. Nicholls congratulated the author for his work on an important subject. It would be highly advantageous to be able to state from the analysis of a soured milk what was the freezing-point before souring; but, as this might concern a charge of added water, it was necessary to examine critically any method which claimed this. The constituents contributing to the freezing-point could be divided into (*a*) lactose, (*b*) soluble salts and (*c*) non-protein nitrogenous compounds and colloidal complexes. As regards (*a*), there were well-known corrections which could be applied for lactose lost during souring. Since experience had shown that the application of these corrections gave a very good result for the non-fatty solids originally present, it could be accepted that the original lactose was determinable. As regards (*b*), the soluble ash was known to be less than the total of the salts which were soluble in the original milk For instance, the soluble ash contained very little phosphates, whereas milk always contained phosphates in solution. Presumably the calcium which had been in combination with casein and with citric acid in the milk rendered the phosphates in the ash insoluble. Again, fresh milk had a *p*H of about 6·5, whereas the ash was strongly alkaline. Could it be assumed that the depression of freezing-point of the soluble ash always bore a constant relation to the depression due to the originally soluble salts? In other words, could it be assumed that a change in chlorides to compensate for a variation in lactose was always accompanied by a corresponding change in the phosphates? In this connection it might be mentioned that chlorides were invariably lost on ashing milk; but this was not significant from the present point of view, since the change of a small amount of salt to sodium carbonate did not materially affect the freezing-point. As regards (*c*) there was no information how these constituents varied with change in composition of the milk; but the formula proposed to be used implied that they were proportional to the lactose. It might, however, be found that the depression of freezing-point due to these substances was for practical purposes a constant.

As far as he had gone the author had shown that his formula gave results within about 3% of the correct figure. This was a reasonably close concordance, but it required confirmation on a much larger number of samples. The author had the unique opportunity of being able to select samples of widely differing compositions and if, at a later date, he could present results on a wide range, the Society would be greatly indebted to him.

Mr. G. Taylor enquired whether the author's formula was suitable for the examination of milk from a cow suffering from sub-clinical mastitis, where the effect of such condition on the composition of the milk was a substantial decrease in the amount of lactose accompanied by an equally substantial increase in the amount of chlorides, while at the same time the freezing-point depression was normal.

Dr. J. G. A. Griffiths said that if an analyst was requested to report on the possibility of added water in a sample of milk, and the sample had undergone decomposition as a result of being kept for many weeks, it was evidently of value to be able to deduce the freezing-point of the original milk from data derived from the sour sample. The author was able to infer, within ±3%, the freezing-point of the original milk by a method which appeared to be less tedious than, but as accurate as, that of H. J. Evans (Analyst, 1936, **61**, 666). For some purposes this 3% discrepancy was large; for example, a sour milk with an inferred original freezing-point of −0·525° (Hortvet) might, in fact, have had any freezing-point between −0·540° and 0·510° and therefore it would not be possible on the basis of inferred freezing-point alone to determine whether the milk was genuine or contained as much as 3% of added water. The author should feel encouraged to continue his investigations with the object of diminishing this margin of uncertainty.

Mr. Macdonald, replying to Dr. Nicholls, heartily agreed that before using the method as an official test it would be essential to examine a much larger number of samples, and that he would be only too pleased to do so when he had the time. While the soluble ash derived from a fresh milk was composed almost entirely of alkali chlorides, the soluble ash from sour milk contained some proportion of alkali phosphates. Replying to Dr. Nicholls, Mr. Taylor, and Dr. Monier-Williams, he said that the formula used was applicable to milks low in lactose and high in chlorides as shown, for example, by Sample Nos. 19 and 20 in Table III, and Nos. 17 and 18 in Table IV. The author agreed with Dr. Griffiths that a calculated figure of −0·530° C. might be difficult to interpret in a legal sense, since the limits of accuracy were ±0·015°C. but, at the moment, there appeared to be no method available which would yield results of a greater order of accuracy.

Reaction of Diazotised *p*-Nitraniline with Phenols: Detection of Tricresyl Phosphate in Edible Oil

By E. COLLINS

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

In 1938 an epidemic occurred in Mauritius which was believed to be due to the contamination of imported edible oil by tricresyl phosphate derived from second-hand drums.[1] Unfortunately, none of the oil in question was ever available for analysis, but a routine examination of oil imported in drums (mainly arachis, soya and mustard) was begun by the Medical and Health Department.

At first the rapid test previously[1] described was used. Five drops of the oil are saponified with alcoholic potash and to the diluted mixture is added a soln. of diazotised *p*-nitraniline in dil. acetic acid. The mixture must remain alkaline. With uncontaminated oils, variable yellow or brown "blank" colours are obtained, whilst the *o*- and *m*-cresols formed by the hydrolysis of tricresyl phosphate yield red azo-colours.

Although the test is fairly sensitive (0·01% in favourable conditions), it was not found completely satisfactory in use, since (*a*) the blank colours are sufficiently intense to mask small amounts of cresols, and (*b*) it can readily be shown that the conversion of cresols to azo-colours is under these conditions by no means complete.

In the present paper a modified test is described which has proved considerably more reliable. Although taking longer than the original rapid test, its much greater sensitivity (*ca.* 5 p.p.m.) permits the examination of mixed samples in routine work. The final conversion of the cresols to azo-colours is carried out by means of an alkaline diazo-reagent (stable diazotate), whereby the coupling reaction takes place at controllable speed; by adjustment of *p*H and time of reaction this permits a discrimination between the various colour-producing substances present, which is not generally possible when the coupling is carried out instantaneously in the usual way with an acid diazo-reagent (labile diazotate). These reactions have been very little studied from the analytical point of view; several methods which have been put forward for the determination of phenols in water, for example, by means of diazotised *p*-nitraniline specify rapid coupling by means of the acid reagent under arbitrary conditions of *p*H, etc.; if these conditions are such as will secure complete coupling of the more slowly reacting phenols (*e.g.*, phenol itself), then, for example, the coupling of α-naphthol, which reacts approx. 10 times as rapidly as phenol, is likely to proceed beyond the ordinary stage with the production of off-colours.

Reactions between Diazotised *p*-Nitraniline and Phenols—It is not proposed to discuss the disputed question of the structure of the diazo-compounds, but for descriptive purposes the terminology of Hantzsch is used.

Paranitraniline, $NO_2.C_6H_4.NH_2$ (yellow), when diazotised in acid soln., yields salts of the diazonium ion $NO_2.C_6H_4.N_2^+$ (colourless). When this soln. is run into an excess of alkali, the diazonium salt is converted in rapid succession into the labile, and then into the stable diazotate. These two compounds are presumably ionised in soln. and are considered to be derived from the syn- and anti- forms of the diazohydroxide $NO_2.C_6H_4N : NOH$. The labile form, which has thus only a transient existence, reacts extremely rapidly with phenols in moderately alkaline soln., whilst the stable form, which can exist unchanged in alkaline soln. for prolonged periods, reacts very much more slowly. The rate of coupling of both with phenols falls off with increasing alkalinity. It is shown below that for the stable compound the rate of coupling with phenol molecules is proportional to the hydrogen ion concn., and probably this applies also to the labile compound. In both compounds this effect is enhanced at *p*H's above *ca.* 10 by the removal of phenol molecules as ions. It is commonly stated in text-books that the stable diazotates do not couple with phenols, but this is certainly not true for the acid substituted compounds derived from *p*-nitraniline and sulphanilic acid.

The above reactions may be illustrated by the following expt. Into three 50-ml Nessler cylinders are placed respectively 5 ml of (1) an approximately equimolecular $Na_2CO_3/NaHCO_3$ buffer, (2) dil. Na_2CO_3 soln., (3) *N* NaOH. To each is added 50 μg of *o*-cresol and the soln. is diluted to the mark. The tubes now contain 1 p.p.m. solns. of *o*-cresol with approx. *p*Hs of 10, 11·5 and 13 respectively. Two ml of acid diazo-reagent (a 0·03% soln. of *p*-nitraniline in *N*/10 hydochloric acid diazotised for use by decolorising with a crystal of sodium nitrite; cooling is not necessary) are blown into each of the tubes from a pipette with a fine tip, and the soln. are mixed. In the first tube the *o*-cresol couples instantaneously and completely, with production of a red azo-colour. In the second tube the coupling is instantaneous, but only about 1/4 complete; while in the third tube there is no production of azo-colour, but a pale yellow colour develops in a few sec. as the reagent reverts to the stable diazotate. This illustrates the inhibitory effect of alkali on the fast coupling reaction with the labile diazotate, and explains why the rapid test for tricresyl phosphate in oil fails for very small quantities; the soln. contains an excess of free alkali from the saponified mixture. The coupling in the test on oils may be rendered more complete by adjustment of *p*H, but this serves little practical purpose, since the blank colours are similarly enhanced.

If the three tubes are allowed to stand, the colour in the second tube slowly increases by reaction of unchanged cresol with the stable diazotate, and eventually practically complete conversion into azo-colour is observed. The half-period of the reaction at this *p*H is of the order of hours. In the third tube the reaction is unobservably slow and the contents remain pale yellow, while in the first tube there is a slow deterioration of the red colour due to attack on the azo-colour by the excess reagent and to decomposition of the latter; the stability of the stable diazotate falls off with decreasing alkalinity.

REACTION BETWEEN PHENOLS AND STABLE *p*-NITROBENZENE DIAZOTATE—This reaction, which is used in the test described below, may be demonstrated directly by a modification of the expts. just described. Into a 50-ml Nessler cylinder is placed a little $Na_2CO_3/NaHCO_3$ buffer; this is diluted with water, and 2 ml of the acid diazo-reagent are run in. After 1 min. for reversion of the reagent to stable form, 50 μg of *o*-cresol are added, and the mixture is diluted to the mark. There is no instantaneous production of colour when the cresol is added, but the slow reaction at once sets in; the rate of increase of the red colour shows that the half-period is of the order of 10 min. The kinetics of this reaction have been studied colorimetrically, using different buffer mixtures and varying amounts of phenols and reagent; for phenol itself, *o*- and *m*-cresols, and α- and β-naphthols, at *p*H's below those at which they are appreciably ionised, the rate of change of concn. of phenol, *i.e.*, rate of increase of azo-colour, was found to be closely proportional to the product of the concns. of unchanged phenol, excess reagent and hydrogen ion. When, as in the test to be described, a standard quantity of composite buffer-diazotate reagent is used in a standard total volume, the concns. of diazotate and hydrogen ion are effectively constant throughout the course of the reaction, provided that the amount of phenol is small, and so the rate of reaction at any instant is proportional to the concn. of phenol remaining, and the reaction thus becomes kinetically unimolecular. In consequence, the time taken for the conversion of one half, or any other arbitrary fraction, of the phenol into azo-colour is independent of the amount present, and is a characteristic property of the particular phenol concerned. As shown in the table below, these times vary so widely as to be of considerable value in analysis.

FORMATION OF LAKES WITH MAGNESIUM HYDROXIDE—If to the solns. obtained in the coupling expts. mentioned above a magnesium salt is added followed by a small excess of sodium hydroxide soln. the red azo-colour (*p*-nitrobenzene-azo-*o*-cresol) is strongly adsorbed on to the pptd. $Mg(OH)_2$ as a blue lake. This reaction appears to be a general one for azo-colours derived from diazotised *p*-nitraniline and is utilised in several well-known organic reagents for magnesium; the colour is almost always some shade of blue, though for the derivative of phenol itself the lake is a fine violet.

By centrifuging in a conical tube practically the whole of the azo-colour is concentrated as lake on the deposit of $Mg(OH)_2$ and thus separated from the excess of yellow reagent, which is not adsorbed. By reducing the amount of magnesium the staining of the deposit may be made an extremely sensitive test for phenols. Most of the azo-colours derived from phenols show indicator properties; the colour changes may be observed by pouring off the supernatant layer of liquid, dissolving the $Mg(OH)_2$ ppt. in dil. acetic acid, and then making alkaline with ammonia.

DETECTION OF TRICRESYL PHOSPHATE IN EDIBLE OIL—Into a 50–55-ml sugar inversion flask put 2·5 ml of oil and 3 ml of ethyl alcohol, mix by shaking, and add 2 ml of saturated aqueous sodium hydroxide soln. Saponfication begins in the cold; heat on a water-bath until condensing alcohol vapour begins to ascend the neck of the flask and continue to heat cautiously while adding water at intervals, drop by drop, until the solid soaps have just dissolved and the saponified mixture is perfectly clear. Add a further 1 ml of water and heat over the water-bath for a further 5 min. at such a rate that alcohol vapour just does not escape from the top of the flask, which should be covered. Add 10 ml of water, cool, and add 20 ml of sodium chloride soln. (25 g/100 ml). Without mixing, make the volume up to 54 ml with water, pour the contents into a 100-ml conical flask, close this with a rubber stopper, and shake *vigorously* for *ca.* 2 mins. The pptd. soaps are reduced to a finely divided granular suspension in a clear aqueous phase. Pour back a little of the mixture into the original flask and shake to remove any soap adhering to the walls. Filter the whole into a 50-ml cylinder; the filtration is rapid and the filtrate is clear and bright. With a paper of moderate size about 45 ml can be collected. Place 40 ml of filtrate ($\equiv$ 2 ml of oil, allowing 4 ml of pptd. soaps) in the flask of a Reichert–Meissl apparatus, add methyl orange, and make acid with a measured vol. (x ml) of dil. sulphuric acid. Add $(20-x)$ ml of water and a few glass beads and distil 40 ml (*i.e.*, 2/3) into a Nessler cylinder marked at this level. On account of the enhancement of their partial pressures by the salts present, distillation of the lower phenols is practically complete.

Preparation of alkaline diazo-reagent—Blow 2 ml of the acid diazo-reagent (see above) from a pipette with a fine jet into 4 ml of $M/2$ sodium carbonate in a test-tube. It is essential that the reagent should be delivered directly into the excess of alkali to avoid intermediate *p*H values, which cause auto-decomposition. Add, with mixing, 4 ml of $M/2$ sodium

bi-carbonate soln. This reagent is moderately stable (*e.g.*, for ½ to 1 hour at tropical laboratory temperatures). Add the whole to the distillate in the Nessler cylinder, making 50 ml in all, and observe the colour changes.

With uncontaminated oils weak yellow blank colours usually develop in the course of the first minute or so; occasionally these colours are pale orange or brown, but they are invariably far weaker than blank colours obtained on the undistilled saponified mixture. By continued action of the reagent they usually fade somewhat. With oils to which 0·01% of commercial tricresyl phosphate has been added, however, the coupling reaction with *o*- and *m*-cresols immediately sets in, with the production of the red azo-colours; since for both cresols the half-period is about 9 min., the reaction is ½, ¾, ⅞, etc., complete after about 9, 18, 27, etc., min.; in 20 min. the soln. is deep red.

TRIALS OF THE ABOVE METHOD—When 0·001% of commercial tricresyl phosphate was added to samples of the following oils: soya, mustard, olive, almond, arachis, cottonseed, sesame, and cocoa-butter, the distillates in each instance gave a pronounced orange-red colour at the end of 20 min.; blank colours on the original oils were never more than weak yellow or brown. The useful limit of sensitivity of the test is thus about 0·0005%, *i.e.*, it is approx. 20 times more sensitive than the rapid test. The limit of sensitivity is principally determined by the intensity of the blank colours; distillation seems to be the only way of reducing these to really low values. The test in its present form does not work effectively for oils whose soaps are not pptd. in a granular state by sodium chloride (*e.g.*, coconut, castor). Phenol, and α- and β-naphthols (0·001% in cottonseed oil) each gave pronounced positive reactions when the distillates were allowed to couple for the appropriate time (about twice the half-period given in the table below) with the alkaline diazo-reagent. The magnesium hydroxide test may be applied to coupled distillates containing azo-colour by adding, to 10 ml, 0·2 ml of magnesium sulphate ($7H_2O$) soln. (25 g/100 ml) followed by a small excess of sodium hydroxide and centrifuging. With uncontaminated oils the ppt. is stained a weak bluish-grey; with 0·001% of tricresyl phosphate the colour is a pronounced blue. The test is chiefly useful for distinguishing phenol by means of its violet lake, and for separating traces of azo-dye from the reagent in order to observe their indicator properties.

QUANTITATIVE APPLICATION—The recovery of phenol, *o*- and *m*-cresols, and therefore probably *p*-cresol, in the distillate is practically complete, since colorimetric determinations of the first three of these compounds in distillates from oils to which known quantities had been added gave quantitative results when corrections were made for blank colours; with α- and β-naphthols, however, there was incomplete recovery, evidently on account of their much lower volatility. These determinations were carried out by the fast coupling reaction, *i.e.*, addition of acid diazo-reagent to distillates buffered with $Na_2CO_3/NaHCO_3$. Since *p*-cresol reacts somewhat irregularly under the conditions described, and since it may be present in variable proportions in commercial tricresyl phosphate, it is evidently not possible to state the exact proportion of this compound in an oil by colorimetric tests on the distillates. However, if direct examination of the oil showed that cresols were present as this particular ester, then by making the arbitrary assumption that the ester was derived only from the *o*- and *m*-isomers, whose azo-colours have practically the same shade and intensity, a colorimetric determination on the lines suggested by the test would enable a minimum figure to be given for administrative purposes. This paper, however, is more concerned with the actual detection of minute quantities of tricresyl phosphate in oil, and in fact no such contamination, among many hundreds of samples imported in drums, has been observed since 1938.

In Table I below are collected relevant data for six simple phenols. The half-periods are for the arbitrary conditions of the test at a temp. of *ca.* 27° C.

TABLE I

Compound	Half-period (min.)	Azo-colour		
		Alkali	Acid	$Mg(OH)_2$ lake
Phenol	31	orange	yellow	violet
o-Cresol	9	red	,,	blue
m-Cresol	9	,,	,,	,,
p-Cresol	slow reaction	purple-red	,,	,,
α-Naphthol	approx. 2·5	bluish-purple	brown	sky-blue
β-Naphthol	approx. 6	orange-red, insol.	no change	not formed

SUMMARY—(1) The rapid test for tricresyl phosphate in edible oil[1] fails for low concns. on account of strong blank colours and incomplete conversion of the cresols to azo-colours.

(2) The nature of the coupling reactions between the phenols and diazotised *p*-nitraniline is discussed, and a modified test is described of considerably greater sensitivity and reliability; the test involves the removal of soaps from the saponified mixture by salting out, distillation of the acidified filtrate, and coupling the distillate with stable *p*-nitrobenzene diazotate in alkaline buffer soln.

(3) The test is a general one for the lower phenols and their esters; the azo-colour formed can be separated and identified by pptn. as lakes on magnesium hydroxide.

I am obliged to the Director, Medical and Health Department, Mauritius, for permission to publish this paper.

REFERENCE

1. ANALYST, 1938, **63**, 813.

GOVERNMENT CHEMIST'S LABORATORY
REDUIT, MAURITIUS

January, 1945

The Absorption of Diphenyl and *o*-Phenyl-phenol by Oranges from Treated Wraps

BY R. G. TOMKINS AND F. A. ISHERWOOD

ROTTING of citrus fruits due to green mould can be reduced by wrapping the fruit in paper treated with the volatile fungicides diphenyl[1,2,3] and *o*-phenyl-phenol.[4,5,6]

Methods of estimating the small amounts of these fungicides absorbed by oranges from treated wraps have now been devised and some of the factors affecting the extent of absorption are discussed below.

Oranges were wrapped in papers impregnated with diphenyl, or *o*-phenyl-phenol and hexamine, and then stored in partly-open tins at constant temperature. The amount of diphenyl (or *o*-phenyl-phenol) taken up was estimated at intervals. In most of the expts. each treated wrap contained about 100 mg of diphenyl or *o*-phenyl-phenol, which is more than is required to reduce wastage. Consequently the quantities of the fungicides absorbed by the fruit were probably greater in our experiments than would be expected under commercial conditions.

ESTIMATION OF DIPHENYL IN ORANGES—A sample (4 or 5) of the oranges which had been stored in diphenyl-treated wraps was weighed and the peel was separated from the pulp. The tissue (peel or pulp) finely minced, was then steam-distilled in the usual manner until about 2 litres of distillate had been collected (2 hr.). The distillate was extracted three times with chloroform (90 ml), which removed both the diphenyl and any orange oil present. Of the chloroform soln. 45 ml, containing about 2 ml of oil, were shaken in a separating funnel with sulphuric acid (sp.gr. 1·84, 45 ml). Moderately vigorous shaking of the mixture was necessary for the efficient destruction and removal of the orange oil, but the formation of a too persistent emulsion should be avoided. After standing for 20 min. the mixture separated into two layers, the upper light yellow and the lower dark red. The latter (sulphuric acid) was removed, and the upper layer (chloroform) again shaken with an equal vol. of sulphuric acid (sp.gr. 1·84). This procedure was repeated four times, chloroform being added to the upper layer when necessary to maintain its vol. at 45 ml. After the last treatment the chloroform soln. was shaken with water (120 ml in two portions), dried over anhydrous calcium chloride and filtered. The colour of the resulting soln. was a very pale yellow, indicating the almost complete destruction of the orange oil. The diphenyl in this solution was then estimated according to Mulliken 1904,[7] "Drop a hard lump of sublimed aluminium chloride weighing 0·2–0·3 g into a clean 6–8 in. test-tube that has just been taken from a hot drying-oven. Stopper the tube loosely. Hold it in a nearly horizontal position, and by means of a small flame placed under one end slowly sublime the chloride until it forms a thin light yellow coating covering a considerable portion of the glass surface. Allow to cool. Drop in 0·5 ml of a soln. containing 0·05 g of the hydrocarbon dissolved in 2·5 ml of chloroform. Stopper the tube tightly. Lay it on to its side upon a sheet of white paper that rests upon and partly covers the colour standard. Then roll it back and forth so that the solution shall flow over and wet all parts of the sublimate. Observe the colour after a few seconds, and again after 15–20 minutes." The colour given by diphenyl was blue. (Orange oil, if present, completely masked the colour given by the diphenyl.) The chloroform soln. of the diphenyl obtained from the oranges was diluted until 0·5 ml gave a similar colour intensity under standard conditions to that produced by 0·5 ml of chloroform containing 0·05 mg of diphenyl. Distilled dry chloroform was used, and every precaution taken to exclude traces of moisture.

It was found convenient to take a large fragment of aluminium chloride and break it into smaller pieces under chloroform to ensure fresh substance for the test.

The method must be considered only roughly quantitative, the results being subject to an error of about 20%, as shown in Table I.

TABLE I

Procedure	Amount of diphenyl added mg	Amount of diphenyl recovered mg
1. Added to $CHCl_3$, treated with H_2SO_4, etc.	5	4·5
	10	10
	20	16
2. Added to 2 ml* of oil, the oil dissolved in $CHCl_3$ and then treated with H_2SO_4, etc.	5	4·5
	10	8·4
	20	14
3. Added to H_2O, steam-distilled, and distillate extracted with $CHCl_3$, etc.	10	8·9
4. Added to orange oil* (4 ml) steam-distilled and distillate extracted with $CHCl_3$, etc.	10	9·5
5. Added to minced peel from 5 oranges, steam-distilled, and distillate extracted with $CHCl_3$, etc.	5	4·8
	10	8·9
	20	18·2
6. Orange* oil (2 ml) steam-distilled, etc.	nil	nil

* The orange oil had been previously prepared from orange peel by steam-distillation.

WRAPS—The treated wraps (2 or 3, each approx. 10 in. × 10 in.) were extracted with chloroform (3 × 30 ml) and the amount of diphenyl present was determined as described above. As no orange oil was present, the treatment with conc. sulphuric acid was omitted.

ESTIMATION OF *o*-PHENYL-PHENOL IN ORANGES—A sample (4 or 5) of the oranges which had been stored in *o*-phenyl-phenol treated wraps was weighed and the peel was separated from the pulp. The finely-minced tissue (pulp or peel) was then steam-distilled in the usual manner until the volume of the distillate collected was about two litres (2 hr.). The distillate was acidified (5 ml of 2 *N* hydrochloric acid) and then shaken vigorously with light petroleum (10 ml, B.P. 40–60° C.) in a separating funnel. After standing a short time the mixture separated into two layers; the light petroleum layer was removed and the aqueous layer was again shaken with light petroleum (10 ml). It was found that at least ten extractions with light petroleum were necessary to remove all the *o*-phenyl-phenol from the aqueous phase. The combined light petroleum extracts were then shaken vigorously with sodium hydroxide soln. (*N*/10, 33 ml) in a separating funnel. After the mixture had stood for a short time for it to separate, the sodium hydroxide was removed, and the light petroleum soln. was again shaken with fresh sodium hydroxide (*N*/10, 33 ml). This procedure was repeated once more. The *o*-phenyl-phenol now in solution in the sodium hydroxide soln., was estimated colorimetrically by coupling with diazotised sulphanilic acid as described by Hanke and Kressler[8] for phenols and related compounds The colour was compared with that produced by a standard solution of *o*-phenyl-phenol (0·01 mg/ml) under similar conditions.

Table II shows the degree of accuracy which may be expected by this method:

TABLE II

Procedure	Amount of *o*-phenyl-phenol added mg	Amount of *o*-phenyl-phenol found mg
1. Added to light petroleum (100 ml.) and extracted with sodium hydroxide soln.	5	5
	10	10
	20	20
2. Added to water (2 litres), and extracted with light petroleum. The light petroleum extracted with sodium hydroxide soln.	10	8·6
3. Added to water, steam distilled, and the distillate extracted with light petroleum, etc.	5	4·0
	10	8·3
	20	16·8
4. Added to orange oil (4 ml) dissolved in light petroleum and extracted with sodium hydroxide soln.	5	5
	10	10
	20	20
5. Added to the peel (minced) from 5 oranges (allowed to stand overnight) and then steam-distilled, etc., as described in the full procedure.	5	3·75
	10	9·1
	20	16·9
6. Untreated orange peel (5 oranges).	nil	nil

WRAPS—The treated wraps (2 or 3, approx. 10 in. × 10 in.) were extracted with sodium hydroxide soln. (*N*/10, 100 ml in two portions). The aqueous soln., which contained the sodium salt of *o*-phenyl-phenol, was acidified and extracted with light petroleum as described above.

AMOUNTS OF DIPHENYL OR *o*-PHENYL-PHENOL RECOVERED FROM ORANGES WRAPPED IN PAPER IMPREGNATED WITH DIPHENYL OR *o*-PHENYL-PHENYL AND HEXAMINE—The effects of temperature of storage and of the amount of fungicide in the wrap are shown in Tables III, IV, VI and VII. Practically no diphenyl or *o*-phenyl-phenol (less than 0·1 mg) was recovered from the pulp, the amounts recorded below having been recovered from the peel.

These and other expts. showed that both fungicides are absorbed by oranges from treated wraps in quantities which were related to the concentration in the wraps, and duration of storage. In most expts. absorption increased with temperature. The amounts absorbed also varied with oranges from different countries and was about 4 to 20 mg of diphenyl or 20 to 30 mg of *o*-phenyl-phenol per 100 g. Aeration of the fruit after removal from treated wraps resulted in a very slow loss of the absorbed fungicides (Tables V and VIII).

TABLE III

UPTAKE (mg per orange) OF DIPHENYL BY JAFFA ORANGES
(Each wrap containing 100 mg of diphenyl)

Temperature of storage °C.	Days					
	14	27	34	48	62	71
18	3·2	10·5	4·8	6·4	7·2	8·0
10	4·0		6·4	8·0	8·9	9·4
5	2·4		3·9	4·5	6·5	7·3

TABLE IV

UPTAKE OF DIPHENYL (mg per orange) AT 10° C. FROM WRAPS CONTAINING DIFFERENT AMOUNTS OF DIPHENYL

Amount of diphenyl in wraps	Days				
	14	36	48	62	71
100 mg per wrap ..	4·0	6·4	8·0	8·9	9·4
50 ,, ,, ,, ..	2·4	4·0	5·2	5·6	6·7
20 ,, ,, ,, ..	1·9	2·8	2·8	2·9	2·8

TABLE V

LOSS OF DIPHENYL FROM FRUIT PREVIOUSLY WRAPPED IN DIPHENYL TREATED WRAPS
(The fruit was stored in a desiccator through which a slow stream of air was passing)

	Days in air				
	0	11	17	23	36
Diphenyl, mg per orange	7·6	7·2	4·6	4·6	4·8

The odour of diphenyl soon disappeared from these oranges.

TABLE VI

UPTAKE (mg per orange) OF *o*-PHENYL-PHENOL BY ORANGES AT DIFFERENT TEMPERATURES
Each wrapcontained 95 mg *o*-phenyl-phenol plus 25 mg hexamine

Temperature of storage °C.	Days				
	17	42	57	68	77
18	4·8	9·1	11·4	10·0	10·0
10	2·4	4·6	5·0	8·9	8·0
5	3·0	4·0	4·0	4·4	5·0

TABLE VII

UPTAKE OF *o*-PHENYL-PHENOL BY ORANGES FROM WRAPS CONTAINING DIFFERENT AMOUNTS OF *o*-PHENYL-PHENOL AND HEXAMINE. TEMPERATURE 10° C.

Amount of *o*-phenyl-phenol and hexamine per wrap	Days				
	17	41	57	68	76
96 mg of *o*-phenyl-phenol + 25 mg of hexamine	2·4	4·6	5·0	8·9	8·0
48 ,, ,, + 12·5 ,, ,,	3·0	5·7	3·3	4·2	4·4
24 ,, ,, + 6·2 ,, ,,	1·6	2·9	2·5	3·3	3·4
85 ,, ,, + 30 ,, ,,	4·3	8·1	8·3	—	8·5

TABLE VIII

LOSS OF *o*-PHENYL-PHENOL FROM FRUIT PREVIOUSLY STORED IN *o*-PHENYL-PHENOL TREATED WRAPS (the fruit was stored in a desiccator through which a slow stream of air was passing)

	Days in air				
	0	12	16	22	37
Amount of *o*-phenyl-phenol, mg per orange ..	6·7	7·7	6·7	10·0	5·7

TABLE IX

EXAMPLES OF AMOUNTS OF DIPHENYL OR *o*-PHENYL-PHENOL FOUND IN THE PEEL AND PULP (mg per orange)

Diphenyl		*o*-Phenyl-phenol	
Peel	Pulp	Peel	Pulp
6·1	nil	17·4	nil
4·8	0·1	12·8	0·04
4·7	nil	10·0	nil
4·6	0·11	10·0	,,
4·2	0·1	6·65	0·03
3·4	0·17	5·7	nil
1·8	nil		

COMMERCIAL STORAGE WITH TREATED WRAPS—Three cases of Jaffa oranges (each orange wrapped in a paper containing diphenyl) were sent from Palestine to England. The amount of diphenyl originally included in the papers is not known. No diphenyl was found in the papers on arrival in this country, but the amounts recovered from the fruit taken from each of the three cases was 3·9, 4·2 and 3·8 mg per orange respectively. The average weight of the oranges was 230 g and the average weight of the peel 109 g. The pulp was found to contain no diphenyl. Thus the concentration for the whole orange was 17 p.p.m., while the peel contained about 37 p.p.m.

MARMALADE FROM ORANGES PREVIOUSLY STORED IN TREATED WRAPS—Sour oranges were stored for three weeks in treated wraps, and then made into marmalade (8 sour oranges, 2 lemons, 7½ lb. of sugar and 8 pints of water made 12 lb. of marmalade). The amount of diphenyl originally present in the 8 sour oranges (1265 g) was estimated to be 48 mg; of this, less than 0·6 mg remained in the marmalade (1 part per 10,000,000).

In a similar expt. with *o*-phenyl-phenol, 25 mg remained of 84 mg originally present in the 8 sour oranges, which weighed 1253 g (4·5 parts per 1,000,000).

SUMMARY—Methods have been devised for the estimation of diphenyl and *o*-phenyl-phenol absorbed by oranges from treated wraps. The amounts of diphenyl and *o*-phenyl-phenol found to be absorbed from wraps containing 100 mg of these substances were 4–20 mg of diphenyl or 20 to 30 mg of *o*-phenyl-phenol per 100 g of peel (= approx. 1 orange).

The work described above was carried out as part of the programme of the Food Investigation Board. It is published by permission of the Department of Scientific and Industrial Research.

We are indebted to Dr. J. R. Nicholls and Mr. A. G. Grimwade, of the Government Laboratory, for advice and help in devising the methods for the determination of diphenyl and *o*-phenyl-phenol. Mr. I. Sharman helped with the experimental work.

REFERENCES

1. Tomkins, R. G., Report of Food Investigation Board for the Year 1935, p. 129.
2. Farkas, A., *Hadar*, 1938, **11**, [9], 241; *Id.*, 1939, **12**, [8–10], 227.
3. Ramsey, G. B., Smith, M. A., and Herberg, B. G., *Bot. Gaz.*, 1944, **106**, 74.
4. Tomkins, R. G., Report of Food Investigation Board for the Year 1936, p. 146.
5. —— Report of Food Investigation Board for the Year 1938, p. 186.
6. Van der Plank, J. E., Rattray, J. M., and Van Wyk, G. F., *J. Pom. and Hort. Sci.*, 1940, **18**, 135.
7. Mulliken, S. P., "*The Identification of Pure Organic Compounds*," Vol. I, 1941, John Wiley & Sons, New York.
8. Hanke, M. T., and Kressler, K. K., *J. Biol. Chem.*, 1927, **50**, 235–269.

LOW TEMPERATURE STATION FOR RESEARCH IN BIOCHEMISTRY AND BIOPHYSICS, UNIVERSITY OF CAMBRIDGE, AND DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

June, 1945

The Toxicity of Diphenyl and *o*-Phenyl-phenol

By F. C. MACINTOSH

The data presented by Tomkins and Isherwood[1] leave little doubt that only very small amounts of either diphenyl or *o*-phenyl-phenol can be consumed by persons eating oranges stored in wraps treated with one of these substances. The tests on animals described below show, in addition, that both these compounds are of very low toxicity, and that consequently the use of such wraps involves no danger to the health of the consumer.

Ingestion of Single Doses—(*a*) Diphenyl suspended in gum acacia soln. or dissolved in nut oil has been given by stomach tube to cats in doses up to 1 g/kg of body weight without causing any visible ill-effect.

(*b*) *o*-Phenyl-phenol, similarly administered, was more toxic, the mean lethal dose being about 0·5 g/kg for cats, and about 3 g/kg for rats. The picture of acute intoxication was one of general depression, with death following through respiratory failure; in cats haemorrhage was observed *post mortem* in the lungs, liver, alimentary canal and myocardium. Sub-lethal doses of *o*-phenyl-phenol usually produced no obvious signs of poisoning.

Ingestion of Repeated Doses—With many aromatic compounds the effects produced by their repeated administration are quite unlike those produced by a single dose; the blood-forming tissues, in particular, are often poisoned by prolonged exposure to concentrations far below those immediately lethal. For this reason it is always necessary to determine the chronic as well as the acute toxicity of any substance which may be administered repeatedly to man. Young rats are suitable test objects for this purpose, since any cumulative effect of the substances might be manifested in a departure from the normal growth rate.

(*a*) *Diphenyl*—Sixty male white rats, weighing between 35 and 75 g, were divided into 4 equal groups of the same mean weight and fed *ad libitum* on the stock laboratory diet. Each rat was given 0·005 ml of nut oil per g of body weight daily by mouth and was weighed every second day. Group 1 received no diphenyl. The oil administered to groups 2, 3 and 4 contained 0·4, 4 and 40 mg/ml respectively of diphenyl; the daily intake of the compound was thus 2, 20, 200 mg/kg for these three groups. There was no significant difference between the mean growth rates of the 4 groups during the 4 weeks of the test, and no animal showed any sign of ill-health attributable to the diphenyl feeding. At the end of the test, the blood of 6 rats from Group 1, and 6 rats from Group 4, was examined. Haemoglobin content, total white cell count and differential white cell count were normal in all cases.

(*b*) *o-Phenyl-phenol*—The effect of chronic administration of this substance was tested in an expt. on 60 young male white rats lasting 32 days; the method of administration and the dosage levels were exactly the same as in the diphenyl expt. The mean growth rate was again practically identical in the 4 groups; there were no signs of ill-health attributable to the *o*-phenyl-phenol, and examination of the blood of 8 rats each from Groups 1 and 4 at the end of the expt. showed no significant effect of the treatment on the haemoglobin or white cell level.

Parenteral Administration—(*a*) *Diphenyl*—No sign of irritation followed the injection of 0·5 ml of a 4% soln. into the skin of a rabbit's ear, or the rubbing of such a soln. into the skin of the forearm of 2 human subjects. The intraperitoneal injection of 0·5 g/kg into a rabbit in the form of an oily solution or acacia-saline suspension had no ill-effect.

(*b*) *o-Phenyl-phenol*—The tests for cutaneous irritation described for diphenyl were negative for this compound also. By intraperitoneal injection *o*-phenyl-phenol is somewhat more toxic than by mouth, the mean lethal dose for rats being about 1·5 g/kg with the oily solution and about 0·5 g/kg with the acacia-saline suspension.

Inhalation of Vapour—The vapour pressure of both compounds is, at ordinary temperatures, so low that the risk of a toxic quantity entering the body through the lungs seems remote. A mouse kept for 6-hr periods on 2 successive days in air saturated with diphenyl vapour at 20° C. showed signs of discomfort during the exposure, but appeared normal soon after being removed from the chamber, and showed no sign of injury later. A similar expt. with *o*-phenyl-phenol, at 25° C., gave the same result.

These expts. emphasise the low toxicity of both compounds, especially of diphenyl, which appears to be pharmacologically nearly inert. The doses given to the animals in these tests were, of course, far greater than would ever be ingested by anyone eating oranges from treated wraps. Thus, supposing that the peel of such oranges contained uniformly

20 mg/100 g of diphenyl or 30 mg/100 g of *o*-phenyl-phenol (the highest concentrations found by Tomkins and Isherwood) and supposing also that the rat and man are equally sensitive to these compounds, then a 60-kg man eating the peels of *ca.* 400 oranges a day would still not be getting enough preservative to do him any harm. The amounts of the substances in orange pulp and in marmalade made from treated oranges are still more negligible. The low toxicity of the compounds when given by injection or inhalation shows that the handling of treated oranges and wraps is not dangerous to health.

REFERENCE

1. Tomkins, R. G., and Isherwood, F. A., ANALYST, 1945, **70**, 330.

NATIONAL INSTITUTE FOR MEDICAL RESEARCH
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June, 1945

Errata—POLAROGRAPHIC DETERMINATION OF LEAD IN BRASSES AND BRONZES, June issue, p. 250.

l. 26–27. *For* "potassium cyanide" *read* "sodium hydroxide."
l. 28. *For* "sodium hydroxide" *read* "potassium cyanide."

Notes

DETERMINATION OF SMALL AMOUNTS OF ALUMINIUM IN WATER BY MEANS OF HAEMATOXYLIN

IN a recent paper[1] I referred to the necessity of slightly acidifying the sample before applying the test so as to dissolve any particles of suspended aluminium hydroxide. Later Strafford and Wyatt[2] stressed the same point, but they stated that the amount of acid used in my method is still sometimes insufficient to dissolve "aged" and coagulated hydroxide: they recommended the use of a greater initial concn. of acid and they also found that this was necessary to decompose the complex aluminium compounds which they had encountered in certain waters.

To check this point, I have recently made a few duplicate determinations on samples containing coagulated aluminium hydroxide, using my original method and also a slightly modified technique employing the higher initial acid concn. used by Strafford and Wyatt. The following results were obtained, expressed in p.p.m. of aluminium.

	Sample No.			
Preliminary treatment	1	2	3	4
Using 0·5 ml of 0·5 *N* HCl per 25 ml of sample	0·30	0·21	0·21	3·7
Using 5 ml of *N* HCl per 25-ml sample at b.p.	0·40	0·25	0·23	3·7

Samples 1, 2 and 3 were river-derived water containing residual coagulated aluminium hydroxide (several days old). Sample 4 was a similar water which had been coagulated in the laboratory 18 hr. previously and tested after dilution (1 : 9). All the samples were shaken very vigorously before testing. It will be seen that with samples 2, 3 and 4 the results obtained by the two procedures were very similar, whilst with sample 1 the higher initial acidity gave an appreciably higher figure for aluminium content.

It seems likely that the ease with which any coagulum dissolves in acid is dependent on how far it has previously been disrupted by shaking and to what extent it is laden with organic matter. Since such factors may cause disagreement in results, it is evidently safer to use the higher initial acidity, and to this end my method may be simply modified as follows.

Add to 25 ml of the mixed sample, in a small flask, 5·0 ml of *N* hydrochloric acid and heat to incipient boiling. Remove from the flame and add 4·75 ml of *N* sodium carbonate and re-heat to b.p. to expel carbon dioxide. Cool to room temperature, transfer to a 100-ml Nessler glass and proceed as in my original method (*loc. cit.*, p. 210) but without any further addition of acid. The same procedure must of course be followed in the preparation of the standards.

REFERENCES

1. Houghton, G. U., ANALYST, 1943, **68**, 208.
2. Strafford, N., and Wyatt, P. F., ANALYST, 1943, **68**, 319.

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March, 1945

ARTIFICIAL COLOUR STANDARDS FOR USE WITH HOUGHTON'S METHOD OF DETERMINING ALUMINIUM

RECENTLY we have had occasion to carry out large numbers of aluminium tests by Houghton's method[1]; after colour development the soln. containing the aluminium was diluted to 50 ml with water.

To avoid the use of large numbers of standards, the preparation of artificial colour standards for this test has been investigated. As a result, it has been shown that standards of the following composition are stable for at least 4 days under ordinary laboratory conditions. When compared with the corresponding colours obtained on direct application of Houghton's test to known aluminium solns., the colour comparison was very satisfactory indeed when the light from an 80 watt Mazda Fluorescent Tube was used.

For the standards, 0·1% w/v soln. of the three dyestuffs Solochrome Violet R.S., Soledon Golden Yellow R.K.S. and Solacet Fast Blue 2 B.S. are prepared and stored in darkened bottles. Immediately before use, a 0·005% w/v soln. of Solochrome Violet R.S., and 0·01% w/v solns. of Soledon Golden Yellow R.K.S. and Solacet Fast Blue 2 B.S. are prepared by dilution of the above solns.

The aluminium standards are prepared by mixing the following volumes of the dyestuff solns. and diluting to 50 ml.

Al μg	Soln. of Solochrome Violet R.S. (0·005% w/v) ml	Soln. of Soledon Golden Yellow R.K.S. (0·01% w/v) ml	Soln. of Solacet Fast Blue 2 B.S. (0·01% w/v) ml
blank	2·43	5·57	1·81
1	2·23	4·57	2·41
2	2·03	3·97	3·01
3	2·03	3·57	3·61
4	2·03	3·17	4·11
5	2·23	2·77	4·61
6	2·43	2·37	5·01
7	2·51	2·17	5·27
8	2·65	2·07	5·80
9	2·78	1·97	6·13
10	2·92	1·87	6·46

For all ordinary purposes the above additions may be made correct to the second place of decimals.

The 0·01% w/v solns. of the three dyestuffs used have been examined in the Spekker Absorptiometer, with the following results.

Water blank and 1-cm cell

Dyestuff	Filter	Indicator drum reading
Solochrome Violet R.S.	Green Glass No. 5	0·631
Soledon Golden Yellow R.K.S. ..	Dark Blue Glass No. 7	0·549
Solacet Fast Blue 2 B.S.	Red Glass No. 1	0·955

REFERENCE

1. Houghton, G. U., ANALYST, 1943, **68**, 208.

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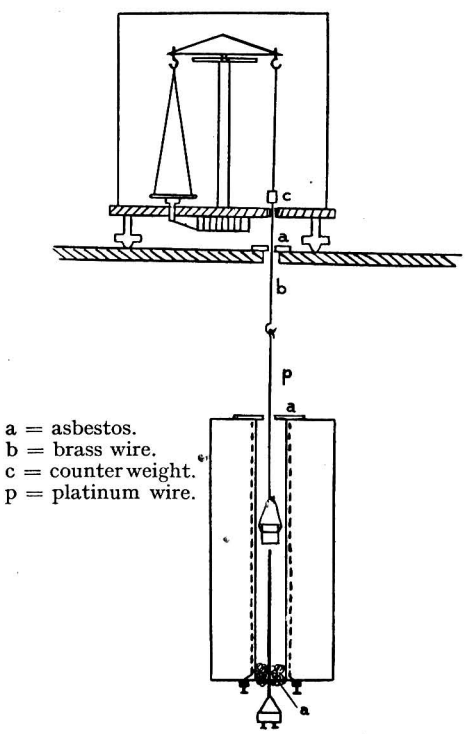
July, 1945

USE OF THE THERMAL BALANCE FOR IGNITING OR DRYING ANALYTICAL PRECIPITATES



a = asbestos.
b = brass wire.
c = counter weight.
p = platinum wire.

Fig. 1.

THE thermal balance has already proved its utility as an instrument for studying the rate of thermal decomposition of solids, but we have found that it may be applied with advantage to the ignition or drying of analytical ppts. The technique permits the crucible and its contents to be weighed while it is being heated in an electric furnace at a known temperature. This is a particular advantage where the product is hygroscopic or reactive, *e.g.*, Al_2O_3 from aluminium hydroxide or CaO from calcium oxalate; furthermore, the attainment of constant weight can be detected with ease and certainty, so that excessively long or high temperature heating can be avoided. Since the temperature of the furnace can be accurately determined, it is possible to arrest the decomposition at some intermediate stage if this is quicker or more convenient; *e.g.*, calcium oxalate can be taken to constant weight at 500° C., when it is converted into calcium carbonate quantitatively. The use of the desiccator is of course eliminated.

Following are example of times and temperatures required for attainment of constant weight.

$Al(OH)_3 \longrightarrow Al_2O_3$	1 hr.	at 900° C.
$Fe(OH)_3 \longrightarrow Fe_2O_3$	45 min.	at 850° C.
$CaC_2O_4 \longrightarrow CaCO_3$	20 ,,	,, 500° C.
$CaCO_3 \longrightarrow CaO$	40 ,,	,, 900° C.
$BaSO_4$	30 min.	at 650° C.
$PbSO_4$ (alcohol washed)	15 ,,	,, 130° C.
$Cu(CNS)_2$,, ,,	30 ,,	,, 130° C.
AgCl	30 ,,	,, 130° C.
$MgNH_4PO_4.6H_2O \longrightarrow Mg_2P_2O_7$	60 ,,	,, 950° C.

The following arrangement (see Fig. 1), in which a standard analytical balance can be readily adapted without any permanent alteration, has been found convenient. The pan and pan support are removed from one arm of the balance, leaving a hole in the base of the balance through which the brass suspension wire (say, 18 S.W.G.) can pass. The latter ends in a hook which is centred *ca.* 1 in. above the top of the vertical tubular furnace, say of 2 in. internal diameter. The crucible is held in a suitable cradle of platinum or nichrome wire, which can be hung from the brass hook so that it comes about half way down the furnace. The thermocouple is inserted from the base so as to terminate approximately $\frac{1}{2}$ in. below the bottom of the crucible; the bottom opening of the furnace should be plugged with asbestos wool to eliminate convection currents which otherwise upset the swinging of the balance. An iron or silica tube can also be inserted beside the thermocouple, so that a gentle air-stream can be passed through the furnace, if required, for sweeping out any gaseous products of decomposition or for cooling the furnace.

At temperatures above *ca.* 600° C. slight irregularities of swing may occur from time to time owing to small convection effects, but they are easily detected and it is only necessary to wait a few seconds for the balance to resume its regular oscillation before recording the weight. Uncertainties arising from these convection effects rarely exceed 0·0002 g.

The buoyancy of the hot air is, of course, less than that of air at room temp.; since, however, the crucible is weighed at the same temperature before and after adding the precipitate, this buoyancy effect cancels out.

We have found it convenient to set up two thermal balances, one to operate with a home-made heater designed for temperatures from 100° to 150° C., the other with a standard laboratory furnace for the range 150° to 1000° C. These have proved very useful in an undergraduate quantitative course, as well as in ordinary analytical work.

REFERENCES

Šplíchal, J., *Coll. Czech. Chem. Comm.*, 1937, **9**, 302.
Orosco, E., *Ministerio trabalho. ind. com., Inst. nach. tech.* (Rio de Janiero), 1940, 33 pages.
Oshima, Y., and Fukuda, Y., *J. Soc. Chem. Ind. Japan*, 1930, **33**, 251.
Dubois, M. P., *Bull. Soc. chim.*, 1936, [v], **3**, 1178.
Somiya, T., *J. Soc. Chem. Ind. Japan*, 1928, **31**, 217.

WASHINGTON SINGER LABORATORIES
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May, 1945

ESTIMATION OF SACCHARIN IN TABLETS

THE rapid estimation of saccharin in tablets for routine analysis is generally carried out by the method of the British Pharmacopoeia, 1932, using whole tablets.

Difficulties are sometimes encountered and inaccuracies incurred by excessive frothing of the solution during the ammonia distillation owing to the presence of saponifiable lubricants, such as stearic acid, tallow, waxes, etc., employed in the manufacture of the tablets. Froth-repressing substances, such as amyl alcohol, are of little use.

This difficulty can be readily overcome by adding to the alkali solution, in suitable proportions, barium, calcium or other hydroxides capable of forming insoluble salts with higher fatty acids.

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July, 1945

Ministry of Food

STATUTORY RULES AND ORDERS*

1945—No. 911. The Fruit Pulp Order, 1945. Dated July 24, 1945. Price 2d.

This Order, apart from adjustments in maximum prices, re-enacts in substance the provision of the Fruit Pulp Order, 1944. It preserves certain licences granted under the 1944 Order.

In this Order "fruit pulp" and "fruit purée" mean fruit pulp and fruit pureé manufactured in the United Kingdom, and "purée" includes paste.

FOOD LABELS AND ADVERTISEMENTS. CLAIMS REGARDING VITAMIN AND MINERAL CONTENTS†

WHERE a food advertisement or label claims or suggests that the food concerned contains vitamins or minerals it must, under the Labelling of Food (No. 2) Order, 1944, disclose the quantity of each of the vitamins or minerals claimed to be present. A mere disclosure of the quantity, however, is not sufficient to prevent the advertisement or label from being misleading within the meaning of Regulation 1 of the Defence (Sale of Food) Regulations, 1943, if statements or suggestions are made in it which imply that the food has a nutritional or dietary value in consequence of the presence of these vitamins or minerals which is in fact not warranted by the quantities present.

A decision as to whether any particular statement is misleading can, of course, be given only in a Court of Law, but to assist traders and, indeed, in response to many requests from traders for guidance in preparing

* Obtainable from H.M. Stationery Office.

† P.N. 3711. July 17, 1945.

their advertisements and labels, the Ministry of Food has prepared a statement which is intended to indicate the limits of what may reasonably be claimed for a food according to the quantity of vitamins or minerals it contains.

The dietary value of vitamins and minerals obviously depends not only on the amount present in a given quantity of the particular food, but on the amount present in that quantity which a consumer takes in a day. In other words, the amount which an average consumer may reasonably be expected to consume daily should contain not merely a significant quantity of the vitamin or mineral in question, but a quantity sufficient in the light of modern nutritional science to justify whatever reference is made to it in the advertisement or on the label.

The statement, which is given below, has been prepared after consideration of recommendations by the Medical Research Council, and the Ministry of Food suggests that it should form a code of practice to ensure that references in advertisements and labels are not misleading.

SUGGESTED CODE OF PRACTICE IN FRAMING LABELS AND ADVERTISEMENTS

1. Unless the amount of the food that would ordinarily be consumed in one day contains at least *one-sixth of the daily requirement* of the vitamin or mineral; (*a*) no claim based on its presence should be made, and (*b*) no reference to its presence is justified in any advertisement for the general public, or on any label. (The inclusion of a mineral salt in the statement of ingredients required by the Labelling of Food Order will not of itself be regarded as implying the presence of a scheduled mineral.)

2. Unless the amount of the food that would ordinarily be consumed in one day contains at least *one-half of the daily requirement* of the vitamin or mineral, no claims are justified which imply that the food is a "rich" or "excellent" source of the vitamin or mineral.

3. Unless the amount of the food that would ordinarily be consumed in one day contains at least the *full daily requirement* of the vitamin or mineral, no reference is justified to the value of the food for the prevention or cure of disease due to the lack of the vitamin or mineral present in the food.

4. Claims based on the presence of more than one vitamin or mineral should not be made unless each such vitamin or mineral is present in the proportion necessary to justify the claim.

5. There is no evidence of a deficiency of phosphorus in the ordinary mixed diet of this country. Statements in advertisements or on labels suggesting the contrary or that it is desirable to supplement the dietary phosphorus intake should not be made.

6. The following table, which includes the figures laid down by the Third Session of the Technical Commission on Nutrition, 1937, of the League of Nations Health Organisation, shows the normal daily requirement of the vitamins and minerals scheduled in the Labelling of Food Order:

Vitamin A	3,000 I.U.	Calcium	0·75 g
,, B_1	300 ,,	Iodine	0·1 mg
,, B_2	1·8 mg	Iron	10·0 ,,
Nicotinic acid	12·0 ,,	Phosphorus	0·75 g
Vitamin C	30·0 ,,		
,, D	500 I.U.		

The statement refers to the foods commonly consumed by normal healthy adults and may not in all cases be applicable to foods used for special purposes.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Monoglyceride in Fats and Oils by Oxidation with Periodic Acid. W. D. Pohle, V. C. Mehlenbacher and J. H. Cook (*Oil and Soap*, 1945, **22**, 115–119)—A procedure for the determination of monoglycerides in oils and fats has been developed in which periodic acid is allowed to react with the sample and the excess determined iodimetrically. *Method*—Weigh accurately into a 150-ml glass stoppered flask a quantity of the sample containing about 0·15 g of monoglyceride (10 g of samples containing 1% or less). Introduce into the flask containing the sample 25 ml of a reagent made by dissolving 5 g of periodic acid in 200 ml of water and adding 800 ml of glacial acetic acid; this reagent should be stored in a dark, glass-stoppered bottle. If the sample is solid, liquefy it by warming on a boiling-water-bath, 1–2 min. usually being sufficient. The temp. should not be allowed to exceed 60° C.; no heat should be applied if the sample is liquid. Shake the liquid mixture for 30–60 sec., rinse the stopper and the walls of the flask with a few ml of glacial acetic acid and leave for 30 min. at room temp. (34° C. or less). Then add 10 ml of a 20% aqueous soln. of potassium iodide and titrate the liberated iodine with 0·1 *N* sodium thiosulphate, using starch soln. as indicator. Make a blank determination on the oxidising soln. without the sample at the same time and in the same manner. The % of monoglyceride in the sample is given by

$$\frac{(x - y) \times C \times 100}{W \times 10{,}000}$$

Where x = ml of 0·1 *N* sodium thiosulphate for blank: y = ml of sodium thiosulphate for sample: C = one half the molec. weight of the monoglyceride: W = weight of sample in g. The molec. weight used in the calculation may be selected according to the type of material being examined. In many instances calculating to monostearin or monoolein is sufficiently accurate. The average molecular weights of the monoglycerides of the fatty acids of a number of fats and oils are given as follows. Coconut oil, 281·8; cottonseed oil, tallow, palm oil and soyabean oil may be taken as 354·5. The presence of protein, cellulose material or glycerol in the sample causes high results, and these substances must be removed. Filtration is usually sufficient to free ordinary oils and fats from the first two, while glycerol may be removed by washing with 20% sodium chloride soln. Figures are quoted which indicate that di- and triglycerides do not react

in the test nor influence the reaction of monoglycerides with periodic acid. Glycol monoesters are also without effect. It is claimed that the method is accurate to within ±0·6% when tested on pure, repeatedly recrystallised monoglycerides and that individual analyses of commercial products were within ±0·6% of the average of all tests. Individual analyses of products containing 2% or less of monoglyceride were usually within ±0·1% of the average. J. A.

Weakening of Self-raising Flour on Storage. T. H. Fairbrother (*Food Manufacture*, 1945, **20**, 133–139)—Under a recent Government Order (S.R.& O., 1944, No. 44), self-raising flour is now subject to a limit with regard to CO_2 content. It is shown that: (1) The methods in the Order are given in too little detail, resulting in considerable variations in results, especially in the determination of residual CO_2. This has led to samples really satisfactory in CO_2 content being condemned by some analysts owing to faulty analytical technique. (2) Self-raising flours gradually "run down" during storage especially under unfavourable conditions. With regard to (1), in order to ensure more uniform and accurate results among analysts, it is recommended that the method should definitely state (*a*) amount of water, (*b*) amount of flour, (*c*) type of stirring or rotation, (*d*) method of addition of flour to water, (*e*) period of final boiling. Three methods are given of measuring the CO_2 evolved: (A) absorption on soda-lime in U-tubes—the normal gravimetric method; (B) gasometric method—employing a slight variation of the well-known Chittick apparatus; (C) absorption of the CO_2 evolved in a column of glass beads wetted by baryta (50 ml of *N*/5), and back-titrating the unused baryta with *N*/5 HCl. Method (B) is the quickest and simplest, but all three methods give satisfactory results provided the necessary attention is paid to detail. In the residual CO_2 determination it is recommended that the ratio of water to flour should be at least 20 : 1, and that after very thorough mixing the suspension should be immersed *in* a boiling water-bath for 20 min., followed by boiling on a double asbestos gauze for 3 min., care being taken (by continuous and fairly vigorous stirring) not to char the sample. This procedure should get rid of all the evolved CO_2 from the suspension and should give a correct residual figure. To test the "running down" of self-raising flours on storage, a uniform sample of self-raising flour was distributed in 3 types of bag. One series was kept in a humidity cabinet (70–100% relative humidity, 52°–85° F.); the other series was packed in commercial cardboard containers and stored in the laboratory storeroom. On analysis the following results were obtained.

	On November 1, 1944			On February 1, 1945		
	Total CO_2 %	Residual CO_2 %	Available CO_2 %	Total CO_2 %	Residual CO_2 %	Available CO_2 %
Humidity cabinet:						
Cellophane, gummed ..	0·548	0·040	0·508	0·380	0·043	0·337
,, Gloy	0·550	0·040	0·510	0·398	0·047	0·351
Paper, gummed	0·574	0·045	0·529	0·390	0·050	0·340
Stored in laboratory:						
Cellophane, gummed ..	0·548	0·040	0·508	0·512	0·038	0·474
,, Gloy	0·550	0·040	0·510	0·530	0·038	0·492
Paper, gummed	0·574	0·045	0·529	0·536	0·044	0·492

The results show that a sample may lose sufficient CO_2 on storage to be outside the required limits of the Order (although it may still give a satisfactory baking result). Acid calcium phosphate, the most common acid ingredient in self-raising flour, is known to react with the bicarbonate in the flour in the ordinary dry state during storage, and this reaction increases rapidly with increasing moisture content and temperature of storage. W. M.

Preliminary Investigation of the Application of the Karl Fischer Reagent to the Determination of Moisture in Cereals and Cereal Products. R. H. Fosnot and R. W. Haman (*Cereal Chem.*, 1945, **22**, 41–48)—Fischer (1935) developed a chemical method of moisture determination by using a complex reagent consisting of pyridine, methanol, sulphur dioxide and iodine which possessed the desired specificity for water. The reaction cannot be definitely expressed stoichiometrically, but it is believed to progress in two steps. First, the iodine, sulphur dioxide and pyridine combine with water to give the 1 : 1 complex of pyridine and sulphur trioxide, which then reacts with the methanol to give the pyridine salt of methyl sulphuric acid. Most materials, liquid and solid, can be analysed by this reagent except inorganic alkalis. In short, the method consists in adding an excess of reagent to the material under test (an excess is denoted by the brown colour of iodine) and back-titrating the excess with a soln. of water in methanol. With colourless materials the end-point may be observed visually as the colour changes from iodine brown to a bright yellow. The colour change is, however, gradual, progressing from red through orange, and an exact end-point is difficult to determine. There is a definite potential change at the end-point and the reaction lends itself admirably to electrometric measurement. Two methods of this type have been employed. The first uses one electrode of platinum and one of tungsten, and the potential change is measured with a suitable galvanometer (Almy, Griffin and Wilcox, *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 392–396). The second uses two platinum electrodes with an impressed voltage across them, the end-point being indicated by a sudden decrease of the current to zero. A "magic eye" electronic tube instrument is very convenient for this purpose, and a suitable instrument has been described by McKinney and Hall (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 460).

The Fischer reagent is made up as follows. Pass into 700 ml of pyridine (Eastman Reagent No. 214-H) sulphur dioxide until 380 g have been added. Cool, and add 1 litre of pyridine and 200 ml of anhydrous methanol. Cool in an ice-bath and

slowly add 500 g of iodine with occasional shaking. To prepare anhydrous methanol leave industrial methanol over Drierite for several days and then distil the methanol, taking precautions to prevent contamination with moisture. To prepare the standard water solution add 5 ml of water to 1 litre of dry methanol.

Standardisation—(1) The reagent is standardised against the standard water soln., using 15 to 20 ml of the reagent. This factor R is expressed as follows:

$$R = \frac{\text{ml of standard water soln.}}{\text{ml of Fischer reagent}}.$$

(2) A factor "b" is determined; this is the correction for the amount of water in the 25 ml of methanol used as solvent for the sample. Titrate 25 ml of the methanol by adding excess of Fischer reagent and back-titrating with standard water soln.

$$\text{"b"} = (\text{ml of Fischer reagent} \times \text{R}) - \text{ml of standard water soln.}$$

(3) A factor "W," the water factor, is determined by weighing 0·03 to 0·15 g of water (weighing pipette) into 25 ml of methanol and titrating as in (2).

$$\text{"W"} = \frac{\text{g of water weighed}}{(\text{ml of Fischer reagent} \times \text{R}) - (\text{ml of standard water soln.}) - \text{"b"}}$$

The Fischer reagent gradually deteriorates, and "R" and "b" should be determined daily. "W" usually remains constant and need only be determined on each fresh batch of standard water soln.

Determination—To a suitable quantity of the sample (containing *ca.* 100 mg of water) add 25 ml of dry methanol. Heat the mixture to b.p. on a water-bath. Cool, add the Fischer reagent until there is an excss of about 2 ml, and after a suitable time back-titrate with standard water soln. The moisture content is given by the following formula:

$$\%\ \text{moisture} = \frac{[(\text{ml of Fischer reagent} \times \text{R}) - (\text{ml of std. water soln.}) - \text{"b"}]\ \text{W} \times 100}{\text{weight of sample}}$$

It has been found that the critical factors for cereals are: length of time of contact with the Fischer reagent and particle size. About 60 min. contact time is required with most cereals, ground to pass a 0·5 mm screen. With finer grinds the contact time may be slightly reduced. These conditions muat be determined for the substance under test. The results are reproducible to approx. 0·2% of moisture. Typical results obtained by this method, which is claimed to save time as compared with normal oven determinations, are given below:

Material	Moisture content, % Fischer		Oven
Corn flakes	9·3		9·7
Wheat flour	12·1	(5 hr. drying)	11·4
		(8 ,, ,,)	11·6
Oat flour	8·1		8·1
Soy ,,	6·0		5·9
Wheat gluten	7·6		7·5
Wheat	7·1		7·0
Barley	7·3		7·3
Dried eggs	3·5		3·7
Malted milk	4·0		4·1
Malt syrup	20·0	(Pycnometer)	20·1
Condensed whole milk	62·0		62·3
,, skim ,,	73·5		73·3

W. M.

Preparation and Properties of Sulphonacetamides. Method for the Separation of Sulphonamides from N-Alkylsulphonamides. H. T. Openshaw and F. S. Spring (*J. Chem. Soc.*, 1945, 234–236)—Aromatic hydrocarbons and ethers, aryl chlorides and bromides are frequently identified by conversion into the sulphonamide after chlorosulphonation. A method of characterising sulphonamides and of estimating their molecular weights is described, in which they are acetylated by the method of Noelting (*Ber.*, 1875, **8**, 598) and the resulting sulphonacetamide is titrated with standard alkali, using phenolphthalein as indicator. *Method*—Heat 1 part of the sulphonamide with 2·5 parts of acetyl chloride under reflux for 30 min.; if solution is not complete in 5 min., add up to 2·5 parts of glacial acetic acid. Remove the excess of acetyl chloride, add the cold reaction mixture to water, collect the product, wash with water and dissolve in warm sodium bicarbonate soln. Filter, acidify with acetic acid and recrystallise the pptd. sulphonacetamide from aqueous alcohol. Determine the equivalent by dissolving *ca.* 0·3 g of the acetyl derivative (accurately weighed) in 50 ml of 50% alcohol and titrating with 0·1 *N* sodium hydroxide with phenolphthalein as indicator. Seventeen sulphonacetamides have been prepared; their m.p. are as follows:

	°C.
Benzene-	124–125
Toluene-*p*-	137
Toluene-α-	130
p-Ethylbenzene-	97
2 : 4 : 5-Trimethylbenzene-	155
2 : 4 : 6-Trimethylbenzene-	165·5
Naphthalene-1-	185
Naphthalene-2-	145–146
Tetralin-6-	138
p-Cymene-?-	149
p-Methoxybenzene-	140
p-Ethoxybenzene-	151·5
m-Nitrobenzene-	189
p-Nitrobenzene-	192
p-Bromobenzene-	202–203
2 : 5-Dichlorobenzene-	214
2 : 5-Dibromobenzene-	228

N-alkylsulphonamides are acetylated as described above except that, since they are insoluble in sodium bicarbonate soln., the crude product is crystallised directly from aqueous alcohol. The melting points of the 10 N-alkylsulphonacetamides described are as follows:

$Ar.SO_2.NR.COCH_3$

Ar	R	m.p.°C.
Benzene-	Benzyl	76
p-Toluene-	Benzyl	98
p-Bromobenzene-	Methyl	94
p-Bromobenzene-	Ethyl	88·5–89·5
p-Bromobenzene-	Benzyl	88–89
m-Nitrobenzene-	Methyl	132
m-Nitrobenzene-	Ethyl	89
β-Naphthalene-	Methyl	82
β-Naphthalene-	Ethyl	76
β-Naphthalene-	Benzyl	168·5–169

Separation of mixtures of a sulphonamide and a N-alkylsulphonamide can be achieved as follows. Acetylate the mixture as described above. Pour the cold reaction mixture into cold water, collect the product, wash with water and digest with warm sodium bicarbonate soln. Filter off the insoluble N-alkylsulphonacetamide and recrystallise from aqueous alcohol. Acidify the filtrate with acetic acid, collect the pptd. sulphonacetamide and recrystallise from aqueous alcohol. Sulphonacetamides

can be hydrolysed by heating under reflux with 5% aqueous potassium hydroxide soln. for 45 min. N-alkylsulphonacetamides require treatment with 5% aqueous-alcoholic potassium hydroxide soln. for 1 hr. J. A.

Analysis and Fatty Acid Composition of Tobacco Seed Oils. R. W. Riemenschneider, R. M. Speck and E. G. Beinhart (*Oil and Soap*, 1945, **22**, 120–122)—Twelve samples of oil extracted from the seeds of a number of different types and varieties of tobacco plant have been examined. The figures obtained are presented in a table which is summarised as follows: oil content, 35·1–43·4% (av. 39·24); n_D^{25}, 1·4738–1·4743 (av. 1·47397); free fatty acids, 0·14–3·76% (av. 1·34); iodine val. (Wijs), 139·5–145·9 (av. 142·3); thiocyanogen value, 78·3–81·6 (av. 80·0). The thiocyanogen values were determined with 0·1 *N* thiocyanogen soln. with an absorption time of 24 hr. at 21° C. The composition of the fatty acids of the oils was calculated from the thiocyanogen and iodine values, the results being summarised as follows: oleic, 12·0–19·2% (av. 15·0); linolic, 71·7–77·0% (av. 74·7); saturated, 8·5–12·2% (av. 10·3). The linolic acid content of two samples of fatty acid was determined spectrophotometrically, with results 3·0 and 5·4% higher than those by the thiocyanometric method. A more complete analysis of the fatty acid constituents of one sample of flue-cured tobacco seed oil was carried out by fractionation of the methyl esters and low temp. crystallisation of the distilled ester fractions. The results obtained by this method accorded well with those determined directly on the oil. The saturated acids consisted of palmitic and stearic acids in the proportions of 7 and 3% respectively of the total fatty acids. This sample of oil contained 0·043% of tocopherol. J. A.

Investigations of the Seed Oils of some Sudan *Mimosaceae*. D. N. Grindley (*J. Soc. Chem. Ind.*, 1945, **64**, 152)—The seeds of this family contain very little fixed oil. The constants, etc., for these oils, which were extracted with light petroleum, were as follows.

Species:	*Acacia albida*	*A. sieberiana*	*A. verek*	*A. mellifera*	*A. seyel*	*A. arabica*	*Albizzia lebbek*
Native name:	Heraz	Kuk	Hashab	Kitter	Talh	Sunt	Lebbek
Wt. of 100 seeds, g ..	7·94	21·96	7·76	5·46	9·11	10·78	8·14
Oil, %	2·74	3·99	7·78	7·89	6·48	5·46	6·85
n_D^{40}	1·4691	1·4676	1·4674	1·4650	1·4685	1·4679	1·4668
Iodine val.	96·3	102·5	98·8	74·8	97·1	98·5	84·8
Thiocyanogen val. ..	69·6	67·1	68·1	57·3	66·3	69·2	62·4
Sapon. val.	186·0	188·6	187·0	186·6	188·3	187·7	185·8
Unsap. matter, % ..	3·86	3·37	3·50	2·59	2·83	3·63	2·52

The fatty acids in most of these oils consist of 20 to 30% of saturated acids, including *ca.* 3% of higher acids (arachidic, behenic and lignoceric), the balance being a mixture of oleic and linolic acids in ratios from *ca.* 2 : 1 to 3 : 4 according to species. E. B. D.

Nature and Specificity of the Vitali-Morin Reaction for Solanaceous Alkaloids. W. O. James and M. Roberts (*Quart. J. Pharm.*, 1945, **18**, 29–35)—The Vitali-Morin reaction, in which a few mg of the substance under test are evaporated to dryness with *ca.* 0·2 ml of fuming nitric acid, the residue is dissolved in acetone, and the soln. is treated dropwise with a 3% soln. of potassium hydroxide in methyl alcohol, is shown to be a special example of the colour reaction of polynitrobenzene derivatives with alkalis. In the reaction with the solanaceous alkaloids it is considered that at least two nitro groups are introduced into the benzene ring in the tropic acid part of the molecule, since mononitrobenzene derivatives give no colour in the test and trinitro compounds produce colours with a strong absorption at the blue end of the spectrum, which is lacking in the purple colour derived from atropine. It is shown that acetone enhances the production and stabilisation of the colours, especially the blues characteristic of dinitro compounds, and it is stated that such colours may be used to identify dinitro compounds in simple combinations. With the procedure employed, aliphatic and heterocyclic substances do not give colours soluble in acetone, while, of the aromatic series, relatively few compounds give the reaction. Hydrocarbon side chains enhance it, but other groups, especially hydroxyl and carboxyl, reduce or destroy it. The formation of intense purple colours in the Vitali-Morin reaction appears to be associated with the grouping $C_6H_5.CH.X.COOR$, provided that X is not hydroxyl, and R implies esterification of the carboxyl group. The only substances recognised in plant extracts giving such strong reactions are esters of tropic and truxillic acids. Weaker reactions are given by mandelic acid, $C_6H_5.CHOH.COOH$, and its derivatives, provided that the secondary alcohol group is esterified. This appears to occur naturally only in the mandelonitrile glycosides (*e.g.*, amygdalin). J. A.

Biochemical

Micro-estimation of Glycocyamine and Arginine by Means of a Synthetic Ion Exchange Resin for Chromatographic Separation. E. A. H. Sims (*J. Biol. Chem.*, 1945, **158**, 239–245)—Weber's method (*J. Biol. Chem.*, 1930, **86**, 217; 1930, **88**, 353), and several published modifications of it, are based on the observation that glycocyamine and arginine are the only common biological substances that yield a colour in the Sakaguchi reaction and that they can be separated from one another by adsorption on Permutit. One disadvantage of this method is that variations are obtained when different batches of Permutit are used. Amberlite IR–100–H is now recommended in place of Permutit, in conjunction with Dubnoff's method (*J. Biol. Chem.*, 1941, **141**, 711) for colour development. Dilute the sample of urine 6-fold and serum ultra-filtrates 2-fold, and adsorb the arginine and glycocyamine on a column of Amberlite IR–100–Na. The adsorbent is prepared from the analytical grade of the resin, IR–100–H, as follows. Treat each 1 g in a suitable column with 10 ml of 5% sodium chloride soln., allowing at least 10 hr. total downflow time. Wash the column with distilled water and store the resin in the refrigerator

in 0·3% sodium chloride soln. It remains efficient for at least 6 months. For the analytical procedure the rate of flow down the column should be retarded so that 5-ml portions of the soln. to be analysed require 20 min. or slightly longer to pass through. This can be done conveniently in the apparatus shown in Fig. 1. The column A is

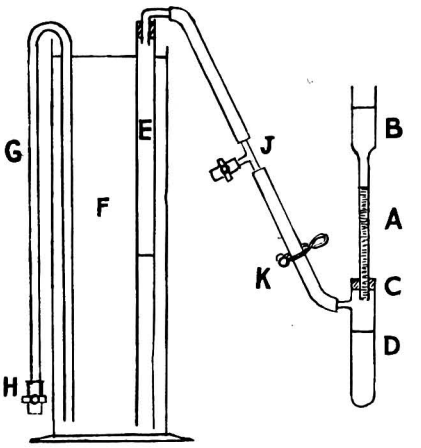
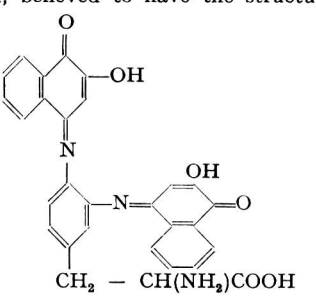


Fig. 1.

about 12·5 cm. long and has an internal diameter of 4 mm, so that 1 ml of fluid forms a column about 10 cm long. Insert a cotton-wool plug in the constricted tip of the tube and, while the outlet is blocked with the finger, fill the column and reservoir B with 0·3% sodium chloride soln., and add the resin, repeatedly tapping the tube to ensure uniform packing, until it reaches the 7·5-cm mark etched on the column. With the thumb over the top of the reservoir, attach the column by means of the rubber stopper C to collecting tube D. This tube carries a side arm with a length of rubber-tubing temporarily closed by means of the pinchcock K. Connect the other end of the tubing to a piece of glass tubing E of about the same diameter as the collecting tube, and insert this into a cylinder of water F, the level of which can be adjusted by the siphon G and screw-clip H. Remove all the saline above the neck of the reservoir B and replace by 5 ml of the soln. to be analysed. Fill the cylinder F with water, and by means of the T-tube J, blow air into the system until the distance between the levels of water in E and F is equal to the height of the fluid in A and B. Now open the pinchcock K, and regulate the downflow in the column by siphoning off the water from cylinder F at the proper speed. When the soln. to be analysed has passed nearly through the column, close the pinchcock K, fill the reservoir B with 0·3% sodium chloride soln., and add enough water to the cylinder F to re-establish equilibrium. Repeat until 13 ml have passed through the column. Dilute the filtrates to 15 ml and transfer 4 ml to a colorimeter tube. Cool the tube in an ice-bath, add 1·2 ml of freshly prepared α-naphthol-urea reagent (prepared by mixing 0·2 ml of a 0·2% alcoholic soln. of pure α-naphthol and 1·5 ml of a 16% aqueous urea soln.). Mix and allow the tube to stand in the ice-bath for 10 to 15 min., and then add 0·72 ml of a sodium hypobromite soln. made by adding 0·66 ml of liquid bromine to 100 ml of 5% sodium hydroxide soln. Mix immediately and evaluate the colour at intervals in an Evelyn photoelectric colorimeter with a 6-ml aperture and a 540 *mμ* filter. The maximum colour which develops at the end of 20 min. is directly proportional to the concn. of glycocyamine up to 1·6 mg per 100 ml, but the blanks become most uniform after 60 min., at which time the colour produced by glycocyamine is also stable and intense. Readings, therefore, are best taken 60 min. after addition of the hypobromite. The results are calculated from the values obtained with standard solns. of glycocyamine in 0·3% sodium chloride soln. Recoveries of glycocyamine added to solns. of arginine or to human urine were almost quantitative. F. A. R.

Determination of Phenylalanine in Proteins. W. C. Hess and M. X. Sullivan (*Arch. Biochem.*, 1944, **5**, 165–173)—Phenylalanine can be determined by conversion into 3 : 4-dinitro-phenylalanine, reduction to 3 : 4-diamino-phenylalanine and combination in slightly acid soln. with 1 : 2-naphthaquinone-4-sodium sulphonate to yield a red compound, believed to have the structure:



To 5 mg of the amino acid add 2·0 ml of nitrating mixture (10 g of sodium nitrate in 100 ml of conc. sulphuric acid) as described by Block and Bolling (*J. Biol. Chem.*, 1939, **129**, 1), heat for 20 min. on the steam-bath, add 10 ml of water and about 100 mg of zinc powder. Heat on the steam-bath for 15 min. and leave at room temp. for 30 min. Dilute to 20 ml and filter, and to 1·0 ml of the filtrate add 1·0 ml of 0·25% 1 : 2-naphthaquinone-4-sodium sulphonate soln. Leave for 15 min. at room temp., add 3 ml of 95% ethanol and estimate the colour of the resulting soln. in a Klett-Summerson photoelectric colorimeter with a 54 filter. Subtract from the result the reading of the naphthaquinone blank. The average of 16 separate determinations of a soln. containing 0·125 mg of pure phenylalanine was 0·122 mg, with a standard deviation of 0·004 mg. The colour was directly proportional to the concentration. The smallest amount of phenylalanine that can be estimated by this method is 6 μg. Tryptophan and tyrosine interfere with the method, but they can be destroyed by addition of a 2·5% soln. of potassium permanganate, drop by drop, with stirring, until a rose colour persists for at least 1 min. The soln. is then evaporated to an oil on the water-bath and the determination is carried out as described above. With this modification a recovery of 97·6% of phenylalanine was obtained in presence of equal weights of tryptophan and tyrosine. With a soln. of phenylalanine containing twice its weight of cystine, methionine, leucine, *iso*leucine, arginine, glutamic acid, alanine, glycine, proline and valine recoveries of 96·2% and 97·3% were obtained with and without addition of permanganate respectively. In presence of benzoic acid, *p*-aminobenzoic acid, tyrosine, tryptophan and histidine, the recovery, after addition of permanganate, was 101·8%.

Estimation in proteins—Proteins were hydrolysed with 7 *N* sulphuric acid, 20% hydrochloric acid or 5 *N* sodium hydroxide. *Acid hydrolysis*—To 200–500 mg of protein, add 2·0 ml of either acid and 0·5 ml of butyl alcohol to prevent foaming. Heat under reflux in an oil-bath at 125–130° C., cool, dilute the hydrolysate to 30 ml and filter. Put 6-ml aliquots in glass evaporating dishes, add 2·5% potassium permanganate soln. dropwise with stirring until the rose colour persists for at least 1 min. and concentrate the soln. to an oil. Evaporate 2·5 ml of a 0·1% soln. of phenylalanine in 0·5 *N* sulphuric acid in the same way. Add 2 ml of nitrating mixture to each of the residues, heat for 20 min. on the steam-bath, and add 10 ml of water and about 100 mg of zinc powder. Heat on the water-bath for 15 min. and leave at room temp. for 30 min. Dilute to 20 ml, filter and estimate the colour as described above. Use two blanks, one to compensate for the colour of the filtrate (prepared by adding 1 ml of water to 1 ml of filtrate followed by 3 ml of 95% ethanol) and the other to compensate for the colour of the naphthaquinone reagent (prepared by substituting 1 ml of water for 1 ml of the filtrate). *Alkaline hydrolysis*—Add 2 ml of 5 *N* sodium hydroxide and 0·5 ml of butyl alcohol to 200–500 mg of the protein. Heat at 125–130° C. as in the acid hydrolysis, add 1·7 ml of 14 *N* sulphuric acid, dilute to 30 ml with water and filter. Add permanganate soln., evaporate to dryness and proceed as described above. In general, the values obtained by the three methods of hydrolysis agreed well; hydrolysis with sodium hydroxide gave slightly higher results than with sulphuric acid and appreciably higher results than with hydrochloric acid. F. A. R.

Microbiological Estimation of Glutamic Acid. C. M. Lyman, K. A. Kuiken, L. Blotter and F. Hale (*J. Biol. Chem.*, 1945, **157**, 395–405)—The medium previously described by Kuiken *et al.* (*J. Biol. Chem.*, 1943, **151**, 615; ANALYST, 1944, **69**, 156) was used, except that the glutamic acid was omitted and the equivalent of 0·25 mg of glutamine per tube was added. After the autoclaving and inoculation the tubes were incubated at 35° C. for 72 hr., and the lactic acid formed was titrated. Pure *l*(+)-glutamic acid was used to prepare standard curves, from which the results were calculated. Proteins were hydrolysed by heating 100-mg samples under a reflux condenser for 24 hr. with 10 ml of 6 *N* hydrochloric acid. The hydrolysates were diluted to about 50 ml, filtered and concentrated to a syrup *in vacuo*, and the residue was dissolved in water, neutralised with sodium hydroxide, and diluted to suitable volume. Foodstuffs were dried *in vacuo* for 5 hr. at 95° C. and then extracted with dry ether for 16 hr. The fat-free samples were then hydrolysed in the same manner as the proteins. The recovery of glutamic acid added to protein hydrolysates ranged from 98 to 105% of the theoretical. F. A. R.

Determination of γ-Tocopherol in Vegetable Oils. G. S. Fisher (*Ind. Eng. Chem., Anal. Ed.* 1945, **17**, 224–227)—No vegetable oil has been reported to contain both β- and γ-tocopherol, and only wheat germ oil has been shown to contain β-tocopherol. If the concn. of either α- or γ-tocopherol can be determined, the concn. of the other can then be calculated by difference from the total tocopherol content, for the determination of which several methods are available. Red solns. are formed when either β- or γ-tocopherol is treated in acetic acid soln. with nitric acid, but α-tocopherol gives only a slightly yellow soln. under these conditions. Since the intensities of both the red colour with γ-tocopherol and the yellow colour with α-tocopherol are proportional to the concn. of the tocopherol, it is possible to determine the amount of γ-tocopherol in presence of α-tocopherol by means of the photoelectric colorimeter and the principle of differential spectral separation. The apparatus consists of an Evelyn colorimeter equipped with filters Nos. 490 and 420. Weigh samples of pure tocopherol or oils free from interfering pigments (≡ 0·20 to 2·00 mg of γ-tocopherol) directly into the colorimeter tube and dilute to exactly 9·6 ml with a mixture of acetic acid and chloroform (3 : 2). With the tube in the colorimeter and filter No. 420 in place adjust the light intensity to give a galvanometer reading of 100. Remove the tube and record the reading of the galvanometer (centre setting). Determine the centre setting for filter No. 490 in the same way. Add 0·4 ml of nitric acid to the tocopherol soln. and shake. Adjust the light intensity to give the centre setting for filter 490 and after 30 sec. insert the tube in the colorimeter and record the reading. Repeat the procedure with filter 420. Obtain the L values corresponding with the recorded galvanometer readings from the equation $L = 2 - \log G$, where G is the galvanometer reading. The absorption of light by the oxidation product of α-tocopherol is too great to allow the general use of single-filter photometry, hence the method of differential spectral separation (Knudson *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 715; Ashley, *Id.*, 1939, **11**, 72) must be used. The necessary equations are

$$L^{490} = K\gamma^{490}C\gamma + K\alpha^{490}C\alpha$$
$$L^{420} = K\gamma^{420}C\gamma + K\alpha^{420}C\alpha$$

whence $$C\gamma = \frac{K\alpha^{490}L^{420} - K\alpha^{420}L^{490}}{K\alpha^{490}K\gamma^{420} - K\gamma^{490}K\alpha^{420}}$$

Cα and Cγ are the concn. of α- and γ-tocopherol in g per 100 ml. L^{490} and L^{420} are the L values determined as already described, and the K's are specific L values determined from known mixtures of α- and γ-tocopherol. In oils the % of γ-tocopherol is 10 × Cγ divided by the wt. of the sample in g. Similar equations can be written for β-tocopherol.

Two types of interference occur with highly pigmented oils and concentrates, one due to the bleaching of pigments (*e.g.*, carotene) by nitric acid making preparation of a suitable blank soln. impossible, the other resulting from the development of red to yellow colours by the action of nitric acid on extraneous coloured or colourless substances. The Parker McFarlane treatment of oils (*Canad. J. Research*, 1940, **18B**, 405; ANALYST, 1941, **66**, 209) before treatment with nitric acid removes both types of interference. Dissolve 1 or 2 g of oil in a 100-ml centrifuge tube in 80 ml of purified light petroleum (Skellysolve B), add 10 to 20 ml of sulphuric acid (85% w/w), stopper the tube with a cork coated with sodium silicate and after inverting the tube several times centrifuge the mixture for *ca.* 5 min. at 1500 r.p.m. Wash the supernatant liquid in another tube with *ca.* 20 ml of 1% potassium hydroxide soln. and centrifuge again for *ca.* 10 min. Place suitable aliquots of the light petroleum layer in the colorimeter and remove the solvent on the steam-bath, preferably in an inert atmosphere. Develop and measure the colour as previously described. This treatment also serves to remove

tocoquinones and peroxides from partly oxidised oils. Since amounts of oil greater than 1·5 g do not form homogeneous solns. with nitric acid, concentrate very dil. solns. of tocopherol by saponification in presence of pyrogallol as protective agent, and extract the unsaponifiable portion with peroxide-free ether. Make the dry ethereal extract up to a suitable vol. (*e.g.*, 100 ml), remove the solvent from an aliquot in the colorimeter tube and develop and measure the colour as previously described. Since the wt. of oil represented by the aliquot is known it is unnecessary to weigh the unsaponifiable matter to obtain the % of γ-tocopherol in the oil. A comparison of the absorption curves of the crude reaction product of pure γ-tocopherol and nitric acid with the corresponding curve of tocopherol red suggests that γ-tocopherol is converted into tocopherol red by oxidation with nitric acid in acetic acid soln. and the position of the maximum shows that 96% of the γ-tocopherol is so converted at room temp. in *ca.* 30 sec. On the other hand the dissimilarity of the absorption curve of the oxidation product of α-tocopherol and the complete absence of any extinction maximum in the region 460 to 480 mμ shows that none of the α-tocopherol is converted into tocopherol red under these conditions. Exact determination of the absorption of the reddish violet product from β-tocopherol was difficult owing to rapid fading of the colour. The method used for determination of total tocopherol was that of Emmerie and Engel (*Rec. Trav. Chim.*, 1938, **57**, 1351; ANALYST, 1939, **64**, 216). Although γ-tocopherol is *ca.* 3 times as active an antioxidant as α-tocopherol, it was found that oils having the highest content of γ-tocopherol are not necessarily the most stable. Apparently other factors, such as degree of unsaturation, have to be considered. The method described presupposes absence of β-tocopherol from the oil, but, so far as is now known, few natural oils contain it. A. O. J.

Report of the 1943-1944 Methods of Analysis Sub-Committee on Riboflavin Assay. J. S. Andrews (*Cereal Chem.*, 1944, **21**, 398–407)—This report deals with the alternative systems of purification of cereal extracts (namely "Florisil" for extraction, and permanganate for oxidation) in the determination of riboflavin fluorimetrically. Enriched flour and bread were studied, and it was found that with flour, direct methods give just as satisfactory results as when "Florisil" or permanganate is employed. With bread, permanganate is fairly satisfactory in removing interfering substances, although results were still rather higher than the calculated figure (3·42 μg/g as against 3·16 μg/g). The use of "Florisil" effects no improvement over the direct procedure, both methods giving results considerably higher than the calculated value (4·08 μg/g as against 3·16 μg/g). Micro-biological estimations agree well with the fluorimetric results for flour and both are in agreement with the calculated value. With bread, the mean of the fluorimetric assays was slightly higher than the microbiological mean value, and in closer agreement with the calculated value.

The methods described are. (1) *Direct reading*—Prepare a standard riboflavin soln. of 1 μg per ml. Weigh into 125-ml Erlenmeyer flasks six 1·5-g samples of enriched flour or bread. Suspend uniformly in 50 ml of 0·1 *N* sulphuric acid. Add the following amounts of standard riboflavin solns.: 0, 2, 4, 6, 8 and 10 ml respectively. Heat by autoclaving for 15 min. at 15 lb. pressure, cool and adjust to *p*H 4·3, using 10% sodium acetate (requires approx. 6·3 ml). Transfer to 100-ml volumetric flasks and make up to 100 ml with distilled water. Mix thoroughly and filter, discarding the first 10 ml of filtrate. Measure fluorescence of aliquots. Prepare a fresh cold soln. (in ice-bath) of 5% hydrosulphite in 2% sodium bicarbonate soln. and add 0·5 ml to each of the above aliquots after the original fluorescence has been determined. Mix quickly and determine the fluorescence (this is the blank reading). (2) *Permanganate treatment*—To 25-ml aliquots of filtrates prepared as for the direct reading add 0·2 ml of 4% potassium permanganate soln. Mix, leave for 1 min. and add 0·2 ml of 3% hydrogen peroxide. Measure the fluorescence. Add 0·5 ml of hydrosulphite soln., mix and again measure the fluorescence (blank). (3) *Florisil treatment*—Pass 20-ml aliquots of the filtrates through the Florisil column (*Cereal Chem.*, 1943, **20**, 614), wash and dry, and elute the riboflavin with pyridine acetic acid soln., collecting 20 ml of eluate. Mix thoroughly and measure the fluorescence. Add 0·5 ml of hydrosulphite solution, mix, and measure the fluorescence (blank). Slight corrections on the blank readings are necessary owing to the increased volume of the blank soln. after treatment with hydrosulphite. W. M.

Rapid Method for the Determination of Riboflavin in Wheat and Wheat Products. A. Hoffer, A. W. Alcock and W. F. Geddes (*Cereal Chem.*, 1944, **21**, 524)—The reagents used in this fluorimetric method are (1) A 25% soln. of potassium chloride in 2% acetic acid. (2) A 0·5% freshly prepared soln. of potassium permanganate. (3) Hydrogen peroxide (0·3%). (4) Solid sodium hydroculphite. (5) Standard riboflavin soln. 1μg/ml. *Method*—Place 0·5 g of the sample in a 50-ml centrifuge tube with 20 ml of the potassium chloride soln. Break up any lumps by means of a glass rod. Place in a water-bath for 30 min. at 70° C., covering to prevent evaporation. Remove from the bath, stir thoroughly and centrifuge at 2500 r.p.m. for at least 10 min. Transfer 5 ml of the supernatant liquid to a cuvette and add 5 ml of water. Add rapidly 1 ml of permanganate soln., and after ½ min. add 1 ml of hydrogen peroxide soln. After the gas bubbles have cleared read the fluorescence (A). Add 1 ml of riboflavin standard and again read the fluorescence (B). Add *ca.* 30 or 40 mg of sodium hydrosulphite followed by further additions of 10 mg each, reading the fluorescence after each addition. When an addition of hydrosulphite fails to reduce the reading that reading is taken as the blank fluorescence (C). The riboflavin content of the sample is calculated as follows:

$$\text{Riboflavin } \mu g/g = \frac{A - 1{\cdot}08C}{1{\cdot}08B - A} \times 8.$$

The use of the factor 1·08 is necessitated by the increase in the volume of the liquid in the cuvette from 12 ml to 13 ml upon the addition of the riboflavin standard. It was shown that the size of the sample had some influence on the result, the smaller the quantity taken the higher the result. The filters used were Corning Nos. 511 and 038 for the incident light and 351 for fluorescent light. Owing to the greater stability of the extracts prepared in this way, as compared with those obtained by adsorption and elution, the riboflavin assays by the present method can be made in diffused daylight. Two or three min. after addition of the sodium hydrosulphite the soln. tends to become opalescent owing to pptn. of sulphur. There is, however, sufficient time to determine the "blanks" before this

occurs. The object of dilution after centrifuging prior to the addition of permanganate is to avoid excessive foaming on addition of hydrogen peroxide. The method has one great advantage, namely, the elimination of absorption on Florisil, with all the hazards attending this step. It is possible to carry out a determination in about 70 min. W. M.

Titrimetric Determination of "*Lactobacillus casei* Factor" and "Folic Acid." L. J. Teply and C. A. Elvehjem (*J. Biol. Chem.*, 1945, **157**, 303–309)—The method of Luckey, Briggs and Elvehjem (*J. Biol. Chem.*, 1944, **152**, 157) has been modified to give greater acid production, thus making it more suitable for the titrimetric assay of folic acid, and the medium previously described for assaying the *Lactobacillus casei* factor has been modified. The modifications are, first, the inclusion in the inoculum medium for *Streptococcus lactis* R of 1·0 mg of solubilised liver extract per 10 ml of medium, and in the medium for *Lactobacillus casei* 0·1 mg per 10 ml; secondly the inclusion in the basal medium for *S. lactis* R of 2·5% sodium citrate as buffer, and in that for *L. casei* 2·0% sodium acetate; thirdly, the addition of alanine, *p*-aminobenzoic acid, and Norit-treated peptone (prepared by adjusting a soln. of peptone (50 g) in water (200 ml) to *p*H 3, stirring with 5 g of Norit for 1 hr., filtering and diluting to 500 ml. It should be tested to determine its effect on the blank titration; the maximum amount which does not cause an excessively high blank (1 to 3 mg per tube) is then included in the medium) to the *L. casei* medium; these constituents may be omitted from the *S. lactis* R medium if desired. The basal medium used for both organisms is as follows.

Sodium acetate, 200 mg (for *L. casei* only); K_2HPO_4, 25 mg; sodium citrate, 250 mg (for *S. lactis* only); casein (acid-hydrolysed), 50 mg; glucose, 200 mg; cystine, 2 mg; tryptophan, 2 mg; adenine, 0·1 mg; guanine, 0·1 mg; uracil, 0·1 mg; xanthine, 0·1 mg; asparagine, 1·0 mg; peptone treated with Norit (see text); *dl*-alanine, 2·0 mg; aneurine, 2 μg; riboflavin, 2 μg; nicotinic acid, 6 μg; pyridoxine, 12 μg; calcium *d* pantothenate, 4 μg; biotin, 0·004 μg; *p*-aminobenzoic acid, 0·1 μg; salts,* 0·05 ml; all in 1 litre.

For plugging the tubes a refined grade of cotton-wool should be used, since Sherwood and Singer (*J. Biol. Chem.*, 1944, **155**, 361) showed that significant amounts of folic acid can be leached from ordinary cotton-wool plugs. Incubate for 30 to 72 hr. and titrate the contents of the tubes, using bromothymol blue as indicator in the *L. casei* assays and thymol blue as indicator in the *S. lactis* R assays. Vitamin B_c was used as a standard. The results obtained by the two methods showed excellent agreement for some foodstuffs, but not for others.

F. A. R.

Organic

Acid Salts of Organic Acids as *p*H Standards. J. C. Speakman and N. Smith (*Nature*, 1945, **155**, 698)—Crystalline acid sodium or potassium salts of monobasic organic acids are suggested as *p*H standards. A solution of such a salt is equivalent to a soln. of the acid half neutralised with a strong base and constitutes a readily prepared buffer soln. with a *p*H value slightly lower than the value of *p*K for the acid. Its general usefulness is restricted by the low solubilities of most organic acids and the limited range of dissociation constants among acids otherwise suitable. Preliminary measurements at 20° C. have shown that *M*/50 potassium hydrogen benzoate soln. has *p*H = 4·15 and potassium hydrogen phenylacetate has *p*H = 4·23 on the scale recommended by Hitchcock and Taylor (*J. Amer. Chem. Soc.*, 1937, **59**, 1812; 1938, **60**, 2710). It is pointed out that potassium hydrogen phthalate is not strictly analogous to these compounds, since phthalic acid is dibasic and it is stated that the buffering capacity of phthalic acid—caustic potash mixtures is at a minimum for the composition corresponding to $KHC_8H_4O_4$, and there is only appreciable buffering because pK_1 and pK_2 differ by less than 2·5 units. Borax, equivalent in soln. to $NaBO_2.HBO_2$, is a true buffer and is an acid salt of the above type. J. A.

Observations on Tests for Supposed α-Dicarbonyl Compounds in Autoxidised Fatty Systems. H. Jasperson, R. Jones and J. W. Lord (*J. Soc. Chem. Ind.*, 1945, **64**, 143–145)—The presence of α-dicarbonyl compounds among the secondary products of oxidation of drying oils was postulated by Elm (*Ind. Eng. Chem.*, 1932, **24**, 1044) and by Morrell *et al.* (*J. Soc. Chem. Ind.*, 1936, **55**, 265T). Two colorimetric tests for these compounds have been proposed, that by Prill (*Oil and Soap*, 1942, **19**, 107) depending on oximation and conversion of the dioximes into nickelous, ferrous or cupric derivatives which give coloured solns. in pyridine or benzene, and that by O'Daniel and Parsons (*Oil and Soap*, 1943, **20**, 72) based on the colour produced on saponification of autoxidised oils. Both tests have been applied to the specific dicarbonyls diacetyl and diketostearic acid and to autoxidised ground-nut oil and methyl linolate.

Prill's Method—Add 2 ml of a freshly prepared 0·3% soln. of hydroxylamine hydrochloride in pyridine to *ca.* 0·5 g of the sample contained in a wide test-tube. Heat for 2 hr. at 80° C., cool and add 0·3 ml of acetone to fix the excess hydroxylamine. Leave for 5 min. and then add 1 ml of a 60% aqueous soln. of sodium potassium tartrate to prevent interference by hydroxylamine and free fatty acids, followed by 1 ml of 5% aqueous ferrous sulphate soln., with shaking. Finally, slowly add 4 ml of saturated aqueous potassium hydroxide soln. to salt out the ferrous derivative into the pyridine layer, which becomes red if the test is positive. Two control tests are made, one without the ferrous sulphate and the other without the sample. The method was tested for diacetyl and diketostearic acid by adding these compounds to fresh deodorised ground-nut oil in amounts ranging from 1 to 12 mg. It was found necessary to use six times the quantities of reagents specified above to maintain an adequate excess. The results indicate that the method is quantitative for dicarbonyl compounds; thus, for 2 mg of diketostearic acid, the colour, measured in a 1-cm cell, was 12 and for 8 mg it was 48 Lovibond red units. Further, the colours given by the two compounds examined, whether alone or in presence of ground-nut oil, are shown to be spectroscopically almost identical.

O'Daniel and Parson's Method—Warm 25 g of oil to 68° C. with 25 ml of abs. alcohol (previously distilled from caustic soda) and 10 ml of 50% aqueous caustic potash soln. with continuous shaking until clear. Measure the colour of the alcoholic soln. of soap in a 5¼-in. cell in Lovibond red units and

* $MgSO_4 7H_2O$, 10 g; NaCl, 0·5 g; $FeSO_4.7H_2O$, 0·5 g; $MnSO_4.2H_2O$, 0·337 g; H_2O, 250 ml.

subtract from it the colour of the oil. When this method was applied to known amounts of diacetyl in a fresh oil substrate, there was a quantitative increase in the red colour produced. With diketostearic acid, however, no colour was formed under the conditions of the test, but prolonged refluxing produced an orange colour.

The tests were applied to autoxidised fatty systems by exposing fresh deodorised ground nut oil and freshly distilled methyl linolate in shallow vessels at 50° C. and at 90° C. and examining samples periodically. The results of these expts., which are summarised in a table, indicate that during the major part of the oxidation of both substances the saponification test shows a closer relationship between the tintometric increase in colour and the decrease in unsaturation than does the oximation test, while the latter gives colours with autoxidised fats which are tintometrically identical with, but spectroscopically different from those given by the reference dicarbonyls. This is considered to be evidence that the ferrous complexes formed with autoxidised fats are of a nature fundamentally different from those from diacetyl and diketostearic acid. Absorption spectra of the colours obtained by applying the saponification method to autoxidised ground-nut oil and methyl linolate suggest that the former contains substances which behave similarly to diacetyl, whilst the latter does not. J. A.

Determination of Water in Hydrocarbon Gases. H. Levin, K. Uhrig and F. M. Roberts (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 212–215)—The method is based upon the observation that when a gas containing moisture is brought into contact with cold dehydrated acetone the water is retained by the acetone, in which it can be determined by reaction with acetyl chloride in presence of pyridine. The apparatus consists essentially of a conical 300-ml flask with a ground-in glass head carrying an inlet tube reaching nearly to the bottom of the flask and connected through stopcocks with a 50-ml sample cylinder fitted with needle valves, a pipette with a stopcock at each end, an air inlet through a drying tube containing anhydrous calcium sulphate (Drierite), a mercury manometer and a vacuum pump. Evacuate the flask and the pipette to a pressure of 1 mm of mercury for 15 min. to remove moisture. Isolate the flask by closing the appropriate taps and admit air into it through the drying tube. Remove the flask from its head and allow 5 ml of dehydrated acetone (*infra*) to be drawn up the inner tube into the pipette. Quickly replace the flask, place the weighed sample cylinder in position, re-evacuate the system (excluding the pipette) for 5 min., again isolate the flask, fill it with dry air and quickly place in it 5 ml of pyridine and 25 ml of dehydrated acetone. Replace the flask and cool it to −57° to −62° C. in a bath of solid carbon dioxide and acetone. After 10 min. evacuate the system and allow the whole of the sample to flow from the cylinder into the flask in 1 or 2 min. through the inner tube, which should not reach the surface of the liquid. Remove frozen water from the delivery tube by admitting acetone from the pipette. Remove the cooling-bath and, without detaching the flask, agitate the contents to ensure extraction of the water from the condensed gases, which are usually not soluble in acetone at the temp. of the cooling-bath unless they are rich in olefines. To remove the gases by evaporation, place the flask in the cooling-bath, evacuate to subatmospheric pressure, remove the bath and continue pumping until most of the gas has evaporated. This can be observed from the appearance of the liquid and from the behaviour of the manometer, which will remain at a constant reading until *ca.* 90% of the gas has evaporated and will then show a decrease of pressure. Cut out the vacuum pump, allow the pressure in the flask to increase spontaneously to +20 mm, and gradually release the gas through the drying tube, maintaining the positive pressure by manipulation of the stopcock connecting the flask with the drying tube. When the flask has attained room temp. remove it from the system and quickly insert a glass stopper. Remove the sample container from the system and draw into it 5 ml of dehydrated acetone, shake for a few min., and add the rinsing to the liquid in the flask. This procedure is necessary because gaseous hydrocarbons may contain more water than the liquefied gases can hold at the same temp.; the excess separating and adhering to the cylinder may otherwise be lost from the water determination. Weigh the sample container to ascertain the wt. of gas used. To the contents of the flask add 10 ml of 0·75 *M* acetyl chloride in dry toluene, shake the flask and allow it to stand for 3 min. Decompose the excess of acetyl chloride by adding 1 ml of abs. alcohol and shaking and, after 5 min., add 25 ml of abs. alcohol to form a homogeneous soln. Titrate the liquid to phenolphthalein indicator with 0·1 *N* sodium hydroxide and correct the result by a blank titration with all the reagents. The difference between the acidity of the liquid and that found in the blank determination is a measure of the water present. If the sample consists of hydrocarbons that are not liquid at the temp. of the cooling-bath, it is passed in at a pressure of *ca.* +20 mm and its sp.gr. and volume are determined. If the water content of the sample is known to be low, the amount of acetyl chloride used may be reduced to avoid high blank values. Acetyl chloride in presence of pyridine reacts quantitatively with water to produce 2 mol. equiv. of acid and with abs. alcohol to produce 1 mol. equiv. (Smith and Bryant, *J. Amer. Chem. Soc.*, 1935, **57**, 841).

Pyridine containing up to 1 mg of water per ml is satisfactory for use. To dehydrate acetone, treat it with calcium chloride for several days, filter and shake the filtrate with excess of pyridine and acetyl chloride. Esterify the excess of acetyl chloride by shaking the mixture with amyl alcohol for 10 min. and fractionally distil the acetone from the higher-boiling ester, with precautions against inclusion of moisture. For each g of water in the acetone use *ca.* 30 ml of pyridine, 10 ml of acetyl chloride and 20 ml of amyl alcohol. The water content may thus be reduced to 0·05 mg per ml, although up to 0·5 mg per ml may be tolerated. A. O. J.

Analysis of the Light Constituents in Crude Petroleum by Low-temperature Fractional Distillation. R. J. Askevold and M. S. Agruss (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 241)—It is often desirable to know the composition of the light gases occurring in crude oils, especially with the increasing use of these in production of aviation spirit. The low-temp. fractionating columns commonly used for gas analysis are not entirely suitable for determination of the small amounts of light constituents of crude oils, and difficulty is also caused by formation of ice in cold portions of the column, with resulting blockage and leakage. The apparatus described permits the use of a sample large enough to provide measurable amounts of butane and lighter gases for distillation in an ordinary low-temp.

fractional distillation column and at the same time removes water from the portion of the sample that enters the column. The auxiliary still, which may be used with any low-temp. column having a still body of 20-ml capacity or more, consists of a 500-ml or litre round-bottomed flask having an inlet tube with stopcock fused into the side near the neck. The neck carries a vertical ground-in column of diam. 24 mm terminating in an adapter for a thermometer reading from 0° to 100° C. Eight in. from the orifice of the flask a side tube of diam. 6 mm leads through a stopcock to a conventional low-temp. fractionating column. Before an analysis the column of the auxiliary still is packed with pellets of sodium hydroxide and a few are placed in the flask to facilitate boiling. Evacuate the entire system to a pressure of less than 1 mm of mercury. Cool the reflux condenser of the fractionating column to below the b.p. of methane, place a Dewar flask containing acetone and "dry ice" round the inlet tube of the fractionator and introduce the sample slowly into the flask. Immediately apply an electric heater to strip light constituents from the crude oil. During distillation water and hydrogen sulphide will be removed in the pellet-packed column, heavier hydrocarbons will condense in the fractionator, and lighter ones will be condensed by the reflux condenser. Heating of the auxiliary still should be slow enough for effective fractionation but should be continuously increased to ensure a slightly higher pressure in the auxiliary still than in the fractionator, to prevent flowing back of cold condensate into the auxiliary column. If the pressure of the fractionator reaches atmospheric pressure, distil methane off at such a rate as to maintain atmospheric pressure. The crude oil may safely be assumed to be free from butane and lighter gases when the temp. at the top of the auxiliary column reaches 55° C. At this point remove the electric heater and close the connection between the two columns. Fractionate the condensate in the usual way to separate methane, ethane, propane, *iso*butane and *n*-butane and then distil the residue of pentanes and heavier hydrocarbons back into the stripped crude oil by cooling the auxiliary still with "dry ice." Calculate the % of each light hydrocarbon on the basis of the water- and hydrogen sulphide-free crude oil by weighing the crude residue. If it is desired to fractionate the stripped residue to obtain further data, transfer the flask containing it to any suitable crude assay column and continue the distillation. Analyses made by the method described agreed well with those made with the regular debutanising equipment. A. O. J.

Determination of Aniline in Alkylanilines. J. Haslam and P. F. Hearn (*J. Soc. Chem. Ind.*, 1945, **64**, 152)—Spencer and Brimley (*J. Soc. Chem. Ind.*, 1945, **64**, 53) have stated that in the determination of dimethylaniline (ANALYST, 1944, **69**, 141) the acid concn. is rather critical and they prefer to neutralise the aliquot of the diluted acetylation mixture (indicator phenolphthalein) and add 10 ml of *N*/10 hydrochloric acid. Results of test investigations, here given, show that it is the concn. of mineral acid which is important; acetic acid has little influence on the test. E. B. D.

Determination of Nitriles by Means of the Karl Fischer Reagent. J. Mitchell, Jr., and W. Hawkins (*J. Amer. Chem. Soc.*, 1945, **67**, 777–778)—The method is based on hydrolysis of the nitrile to the amide, using an excess of water in presence of boron trifluoride and acetic acid, followed by titration of the unused water with Karl Fischer reagent. It is generally suitable for determining normal alkyl cyanides, and quantitative results have been obtained with the normal lower aliphatic nitriles, nitriles of dibasic acids and several aromatic cyanides. Prepare the hydrolytic reagent by dissolving 300 g of dry boron trifluoride gas and 6·5 ml of water in 500 ml of glacial acetic acid ($BF_3.2CH_3COOH$); prepare the Karl Fischer reagent according to Smith, Bryant and Mitchell (*J. Amer. Chem. Soc.*, 1939, **61**, 2407). Weigh the sample, containing up to 10 mg-equivalents of nitrile, into a 250-ml glass-stoppered volumetric flask. Add 20 ml of hydrolysis reagent. Tightly stopper the flask and place it in an oven or water-bath at 80 ± 2° for 2 hr., with duplicate blanks. Remove the flasks, allow them to cool to room temp., place in finely-chopped ice, and add carefully 15 ml of dry pyridine (pyridine combines with the boron fluoride, preventing esterification with methanol during the titration with Karl Fischer reagent). Titrate directly with Karl Fischer reagent. The nitrile group does not interfere, and the sample can be titrated directly for free water. The net titre plus water originally in the sample is a direct measure of the equivalents of cyanide. The following results are quoted.

	Found, weight %		
Nitrile	Nitrile	Water	Total
Acetonitrile	97·6 ± 0·2	2·3	99·9
Propionitrile	98·7 0·2	1·6	100·3
Cyanoacetamide ..	93·6 0·4	0·8	94·4
n-Butyronitrile ..	99·0 0·2	1·2	100·2
n-Valeronitrile ..	99·4 0·0	0·4	99·8
Lauryl cyanide ..	97·7	0·1	97·8
Adiponitrile(*a*) ..	99·8 0·2	0·0	99·8
Sebaconitrile(*a*) ..	100·0 0·4	0·0	100·0
Phenylacetonitrile ..	99·6 0·2	0·2	99·8
m-Toluonitrile ..	100·6 0·2	0·1	100·7
p-Toluonitrile ..	100·8 0·4	0·1	100·9
p-Chlorobenzonitrile ..	97·6 0·3	0·1	97·7
β-Naphthonitrile ..	96·5 0·4	0·1	96·6

(*a*) du Pont; all others Eastman Kodak chemicals used without further purification.

Nitriles which gave low totals were assumed to contain inert impurity. Other nitriles gave incomplete but fairly precise results. *o*-Toluonitrile and α-naphthonitrile gave values of 89·5 ± 0·5 and 80·9 ± 0·2%, respectively. Methyleneamino-acetonitrile gave only 80·9 ± 0·2% reaction, presumably because of the inhibiting influence of the amino group. Cyanoacetonitrile gave values of 88·8 ± 0·9% and cyanoacetic acid only 37·1 ± 1·4%.

Materials which interfere with the Fischer hydroxyl procedure (Bryant, Mitchell and Smith, *J. Amer. Chem. Soc.*, 1940, **62**, 1) interfere with the nitrile technique. Alcoholic hydroxyl esterifies, eliminating an equivalent amount of water; if the hydroxyl content can be determined by an independent method, suitable corrections can be applied (the acetyl chloride method of Smith and Bryant, *J. Amer. Chem. Soc.*, 1935, **57**, 61, can be used in absence of amino-nitrogen). Cyanohydrins are readily hydrolysed. The normal hydroxyl esterification catalyst (100 g of BF_3 per litre) hydrolysed acetaldehyde cyanohydrin to *ca.* 60%; the net increase in water after 2 hr. at 65° C. was equivalent to *ca.* 40% of the hydroxyl. Under the conditions of the general nitrile procedure both reactions are quantitative, giving a net water balance of zero.

Little interference is observed with amines; formamide, acetamide and adipamide gave values of 0·0, 0·3 and 0·2% respectively. E. M. P.

Determination of *p*-Nitrophenol in Full Chrome Leather. R. Ganday (*J. Internat. Soc. Leather Trades' Chem.*, 1945, **29**, 143–145)—Determinations of *p*-nitrophenol in the commercially pure substance, by Koppeschaar's method (*Z. anal. Chem.*, 1876, **15**, 232; *cf. Sutton's Volumetric Analysis*, 1935, p. 415) for phenols gave reliable results for 20 to 30-mg samples. For leather, the method is as follows. Extract 10 g of air-dry leather, cut into pieces *ca.* 1 cm. square, in a Soxhlet extractor with ether (sp.gr. 0·720) for 2 hr. Distil off the ether and evaporate to dryness on the steam-bath, cool and dissolve the residue in 20 ml of light petroleum, b.p. 40–60° C. Pour the soln. into an extraction tube (containing water) which is stoppered at the bottom by a two-holed cork, with cotton-wool plug above it. An outlet tube with tap passes through one hole; through the other is a tube which is bent horizontally, then vertically upwards and again horizontally and narrowed to an opening over a beaker, containing 1 ml of 30% sodium hydroxide soln. Rinse the flask with a further 10 ml of light petroleum. Extract the *p*-nitrophenol by running in a fine jet from the funnel above it. The drops of water should be very small, but emulsions should not pass the cotton-wool plug. Rinse the light petroleum extraction flask with water into the funnel. Cover the beaker and tube with a watch-glass and, when the level of liquid has risen sufficiently, boil the water so that the level is kept constant as fresh water drips in from the tube. Continue the extraction until a little of the soln. obtained from the other outlet tubes gives no further yellow colour with 30% sodium hydroxide soln.; then evaporate the soln. in the beaker down to 20 ml, cool, rinse into a glass bottle with 10 ml of water, add 25 ml of bromate-bromide soln., *ca.* N/10, and 10 ml of conc. hydrochloric acid and stopper quickly with a glass stopper. Shake occasionally for 15 min., add 50 ml of water and 10 ml of 20% potassium iodide soln., and titrate back with *N*/10 sodium thiosulphate; make a blank test on bromate soln. For *N*/10 bromate, 1 ml ≡ 0·003475 g of *p*-nitrophenol. E. B. D

Determination of Nitrogen in Nitriles. E. L. Rose and H. Ziliotto (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 211–212)—The few methods hitherto available for determination of nitrogen in nitriles are not useful when ease of manipulation and rapidity are desired. The procedure here described depends upon the reducing action of hydriodic acid, but, since hydriodic acid is not a practical reagent, a mixture of potassium iodide and conc. sulphuric acid is used. To the sample (≡ 40 to 60 mg of nitrogen) in a digestion flask add 1·5 g of potassium iodide and 30 ml of conc. sulphuric acid and heat the mixture on the steam-bath for 45 min. with occasional shaking. Add 10 g of potassium sulphate, 0·3 g of anhydrous copper sulphate, 0·1 g of selenium and a few glass beads. Heat the mixture gently at first, then boil briskly and continue the digestion for 1 hr. after the mixture becomes of a clear green colour, avoiding reduction of the volume below 20 ml. Remove iodine from the neck of the flask, if necessary, by application of a flame. Cool the mixture, add 250 ml of water and, after further cooling, pour down the side of the flask enough 50% sodium hydroxide soln. (*ca.* 90 ml) to make the liquid alkaline, but avoid mixing the alkali with the acid soln. Add several pellets of zinc to prevent bumping and distil into a measured amount of 0·1 *N* sulphuric acid. Usually 125 to 150 ml of distillate will contain all the ammonia. Titrate the distillate with 0·1 *N* sodium hydroxide, using a mixed indicator containing 0·9 g of sodium alizarin sulphonate and 0·125 g of indigo carmine in 100 ml of water. At the end-point the green colour becomes bluish-grey. Correct the result by a blank. Several nitriles were analysed by this method with good results, and since two of these, *viz.*, acrylonitrile and acetonitrile, boil below 100° C., the applicability of the method to volatile compounds is demonstrated. To establish the accuracy of the method, the nitrogen content of some cyclic and aliphatic nitriles was determined by the Friedrich modification of the Kjeldahl method (Friedrich *et al.*, *Z. physiol. Chem.*, 1933, **216**, 68) adapted to a macro scale, as well as by the proposed method. For this purpose 2 ml of hydriodic acid (sp.gr. 1·7), a pinch of red phosphorus and the weighed sample were heated in a sealed tube at 200° C. for 1 hr. The contents of the tube were then treated as in the Kjeldahl method. Results by the two methods agreed well. Compounds having a nitro group as well as the nitrile group yielded all their nitrogen, but with some types of nitrogen compound requiring special treatment before the Kjeldahl digestion the method was unsuccessful. Naphthalene-β-azo-*p*-dimethyl aniline yielded all its nitrogen, but only part of the nitrogen was obtained from *m*-xylene-azo-β-naphthol. Apparently, substituent groups in certain positions hinder the reduction. Unsuccessful results were obtained also with hydrazine, pyridine and inorganic nitrates. A. O. J.

Inorganic

Determination of Small Amounts of Nickel in Aluminium Alloy (Duralumin). M. D. Trikov and V. A. Lapshina (*Zavod. Lab.*, 1941, **10**, 253–256. Translation in *Metallurgia*, 1945, **31**, 261–262)—Low results were obtained when two recognised procedures, using dimethylglyoxime, were applied to the determination of small amounts (0·005 to 0·1%) of nickel which had been added to solns. of nickel-free aluminium and duralumin. Losses may be caused by high nitrate concn., pptn. from too dilute a soln., use of excess of alcoholic reagent soln., and presence of large amounts of copper in absence of tartaric acid. The modified procedure proposed is as follows. Place 5 g of sample in a 500-ml conical flask and add 50 ml of cold 15% potassium hydroxide soln. After the first vigorous reaction add a further 50 to 70 ml of potassium hydroxide soln., and when hydrogen evolution ceases add 100 ml of water and heat to boiling for 1 to 2 min. Filter off the insoluble matter and wash 6 times with hot water. Dissolve in a mixture of 5 ml of hydrogen peroxide (the strength is not explicitly given.—ABSTRACTOR) and 20 ml of hot diluted hydrochloric acid (1+1) and wash the paper with hot water. Boil the soln. to remove chlorine and evaporate to 20 ml. Cool somewhat, add 15 ml of 25% tartaric acid soln., neutralise with ammonia soln. to form the deep blue copper complex and add 1 to 2 ml excess. Stir and examine the soln. for any sign of a ppt. If one is detected, allow it to coagulate and filter off. Make acid with diluted hydrochloric acid (1+1), evaporate to 50 to 60 ml and cool to 60 to 70° C. Add 5 ml of alkaline dimethylglyoxime soln. (30 g in 1 litre of 3% potassium hydroxide soln.) and then enough ammonia soln. to form the

deep blue complex and 2 ml in excess. If no ppt. appears immediately (less than 0·01% of nickel), rub the sides of the beaker carefully with a glass rod while the beaker is cooling in a stream of water. Keep the soln. warm for 2 hr., filter, wash 3 times with hot* water and redissolve in 10 ml of hot diluted hydrochloric acid (1+1), washing the filter 4 times. Add to the soln. 2 ml of tartaric acid soln., 2 ml of dimethylglyoxime reagent and sufficient ammonia soln. to make the soln. neutral to methyl orange. Add a further 1 ml of ammonia soln. and keep the whole warm for 30 min. Filter through a weighed sintered glass crucible (No. 2 porosity), wash 8 times with hot water, dry for 1 hr. at 110 to 120° C., cool and weigh. Results should be accurate to 0·0002% up to 0·01% of nickel and to 0·005% with 0·05%. L. A. D.

Determination of Iron in Presence of Cobalt. Two-component Colorimetric Method. E. A. Brown (*Ind. Eng. Chem., Anal. Ed.*, **1945**, **17**, 228–230)—The method is an adaptation of the thiocyanate colorimetric method. Two readings are taken with a photoelectric absorptiometer, with different filters, which makes it possible to calculate the iron concn. in presence of large amounts of cobalt. The filters are selected to cover portions of the spectrum where the two ions show their largest differences of absorption. The formula used depends on the coloured ions obeying Beer's law, and on their behaving independently of one another. The constants involved are determined from the electrophotometer curves for separate solutions of iron and cobalt; two sets of curves are obtained, one with a filter giving absorption in the region of 525 $m\mu$ (filter A, green) and one in the region of 425 $m\mu$ (filter B, blue). The formula derived is:—$c_1=(A_2L_B-B_2L_A)/(B_1A_2-B_2A_1)$, where c_1 is the iron concentration, L_A the log transmittancy of the mixed solution with filter A, L_B the same with filter B (direct instrument readings can be used where the electrophotometer has a logarithmic scale), A_1 and B_1 are the slopes of the calibration curves (log transmittancy/concentration) for pure iron solutions with filters A and B, and A_2 and B_2 are the slopes for cobalt solutions. A commercial electrophotometer with 2 cm. cylindrical absorption cells was used.

Method—Iron soln.—Dissolve 0·05 g of electrolytic iron wire in hydrochloric acid, oxidise by boiling with hydrogen peroxide and finally with a slight excess of potassium permanganate, and make up to 1 litre. *Cobalt soln.*—Use a reagent grade cobalt salt and free it from iron by precipitation with ammonia and peroxide; filter, deposit the cobalt electrolycally and redissolve. Make up into solution as chloride to give 1 to 2 mg of cobalt in 1 ml. *Ammonium thiocyanate soln.*, 10%. *Standards*—Take known amounts of iron or cobalt solns. in a 100-ml standard flask. Add 5 ml of hydrochloric acid and make up to roughly 90 ml. Add 5 ml of thiocyanate soln. and make up to volume. Measure the colour immediately with the blue and green filters. Use a blank containing 5 ml of hydrochloric acid, 5 ml of thiocyanate soln. and water to 100 ml for the photometer zero adjustment. Up to 5 p.p.m. of iron were estimated in presence of up to 90 mg of cobalt in 100 ml of soln. with a maximum error of 3%. The effect of fading was eliminated by taking the same time for both standards and samples and by avoiding bright lights. Acetone is not used as a stabiliser as it intensifies the cobalt ion colour.

C. F. P.

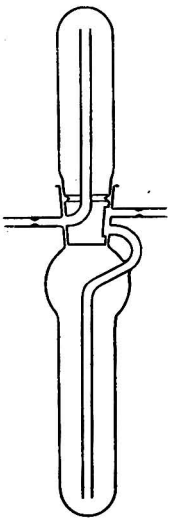
Gravimetric Determination of Silicon in Aluminium Alloys. G. H. Osborn and J. Clark (*Metallurgia*, **1945**, **31**, 230–232)—A method, first proposed by Fuchshuber (*Z. anal. Chem.*, **1939**, **116**, 421) has been modified to give greater accuracy and cover a wider range of silicon contents. It is based upon solution of the sample in a mixture of phosphoric, nitric and sulphuric acids and subsequent evaporation until the yellow soln. becomes colourless. This converts all the silicon directly into silica without introduction of the sodium silicate stage, common to other methods. The addition of cobalt nitrate makes this colour change more decisive, and this, coupled with slight adjustments in the composition of the acid mixture, renders the method suitable for aluminium alloys containing from 1·5% of silicon upwards. *Acid mixture*—Three parts of phosphoric acid (sp.gr. 1·7), 4 parts of nitric acid (sp.gr. 1·4), 1 part of sulphuric acid (sp.gr. 1·84). For contents up to 4% of silicon take a 2-g sample, for contents of 5 to 15% a 1 g, and for contents of 15 to 25% a 0·5-g sample. Dissolve in a covered 800-ml, tall-form beaker in 80 ml of acid mixture (60 ml for a 0·5-g sample). Add 0·1 g of ammonium nitrate to clear colloidal sulphur, allow to cool slightly and then add cautiously 0·1 g of cobalt nitrate dissolved in 100 ml of diluted hydrochloric acid (1+4). Evaporate until the green colour changes to clear blue, and then remove from the hot-plate immediately. Cool, dilute with 40 ml of *boiling* water, boil for 5 to 10 min. and filter on a Whatman 541 paper. Wash free from acid with boiling water, dry, ignite in a platinum crucible and weigh. The purity of the silicon ppt. may be checked by evaporation with hydrofluoric acid, but this is usually unnecessary. If the iron content of the alloy is very low, add 0·2 g of ferric chloride to accentuate the colour change. C. F. P.

Microchemical

Apparatus for Micro-titrations with a Glass Electrode. J. R. Catch, A. H. Cook and J. A. Kitchener (*J. Chem. Soc.*, **1945**, 319–320)—The titration cell is constructed from glass tubing of bore slightly larger than the bulb diameter of the glass electrode. A U-shaped capillary is sealed to the lower end and is filled with 2% agar gel in satd. potassium chloride soln., to allow connection through a salt bridge to a calomel electrode. The e.m.f. is measured on a valve electrometer—*p*H meter. From 2 to 5 mg of the sample are weighed into the cell and dissolved in 0·1 to 0·2 ml of water or other solvent (sufficient to surround the bulb of the glass electrode). The micro-burette is constructed from a 1-ml graduated pipette. Expulsion of acid or alkali is controlled by addition of mercury from a dropping funnel having a capillary outlet. Quantities of reagent of *ca.* 0·01 ml, which can be read to the nearest 0·001 ml, may be delivered without difficulty. After each addition mixing is effected by moving the glass electrode up and down. With $N/20$ reagents, the accuracy in determining the equivalent value of simple compounds (*e.g.*, benzoic acid) is *ca.* 1 part in 500, and a complete titration curve may be constructed, using only micro-quantities of material. J. T. S.

* Washing with *hot* water is usually considered likely to carry some ppt. through the filter.—EDITOR.

Closed Absorption Tube for Micro-determination of Carbon and Hydrogen. A. Langer (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 266)—The tube eliminates the air jacket of the Friedrich design and the cap and sleeve of the Abrahamczik modification (*Mikrochem.*, 1937, **22**, 227). It is 110 mm in overall length, 10 mm in outside diam., and is made of Pyrex glass. The inner capillary tubes are 2 mm, and the side arms 4 mm, in outside diam. A groove ground near the edge of the inner joint prevents squeezing out of lubricant. Before filling (Dehydrite and/or Ascarite) the inside capillaries and ground joint are protected by cotton plugs and a paper sleeve respectively. When the absorbent in the upper part is almost exhausted (after *ca.* 0·2 g of carbon dioxide or 0·1 g of water have been absorbed), this portion of the tube is refilled. After *ca.* 10 partial fillings, the whole tube is cleaned and refilled. An alternative design, in which the small outside loop of the lower capillary is eliminated, is also described. J. T. S.



Titration of Microgram Samples. A. G. Loscalzo and A. A. Benedetti-Pichler (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 187–191)—The technique was developed to permit the use of reagents of customary concn., instead of the more dil. solns. which have been employed previously. Operations are carried out under a low-power microscope fitted with an eyepiece micrometer. The stage carries a "moist chamber" (Benedetti-Pichler and Rachele, *Ind. Eng. Chem., Anal. Ed.*, **1940, 12, 233**), in which reagents are stored and operations are carried out to prevent evaporation errors. The "burette," which has a capacity of 0·05 cu. mm, is made by drawing heavy-walled Pyrex tubing into capillary of 0·5 mm diameter and of *ca.* 0·2 mm bore. The latter is measured and the capillary is cut into 20-cm lengths, each of which is converted into two burettes by heating in the middle and drawing out, preferably by Rachele's device (Benedetti-Pichler and Rachele, *loc. cit.*; the device automatically pulls out the tube while withdrawing it from the zone of heating). Clean air is forced through the burette, while the fine nozzle is immersed in smoking hot paraffin, and is then very slowly withdrawn. The end of the nozzle is then snapped off until an orifice of *ca.* 20 μ is obtained. A reference mark is scratched 3 to 5 mm from the beginning of the taper after the shank adjacent to the taper has been inspected for uniformity. The burette is held horizontally by a rubber-packed gland in a tubular metal holder which is held in the clamp of a mechanical manipulator. The outer end of the holder is connected to a pair of levelling bulbs containing water to enable pressure to be applied, so that outflow of reagent occurs at the desired rate when the burette-tip is immersed in the soln. being titrated, but ceases when the tip is withdrawn. The burette is calibrated by collecting the discharged liquid in a length of capillary, the 0·2 mm bore of which has been measured and is uniform for 5 mm from the freshly-cut end. It is desirable to calibrate with the soln. to be dispensed, by comparing the movement of the meniscus of the soln. in the burette with that produced in the calibration capillary. Though expts. showed that a comparatively large amount of water (*e.g.*, 10% and 24% of the capacity for bores of 0·11 and 0·26 mm respectively) remained on the walls of the burette, significant drainage error was not observed, owing to the low rate of travel of the meniscus (0·005 to 0·08 mm per sec.). Pyrex capillary of *ca.* 0·5 mm bore and *ca.* 0·7 mm outside diam., the end of which is drawn down to 0·2 mm bore, is used as a titration vessel. The soln. to be titrated is placed in the conical portion, and is stirred by moving back and forth by a plunger device attached to the remote end of the capillary. The opening of the titration vessel and the burette tip are viewed and contact of the latter with the soln. to be titrated is made and broken. The standard solns. must be absolutely clear. In such small vols., observations of colour change is difficult. (An average deviation of ±25 parts per 1000 was obtained in titrating 0·5 *M* sodium hydroxide soln. with 0·25 *M* sulphuric acid, even when the former was satd. with methyl red). Acid-base titration, using the indicator system iodide-iodate-starch, and titration of chloride with 0·1 *M* silver nitrate soln., using dichlorofluorescein, allowed the colour change to be observed on the particles and gave a precision of ±8 to ±15 parts per 1000. J. T. S.

Colorimetric Method for the Micro-determination of 2:2-Bis-(*p*-chlorophenyl)-1:1:1-Trichloroethane (DDT). H. A. Stiff, Jnr. and J. C. Castillo (*Science*, 1945, **101**, 440–443)—When DDT is heated in anhydrous pyridine soln. containing xanthydrol (9-hydroxyxanthene) and potassium hydroxide, a red colour develops, which under proper conditions is proportional to the amount of DDT present. The reaction will detect as little as 10 μg of DDT. Expts. with solns. of pure DDT in ether are described. *Xanthydrol-KOH-Pyridine Reagent*—Prepare freshly each day and mix well before using. Place 50 ml of a 0·2% soln. of xanthydrol in clear colourless pyridine in the 500-ml flask of an all-glass reflux apparatus. Heat over an asbestos-centre gauze with a moderate flame. As soon as boiling begins, add directly from the bottle 25 pellets (aver. weight 125 mg) of potassium hydroxide, which must not have undergone deliquescence. Continue boiling, swirling at intervals of 15–20 sec. until the supernatant becomes dark green. Decant hot from the undissolved potassium hydroxide into a dry Pyrex flask, when the green colour disappears. *Procedure*—Transfer the soln. of DDT (up to 200 μg) in ether to a dry test-tube (16 mm × 150 mm) and place the latter in an oil-bath at 120° C. for a few min. to evaporate ether. Remove from the bath, add 2 ml of reagent and replace in the bath for 8 min. Then immerse in cold water for 1 min. and wipe. (During heating, various colours are produced; after cooling, only a red or the initial yellow colour remains.) Add 4 ml of pyridine, mix by inversion, transfer to the dry tube of a photoelectric colorimeter and take the reading within 10 min. of removing from the oil-bath, using a green filter. (A Hellige-Diller Model No. 400 instrument with a green filter No. 520 was used. The cell was a Hellige No. 452-D.) For amounts of DDT up to *ca.* 120 μg the colorimeter reading—concn. graph is linear. Different batches of reagent give constant results, so that a standard curve may be constructed. Water and alcohol inhibit production of the red colour, and both must be absent. J. T. S.

Acrolein Determination by Means of Tryptophan. A Colorimetric Micro Method. S. J. Circle, L. Stone and C. S. Boruff (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 259–262)—Most of the colorimetric methods available for the determination of acrolein have such disadvantages as non-specificity, highly coloured blanks or no absorption max. in the visible range of the spectrophotometric absorption curve. The colour test now presented was suggested by the protein-nitrite-hydrochloric acid test of Voisenet (*Ann. Inst. Pasteur*, 1918, **32**, 476) for acrolein and formaldehyde. Investigation of the reaction showed that tryptophan was a better reagent than the egg-white soln. recommended, and that the trace of nitrite was actually deleterious. Omission of the nitrite gives a colourless blank test and the purity of the colour of the tryptophan-acrolein complex is considerably improved. The two samples of acrolein used presumably contained hydroquinone as polymerisation inhibitor. Portions of these were distilled, and the distillates were collected with and without hydroquinone. Standard solns. of the two original and the two re-distilled samples were prepared containing 100 μg of acrolein per ml of aldehyde-free 95% ethanol. No difference was found in the standard curves made from these four samples. The standard soln. in ethanol retains its strength for several weeks unless hydroquinone is absent when it begins to lose strength in a week or two. A standard soln. of acrolein in water is less stable. The tryptophan soln. (0·01 *M*) was made by dissolving the requisite amount of crystalline (*l*-)- or *dl*-tryptophan in 0·05 *N* hydrochloric acid and is stable when kept in amber-coloured bottles with a few drops of toluene. Solns. of aldehydes other than acrolein were used at a concn. of 0·01 *M*. In order to obtain consistent data the time and temp. of development of the colour and the concn. of the reactants must be standardised, and in studying the effect of variation of a particular constituent on the development of the max. colour the conc. of every other constituent must be held constant. For example, in establishing one of the standard curves, a series of samples was made up in 10-ml volumetric flasks by pipetting into each 0·5 ml of 0·01 *M* tryptophan soln., amounts of acrolein in 95% ethanol (100 μg per ml) ranging from 0 to 2 ml, enough 95% ethanol to make its total vol. in each flask 2 ml and 1·2 ml of water. The flasks were cooled in an ice bath and made up to vol. with ice-cold 12 *N* hydrochloric acid to avoid premature heating on mixing. If desired the order of addition of water and hydrochloric acid may be reversed to give the same results, provided all ingredients are ice-cold, *i.e.*, 6·3 ml of 12 *N* hydrochloric acid may be added to the contents of each flask and the dilution to 10 ml made with water. The colour is developed in a bath at 40° C. for 50 min. in subdued light and the extinction is read in the spectrophotometer (Coleman Model 11 with matched square cuvettes with a path of 13·06 mm, a PC-4 filter and water as the comparison standard). In the analysis the procedure is the same except that acrolein samples of unknown strength must be either concentrated by distillation or diluted (as indicated by a preliminary test) for their concn. to fall within the limits of the standard curve. Since the temp. does not rise above 40° C. on adding the acid at room temp., cooling may be dispensed with, but the results are somewhat less consistent. The reaction was studied with glyoxal, formaldehyde, acetaldehyde, crotonaldehyde and furfural as well as with acrolein. Under the same conditions acrolein reacts faster and develops a colour, with max. sensitivity at 555 mμ, deeper and purer than the colour developed by any of the other aldehydes used. With formaldehyde a deep blue colour with max. absorption at 560 mμ does develop if the reacting mixture stands overnight or if a higher temp. is used. A trace of an oxidising agent, *e.g.*, a nitrite, hastens the reaction with formaldehyde and acetaldehyde, but is not necessary with acrolein. The most favourable ratio of tryptophan to acrolein is *ca.* 2 mol. equiv. to 1 mol. equiv., but this ratio is not critical. With the concn. of tryptophan adopted in the proposed method of analysis (0·005 mg-mol. in 10 ml) no significant error is introduced by excess of tryptophan in the lower range of the standard curves, where the ratio may be as high as 25 mol. equiv. per mol. equiv. Formaldehyde and acetaldehyde in concn. equal to that of acrolein were found to depress the max. extinction value reached. Acrolein cannot therefore be determined in presence of other aldehydes by this method unless their concn. is relatively much less than that of the acrolein. The calibration curves show that in the range 15 to 150 mμ Beer's law is obeyed. A. O. J.

Desiccants in Microchemical Analysis. K. C. Barraclough (*Metallurgia*, 1945, **31**, 269–272)—The author summarises the properties and uses of a number of desiccants and emphasises the fact that the choice of a suitable one depends on the efficiency of drying required. The final absorbent for water in an absorption train should also be used in the preliminary purifying train and must be the most efficient desiccant present. If carbon dioxide is to be determined as well, a layer of the final desiccant used in the train should be packed into the carbon dioxide absorption tube. The desiccants are grouped according to their efficiency; their properties and uses are also discussed. Group I contains the final desiccants, those in Group IV are merely preliminary moisture removers, and those in Groups II and III should be used as preliminary driers to be followed by a Group I reagent. Group I (<0·005 mg of residual water per litre at 30·5° C.). (*a*) Phosphorus pentoxide—probably the most efficient desiccant and capable of leaving a residual moisture content of 0·00065 mg per litre. The tendency to block gas channels as it liquefies may be checked by packing between layers of glass wool, but the use of a preliminary desiccant is preferable. (*b*) Barium oxide—absorption capacity 11% of its own weight. Swells on absorption of water. Can be used for drying ammonia and is efficient at comparatively high temp. (*c*) Anhydrous magnesium perchlorate ("Anhydrone")—absorption capacity 30%. Almost as efficient as (*a*) and is as efficient at 135° C. as at room temp. Reactivated by heating *in vacuo* at 225–250° C. Contracts slightly on absorption of water and does not liquefy. Can be used for drying chlorine, hydrogen chloride, hydrogen sulphide but not ammonia. (*d*) Calcium oxide—can be used for drying ammonia, but affinity for carbon dioxide precludes its use in combustion trains. Traces of silica and alumina lower its absorptive properties. (*e*) Anhydrous calcium sulphate ("Anhydrite" or "Drierite")—absorption capacity 6·6%. Can be regenerated. (*f*) Activated alumina ("Hydralo")—as efficient as (*a*) with a slow gas flow. Can be regenerated and is marketed in an indicating form. Group II (0·01 to 0·02 mg of residual water per litre). Potassium hydroxide. Group III (0·02 to 0·03 mg of residual water per litre). (*a*) Silica gel—can be

repeatedly regenerated and is available in an dicating form. (*b*) Magnesium perchlorate trihydrate ("Dehydrite")—absorptive capacity 15%, neutral in reaction. Efficiency falls rapidly with rise in temp. Group IV (0·1 to 0·3 mg of residual water per litre). (*a*) Dehydrated calcium chloride (fused)—usually contains free lime and is alkaline in reaction. It should not be used, therefore, in carbon dioxide absorption trains. In addition, "ascarite" (asbestos impregnated with sodium hydroxide), a common absorbent for carbon dioxide is itself a more efficient desiccant than calcium chloride. Not recommended for desiccators. Cannot be used for drying ammonia. Is less efficient at 30° than at 0° C. (*b*) Sodium hydroxide. (*c*) Anhydrous barium perchlorate ("Dessichlora")—absorptive capacity 15%. More efficient than calcium chloride at room temp. Can be regenerated by heating at 400° C. (*d*) Zinc chloride. (*e*) "Anhydrous" calcium chloride ($4CaCl_2.H_2O$). (*f*) Granular calcium chloride—alkaline in reaction. (*g*) Anhydrous copper sulphate. Figures available for concentrated sulphuric acid are contradictory, but it is probably a Group I reagent. A little water should be added before use to dissolve sulphur trioxide. Any dissolved sulphur dioxide or carbon dioxide can be removed by passing a current of air through the acid. Acid of 95% strength is still more efficient than calcium chloride. C. F. P.

Physical Methods, Apparatus, etc.

Spectrographic Determination of Sodium, Potassium and Lithium in Portland Cement. A. W. Helz (*J. Res. of Nat. Bur. Stds.*, 1945, **34**, 129–142)—For the determination of sodium and potassium, a small sample of the cement is mixed with three times its weight of a synthetic mineral buffer (60% of silica, 14% of alumina, 20% of calcium carbonate and 6% of lithium carbonate) and approx. one-third its weight of pure silver oxide to serve as an internal standard. The spectrum is excited in a 10 ampere d.c. arc and photographed on Eastman IIR plates which are sensitive to near infra-red radiation. Photometric measurements are made, using the line pairs K 7698·98A, Ag 7687·78A and Na 8194·81A, Ag 8273·52A. Calibration curves are prepared by carrying out the procedure on cements of known alkali content. With potassium contents higher than *ca.* 0·7% of potassium oxide, self-reversal of the potassium lines affects the accuracy of the line-intensity measurements. Where visual inspection of the plate shows that some samples have their potassium lines obviously self-reversed, the analysis is repeated with a much lower ratio of cement to synthetic mineral buffer in the arced sample. This, of course, requires the preparation of an additional calibration curve, using the more dilute mixture. For sodium and potassium contents in the range 0·05% to 1·0%, the probable error for the average of duplicate determinations is 4% of the sodium concn. and 5·8% of the potassium concn. For the lithium determination, equal parts of cement and of a mixture of 70% of graphite and 30% of strontium carbonate are used for the arc sample; the lines Li 3232·61A and Sr 3301·74A are used for the analytical calculations. The method for lithium was developed on synthetic standards; of 30 Portland cements examined, using the method, not one showed the presence of lithium, indicating that the concn. did not exceed *ca.* 0·05%. B. S. C.

Polarographic Determination of Vitamin C in Fruits and Vegetables. W. S. Gillam (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 217–221)—Ascorbic acid in fruits and vegetables may be determined by the polarograph in quantities ranging from 4 to 85 μg/ml of soln. Several extracting media were studied, but the only ones which do not interfere with the anodic wave of the ascorbic acid or render the vitamin unstable are oxalic acid and metaphosphoric acid. For oxalic acid extracts, a potassium biphthalate—sodium hydroxide buffer was found most satisfactory, and with metaphosphoric acid, a mixed phosphate buffer. These supporting electrolytes give diffusion currents proportional to the ascorbic acid concn. when the sample is polarographed between −0·1 and +0·3 volt. The instrument may be calibrated by polarographing solns. containing known amounts of crystalline ascorbic acid whose purity has been determined by iodine titration. With a number of fruit extracts the polarographic method shows good agreement with the visual titration and photometric methods, but special precautions have to be taken to obtain reliable results with some extracts, *e.g.*, from dehydrated beets and onions. B. S. C.

Rapid Photometric Determination of Silicon in Low Alloy and Stainless Steels. D. Rozental and H. C. Campbell (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 222–224)—The colorimetric method of Weihrich and Schwartz (*Arch. Eisenhütten*, 1941, **14**, 501) has been adapted for the rapid determination of silicon in low alloy and stainless steels. *Procedure*—To 200 mg of low alloy steel turnings in a 125-ml conical flask, add 10 ml of 3 *N* hydrochloric acid and 10 ml of 3 *N* nitric acid. Warm on a hot plate until just dissolved, remove immediately and allow to cool. Further heating on the hot plate may cause a change in *p*H owing to continued evaporation. For samples of stainless steel, use 10 ml of 3 *N* hydrochloric acid and only 5 ml of 3 *N* nitric acid, dissolve on the hot plate, add a further 5 ml of the nitric acid and heat for 2 to 3 min. Cool the soln., as obtained by the appropriate method, dilute to 100 ml, filter if the steel contains niobium or tungsten, transfer 25 ml to a 100-ml flask and add 5 ml of 10% ammonium molybdate soln. After 6 min. add 10 ml of 2% sodium fluoride soln., mix well, transfer to a 20-mm cuvette and read the transmission to light of wavelength 420 mμ, using a suitable spectrophotometer (such as the Coleman Model 11). The instrument is previously adjusted to read 100% transmission for distilled water in a similar cell. Prepared calibration curves give the required silicon content. The determination takes about 30 min. Chromium is the only element which interferes, and it does so in direct proportion to the % of chromium present; it is thus easier to make this correction by calculation rather than by the use of blank solns. Phosphorus and iron are decolorised by the sodium fluoride. In various general types of steel, silicon can be determined with an accuracy of ±0·04% of silicon without correcting for chromium or of ±0·02% if the correction is made. B. S. C.

Rapid Method of Evaluating Paper Coatings. W. T. Diefenbach (*Paper Ind.*, 1944, **26**, 992–993)—Coatings and laminations have a characteristic fluorescence in filtered ultra-violet light. Non-uniformity of application is apparent as dark patches or streaks, which are invisible in ordinary light. The resistance to penetration by water vapour is the lower for the samples which show a patchy fluorescence. J. G.

Use of Infra-red Heat in Determining Aniline Points. H. T. Hopkins (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 269)—In the A.S.T.M. version (Serial Designation D 611-43T) of the widely-used aniline-point test, alternate heating and cooling of 20 ml of a mixture of equal parts of aniline and the sample in a glass-jacketed test tube equipped with a stirrer is prescribed. Heat is ordinarily supplied by use of a liquid bath or a flame. The insulating effect of the glass air-jacket is troublesome during the heating period because the mixture is likely to be brought to an unnecessarily high temp. and time is wasted in watching for the appearance of a cloud in the liquid. The use of an infra-red heater reduces the time lag almost to zero, and the thermometer reading begins to alter almost immediately after applying or stopping the heat. With experience the range between max. and min. temp. need not exceed 3° C. The rate of heating is conveniently controlled by changing the position of the infra-red bulb, protection of the apparatus from draughts is not necessary, there is more safety and cleanliness and two expts. can be conducted simultaneously with ease. An additional advantage is that the tube may be vacuum-jacketed. The heating devices recommended are infra-red lamps consisting of 260-watt bulbs with reflectors. With these the working time of each test is reduced to two-thirds of that in the older methods of heating. A. O. J.

Importance and Recognition of the Super-Molecular Structure of Cellulose Hydrate Fibres. H. Siebourg (*Zellwolle, Kunstseide, Seide*, 1941, **46**, 215–223)—Cross-sections of various fibres were examined under the microscope after treatment of the whole fibres (after they had received a preliminary soaking in water) for *ca.* 10 min. with a 50% v/v mixture of acetic acid and acetic anhydride. A reference fibre of known origin which has previously been stained (*e.g.*, with Korinth BK or Brilliant Violet BBK) should be included in each test. With viscose rayons the interior dissolves first and disintegration of the external layers follows. With Schwarza, Lanuso and cuprammonium fibres, all portions of the cross-sections are attacked simultaneously. Fibres spun from the more dilute viscose solns. and those which have been subjected to the least degree of stretching dissolve the more rapidly. J. G.

INAUGURAL MEETING OF THE BIOLOGICAL METHODS GROUP

THE Inaugural Meeting of the Biological Methods Group will be held at 6 p.m. on Wednesday, October 17th, at the Chemical Society's Rooms, Burlington House, Piccadilly, London, W.1. Particulars will be sent by post to all members of the Group. The first three-quarters of an hour will be occupied with Group business and confined to members of the Group. At 6.45 p.m. the meeting will be open to all members of the Society and Mr. A. L. Bacharach, M.A., F.R.I.C., will deliver an address entitled:

"Biological Assay and Chemical Analysis."

PHYSICAL METHODS GROUP

A JOINT Meeting of the Physical Methods Group with the North of England Section of the Society will be held at the Engineers' Club, Albert Square, Manchester, on Saturday, October 20th, at 1.30 p.m. The subject will be Polarographic Analysis and the following short papers will be read.

"An Outline of General Principles of Polarographic Analysis," by W. Cule Davies, D.Sc., Ph.D., A.R.I.C.

"Inorganic Applications, with special reference to Aluminium, Magnesium and Zinc," by A. S. Nickelson, B.Sc., A.R.I.C.

"Applications to the Examination of High Purity Selenium, Nickel and Cobalt Compounds," by R. H. Jones, F.R.I.C.

"Biochemical Applications of Polarographic Analysis," by J. E. Page, B.Sc., Ph.D., F.R.I.C.

It is hoped that ample time will be available for discussion of these papers.

Facilities will be available for a limited number of members to take lunch together in the Engineers' Club at 12.30 p.m., at a charge of 3s. 6d. each. Members wishing to do this should please advise Mr. Arnold Lees, 87, Marshside Road, Southport, before October 6th.

ADVICE TO AUTHORS

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

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

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

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

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

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

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

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

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

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

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

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

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

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

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

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

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

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

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

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

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